Raised intracranial pressure and tissue compliance after large intracerebral hemorrhages in animal models and patients

by

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Abstract

Brain injuries that cause mass effect, including stroke and traumatic brain injury, can increase intracranial pressure (ICP) due to the limited space within the skull. Intracerebral hemorrhage (ICH) is a subtype of stroke in which there is bleeding in the brain. In cases of large bleeds, the bleed and the associated swelling can cause ICP to increase to dangerous levels, possibly leading to brain herniation and/or death. Cranial contents can undergo compliance (a decrease in volume after being subject to the force of increased ICP), but this compliance is limited. Treatments to reduce ICP have mixed effectiveness, and are often not enough to compensate for large bleeds. Animal models are often used to study ICH, and although this is an important way to study ICH pathophysiology and potential treatments, high quality animal research requires an awareness and mitigation of translational problems where possible. In our rodent models, we have found that when ICP is increased after large bleeds, animals can compensate for this bleed by reducing the volume of uninjured brain tissue. Neurons and astrocytes in uninjured regions shrink by up to 50% and pack more densely together. In this thesis, we studied ICP and tissue compliance after ICH with a focus on research translation.

Chapters two and three were both focused on research translation. Chapter two reviewed and summarized current studies on ICP after ICH, including pathophysiology and treatment of increased ICP. We focused on translationally relevant considerations, including model choice, ICP measurement methodology, and ICP analysis. This review highlighted the need for more ICP research in the ICH field. The next chapter examined the use of isoflurane anesthetic in preclinical ICH research. Most ICH patients undergo ICH while conscious, yet almost all animal studies induce ICH under anesthetic for ethical reasons. Anesthetics can impact physiology, including temperature and blood pressure, and can exert neuroprotective effects. In this study, we induced a collagenase ICH in conscious animals using a cannula system. We found that the effects of isoflurane (reduced temperature, reduced blood pressure,

increased blood glucose) were largely transient and normalized quickly after surgery. In cases of large ICH, isoflurane did not affect hematoma volume, lesion volume, or functional deficits.

The following experiment tested the effect of glibenclamide, a sulfonylurea receptor antagonist, on edema after ICH. We hypothesized that this ion channel blocker would prevent cellular edema by preventing ion influx, which would then reduce mass effect. We planned this experiment in advance in the hopes of reducing bias, we used large sample sizes, pre-planned statistical analysis, and published all data with the manuscript. Contrary to our hypothesis, we found that glibenclamide did not reduce edema, lesion volume, or functional deficits.

Chapters five and six examined tissue compliance in translationally relevant rodent models of ICH. Previously, all studies of tissue compliance, despite using different injury models, had all used young, healthy mice. Therefore, we wanted to see if tissue compliance could be observed in a rodent population that better modelled the clinical population of ICH patients. We assessed hematoma volume, edema, ICP, and functional deficits in young and aged spontaneously hypertensive rats (SHRs). We then quantified contralateral hemisphere volume, cortical thickness, and cell volume and density in regions known to undergo tissue compliance. We found that tissue compliance was present across strains, and did not seem to be impaired by hypertension. This finding replicated in aged animals, where we again saw evidence of tissue compliance. These aged SHRs had lower ICP, smaller hematoma volumes, less edema, and a reduced mortality rate compared to young SHRs given the same amount of collagenase. Future research should explore the reasons behind this and exercise caution when using the collagenase model to study ICH in aged animals.

In the final experiment, we retrospectively analyzed CT images of ICH patients stratified by bleed volume at 24 hours post-ICH. We quantified brain tissue and CSF volumes and found that those with larger bleeds had reduced brain and CSF volume after taking gender into account. This preliminary study helps establish the methodology and justify a future clinical trial to characterize tissue compliance in people.

In this thesis, we have demonstrated data to reduce translational concerns regarding the use of anesthetics in preclinical models. Glibenclamide, a sulfonylurea receptor inhibitor, was studied in an attempt to reduce edema and mass effect, but was unsuccessful. We have demonstrated that tissue compliance is evident in aged and hypertensive animal models of ICH, which better represents the clinical ICH population. This work justifies future studies of tissue compliance, as there is still a lot to learn. For example, studies should examine the functional consequences and negative effects of these drastic and transient cellular changes. Additionally, adequately powered clinical trials should investigate tissue compliance in patients using both imaging and neuropathological techniques.

Preface

This thesis is an original work. Experiments were granted research ethics approval by the University of Alberta Biosciences Animal Care and Use Committee ("Rodent Stroke Studies," Animal Use Protocol #960) for the duration of all thesis work.

Chapter 2 of this thesis has been published as Wilkinson C, Kung T, Jickling G, Colbourne F, "A translational perspective on intracranial pressure responses following intracerebral hemorrhage in animal models" in the journal Brain Hemorrhages (2021), volume 2, issue 1. Wilkinson C drafted the initial manuscript to fulfill the candidacy qualification requirement. All authors were involved in the writing and editing of the manuscript prior to submission.

Chapter 3 of this thesis has been published as Wilkinson C, Kalisvaart A, Kung T, Maisey D, Klahr A, Dickson C, Colbourne F, "The collagenase model of intracerebral hemorrhage in awake, freely moving animals: The effects of isoflurane" in the journal Brain Research (2020), volume 1728. Wilkinson C was responsible for experimental design, data collection, data analysis, and manuscript writing. Kalisvaart A was involved in experimental design, data collection, and manuscript writing. Kung T and Maisey D were involved in data collection and manuscript editing. Klahr A and Dickson C assisted with the collection and analysis of EEG data as well as manuscript editing. Colbourne F was the supervisory author and was involved with experimental design, data analysis, and manuscript writing.

Chapter 4 of this thesis has been published as Wilkinson C, Brar P, Balay C, Colbourne F, "Glibenclamide, a Sur1-Trpm4 antagonist, does not improve outcome after collagenase-induced intracerebral hemorrhage" in the journal PLOS One (2019), volume 14, issue 5. Wilkinson C was responsible for experimental design, data collection, data analysis, and manuscript writing.

Brar P and Balay C assisted with data collection and manuscript editing. Colbourne F was the supervisory author and assisted with experimental design, data analysis, and manuscript writing.

Chapters 5-6 of this thesis will be published as Wilkinson C, Kalisvaart A, Kung T, Abrahart A, Khiabani E, Colbourne F, “Tissue Compliance and Intracranial Pressure Responses to Large Intracerebral Hemorrhage in Young and Aged Hypertensive Rats.” All authors assisted with experiment conceptualization and data analysis. Wilkinson C and Kalisvaart A were responsible for data collection.

Dedication

This thesis is dedicated to my parents, Kent and Katia Wilkinson, and my incredibly supportive partner, Chris.

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List of Abbreviations

ANOVA	Analysis of Variance
AWB	Autologous Whole Blood
BBB	Blood-Brain Barrier
BP	Blood Pressure
CA1	Cornu Ammonis-1
CBF	Cerebral Blood Flow
Cl	Chloride
COL	Collagenase
CPP	Cerebral Perfusion Pressure
CSF	Cerebral Spinal Fluid
CT	Computed Tomography
DOACs	Direct Oral Anticoagulants
EEG	Electroencephalogram
Fe	Iron
Gd	Gadolinium
HTS	Hypertonic Saline
ICH	Intracerebral Hemorrhage
ICH-ADAPT	Intracerebral Hemorrhage Acutely Decreasing Arterial Pressure Trial
ICP	Intracranial Pressure
ICP-MS	Inductively Coupled Plasma Mass Spectrometry
INR	International Normalized Ratio
ISO	Isoflurane
IVH	Intraventricular Hemorrhage
K	Potassium
MAP	Mean Arterial Pressure
MCAO	Middle Cerebral Artery Occlusion
MRI	Magnetic Resonance Imaging
Na	Sodium
NDS	Neurological Deficit Scale
NO-ISO	No Isoflurane
OR	Odds Ratio

RGS	Rodent Grimace Scale
RMS	Root Mean Squares
SAH	Subarachnoid Hemorrhage
SHR	Spontaneously Hypertensive Rat
SDR	Sprague Dawley Rat
STICH II	Surgical Trial in ICH II
Sur1-Trpm4	Sulfonylurea Receptor 1-Transient Receptor Potential Melstatin 4
SWS	Slow Wave Sleep
TBI	Traumatic Brain Injury
tPA	Tissue Plasminogen Activator
VISTA ICH	Virtual Trials Archive Intracerebral Hemorrhage
WKR	Wistar Kyoto Rat

Chapter 1:
General Introduction

1.1 Introduction to Stroke

Stroke is a type of brain injury characterized by either a lack of blood supply in the brain (ischemic stroke), bleeding in the brain **(ICH, intracerebral hemorrhage)**, or bleeding in the subarachnoid space **(SAH, subarachnoid hemorrhage)**.[1] Stroke is a devastating injury with high rates of morbidity and mortality, and is a leading cause of disability.[2,3] Within minutes of blood supply being interrupted, various damage mechanisms begin. Cellular injury occurs via many forms of damage, including interrupted energy supply, inflammation, **blood-brain barrier (BBB)** damage, iron toxicity, and others.[4–6] Since stroke is an acute event, there is a need for therapies that limit injury in the hours to days after the event. However, there is no current treatment for intracerebral hemorrhage beyond medical management.[7] Thus, there is great interest in finding a neuroprotective agent that can prevent damage to neurons or boost neuronal repair processes, leading to improved recovery after stroke.

1.2 ICH Epidemiology

In Canada, approximately 62, 000 people have a stroke each year, and intracerebral hemorrhage represents ~10-15% of these cases.[8,9] Primary intracerebral hemorrhage has two causes, hypertension and amyloid angiopathy.[7] Beyond primary causes, secondary intracerebral hemorrhage can be caused by tumours, coagulopathy, and vascular abnormalities (including aneurysms). Hypertensive ICH often leads to non-lobar bleeds, due to the structure of the lenticulostriate arteries.[10] With chronic and uncontrolled hypertension, arteries often undergo degenerative changes, such as atherosclerosis, where vasculature becomes brittle and bleeds become more frequent.[11] In cases of amyloid angiopathy, vasculature is weakened with deposition of β-amyloid protein, leading to increased risk of bleeding.[12] Amyloid angiopathy typically causes lobar bleeding, as cerebral amyloid angiopathy is most commonly observed in cortical vessels and is much less common in the basal ganglia and thalamus.[10,13] Additionally,

multiple demographic and lifestyle factors increase an individual's risk of ICH, which can be divided into non-modifiable, semi-modifiable, and modifiable factors.[14,15]

1.2.1 Non-modifiable Risk Factors

Non-modifiable factors include age, race, and sex. For example, age is the strongest non-modifiable risk factor for stroke.[16] Specifically, incidence of ICH increases with age, the average first-ever stroke occurs at 70 years of age,[17] with ~30% of ICH patients above the age of 80.[18,19] As age increases, the risk of mortality from stroke also increases approximately 5% for each year increase in age.[20,21] Older patients have increased risk of cerebral amyloid angiopathy, hypertension, and atherosclerosis, which can increase both the incidence rate of stroke as well as the initial hematoma volume, leading to worse outcome.[22,23] Risk of stroke is also associated with race. Black and Hispanic individuals have a worse incidence rate of stroke when compared to White individuals.[24] This is believed to be related to the differing rates of hypertension, as well as differential access to healthcare, which can affect risk factors such as diabetes, diet, and lifestyle.[25] Additionally, genetic markers have been identified that may contribute to increased risk of stroke in Asian populations.[26] Specifically, the risk of ICH is increased among Asian populations, and in addition to genetic factors such as a TNF-α polymorphism leading to increased TNF-α levels,[27] the increased rates of diabetes and hypertension observed in this population contributes to greater ICH risk.[28] Sex is another non-modifiable risk factor. Pre-menopause, women typically have a lower stroke risk than men.[16] The cytoprotective effects of estrogen on the cardiovascular system are believed to contribute to this effect.[29] However, estrogen also increases the risk of clotting, and thus, those taking estrogen prescriptions and pregnant women are at increased risk of stroke.[16,29] After menopause, the incidence rate of stroke in women becomes similar to men.[16,30] In later years, women have a higher rate of stroke than men, largely due to the longer expectancy of women.[31] Importantly, many risk factors

interact to differentially affect stroke risk. As previously mentioned, sex effects will vary with age.[30] Although these factors are considered to be non-modifiable, with further research into the mechanisms causing increased risks, treatments could be found to mitigate these risk factors.

1.2.2 Modifiable Risk Factors

Important modifiable risk factors for ICH include smoking, alcohol use, and physical activity.[15] Smoking significantly increases the risk of ICH, particularly lobar ICH.[32] Although some studies suggest nicotinic receptor agonists may be neuroprotective,[33] smoking can acutely increase blood pressure and therefore increase risk of ICH, but smoking does not significantly impact ICH outcomes.[34] Alcohol has a direct linear relationship with risk of intracerebral hemorrhage, where heavy alcohol consumption (e.g., >5 drinks/day) greatly increases risk,[35] but even moderate alcohol consumption (<2 drinks/day) can moderately increase risk.[14,36,37] Alcohol use is often associated with hypertension and generally poor blood pressure regulation.[14] As little as one alcoholic drink per day is associated with decreased brain volume, indicating neuronal loss and/or brain atrophy.[38] On the other hand, physical activity, including leisure activity and active commuting time, is associated with decreased stroke risk.[39] Sedentary behaviour is associated with increased blood pressure and increased risk of diabetes, which likely contributes to the relationship between exercise and stroke risk.[14]

1.2.3 Semi-modifiable Risk Factors

Semi-modifiable risk factors include comorbidities such as diabetes, hypertension, and hypercholesteremia, that can be managed with medical treatment. The single most important risk factor for ICH is hypertension, which leads to vascular damage and a significantly increased risk of bleeding.[40,41] The vasculature undergoes extreme changes when subjected to chronic hypertension, including hypertrophy.[42] With hypertrophy, artery walls thicken and the vascular lumen decreases in size, which can reduce physical stress on the vasculature and protect

cerebral vessels.[43] However, hypertrophy can also limit the ability of the vessel to dilate and the smaller lumen diameter can lead to hypoperfusion.[44] Despite hypertrophy increasing resistance to physical stress, the high blood pressure due to chronic hypertension greatly increases physical stress on the vasculature, which can lead to vascular wall damage and vessel rupture. When cerebral vessels rupture, this causes ICH. Controlling hypertension with pharmacological treatments, such as antihypertensives, can significantly reduce the risk of stroke and other cardiovascular events (OR = 0.79).[45] Similarly, cholesterol levels can affect stroke risk. High blood cholesterol can increase plaque deposits in arteries, causing them to narrow and stiffen, becoming more susceptible to injury and increasing the chance of blood clots.[46] Dyslipidemia, specifically high low-density lipoprotein levels/low high-density lipoprotein levels, can significantly increase stroke risk.[14,47] Interestingly, some studies suggest an inverse correlation between total cholesterol levels and hemorrhagic stroke risk, with the risk of hemorrhagic stroke increasing as cholesterol levels decrease.[14] Diabetes mellitus is another independent risk factor for stroke.[14,36,47] Death from stroke is the cause of ~20% of diabetic deaths.[14] In addition to impaired glucose metabolism and irregular blood glucose levels, people with diabetes often have increased rates of atherosclerosis, hypertension, and abnormal blood lipid levels, all of which contribute to the increased risk of stroke.[36] Medical treatment and lifestyle modification can reduce stroke risk in diabetics, but glycemic control alone does not confer the same risk reduction.[14] Another important risk factor for stroke incidence and stroke outcome is coagulopathy.[48,49] **International normalized ratio (INR)** is a standardized measure of the time it takes blood to clot, with numbers below 1 indicating faster clotting time than normal and numbers above 1 indicating greater bleeding than normal, and an increased risk of hemorrhagic stroke.[50] Anticoagulants are often used to treat atrial fibrillation and reduce risk of thrombotic events, including stroke.[14] **Direct oral anticoagulants (DOACs)** are anticoagulants with lower risk of bleeding events and a more favourable safety profile when compared to warfarin, a vitamin K antagonist that was formerly a front line treatment for atrial fibrillation.[51,52]

Approximately 20% of intracerebral hemorrhagic strokes are associated with anticoagulants,[53,54] although this rate may change as more physicians prescribe DOACs over warfarin.[55] A key factor in medical management of ICH is reversing anticoagulant treatment and reducing the risk of continued bleeding.[56]

1.2.4 Prognosis

Intracerebral hemorrhage has an approximately 40% mortality rate at 30 days and 45% at one year.[57] Independent predictors of mortality include age, initial Glasgow Coma Scale score, comorbidities (such as diabetes, etc.), and ICH volume and location.[58,59] For example, each 10% increase in hematoma volume increases the odds ratio of mortality by 5%.[60] Additionally, for each absolute mL increase in hematoma volume, patients are approximately 7% more likely to not achieve independence.[60] The average hematoma volume is ~ 30 mL in patients.[61] Typically, hematoma volumes of less than 25-30 mL are considered more likely to have a good outcome, and > 30 mL is often associated with a poor outcome (e.g., mortality, persistent functional impairment, modified Rankin scale score of 4-6, which denotes severe disability or death).[60] In Canada, the mortality rate of ICH is ~40%, which has been decreasing over the past few decades.[9,62] Hematomas closer to the brain stem (including pontine and cerebellar hemorrhages) and hematomas that extend into the ventricles are more likely to lead to death.[63] The 30 day mortality rate for infratentorial hemorrhages is approximately 50%.[63] Smaller hemorrhages, particularly in supratentorial regions, are more likely to have a better recovery.[64]

1.3 Pathophysiology of Intracerebral Hemorrhage

Mechanical damage from the blood tearing through brain tissue is often referred to as primary injury.[65] Subsequent injury from the bleed, such as edema, BBB damage, inflammation, and iron toxicity, are considered secondary injury (Figure 1-1).[5,56,66,67] Both primary and secondary damage can cause cell death and impair brain function.[68–71] With primary damage,

the mechanical injury from the force of the bleed can damage neurons and glial cells, interrupting their function, and physically damaging the BBB.[72] This mechanical damage can also tear through blood vessels, leading to additional bleeding and interrupting the blood supply to the brain and potentially leading to ischemic damage. Treatments attempting to limit primary injury, such as blood pressure reduction (evidence level C – low quality or minimal evidence),[73,74] correcting coagulopathy (evidence level B – moderate quality evidence),[75–77] increasing clotting factors (no clear evidence of benefit),[78–80] and tranexamic acid (no clear evidence of benefit),[81] are being studied with mixed results. One major issue with these treatments is timing, as studies are now suggesting that patients benefit most when treatment is delivered within two hours of injury onset, which can often be difficult to achieve in clinical practice.[82] For instance, the time to diagnosis and sending patients to an appropriate hospital (e.g., primary stroke centres) is an impediment to early intervention. Thus, methods to assess stroke type and severity, such as stroke ambulances[83] or portable diagnostic equipment (e.g., electroencephalogram devices[84]), could help improve treatments that limit primary injury.

Secondary injury begins in the minutes to hours after injury and can persist for weeks.[85] Secondary injury is multi-faceted, with many mechanisms interacting and worsening overall injury. For example, edema can worsen BBB damage, which can in turn lead to increases in edema as well as increased infiltration of inflammatory cells and signaling molecules.[6,66] Due to the extended timing of secondary injury, these mechanisms are often targeted for both acute and chronic treatment interventions. These interventions, such as anti-inflammatory agents, aim to reduce cell death and injury from secondary damage and improve functional outcome. Many treatments aiming to reduce secondary injury have demonstrated success in animal models, but thus far, none have translated to success in phase 3 clinical trials.[86] Mechanisms of secondary injury will be discussed in greater detail in section 1.6. Despite the multi-faceted nature of secondary injury, treatments often only target one mechanism of injury. Many speculate that combination treatments, or agents targeting multiple mechanisms, such as hypothermia,[87,88] are

more likely to see success in reducing brain injury and improving functional outcome,[89,90] although combination therapies often increase the risk of side effects.

1.4 Preclinical Stroke Research: Animal Models

Preclinical intracerebral hemorrhage research primarily employs two models of ICH, the **collagenase (COL)** model and the **autologous whole blood (AWB)** model (Figure 1-2).[91–93] The COL model involves injection of bacterial collagenase into the brain, which breaks down the basal lamina of endothelial cells and causes bleeding in the region of injection.[92] In the AWB model, peripheral blood, often from the femoral or tail vein/artery, is injected into the brain.[93] Other, less frequently used ICH models include the micro-balloon model,[72] injection of an inert substance,[94] and injection of blood components (e.g., thrombin, iron, etc.).[67,71] These models are often used to assess isolated effects of a particular component of ICH, such as mass effect or blood component toxicity. However, these models are less representative of clinical ICH and the interactions between different processes. For example, the micro-balloon model can be used to assess mass effect and ICP increases, but does not account for the combined effects of increased ICP and blood components on cell death.

The AWB model and the COL model each have unique strengths and weaknesses. The COL model causes bleeding over the course of hours,[92,95] which is also seen during clinical ICH.[91,96] For instance, approximately 30% of ICH patients will experience hematoma expansion, which is believed to occur due to secondary rupture of blood vessels surrounding the initial hematoma region.[97] However, injection of a foreign substance into the brain can lead to an exaggerated immune response, which may impede clinical translation if the immune response observed differentially affects injury and response to treatments.[98] However, studies have shown that *in vitro*, collagenase itself does not directly lead to neuronal inflammation or cell death.[99,100] The COL model typically leads to hematomas that are spherical in shape, similar to what is seen clinically,[101] with more blood-brain barrier permeability, edema, and inflammation

compared to the AWB model.[91,98] The AWB model avoids this problem as it does not involve injection of any exogenous components. However, the AWB model delivers the blood over the course of minutes, and there is little prolonged bleeding or hematoma expansion.[91] Additionally, it is difficult to induce very severe ICHs with the AWB model, due to technical limitations with blood infusions (e.g., size of the Hamilton syringe, needing to inject the blood before it clots, having blood backflow up the needle track), and therefore a subset of clinical ICHs can be difficult to model.[102] For example, even large volumes of blood (e.g., 160 μL in rat) do not lead to severe injury and mortality.[102] In rats, lesions of <30 mm^3, or <2% percent of brain volume, can be considered mild, whereas >60 mm^3 is considered severe (compared to the average hematoma volume in humans of ~20-30 mL, or ~2% percent of brain volume, and >60 mL is considered severe clinically).[90,103,104] Additionally, rodents do not typically exhibit mortality as high as humans for comparable insults,[105] often surviving comparatively very large bleeds.[102] The reason for this is unknown, but is likely due to the widespread use of young, healthy animals, in addition to other factors, such as species differences in response to large bleeds.

Beyond different surgical models of ICH, there are also different rodent strains that can be used in ICH research. One of the most common rodent strains used in biomedical research are Sprague Dawley rats.[106] However, when modelling co-morbidities, other rodent strains can be used, including the **spontaneously hypertensive rat (SHR)** and the **stroke-prone spontaneously hypertensive rat (SP-SHR)**.[107,108] These animals are bred to have chronically elevated blood pressure, modelling hypertension. Therefore, these rats are expected to have similar vascular abnormalities to hypertensive patients and have a more comparable profile of primary and secondary injury (e.g., more severe neurological deficits,[109] larger inflammatory responses,[110] greater chance of hematoma expansion due to atherosclerosis[111]). Spontaneously hypertensive rats were bred from the **Wistar Kyoto (WKY)** strain of rat, and thus, WKY rats are often used as controls in experiments with SHRs. However, since WKY rats were not fully inbred, they can have much greater variability than inbred strains such as the Sprague Dawley

rats.[112] In the SP-SHR strain, rodents will spontaneously experience strokes.[113] While this is similar to the clinical reality, the spontaneous and unpredictable nature of the injury makes this model impractical for many studies, including studies on neuroprotective treatments. Genetic strains of hypertensive rats are only one way to model hypertension. Acute hypertension can be modelled pharmacologically, using hypertensives such as angiotensin II or a nitric oxide inhibitor such as L-NAME.[114] Dietary interventions, such as a high sodium diet, can also induce hypertension, although this can be much more variable.[115,116] Finally, hypertension can also be induced surgically by unilaterally constricting the renal artery, impairing renal autoregulatory capacity while leaving a functioning kidney to prevent salt and water retention.[116]

1.5 Translational problems in preclinical stroke research

1.5.1 Animal Research

The quality of animal research is a growing concern for both ethical and scientific reasons. Many reviews and guidelines have been published to call for better experimental design, improved standards of reporting, and overall increased research quality.[117–120]

Studies must be planned with a consideration for internal and external validity. A study with internal validity uses appropriate methodology and experimental design such that the research question will be answered, and external factors (e.g., confounding variables) are not likely to influence the observed relationship. Internal validity is threatened when there is a high level of bias, such as when there is a lack of blinding.[121] Conversely, external validity refers to the generalizability of findings. If the sample was randomly selected from an appropriate population, findings can be generalized to that population. If studies frequently exclude participants (e.g., excluding rodents that die from a stroke), or the selection criteria is narrow (e.g., only using young, male rats), the lower the external validity will be.

Study quality can also be improved greatly by taking steps to minimize bias wherever possible.[122] For example, randomizing group assignment and performing behavioural

testing/data analysis without knowing group assignments are two easy ways to reduce the potential for bias.[123] Data shows that ICH[124] and ischemic stroke[125] experiments that do not report allocation concealment find larger effect sizes of treatment than those who properly blind researchers to treatment status. However, in some cases, such as hypothermia, it is not possible to keep the treatment group concealed, and in these cases, it is especially important that other measures are in place to minimize bias.

One suggestion for improving replicability of studies is to introduce measures of standardization between labs.[126] For example, if researchers follow the same standard operating procedures for surgeries, histology, behavioural testing, etc., it will be much simpler to compare results between research labs. As it stands now, slight differences in procedures (e.g., differing anesthetic use) can lead to different results. Performing multi-centre preclinical studies is another suggested measure for improving study quality.[126,127] These studies could have a centralized randomization and data centre, which will minimize bias during experimentation. Fortunately, analysis shows that despite translational failures, preclinical studies do predict stroke pathophysiology and clinical outcomes.[128] Importantly, well designed and robust preclinical studies should better predict therapeutic efficacy.

1.5.2 Translational Research

Despite hundreds of successful neuroprotectants identified in preclinical stroke research, none have translated to clinical success.[129] Still, many of these neuroprotectants have never been tested clinically, and some are still currently under investigation in clinical trials.[130] Of the known translational failures, often multiple factors are suspected to play a role, including experimental design (e.g., lack of appropriate modelling, randomization, blinding), analysis (e.g., errors in statistical methodology), and reporting (e.g., positive publication bias, omitting data).[126,131,132] There are many considerations to be made and steps to be taken when designing and undergoing preclinical research in order to maximize the chances of successful translation.

Modelling considerations are important when designing a translational study. Thought needs to be given about the clinical population that is being modelled, and how our animal models can best align with this population. For example, as discussed in section 1.2, many stroke patients are elderly and have comorbidities, such as diabetes and hypertension, and roughly half of these patients are female.[19] However, many researchers exclusively use young, healthy, male rodents to model this patient population.[131] For example, a recent review found that less than 5% of stroke neuroprotection studies used rodents older than 12 months of age, and ~3% used female animals.[131] The majority of animals used were healthy, and ~5% used animals with comorbid conditions. Therefore, when demonstrating that a treatment will be efficacious in clinical trials, researchers should first determine if treatment efficacy is affected by age, sex, or common comorbidities. This will ensure the treatment is tested in the appropriate patient populations. For example, in ischemic stroke, studies show that treatments that work in healthy rats fail in those with comorbidities.[133,134] Further, therapeutics may interact with drugs taken to treat these comorbid conditions, and yet, this is rarely tested.

An important experimental design consideration is the choice of surgical anesthetic. As most people have strokes while conscious, it is important to consider how using anesthetics might interfere with animal physiology during a stroke, and subsequently, the functional outcome and recovery. Both volatile (e.g., isoflurane) and injectable (e.g., ketamine) anesthetics can affect physiological parameters (e.g., temperature, blood pressure, cerebral blood flow, etc.) that can impact ICH outcome.[135–137] Additionally, evidence suggests some anesthetics can have neuroprotective effects.[138,139] Thus, it is important to include sham surgeries as controls, carefully consider the effects of a chosen anesthetic, and examine interactions between anesthetics and therapeutics.

Additionally, it is important to use an appropriate model of stroke.[91,140,141] For example, which model of stroke will best represent the clinical population of interest? If researchers are interested in modelling severe hemorrhages with substantial edema and **intracranial pressure**

(ICP) increases, the COL model might be preferred over the AWB model.[91] Using injection of blood components, such as thrombin, would be more appropriate for researchers interested at looking at the effects of blood components in isolation.[71] Other important model considerations include stroke severity and location. Often, preclinical studies choose one severity and location for the injury, often the striatum, and comparatively, 25-50% of human ICHs affect the basal ganglia.[142,143] Yet, clinically there is great heterogeneity in stroke presentations.[144] Functional outcome differs depending on the location of the ICH,[145–148] and it is possible that treatments may not have equal efficacy at all ICH locations, although this is rarely considered in animal studies. Additionally, complications may vary by stroke location (e.g., infratentorial ICH is associated with subjective pain).[145] Finally, experimental design and methodology should be carefully reviewed to ensure there is adequate internal and external validity of the experiment so that appropriate conclusions can be drawn.[149]

1.5.3 Statistical Considerations.

Appropriate statistical analyses are needed to ensure data is being interpreted and presented correctly. Many peer-reviewed papers use improper statistical analysis, including using parametric analyses on non-parametric data and using an **analysis of variance (ANOVA)** even when the assumptions are violated.[119,122] The fact that these studies are published after receiving multiple reviews indicates a multi-level problem with statistical knowledge. Ideally, researchers would be able to consult statisticians to assist with data analysis, but this can be cost-prohibitive. Most researchers have some statistical training, yet many are not proficient in statistics and maintain misperceptions (e.g., that the p-value represents the probability of the result being false, that a non-significant p-value means the null hypothesis is true, etc.).[150] Overreliance on p-values without proper understanding can lead to misinterpretation of results. Instead, it may be better to focus on the meaning of results (e.g., effect sizes, implications) rather than a sole focus on statistical significance.[151] Another potential

consideration is to include a statistical review by a statistician or a methodologist as part of the peer review process.[152] Finally, studies need to be fully reported and published, even when some or all findings are null.[153] Often, it can be difficult for researchers to publish negative findings, as they can be seen as less “impactful” or not believed if these findings are contrary to previously published positive findings. Other times, researchers could choose to leave out results that do not fit with their hypothesis or the rest of the findings. Finally, “p-hacking” is a known analysis problem, wherein researchers perform multiple statistical tests on the same data and choose the test that gives them the preferred conclusion.[154] The above situations add bias to the literature, can lead to overstated efficacy, and hurts the chances of translational success.[153] When assessing if a therapy is useful and should be studied clinically, it can be valuable to perform a meta-analysis of current data. However, if negative studies are not published, this will skew the findings of any additional analyses.

1.6 Secondary injury

Secondary injury processes after ICH lead to cell death and contribute to lasting functional deficits.[155] The mechanisms of secondary injury are diverse, and can both independently cause damage as well as interact to worsen the damage of each individual mechanism. The extent of secondary injury can vary by model in animal studies, and due to the lack of studies comparing animal models to clinical findings, it can be difficult to determine which models are most accurate.[91,98] Models should be selected based on the mechanisms being studied and the clinical relevance. For example, studies interested in ongoing bleeding and hematoma expansion should choose the COL model over the AWB model.

The BBB is important in maintaining brain homeostasis and keeping toxic substances out of brain tissue.[156] During ICH, the mechanical force of the blood shears through tissue and damages the BBB, impairing its function. Beyond primary injury, secondary injury processes, including edema and inflammation, can further damage the BBB, including endothelial cells,

astrocytes, and tight junctions, increasing BBB permeability.[156,157] In the peri-hematoma zone, the damage to the BBB is considerable. However, distal regions also show increases in BBB permeability, including the contralateral hemisphere, possibly due to inflammation or angiogenesis.[158] The increases in BBB permeability begin to resolve in the days after ICH in animal models, but in some animals, increased BBB permeability can persist for weeks.[158] Persistent BBB permeability has been noted clinically in ischemic stroke[159] and is believed to exist after ICH,[156] although there is less data in the chronic stages after ICH. In animal studies, the permeability of the BBB is often imaged and quantified using contrast agents or dyes that cannot normally cross the BBB, such as Evans blue or gadolinium.[91,158] The COL model leads to greater BBB permeability than the AWB model.[91] Damage to the blood-brain barrier can allow both inflammatory cells and water into the brain, thereby worsening inflammation and edema.

Edema, or brain swelling, occurs in the minutes to hours following ICH and can persist for days.[160] For comparison, in people, edema after ICH peaks around 1-2 weeks and takes longer to resolve.[161] Edema is commonly divided into two types: cytotoxic and vasogenic. Cytotoxic edema is known as cellular swelling, where water enters neurons and glial cells.[162] This is often due to improper concentration gradients (e.g., excess sodium ions within neurons), which lead to movement of water into cells by osmosis. Concentration gradients can fail due to ischemic damage[160] resulting from hypoperfusion as well as ion dyshomeostasis,[158] which may be caused by BBB damage and blood components in the hematoma. However, evidence for ischemic injury after intracerebral hemorrhage is limited.[102,163,164] Vasogenic edema refers to water entering the extracellular space in the brain, often resulting from BBB damage.[165] Excess water in the brain can also contribute to mass effect, which can increase intracranial pressure (see: 1.7).[166] In animal models of ICH, a substantial portion of edema is derived from blood plasma in the hematoma as the clot retracts.[102,167] The proportion of hematocrit in blood is typically ~45%, but after clot retraction, the hematoma is ~90% hematocrit, reflecting the serum extrusion from the hematoma.[168] In animal studies, brain water content (often using the wet

weight-dry weight method) is typically used as a measure of brain edema. In people, edema is often measured using imaging, specifically **computed tomography (CT)** or diffusion weighted imaging.[169] The COL model typically causes increased edema compared to the AWB model, where the majority of brain water content is located within the hematoma, rather than the peri-hematoma zone.[91,102] In both ischemic stroke and ICH, edema can lead to life-threatening increases in ICP.[165,170] Thus, treatments to acutely reduce edema are being investigated. Diuretics, such as bumetanide, have the potential to reduce brain water content, but results have been mixed.[171–173] Glibenclamide blocks the Sur-1 Trpm-4 channel, which is an ion channel linked to edema formation.[174] Early preclinical and clinical studies have shown promise in using glibenclamide to treat edema after severe ischemic stroke.[175–177] Further work is warranted, particularly to determine if glibenclamide is efficacious after ICH.

The combination of a permeable BBB and movement of water through tissue as the brain swells can contribute to impaired ion concentrations. Normally, the ionic environment of the brain is tightly regulated, as proper neuronal functioning depends on specific ionic gradients.[178] After ICH, we see that ion concentrations are altered, and the severity of impairment is related to distance from the hematoma.[158,179,180] Beyond ion movement associated with edema, the hematoma itself, which has different concentrations of key ions, including sodium, likely contributes to this ion dyshomeostasis in the brain. There is a clear gradient seen, with ion dyshomeostasis becoming less severe as distance from the hematoma increases.[158] The functional implications of ion dyshomeostasis are unclear, and the effect likely varies at different distances from the hematoma along with the concentration gradients. Pharmacological attempts to inhibit ion channels to restore homeostasis have been largely unsuccessful.[173,181]

Even after primary mechanical damage, the blood components themselves are toxic and lead to secondary injury.[182] For example, thrombin itself causes neuronal atrophy and cell death when injected into the brain.[71] Thrombin increases neuronal excitability, leading to seizure activity, disrupts tight junctions, leading to BBB injury, and increases the activity of reactive

oxygen species, contributing to cell death and sub-lethal cellular injury.[71,183,184] Hematoma resolution begins in the days following ICH, and involves red blood cell lysis and phagocytosis by immune cells.[185] Clinically, density changes on CT scans indicate hematoma resolution largely starting at 5-14 days post-ICH and continuing in the days to weeks after.[186] In animal models, we see hematoma resolution begin more quickly, starting at ~3 days post-ICH after the AWB model[185] and between 3-7 days after the collagenase ICH model.[187] Hematoma clearance can persist for weeks after the ICH.[179] Red blood cell lysis releases blood-breakdown products, including hemoglobin, which is further broken down into bilirubin and iron.[188] Cell death is partly attributable to Fenton reactions, where iron reacts with oxygen intermediates, including hydrogen peroxide, leading to free radicals and oxidative stress.[189] The accumulation of free radicals quickly overwhelms endogenous anti-oxidants, such as superoxide dismutase. These free radicals damage cellular components, including deoxyribonucleic acids, proteins, and lipids, leading to cell death. Additionally, iron can cause cell death through ferroptosis, which is cell death dependent on iron and is characterized by the accumulation of lipid peroxidases.[190]

Inflammation is a complex form of secondary injury that can be both harmful and beneficial.[191,192] In response to blood components (red blood cells, thrombin, leukocytes, etc.) in the brain, inflammatory cells immediately respond and begin to accumulate at the site of injury.[193] Microglia, the resident immune cells of the central nervous system, are believed to be the earliest responders. Microglia release cytokines, such as IL-1, IL-6, and TNF-α, which leads to the accumulation of circulating peripheral inflammatory cells, including T-cells, leukocytes, and macrophages.[193] The accumulation of inflammatory cells can cause excess damage. Inflammatory cells produce free radicals and reactive oxygen species that can be lethal to neurons. Further, proinflammatory mediators, such as chemokines and cytokines, can further contribute to damage and increase BBB permeability.[194] Conversely, the inflammatory response can also be beneficial. Macrophages and microglia help remove waste, including dead neurons and blood breakdown products.[192] Microglia can also promote neurogenesis and angiogenesis,

aiding in recovery. One study demonstrated that minocycline, an anti-inflammatory treatment, loses efficacy when treatment is delayed to 7 days after ICH onset.[195] This may be a reason why anti-inflammatory treatments, including minocycline, have had mixed results in both animal studies and clinical trials.[196,197] Due to this complexity, treatments should target the harmful effects of inflammation without dampening the positive responses. This may mean targeting a specific component of the inflammatory cascade, or using a specific treatment timing.

Seizures are believed to occur in approximately 10-20% of ICH patients, and 30% of ICH patients experience subclinical seizures.[198] These seizures can often be missed in patients that do not undergo electroencephalographic monitoring (the majority of patients). Electrographic seizures occur in ~2/3 of animals, and behavioural seizures occur in ~1/2 of animals after the COL model, but are not seen after the AWB model.[199,200] Seizures can lead to increased ICP, potentially aggravating injury (see section 1.7).[201] For example, after traumatic brain injury, seizure activity contributed to prolonged ICP increases and impaired brain metabolism.[202]

1.7 Intracranial pressure after intracerebral hemorrhage

The space in the cranium is limited, due to the rigid skull. Brain injuries that add additional mass into this limited space can cause the ICP to increase.[203] After severe ischemic strokes, such as large vessel occlusion, the added mass due to edema can cause intracranial hypertension.[204] After large ICH, both the hematoma and subsequent edema contribute to mass effect, increasing ICP. Elevated ICP can acutely cause loss of consciousness, lead to worse functional outcome, and can cause mortality.[205–207] The hydrostatic pressure (pressure exerted by fluids, such as edema) can directly lead to neuronal rupture, and studies have shown that the increased pressure can lead to oxidative damage and apoptosis.[208,209] **Cerebral perfusion pressure (CPP)**, ICP, and **mean arterial pressure (MAP)** are directly related through the formula: CPP = MAP – ICP. However, this formula is simplistic and limited, as it does not take into account venous blood volume or pressure gradients in arterial blood.[210] Therefore, when

ICP is increased, this can impair **cerebral blood flow (CBF)**, potentially causing ischemic damage.[211] Additionally, when compliance mechanisms are exhausted, elevated ICP can lead to brain herniation, which can include lethal compression of the brainstem.[212] Therefore, significantly elevated ICP is concerning and often requires ICP management strategies.

1.8 Current ICP management strategies

Although there are currently no treatments that reliably normalize ICP after ICH, there are treatments and ICP management strategies that have modest effect sizes and mixed results in clinical trials.[213–217] Current ICH management guidelines[56] state that evidence is limited in support of therapies, but still provide recommendation for ICP management. These recommendations include: **cerebrospinal fluid (CSF)** drainage, osmotic agents, decompressive craniectomy, sedation, barbiturates, hypothermia, and surgical hematoma removal.[56] The listed recommendations are based on limited evidence, and often not level 1 evidence (multiple randomized controlled trials and meta-analyses demonstrating efficacy). Draining CSF removes mass from the cranial compartment, aiding in lowering ICP.[218] Pharmacological agents to reduce CSF production, such as acetazolamide, have also been tested, with mixed results.[219,220] Hyperosmotic agents, such as hypertonic saline and mannitol, draw water out of the brain via osmosis, thereby reducing ICP.[221] Removing a portion of the skull does not remove any mass from the cranium, but does give the brain space to swell and prevents herniation.[222] Pharmacological agents such as barbiturates and sedatives slow down metabolism of the central nervous system, reducing CBF and cerebral blood volume, reducing cranial contents.[223,224] Similarly, therapeutic hypothermia also reduces metabolism, lowers CBF, and has been shown to reduce ICP.[225,226] Some studies also demonstrate that hypothermia reduces edema, but this finding is inconsistent.[227–229] One study posits that despite the inconsistent effects on edema lowering, therapeutic hypothermia may reduce ICP by reducing CSF outflow resistance, although data to support this theory is limited.[230] Finally, surgical

hematoma removal (via stereotaxic or endoscopic methods) is theorized to reduce ICP by removing mass from the skull, but studies assessing ICP after hematoma removal are rare. Animal studies and clinical trials on ICP lowering treatments will be discussed in more detail in Chapter 2.

1.9 ICP regulation and compliance mechanisms

When ICP is elevated, compliance mechanisms exist to accommodate added mass and lower ICP. This is known as intracranial compliance.[231] Originating in 1783, the Monro-Kellie doctrine states that since the skull is a rigid structure and the brain is incompressible, any change in blood, brain, or CSF volume necessitates a compensatory change to accommodate the added mass.[232] For example, the volume of CSF is reduced (e.g., drainage increases and production decreases), and venous blood volume in the brain is reduced, typically via the external jugular veins.[212] Clearing these fluids creates additional space within the cranium and can lower ICP. Therefore, in an uninjured brain, typically we see that relatively large changes in volume lead to small changes in ICP due to this accommodation. However, there is a limit to these compliance mechanisms, due to the limited volume of CSF and venous blood. For example, cerebral blood volume is 1-2 mL/100g of tissue, approximately 70% of which is venous blood.[233,234] Therefore, after a certain volume of added mass, these compliance mechanisms become exhausted, and the volume added is beyond the zone of cerebral autoregulation, leading to greater increases in ICP.[212] Cerebral autoregulation refers to the maintenance of consistent cerebral blood flow despite changing blood pressure.[235] Originally, it was thought that CBF would be maintained from arterial pressures ranging from 60-150 mmHg.[236] However, the zone of cerebral autoregulation is not as wide as previously thought (5-10 mmHg, vs the 90 mmHg range originally proposed), and the range varies greatly between individuals.[237] Therefore, it is unclear how well cerebral blood flow might be maintained when accommodating an ICP challenge. It was previously believed that after the CSF and blood

compliance was exhausted, then continually elevated ICP would lead to brain herniation and ischemia. However, recent data suggests that the skull itself may be able to expand to a small degree (<10 μM in rodents).[238] Further, we have recently found that the parenchymal tissue itself can contribute to compliance,[102,166] contrary to the Monro Kellie doctrine.[232] After large ICH and severe ischemic stroke, neurons in uninjured brain regions, such as the contralateral hemisphere and distal structures, reduce their volume and increase their packing density, leading to an overall reduction in contralateral hemisphere volume.

1.10 Tissue compliance: what we currently know

Tissue compliance (increased packing density and decreased volume of neurons) as an ICP compliance mechanism could be a life-saving compliance mechanism occurring as other forms of compliance reach their limits after severe mass effect.[166] The additional space savings could prevent brain herniation and death. In rodent studies, we see that neurons start to decrease in volume at 6 hrs post-ICH (unpublished data), and by 24 hrs they have decreased volume by about 40-50%. These cells then return to their normal volume by 7 days post-ICH.[102,166] At this time, we have no evidence of cell death in these neurons between 1-7 days post-ICH, as fluoro jade C staining did not reveal any fluoro jade positive cells (indicative of cell death) outside of the injured area. Therefore, these cells are not decreasing in volume due to apoptosis. We have seen evidence of tissue compliance across regions and hemispheres. Thus far, this includes hippocampal CA1 and sensory cortex pyramidal neurons in both hemispheres as well as medium-sized striatal neurons in the contralateral hemisphere. The decrease in cell volume is not just due to widespread changes in water content (e.g., dehydration), as muscle water content and cerebellum water content is unaffected. There appears to be a threshold ICH volume where tissue compliance begins. In the AWB model, tissue compliance was observed only after the largest of three insult severities, indicating this could be a phenomenon that engages at a certain threshold, rather than gradually as the hematoma volume increases.[102]

However, higher powered studies are needed to determine if this threshold exists and what the threshold is.

This is not an ICP compliance mechanism that is limited to one model of ICH. Thus far, we have observed tissue compliance in both the COL model and the AWB model of ICH as well as the suture occlusion model of **middle cerebral artery occlusion (MCAO)**.[102,166] All of these models contain mass effect, either through brain edema or through both edema and the hematoma. Therefore, it is not an ICH specific property (e.g., blood components) that is leading to tissue compliance. The COL and AWB model have similar ICP profiles, but the extent of ICP increase is dependent on the hematoma and edema volume.[102,239] Since the COL model has prolonged bleeding over hours, the peak ICP may be more delayed compared to the AWB model. The suture occlusion model of MCAO causes a similar increase in ICP, gradually but steadily increasing over the first day as edema forms.[240] Finally, tissue compliance was also investigated in a neonatal model of hypoxic-ischemic encephalopathy.[166] In this model, we see large amounts of edema, however, the ICP responses may differ, as the sutures in the skull have not fully fused. Because of this, we do not have the same closed system that exists in adult brain injury. In this model, we saw little evidence of tissue compliance, possibly due to differing ICP levels.

Thus far, all data on tissue compliance after ICH has been done in young, healthy rodent models. Therefore, this may be a phenomenon specific to rodents that humans are not capable of. Alternatively, this may be only observed in young and healthy animals and people, and may not be a compliance mechanism that older patients with comorbidities (such as diabetes and hypertension) are capable of. There is some evidence that suggest humans may be capable of tissue compliance. A small clinical study demonstrated that neuronal density increased in patients that had large brain tumours.[241] This suggests that humans may also use tissue compliance in cases of mass effect in order to reduce ICP. This case differs from ICH in that

tumour development involves a slow growing mass, whereas ICH and edema development occur rapidly and would leave less time for tissue compliance to take effect.

However, the drastic and acute changes that these neurons undergo, despite not being lethal under the conditions we examined, may have consequences that are not currently known. For example, neuronal functioning may be impacted through changes to the ionic environment or from subcellular damage. Imaging data using x-ray fluorescence shows that regions around the hematoma (the peri-hematoma zone) have impaired ion concentrations (e.g., increased chloride and decreased potassium) that are most drastic closest to the hematoma.[158,179] These studies have not found any large-scale impairments in contralateral regions, but it is important to note that this was done using a moderate size ICH that may not have caused substantially increased ICP and tissue compliance. It is possible that in the setting of tissue compliance, we would see ion concentration differences in areas of large cell volume changes. Additionally, it is possible that these ion concentration changes would be taking place on a much smaller scale than the impairments seen in the peri-hematoma zone, and thus may require finer resolution to quantify.[242] Subcellular damage, such as damage to mitochondria or other organelles, could be impairing neuronal function. Previous research has noted mitochondrial injury in neurons that have undergone these extreme volume reductions.[166] Therefore, even though these neurons survive, there still may be cellular damage that could lead to impairments in functioning (e.g., less ATP production). It is also possible that in some settings, the impacts to neuronal functioning could be lethal (e.g., depression in brainstem neuron function). Therefore, more work needs to be done to explore both the benefits and consequences of tissue compliance.

1.11 Tissue compliance: what we hope to learn

Many important questions still remain about tissue compliance in response to ICP challenges. What is the mechanism behind tissue compliance? Many cells naturally regulate volume using osmotic volume regulation, decreasing volume by extruding ions and osmolytes

(e.g., taurine).[194,243,244] It is possible that ion channels (e.g., chloride channels, the sodium potassium pump),[245,246] aquaporins, and mechanically sensitive channels (e.g., TRPV4) are implicated in the rapid and drastic cell volume decreases seen after ICH.[247,248] Many of these ion channels (e.g., Sur-1 Trpm-4) that can contribute to cell volume regulation are also implicated in cytotoxic edema formation after ICH. Therefore, further study of these channels and the therapeutic implications of inhibiting them are warranted. Another key question about tissue compliance is if it is helpful or harmful? If both, a key research objective is to find a way to minimize side effects and negative consequences, while keeping the potentially lifesaving ICP lowering effects. Other main research objectives include determining which populations undergo tissue compliance, and which factors are related to tissue compliance? For example, it is important to determine if tissue compliance is observed in clinical populations. Perhaps there are sub-populations that cannot undergo tissue compliance, such as patients with chronic hypertension, elderly patients, or female patients. For example, age-related tissue atrophy may play a role in ICP responses.[249] Additionally, estrogen is known to affect cell volume regulation, and therefore, the estrous cycle in females may impact tissue compliance.[250] Knowing which factors influence this phenomenon can give insights into the mechanisms behind tissue compliance. For example, if there are age-related impairments in cell volume regulation,[251] this could be expressed as reduced tissue compliance in the elderly, and the reasons behind this can be further explored. Knowing the mechanisms behind tissue compliance could potentially allow clinicians to induce tissue compliance in patients if they do not undergo tissue compliance on their own. Additionally, mechanistic insights can help lead to treatments limiting dangerous side effects. Finally, it is important to know how existing therapies impact tissue compliance. For example, hypertonic saline can be used to lower ICP,[216] but this may impact cellular responses, cell volume regulation mechanisms, and therefore, tissue compliance.

In summary, much more research is needed in the area of tissue compliance, but so far, current research is promising. Tissue compliance represents a potentially life-saving mechanism

of ICP compliance, but such drastic changes to the brain environment likely have consequences. Exploring both clinical research and preclinical research on the factors that influence tissue compliance (e.g., age, sex, and hypertension) is an important first step to guide future research. These studies will pave the way for additional mechanistic and therapeutic studies on tissue compliance after stroke.

1.12 References

1. Barber PA, Zhang J, Demchuk AM, Hill MD, Buchan AM. Why are stroke patients excluded from TPA therapy? An analysis of patient eligibility. *Neurology*. 2001;56(8):1015-1020. doi:10.1212/WNL.56.8.1015
2. Avan A, Hachinski V. Stroke and dementia, leading causes of neurological disability and death, potential for prevention. *Alzheimer's Dement*. 2021;17(6):1072-1076. doi:10.1002/alz.12340
3. Kapral MK, Fang J, Hill MD, et al. Sex differences in stroke care and outcomes: Results from the Registry of the Canadian Stroke Network. *Stroke*. 2005;36(4):809-814. doi:10.1161/01.STR.0000157662.09551.e5
4. Wu H, Wu T, Xu X, Wang J, Wang J. Iron toxicity in mice with collagenase-induced intracerebral hemorrhage. *J Cereb Blood Flow Metab*. 2011;31(5):1243-1250. doi:10.1038/jcbfm.2010.209
5. Duan X, Wen Z, Shen H, Shen M, Chen G. Intracerebral Hemorrhage, Oxidative Stress, and Antioxidant Therapy. *Oxid Med Cell Longev*. 2016;2016:1203285. doi:10.1155/2016/1203285
6. Nadeau CA, Dietrich K, Wilkinson CM, et al. Prolonged Blood-Brain Barrier Injury Occurs After Experimental Intracerebral Hemorrhage and Is Not Acutely Associated with Additional Bleeding. *Transl Stroke Res*. 2019;10(3):287-297. doi:10.1007/s12975-018-0636-9
7. Hemphill JC, Greenberg SM, Anderson CS, et al. Guidelines for the Management of Spontaneous Intracerebral Hemorrhage: A Guideline for Healthcare Professionals from the American Heart Association/American Stroke Association. *Stroke*. 2015;46(7):2032-2060. doi:10.1161/STR.0000000000000069
8. Boulanger JM, Lindsay MP, Gubitz G, et al. Canadian Stroke Best Practice Recommendations for Acute Stroke Management: Prehospital, Emergency Department,

and Acute Inpatient Stroke Care, 6th Edition, Update 2018. *Int J Stroke*. 2018;13(9):949-984. doi:10.1177/1747493018786616

9. Shoamanesh A, Patrice Lindsay M, Castellucci LA, et al. Canadian stroke best practice recommendations: Management of Spontaneous Intracerebral Hemorrhage, 7th Edition Update 2020. *Int J Stroke*. 2021;16(3):321-341. doi:10.1177/1747493020968424
10. Matsukawa H, Shinoda M, Fujii M, et al. Factors associated with lobar vs. non-lobar intracerebral hemorrhage. *Acta Neurol Scand*. 2012;126(2):116-121. doi:10.1111/j.1600-0404.2011.01615.x
11. Sutherland GR, Auer RN. Primary intracerebral hemorrhage. *J Clin Neurosci*. 2006;13(5):511-517. doi:10.1016/j.jocn.2004.12.012
12. Mehndiratta P, Manjila S, Ostergard T, et al. Cerebral amyloid angiopathy-associated intracerebral hemorrhage: Pathology and management. *Neurosurg Focus*. 2012;32(4). doi:10.3171/2012.1.FOCUS11370
13. Yamada M. Cerebral amyloid angiopathy: Emerging concepts. *J Stroke*. 2015;17(1):17-30. doi:10.5853/jos.2015.17.1.17
14. Boehme AK, Esenwa C, Elkind MSV. Stroke Risk Factors, Genetics, and Prevention. *Circ Res*. 2017;120(3):472-495. doi:10.1161/CIRCRESAHA.116.308398
15. Ariesen MJ, Claus SP, Rinkel GJE, Algra A. Risk factors for intracerebral hemorrhage in the general population: A systematic review. *Stroke*. 2003;34(8):2060-2065. doi:10.1161/01.STR.0000080678.09344.8D
16. Roy-O'Reilly M, McCullough LD. Age and sex are critical factors in ischemic stroke pathology. *Endocrinology*. 2018;159(8):3120-3131. doi:10.1210/en.2018-00465
17. Li C, Baek J, Sanchez BN, Morgenstern LB, Lisabeth LD. Temporal trends in age at ischemic stroke onset by ethnicity. *Ann Epidemiol*. 2018;28(10):686-690.e2. doi:10.1016/j.annepidem.2018.07.010
18. An SJ, Kim TJ, Yoon BW. Epidemiology, risk factors, and clinical features of intracerebral

hemorrhage: An update. *J Stroke*. 2017;19(1):3-10. doi:10.5853/jos.2016.00864

19. Ikram MA, Wieberdink RG, Koudstaal PJ. International epidemiology of intracerebral hemorrhage. *Curr Atheroscler Rep*. 2012;14(4):300-306. doi:10.1007/s11883-012-0252-1
20. Arboix A, Vall-Llosera A, García-Eroles L, Massons J, Oliveres M, Targa C. Clinical features and functional outcome of intracerebral hemorrhage in patients aged 85 and older. *J Am Geriatr Soc*. 2002;50(3):449-454. doi:10.1046/j.1532-5415.2002.50109.x
21. Mogensen UB, Olsen TS, Andersen KK, Gerds TA. Cause-specific mortality after stroke: Relation to age, sex, stroke severity, and risk factors in a 10-year follow-up study. *J Stroke Cerebrovasc Dis*. 2013;22(7). doi:10.1016/j.jstrokecerebrovasdis.2012.04.006
22. Oyama N, Gona P, Salton CJ, et al. Differential impact of age, sex, and hypertension on aortic atherosclerosis: The Framingham heart study. *Arterioscler Thromb Vasc Biol*. 2008;28(1):155-159. doi:10.1161/ATVBAHA.107.153544
23. Dandapani BK, Suzuki S, Kelley RE, Reyes-Iglesias Y, Duncan RC. Relation between blood pressure and outcome in intracerebral hemorrhage. *Stroke*. 1995;26(1):21-24. doi:10.1161/01.STR.26.1.21
24. Sacco RL, Boden-Albala B, Abel G, et al. Race-ethnic disparities in the impact of stroke risk factors the Northern Manhattan stroke study. *Stroke*. 2001;32(8):1725-1731. doi:10.1161/01.STR.32.8.1725
25. Carson AP, Howard G, Burke GL, Shea S, Levitan EB, Muntner P. Ethnic differences in hypertension incidence among middle-aged and older adults: The multi-ethnic study of atherosclerosis. *Hypertension*. 2011;57(6):1101-1107. doi:10.1161/HYPERTENSIONAHA.110.168005
26. Tsai MH, Kuo CW, Lin TK, et al. Ischemic Stroke Risk Associated with Mitochondrial Haplogroup F in the Asian Population. *Cells*. 2020;9(8). doi:10.3390/cells9081885
27. Wu J cang, Zhang X, Wang J hao, et al. Gene polymorphisms and circulating levels of the TNF-alpha are associated with ischemic stroke: A meta-analysis based on 19,873

individuals. *Int Immunopharmacol*. 2019;75. doi:10.1016/j.intimp.2019.105827

28. Krishnan K, Beishon L, Berge E, et al. Relationship between race and outcome in Asian, Black, and Caucasian patients with spontaneous intracerebral hemorrhage: Data from the Virtual International Stroke Trials Archive and Efficacy of Nitric Oxide in Stroke trial. *Int J Stroke*. 2018;13(4):362-373. doi:10.1177/1747493017744463
29. Hurn PD, Brass LM. Estrogen and stroke: A balanced analysis. *Stroke*. 2003;34(2):338-341. doi:10.1161/01.STR.0000054051.88378.25
30. Howard VJ, Madsen TE, Kleindorfer DO, et al. Sex and Race Differences in the Association of Incident Ischemic Stroke with Risk Factors. *JAMA Neurol*. 2019;76(2):179-186. doi:10.1001/jamaneurol.2018.3862
31. Caso V, Paciaroni M, Agnelli G, et al. Gender differences in patients with acute ischemic stroke. *Womens Health (Lond Engl)*. 2010;6(1):51-57. doi:10.2217/whe.09.82
32. Zia E, Pessah-Rasmussen H, Khan FA, et al. Risk factors for primary intracerebral hemorrhage: A population-based nested case-control study. *Cerebrovasc Dis*. 2006;21(1-2):18-25. doi:10.1159/000089589
33. Chen CJ, DIng D, Ironside N, et al. Cigarette smoking history and functional outcomes after spontaneous intracerebral hemorrhage. *Stroke*. 2019;50(3):588-594. doi:10.1161/STROKEAHA.118.023580
34. Juvela S, Hillbom M, Palomaki H. Risk factors for spontaneous intracerebral hemorrhage. *Stroke*. 1995;26(9):1558-1564. doi:10.1161/01.STR.26.9.1558
35. Chen CJ, Brown WM, Moomaw CJ, et al. Alcohol use and risk of intracerebral hemorrhage. *Neurology*. 2017;88(21):2043-2051. doi:10.1212/WNL.0000000000003952
36. Choudhury MJH, Chowdhury MTI, Nayeem A, Jahan WA. Modifiable and Non-Modifiable Risk Factors of Stroke: A Review Update. *J Natl Inst Neurosci Bangladesh*. 2015;1(1):22-26. doi:10.3329/jninb.v1i1.22944
37. Larsson SC, Wallin A, Wolk A, Markus HS. Differing association of alcohol consumption

with different stroke types: A systematic review and meta-analysis. *BMC Med*. 2016;14(1). doi:10.1186/s12916-016-0721-4

38. Daviet R, Aydogan G, Jagannathan K, et al. Associations between alcohol consumption and gray and white matter volumes in the UK Biobank. *Nat Commun*. 2022;13(1):1-11. doi:10.1038/s41467-022-28735-5
39. Hu G, Sarti C, Jousilahti P, Silventoinen K, Barengo NC, Tuomilehto J. Leisure time, occupational, and commuting physical activity and the risk of stroke. *Stroke*. 2005;36(9):1994-1999. doi:10.1161/01.STR.0000177868.89946.0c
40. Brott T, Thalinger K, Hertzberg V. Hypertension as a risk factor for spontaneous intracerebral hemorrhage. *Stroke*. 1986;17(6):1078-1083. doi:10.1161/01.STR.17.6.1078
41. de Oliveira Manoel AL, Goffi A, Zampieri FG, et al. The critical care management of spontaneous intracranial hemorrhage: A contemporary review. *Crit Care*. 2016;20(1). doi:10.1186/s13054-016-1432-0
42. Egan BM. Adaptive changes in cerebral blood vessels during chronic hypertension. *J Hypertens*. 1992;10(4):399. doi:10.1097/00004872-199204000-00013
43. Hart MN, Heistad DD, Brody MJ. Effect of chronic hypertension and sympathetic denervation on wall/lumen ratio of cerebral vessels. *Hypertension*. 1980;2(4):419-423. doi:10.1161/01.HYP.2.4.419
44. Cipolla MJ, Liebeskind DS, Chan SL. The importance of comorbidities in ischemic stroke: Impact of hypertension on the cerebral circulation. *J Cereb Blood Flow Metab*. 2018;38(12):2129-2149. doi:10.1177/0271678X18800589
45. Webb AJ, Fischer U, Mehta Z, Rothwell PM. Effects of antihypertensive-drug class on interindividual variation in blood pressure and risk of stroke: a systematic review and meta-analysis. *Lancet*. 2010;375(9718):906-915. doi:10.1016/S0140-6736(10)60235-8
46. Amarenco P, Lavallée PC, Labreuche J, Touboul PJ. Stroke prevention, blood cholesterol and statins. *Acta Neurol Taiwan*. 2018;27(4):90-106. doi:10.1201/b14239-8

47. Cui Q, Naikoo NA. Modifiable and non-modifiable risk factors in ischemic stroke: A meta-analy. *Afr Health Sci*. 2019;19(2):2121-2129. doi:10.4314/ahs.v19i2.36

48. Lind M, Fahlén M, Kosiborod M, Eliasson B, Odén A. Variability of INR and its relationship with mortality, stroke, bleeding and hospitalisations in patients with atrial fibrillation. *Thromb Res*. 2012;129(1):32-35. doi:10.1016/j.thromres.2011.07.004

49. Waddy SP. Disorders of coagulation in stroke. *Semin Neurol*. 2006;26(1):57-64. doi:10.1055/s-2006-933309

50. Odén A, Fahlén M, Hart RG. Optimal INR for prevention of stroke and death in atrial fibrillation: A critical appraisal. *Thromb Res*. 2006;117(5):493-499. doi:10.1016/j.thromres.2004.11.025

51. Xu Y, Gomes T, Mamdani M, et al. Direct Oral Anticoagulant- or Warfarin-Related Major Bleeding: Characteristics, Reversal Strategies, and Outcomes From a Multicenter Observational Study. *Chest*. 2017;152(1):81-91. doi:10.1016/j.chest.2017.02.009

52. Connolly SJ, Ezekowitz MD, Yusuf S, et al. Dabigatran versus Warfarin in Patients with Atrial Fibrillation. *N Engl J Med*. 2009;361(12):1139-1151. doi:10.1056/nejmoa0905561

53. Schols AMR, Schreuder FHBM, Van Raak EPM, et al. Incidence of oral anticoagulant-associated intracerebral hemorrhage in the Netherlands. *Stroke*. 2014;45(1):268-270. doi:10.1161/Strokeaha.113.003003

54. Flaherty ML, Kissela B, Woo D, et al. The increasing incidence of anticoagulant-associated intracerebral hemorrhage. *Neurology*. 2007;68(2):116-121. doi:10.1212/01.wnl.0000250340.05202.8b

55. Kurogi R, Nishimura K, Nakai M, et al. Comparing intracerebral hemorrhages associated with direct oral anticoagulants or warfarin. *Neurology*. 2018;90(13):e1143-e1149. doi:10.1212/WNL.0000000000005207

56. Hemphill JC, Greenberg SM, Anderson CS, et al. Guidelines for the Management of Spontaneous Intracerebral Hemorrhage: A Guideline for Healthcare Professionals from

the American Heart Association/American Stroke Association. *Stroke*. 2015;46(7):2032-2060. doi:10.1161/STR.0000000000000069

57. Fernando SM, Qureshi D, Talarico R, et al. Intracerebral Hemorrhage Incidence, Mortality, and Association with Oral Anticoagulation Use: A Population Study. *Stroke*. Published online 2021:1673-1681. doi:10.1161/STROKEAHA.120.032550

58. Cheung RTF, Zou LY. Use of the original, modified, or new intracerebral hemorrhage score to predict mortality and morbidity after intracerebral hemorrhage. *Stroke*. 2003;34(7):1717-1722. doi:10.1161/01.STR.0000078657.22835.B9

59. Broderick JP, Brott TG, Duldner JE, Tomsick T, Huster G. Volume of intracerebral hemorrhage: A powerful and easy-to-use predictor of 30-day mortality. *Stroke*. 1993;24(7):987-993. doi:10.1161/01.STR.24.7.987

60. Lopresti MA, Bruce SS, Camacho E, et al. Hematoma volume as the major determinant of outcomes after intracerebral hemorrhage. *J Neurol Sci*. 2014;345(1):3-7. doi:10.1016/j.jns.2014.06.057

61. Fujii Y, Takeuchi S, Sasaki O, Minakawa T, Tanaka R. Multivariate analysis of predictors of hematoma enlargement in spontaneous intracerebral hemorrhage. *Stroke*. 1998;29(6):1160-1166. doi:10.1161/01.STR.29.6.1160

62. Specogna A V., Patten SB, Turin TC, Hill MD. Cost of spontaneous intracerebral hemorrhage in Canada during 1 decade. *Stroke*. 2014;45(1):284-286. doi:10.1161/Strokeaha.113.003276

63. Fallenius M, Skrifvars MB, Reinikainen M, et al. Spontaneous Intracerebral Hemorrhage: Factors Predicting Long-Term Mortality after Intensive Care. *Stroke*. 2019;50(9):2336-2343. doi:10.1161/STROKEAHA.118.024560

64. Tuhrim S, Horowitz DR, Sacher M, Godbold JH. Volume of ventricular blood is an important determinant of outcome in supratentorial intracerebral hemorrhage. *Crit Care Med*. 1999;27(3):617-621. doi:10.1097/00003246-199903000-00045

65. Diringer MN. Intracerebral hemorrhage: Pathophysiology and management. *Crit Care Med*. 1993;21(10):1591-1603. doi:10.1097/00003246-199310000-00032

66. Thiex R, Tsirka SE. Brain edema after intracerebral hemorrhage: mechanisms, treatment options, management strategies, and operative indications. *Neurosurg Focus*. 2007;22(5):E6. doi:10.3171/foc.2007.22.5.7

67. Caliaperumal J, Ma Y, Colbourne F. Intra-parenchymal ferrous iron infusion causes neuronal atrophy, cell death and progressive tissue loss: Implications for intracerebral hemorrhage. *Exp Neurol*. 2012;237(2):363-369. doi:10.1016/j.expneurol.2012.07.001

68. Nguyen AP, Huynh HD, Sjovold SB, Colbourne F. Progressive brain damage and alterations in dendritic arborization after collagenase-induced intracerebral hemorrhage in rats. *Curr Neurovasc Res*. 2008;5(3):171-177. doi:10.2174/156720208785425710

69. Fulda S, Gorman AM, Hori O, Samali A. Cellular stress responses: Cell survival and cell death. *Int J Cell Biol*. Published online 2010. doi:10.1155/2010/214074

70. Felberg RA, Grotta JC, Shirzadi AL, et al. Cell death in experimental intracerebral hemorrhage: The “black hole” model of hemorrhagic damage. *Ann Neurol*. 2002;51(4):517-524. doi:10.1002/ana.10160

71. Caliaperumal J, Brodie S, Ma Y, Colbourne F. Thrombin Causes Neuronal Atrophy and Acute but not Chronic Cell Death. *Can J Neurol Sci*. 2014;41(6):714-720. doi:10.1017/cjn.2014.105

72. Sinar EJ, Mendelow AD, Graham DI, Teasdale GM. Experimental intracerebral hemorrhage: Effects of a temporary mass lesion. *J Neurosurg*. 1987;66(4):568-576. doi:10.3171/jns.1987.66.4.0568

73. Tsivgoulis G, Katsanos AH, Butcher KS, et al. Intensive blood pressure reduction in acute intracerebral hemorrhage: A meta-analysis. *Neurology*. 2014;83(17):1523-1529. doi:10.1212/WNL.0000000000000917

74. Qureshi AI, Palesch YY, Martin R, et al. Effect of systolic blood pressure reduction on

hematoma expansion, perihematomal edema, and 3-month outcome among patients with intracerebral hemorrhage: Results from the antihypertensive treatment of acute cerebral hemorrhage study. *Arch Neurol*. 2010;67(5):570-576. doi:10.1001/archneurol.2010.61

75. Goldstein JN, Thomas SH, Frontiero V, et al. Timing of fresh frozen plasma administration and rapid correction of coagulopathy in warfarin-related intracerebral hemorrhage. *Stroke*. 2006;37(1):151-155. doi:10.1161/01.STR.0000195047.21562.23
76. Steiner T, Rosand J, Diringer M. Intracerebral hemorrhage associated with oral anticoagulant therapy: Current practices and unresolved questions. *Stroke*. 2006;37(1):256-262. doi:10.1161/01.STR.0000196989.09900.f8
77. Kirshner H, Schrag M. Management of Intracerebral Hemorrhage: Update and Future Therapies. *Curr Neurol Neurosci Rep*. 2021;21(10). doi:10.1007/s11910-021-01144-9
78. Diringer MN, Skolnick BE, Mayer SA, et al. Thromboembolic events with recombinant activated factor vii in spontaneous intracerebral hemorrhage: Results from the factor seven for acute hemorrhagic stroke (FAST) trial. *Stroke*. 2010;41(1):48-53. doi:10.1161/STROKEAHA.109.561712
79. Mayer SA. Recombinant activated factor VII for acute intracerebral hemorrhage. In: *Stroke*. ; 2007. doi:10.1161/01.STR.0000254499.46122.22
80. Gladstone DJ, Aviv RI, Demchuk AM, et al. Effect of Recombinant Activated Coagulation Factor VII on Hemorrhage Expansion among Patients with Spot Sign-Positive Acute Intracerebral Hemorrhage: The SPOTLIGHT and STOP-IT Randomized Clinical Trials. *JAMA Neurol*. 2019;76(12):1493-1501. doi:10.1001/jamaneurol.2019.2636
81. Ovesen C, Jakobsen JC, Gluud C, et al. Tranexamic Acid for Prevention of Hematoma Expansion in Intracerebral Hemorrhage Patients with or without Spot Sign. *Stroke*. Published online 2021:2629-2636. doi:10.1161/STROKEAHA.120.032426
82. Broderick JP. Haemostatic treatment for intracerebral haemorrhage. *Lancet*. 2018;391(10135):2081-2082. doi:10.1016/S0140-6736(18)31040-7

83. Shuaib A, Amlani S, Kalashyan H, et al. Mobile stroke unit triage of patients with a suspected stroke: A novel solution to reducing suspected stroke admissions in busy emergency departments. *BMJ Innov*. 2018;4(2):54-59. doi:10.1136/bmjinnov-2017-000240

84. Wilkinson CM, Burrell JI, Kuziek JWP, Thirunavukkarasu S, Buck BH, Mathewson KE. Predicting stroke severity with a 3-min recording from the Muse portable EEG system for rapid diagnosis of stroke. *Sci Rep*. 2020;10(1). doi:10.1038/s41598-020-75379-w

85. Belur PK, Chang JJ, He S, Emanuel BA, Mack WJ. Emerging experimental therapies for intracerebral hemorrhage: Targeting mechanisms of secondary brain injury. *Neurosurg Focus*. 2013;34(5). doi:10.3171/2013.2.FOCUS1317

86. Crilly S, Withers SE, Allan SM, Parry-Jones AR, Kasher PR. Revisiting promising preclinical intracerebral hemorrhage studies to highlight repurposable drugs for translation. *Int J Stroke*. 2021;16(2):123-136. doi:10.1177/1747493020972240

87. Baker TS, Durbin J, Troiani Z, et al. Therapeutic hypothermia for intracerebral hemorrhage: Systematic review and meta-analysis of the experimental and clinical literature. *Int J Stroke*. Published online 2021. doi:10.1177/17474930211044870

88. Liddle LJ, Kalisvaart ACJ, Abrahart AH, Almekhlafi M, Demchuk A, Colbourne F. Targeting focal ischemic and hemorrhagic stroke neuroprotection: Current prospects for local hypothermia. *J Neurochem*. 2022;160(1):128-144. doi:10.1111/jnc.15508

89. Sindelar BD, Patel V, Chowdhry S, Bailes JE. A Case Report in Hemorrhagic Stroke: A Complex Disease Process and Requirement for a Multimodal Treatment Approach. *Cureus*. Published online 2018. doi:10.7759/cureus.2976

90. Adeoye O, Broderick JP. Advances in the management of intracerebral hemorrhage. *Nat Rev Neurol*. 2010;6(11):593-601. doi:10.1038/nrneurol.2010.146

91. MacLellan CL, Silasi G, Poon CC, et al. Intracerebral hemorrhage models in rat: comparing collagenase to blood infusion. *J Cereb Blood Flow Metab*. 2008;28:516-525.

doi:10.1038/sj.jcbfm.9600548

92. Rosenberg G a., Mun-Bryce S, Wesley M, Kornfeld M. Collagenase-induced intracerebral hemorrhage in rats. *Stroke*. 1990;21(5):801-807. doi:10.1161/01.STR.21.5.801

93. Bullock R, Mendelow AD, Teasdale GM, Graham DI. Intracranial haemorrhage induced at arterial pressure in the rat. Part 1: Description of technique, ICP changes and neuropathological findings. *Neurol Res*. 1984;6(4):184-188. doi:10.1080/01616412.1984.11739687

94. Krenzlin H, Gresser E, Jussen D, et al. The Cerebral Thrombin System Is Activated after Intracerebral Hemorrhage and Contributes to Secondary Lesion Growth and Poor Neurological Outcome in C57Bl/6 Mice. *J Neurotrauma*. 2020;37(12):1481-1490. doi:10.1089/neu.2019.6582

95. Krafft PR, Rolland WB, Duris K, et al. Modeling intracerebral hemorrhage in mice: Injection of autologous blood or bacterial collagenase. *J Vis Exp*. 2012;(67). doi:10.3791/4289

96. Brouwers HB, Greenberg SM. Hematoma expansion following acute intracerebral hemorrhage. *Cerebrovasc Dis*. 2013;35(3):195-201. doi:10.1159/000346599

97. Dowlatshahi D, Demchuk AM, Flaherty ML, Ali M, Lyden PL, Smith EE. Defining hematoma expansion in intracerebral hemorrhage: Relationship with patient outcomes. *Neurology*. 2011;76(14):1238-1244. doi:10.1212/WNL.0b013e3182143317

98. Xue M, Del Bigio MR. Comparison of brain cell death and inflammatory reaction in three models of intracerebral hemorrhage in adult rats. *J Stroke Cerebrovasc Dis*. 2003;12(3):152-159. doi:10.1016/S1052-3057(03)00036-3

99. Matsushita K, Meng W, Wang X, et al. Evidence for apoptosis after intracerebral hemorrhage in rat striatum. *J Cereb Blood Flow Metab*. 2000;20(2):396-404. doi:10.1097/00004647-200002000-00022

100. Chu K, Jeong SW, Jung KH, et al. Celecoxib induces functional recovery after

intracerebral hemorrhage with reduction of brain edema and perihematomal cell death. *J Cereb Blood Flow Metab*. 2004;24(8):926-933. doi:10.1097/01.WCB.0000130866.25040.7D

101. Sims JR, Gharai LR, Schaefer PW, et al. ABC/2 for rapid clinical estimate of infarct, perfusion, and mismatch volumes. *Neurology*. 2009;72(24):2104-2110. doi:10.1212/WNL.0b013e3181aa5329
102. Williamson MR, Colbourne F. Evidence for Decreased Brain Parenchymal Volume After Large Intracerebral Hemorrhages: a Potential Mechanism Limiting Intracranial Pressure Rises. *Transl Stroke Res*. 2017;8(4):386-396. doi:10.1007/s12975-017-0530-x
103. MacLellan CL, Auriat AM, McGie SC, et al. Gauging recovery after hemorrhagic stroke in rats: Implications for cytoprotection studies. *J Cereb Blood Flow Metab*. 2006;26(8):1031-1042. doi:10.1038/sj.jcbfm.9600255
104. Ziai WC, Carhuapoma JR. Intracerebral Hemorrhage. *Contin Lifelong Learn Neurol*. 2018;24(6):1603-1622. doi:10.1212/CON.0000000000000672
105. Adeoye O, Clark JF, Khatri P, Wagner KR, Zuccarello M, Pyne-Geithman GJ. Do Current Animal Models of Intracerebral Hemorrhage Mirror the Human Pathology? *Transl Stroke Res*. 2011;2(1):17-25. doi:10.1007/s12975-010-0037-1
106. Pan H, Guo R, Zhu J, et al. A gene catalogue of the Sprague-Dawley rat gut metagenome. *Gigascience*. 2018;7(5). doi:10.1093/gigascience/giy055
107. Yamori Y, Horie R, Handa H, Sato M, Fukase M. Pathogenetic similarity of strokes in stroke-prone spontaneously hypertensive rats and humans. *Stroke*. 1976;7(1):46-53. doi:10.1161/01.STR.7.1.46
108. Okamoto K, Aoki K. Development of a strain of spontaneously hypertensive rats. *Jpn Circ J*. 1963;27(3):282-293. doi:10.1253/jcj.27.282
109. Wu G, Bao X, Xi G, Keep RF, Thompson BG, Hua Y. Brain injury after intracerebral hemorrhage in spontaneously hypertensive rats: Laboratory investigation. *J Neurosurg*.

2011;114(6):1805-1811. doi:10.3171/2011.1.JNS101530

110. Sun L, Gao YH, Tian DK, et al. Inflammation of different tissues in spontaneously hypertensive rats. *Sheng Li Xue Bao*. 2006;58(4):318-323.

111. Koletsky S. Obese spontaneously hypertensive rats-A model for study of atherosclerosis. *Exp Mol Pathol*. 1973;19(1):53-60. doi:10.1016/0014-4800(73)90040-3

112. Kurtz TW, Curtis Morris R. Biological variability in wistar-kyoto rats implications for research with the spontaneously hypertensive rat. *Hypertension*. 1987;10(1):127-131. doi:10.1161/01.HYP.10.1.127

113. Smeda JS, VanVliet BN, King SR. Stroke-prone spontaneously hypertensive rats lose their ability to auto-regulate cerebral blood flow prior to stroke. *J Hypertens*. 1999;17(12):1697-1705. doi:10.1097/00004872-199917120-00006

114. Badyal DK, Lata H, Dadhich AP. Animal models of hypertension and effect of drugs. *Indian J Pharmacol*. 2003;35(6):349-362.

115. Gomes PM, Sá RWM, Aguiar GL, et al. Chronic high-sodium diet intake after weaning lead to neurogenic hypertension in adult Wistar rats. *Sci Rep*. 2017;7(1). doi:10.1038/s41598-017-05984-9

116. Lerman LO, Chade AR, Sica V, Napoli C. Animal models of hypertension: An overview. *J Lab Clin Med*. 2005;146(3):160-173. doi:10.1016/j.lab.2005.05.005

117. Smith AJ, Clutton RE, Lilley E, Hansen KEA, Brattelid T. PREPARE: guidelines for planning animal research and testing. *Lab Anim*. 2018;52(2):135-141. doi:10.1177/0023677217724823

118. Kilkenny C, Browne WJ, Cuthill IC, Emerson M, Altman DG. Improving bioscience research reporting: The arrive guidelines for reporting animal research. *Animals*. 2013;4(1):35-44. doi:10.3390/ani4010035

119. Ioannidis JPA. Why most published research findings are false. In: *Getting to Good: Research Integrity in the Biomedical Sciences*. ; 2018:2-8.

doi:10.1371/journal.pmed.0020124

120. Fisher M, Feuerstein G, Howells DW, et al. Update of the stroke therapy academic industry roundtable preclinical recommendations. *Stroke*. 2009;40(6):2244-2250. doi:10.1161/STROKEAHA.108.541128

121. Andrade C. Internal, external, and ecological validity in research design, conduct, and evaluation. *Indian J Psychol Med*. 2018;40(5):498-499. doi:10.4103/IJPSYM.IJPSYM_334_18

122. Dirnagl U. Bench to bedside: The quest for quality in experimental stroke research. *J Cereb Blood Flow Metab*. 2006;26(12):1465-1478. doi:10.1038/sj.jcbfm.9600298

123. Macleod MR, Fisher M, O'Collins V, et al. Good laboratory practice: preventing introduction of bias at the bench. *Stroke*. 2009;40(3). doi:10.1161/STROKEAHA.108.525386

124. Frantzias J, Sena ES, MacLeod MR, Salman RAS. Treatment of intracerebral hemorrhage in animal models: Meta-analysis. *Ann Neurol*. 2011;69(2):389-399. doi:10.1002/ana.22243

125. Crossley NA, Sena E, Goehler J, et al. Empirical evidence of bias in the design of experimental stroke studies: A metaepidemiologic approach. *Stroke*. 2008;39(3):929-934. doi:10.1161/STROKEAHA.107.498725

126. Bosetti F, Koenig JI, Ayata C, et al. Translational Stroke Research: Vision and Opportunities. In: *Stroke*. Vol 48. ; 2017:2632-2637. doi:10.1161/STROKEAHA.117.017112

127. Dirnagl U, Fisher M. International, multicenter randomized preclinical trials in translational stroke research: It's time to act. *J Cereb Blood Flow Metab*. 2012;32(6):933-935. doi:10.1038/jcbfm.2012.51

128. Dirnagl U, Endres M. Found in translation: Preclinical stroke research predicts human pathophysiology, clinical phenotypes, and therapeutic outcomes. *Stroke*.

2014;45(5):1510-1518. doi:10.1161/STROKEAHA.113.004075

129. Gladstone DJ, Black SE, Hakim AM. Toward wisdom from failure: Lessons from neuroprotective stroke trials and new therapeutic directions. *Stroke*. 2002;33(8):2123-2136. doi:10.1161/01.STR.0000025518.34157.51

130. Chamorro Á, Lo EH, Renú A, Van Leyden K, Lyden PD. The future of neuroprotection in stroke. *J Neurol Neurosurg Psychiatry*. 2021;92(2):129-135. doi:10.1136/jnnp-2020-324283

131. Liddle LJ, Ralhan S, Ward DL, Colbourne F. Translational Intracerebral Hemorrhage Research: Has Current Neuroprotection Research ARRIVEd at a Standard for Experimental Design and Reporting? *Transl Stroke Res*. 2020;11(6):1203-1213. doi:10.1007/s12975-020-00824-x

132. MacLellan CL, Paquette R, Colbourne F. A critical appraisal of experimental intracerebral hemorrhage research. *J Cereb Blood Flow Metab*. 2012;32(4):612-627. doi:10.1038/jcbfm.2012.8

133. Sena ES, Briscoe CL, Howells DW, Donnan GA, Sandercock PAG, MacLeod MR. Factors affecting the apparent efficacy and safety of tissue plasminogen activator in thrombotic occlusion models of stroke: Systematic review and meta-analysis. *J Cereb Blood Flow Metab*. 2010;30(12):1905-1913. doi:10.1038/jcbfm.2010.116

134. O'Collins VE, Donnan GA, MacLeod MR, Howells DW. Hypertension and experimental stroke therapies. *J Cereb Blood Flow Metab*. 2013;33(8):1141-1147. doi:10.1038/jcbfm.2013.88

135. Constantinides C, Murphy K. Molecular and Integrative Physiological Effects of Isoflurane Anesthesia: The Paradigm of Cardiovascular Studies in Rodents using Magnetic Resonance Imaging. *Front Cardiovasc Med*. 2016;3. doi:10.3389/fcvm.2016.00023

136. Sicard K, Shen Q, Brevard ME, et al. Regional cerebral blood flow and BOLD responses in conscious and anesthetized rats under basal and hypercapnic conditions: Implications

for functional MRI studies. *J Cereb Blood Flow Metab*. 2003;23(4):472-481. doi:10.1097/01.WCB.0000054755.93668.20

137. Yang C-F, Yu-Chih Chen M, Chen T-I, Cheng C-F. Dose-dependent effects of isoflurane on cardiovascular function in rats. *Tzu Chi Med J*. 2014;26(3):119-122. doi:10.1016/J.TCMJ.2014.07.005

138. Statler KD, Alexander H, Vagni V, et al. Isoflurane exerts neuroprotective actions at or near the time of severe traumatic brain injury. *Brain Res*. 2006;1076(1):216-224. doi:10.1016/j.brainres.2005.12.106

139. Gigante PR, Appelboom G, Hwang BY, et al. Isoflurane preconditioning affords functional neuroprotection in a murine model of intracerebral hemorrhage. In: *Acta Neurochirurgica, Supplementum*. Springer-Verlag Wien; 2011:141-144. doi:10.1007/978-3-7091-0693-8_23

140. Fluri F, Schuhmann MK, Kleinschnitz C. Animal models of ischemic stroke and their application in clinical research. *Drug Des Devel Ther*. 2015;9:3445-3454. doi:10.2147/DDDT.S56071

141. MacRae I. Preclinical stroke research - Advantages and disadvantages of the most common rodent models of focal ischaemia. *Br J Pharmacol*. 2011;164(4):1062-1078. doi:10.1111/j.1476-5381.2011.01398.x

142. Ruíz-Sandoval JL, Cantú C, Barinagarrementeria F. Intracerebral hemorrhage in young people: Analysis of risk factors, location, causes, and prognosis. *Stroke*. 1999;30(3):537-541. doi:10.1161/01.STR.30.3.537

143. Cho DY, Chen CC, Lee WY, Lee HC, Ho LH. A new Modified Intracerebral Hemorrhage Score for treatment decisions in basal ganglia hemorrhage-a randomized trial. *Crit Care Med*. 2008;36(7):2151-2156. doi:10.1097/CCM.0b013e318173fc99

144. Karthikeyan S, Jeffers MS, Carter A, Corbett D. Characterizing Spontaneous Motor Recovery Following Cortical and Subcortical Stroke in the Rat. *Neurorehabil Neural*

Repair. 2019;33(1):27-37. doi:10.1177/1545968318817823

145. Chen R, Wang X, Anderson CS, et al. Infratentorial Intracerebral Hemorrhage: Relation of Location to Outcome. *Stroke*. 2019;50(5):1257-1259. doi:10.1161/STROKEAHA.118.023766

146. Delcourt C, Sato S, Zhang S, et al. Intracerebral hemorrhage location and outcome among INTERACT2 participants. *Neurology*. 2017;88(15):1408-1414. doi:10.1212/WNL.0000000000003771

147. Samarasekera N, Fonville A, Lerpiniere C, et al. Influence of intracerebral hemorrhage location on incidence, characteristics, and outcome: Population-based study. *Stroke*. 2015;46(2):361-368. doi:10.1161/STROKEAHA.114.007953

148. Sreekrishnan A, Dearborn JL, Greer DM, et al. Intracerebral Hemorrhage Location and Functional Outcomes of Patients: A Systematic Literature Review and Meta-Analysis. *Neurocrit Care*. 2016;25(3):384-391. doi:10.1007/s12028-016-0276-4

149. Henderson VC, Kimmelman J, Fergusson D, Grimshaw JM, Hackam DG. Threats to Validity in the Design and Conduct of Preclinical Efficacy Studies: A Systematic Review of Guidelines for In Vivo Animal Experiments. *PLoS Med*. 2013;10(7). doi:10.1371/journal.pmed.1001489

150. Munafò MR, Nosek BA, Bishop DVM, et al. A manifesto for reproducible science. *Nat Hum Behav*. 2017;1(1). doi:10.1038/s41562-016-0021

151. Hojat M, Xu G. A visitor's guide to effect sizes - Statistical significance versus practical (clinical) importance of research findings. *Adv Heal Sci Educ*. 2004;9(3):241-249. doi:10.1023/B:AHSE.0000038173.00909.f6

152. Vilaró M, Cortés J, Selva-O'Callaghan A, et al. Adherence to reporting guidelines increases the number of citations: The argument for including a methodologist in the editorial process and peer-review. *BMC Med Res Methodol*. 2019;19(1). doi:10.1186/s12874-019-0746-4

153. Sena ES, Bart van der Worp H, Bath PMW, Howells DW, Macleod MR. Publication bias in reports of animal stroke studies leads to major overstatement of efficacy. *PLoS Biol*. 2010;8(3). doi:10.1371/journal.pbio.1000344

154. Head ML, Holman L, Lanfear R, Kahn AT, Jennions MD. The Extent and Consequences of P-Hacking in Science. *PLoS Biol*. 2015;13(3). doi:10.1371/journal.pbio.1002106

155. Xi G, Fewel ME, Hua Y, Thompson BG, Hoff JT, Keep RF. Intracerebral hemorrhage: Pathophysiology and therapy. *Neurocrit Care*. 2004;1(1):5-18. doi:10.1385/NCC:1:1:5

156. Keep RF, Xiang J, Ennis SR, et al. Blood-brain barrier function in intracerebral hemorrhage. *Acta Neurochir Suppl*. 2008;105(105):73-77. doi:10.1007/978-3-211-09469-3_15

157. Schoknecht K, David Y, Heinemann U. The blood-brain barrier-Gatekeeper to neuronal homeostasis: Clinical implications in the setting of stroke. *Semin Cell Dev Biol*. 2015;38:35-42. doi:10.1016/j.semcdb.2014.10.004

158. Nadeau CA, Dietrich K, Wilkinson CM, et al. Prolonged Blood-Brain Barrier Injury Occurs After Experimental Intracerebral Hemorrhage and Is Not Acutely Associated with Additional Bleeding. *Transl Stroke Res*. 2019;10(3):287-297. doi:10.1007/s12975-018-0636-9

159. Merali Z, Huang K, Mikulis D, Silver F, Kassner A. Evolution of blood-brain-barrier permeability after acute ischemic stroke. *PLoS One*. 2017;12(2). doi:10.1371/journal.pone.0171558

160. Ironside N, Chen CJ, Ding D, Mayer SA, Connolly ES. Perihematomal Edema after Spontaneous Intracerebral Hemorrhage. *Stroke*. 2019;50(6):1626-1633. doi:10.1161/STROKEAHA.119.024965

161. Staykov D, Wagner I, Volbers B, et al. Natural course of perihemorrhagic edema after intracerebral hemorrhage. *Stroke*. 2011;42(9):2625-2629. doi:10.1161/STROKEAHA.111.618611

162. Rungta RL, Choi HB, Tyson JR, et al. The cellular mechanisms of neuronal swelling underlying cytotoxic edema. *Cell*. 2015;161(3):610-621. doi:10.1016/j.cell.2015.03.029

163. Prabhakaran S, Naidech AM. Ischemic brain injury after intracerebral hemorrhage: A critical review. *Stroke*. 2012;43(8):2258-2263. doi:10.1161/STROKEAHA.112.655910

164. Qureshi AI, Wilson DA, Hanley DF, Traystman RJ. No evidence for an ischemic penumbra in massive experimental intracerebral hemorrhage. *Neurology*. 1999;52(2):266-272. doi:10.1212/wnl.52.2.266

165. Thiex R, Tsirka SE. Brain edema after intracerebral hemorrhage: mechanisms, treatment options, management strategies, and operative indications. *Neurosurg Focus*. 2007;22(5). doi:10.3171/foc.2007.22.5.7

166. Kalisvaart ACJ, Wilkinson CM, Gu S, et al. An update to the Monro–Kellie doctrine to reflect tissue compliance after severe ischemic and hemorrhagic stroke. *Sci Rep*. 2020;10(1). doi:10.1038/s41598-020-78880-4

167. Butcher KS, Baird T, MacGregor L, Desmond P, Tress B, Davis S. Perihematomal edema in primary intracerebral hemorrhage is plasma derived. *Stroke*. 2004;35(8):1879-1885. doi:10.1161/01.STR.0000131807.54742.1a

168. Hoff JT, Xi G. Brain edema from intracerebral hemorrhage. *Acta Neurochir Suppl*. 2003;(86):11-15. doi:10.1007/978-3-7091-0651-8_3

169. Wu S, Yuan R, Wang Y, et al. Early prediction of malignant brain edema after ischemic stroke: A systematic review and meta-analysis. *Stroke*. 2018;49(12):2918-2927. doi:10.1161/STROKEAHA.118.022001

170. Dostovic Z, Dostovic E, Smajlovic D, Ibrahimagic OC, Avdic L. Brain Edema After Ischaemic Stroke. *Med Arch (Sarajevo, Bosnia Herzegovina)*. 2016;70(5):339-341. doi:10.5455/medarh.2016.70.339-341

171. Walcott BP, Kahle KT, Simard JM. Novel Treatment Targets for Cerebral Edema. *Neurotherapeutics*. 2012;9(1):65-72. doi:10.1007/s13311-011-0087-4

172. Wang G, Huang H, He Y, Ruan L, Huang J. Bumetanide protects focal cerebral ischemia-reperfusion injury in rat. *Int J Clin Exp Pathol*. 2014;7(4):1487-1494.

173. Wilkinson CM, Fedor BA, Aziz JR, et al. Failure of bumetanide to improve outcome after intracerebral hemorrhage in rat. *PLoS One*. 2019;14(1):1-20. doi:10.1371/journal.pone.0210660

174. Simard JM, Woo SK, Schwartzbauer GT, Gerzanich V. Sulfonylurea Receptor 1 in Central Nervous System Injury: A Focused Review. *J Cereb Blood Flow Metab*. 2012;32(9):1699-1717. doi:10.1038/jcbfm.2012.91

175. Caffes N, Kurland DB, Gerzanich V, Marc Simard J. Glibenclamide for the treatment of ischemic and hemorrhagic stroke. *Int J Mol Sci*. 2015;16(3):4973-4984. doi:10.3390/ijms16034973

176. Simard JM, Tsymbalyuk N, Tsymbalyuk O, Ivanova S, Yurovsky V, Gerzanich V. Glibenclamide Is Superior to Decompressive Craniectomy in a Rat Model of Malignant Stroke. *Stroke*. 2010;41(3):531-537. doi:10.1161/STROKEAHA.109.572644

177. Sheth K, Elm J, Cheung K, et al. Abstracts for the 10th World Stroke Congress, 2016. *Int J Stroke*. 2016;11(3_suppl):4-288. doi:10.1177/1747493016670567

178. Halnes G, Østby I, Pettersen KH, Omholt SW, Einevoll GT. Electrodiffusive Model for Astrocytic and Neuronal Ion Concentration Dynamics. *PLoS Comput Biol*. 2013;9(12). doi:10.1371/journal.pcbi.1003386

179. Williamson MR, Dietrich K, Hackett MJ, et al. Rehabilitation Augments Hematoma Clearance and Attenuates Oxidative Injury and Ion Dyshomeostasis After Brain Hemorrhage. *Stroke*. 2016;48(1):195-203. doi:10.1161/STROKEAHA.116.015404

180. Xi G, Keep RF, Hoff JT. Erythrocytes and delayed brain edema formation following intracerebral hemorrhage in rats. *J Neurosurg*. 1998;89(6):991-996. doi:10.3171/jns.1998.89.6.0991

181. Wilkinson CM, Brar PS, Balay CJ, Colbourne F. Glibenclamide, a Sur1-Trpm4 antagonist,

does not improve outcome after collagenase-induced intracerebral hemorrhage. *PLoS One*. 2019;14(5). doi:10.1371/journal.pone.0215952

182. Dang G, Yang Y, Wu G, Hua Y, Keep RF, Xi G. Early Erythrolysis in the Hematoma After Experimental Intracerebral Hemorrhage. *Transl Stroke Res*. 2017;8(2):174-182. doi:10.1007/s12975-016-0505-3

183. Pleșeru A-M, Mihailă RG. the Role of Thrombin in Central Nervous System Activity and Stroke. *Med Pharm Reports*. 2018;91(4):368-371. doi:10.15386/cjmed-973

184. Nguyen A, Huynh H, Sjovold S, Colbourne F. Progressive Brain Damage and Alterations in Dendritic Arborization after Collagenase-Induced Intracerebral Hemorrhage in Rats. *Curr Neurovasc Res*. 2008;5(3):171-177. doi:10.2174/156720208785425710

185. Cao S, Zheng M, Hua Y, Chen G, Keep RF, Xi G. Hematoma Changes during Clot Resolution after Experimental Intracerebral Hemorrhage. *Stroke*. 2016;47(6):1626-1631. doi:10.1161/STROKEAHA.116.013146

186. Takasugi S, Ueda S, Matsumoto K. Chronological changes in spontaneous intracerebral hematoma — an experimental and clinical study. *Stroke*. 1985;16(4):651-658. doi:10.1161/01.STR.16.4.651

187. Wang J, Wang G, Yi J, et al. The effect of monascin on hematoma clearance and edema after intracerebral hemorrhage in rats. *Brain Res Bull*. 2017;134:24-29. doi:10.1016/j.brainresbull.2017.06.018

188. Huang FP, Xi G, Keep RF, Hua Y, Nemoianu A, Hoff JT. Brain edema after experimental intracerebral hemorrhage: Role of hemoglobin degradation products. *J Neurosurg*. 2002;96(2):287-293. doi:10.3171/jns.2002.96.2.0287

189. Clark JF, Loftspring M, Wurster WL, et al. Bilirubin oxidation products, oxidative stress, and intracerebral hemorrhage. *Acta Neurochir Suppl*. 2008;(105):7-12. doi:10.1007/978-3-211-09469-3_2

190. Zille M, Karuppagounder SS, Chen Y, et al. Neuronal Death after Hemorrhagic Stroke in

Vitro and in Vivo Shares Features of Ferroptosis and Necroptosis. *Stroke*. 2017;48(4):1033-1043. doi:10.1161/STROKEAHA.116.015609

191. Lattanzi S, Cagnetti C, Provinciali L, Silvestrini M. Neutrophil-to-Lymphocyte Ratio Predicts the Outcome of Acute Intracerebral Hemorrhage. *Stroke*. 2016;47(6):1654-1657. doi:10.1161/STROKEAHA.116.013627
192. Zhang Z, Zhang Z, Lu H, Yang Q, Wu H, Wang J. Microglial Polarization and Inflammatory Mediators After Intracerebral Hemorrhage. *Mol Neurobiol*. 2017;54(3):1874-1886. doi:10.1007/s12035-016-9785-6
193. Zhou Y, Wang Y, Wang J, Anne Stetler R, Yang QW. Inflammation in intracerebral hemorrhage: From mechanisms to clinical translation. *Prog Neurobiol*. 2014;115(C):25-44. doi:10.1016/j.pneurobio.2013.11.003
194. Khanna A, Kahle KT, Walcott BP, Gerzanich V, Simard JM. Disruption of Ion Homeostasis in the Neurogliovascular Unit Underlies the Pathogenesis of Ischemic Cerebral Edema. *Transl Stroke Res*. 2014;5(1). doi:10.1007/s12975-013-0307-9
195. Wasserman JK, Zhu X, Schlichter LC. Evolution of the inflammatory response in the brain following intracerebral hemorrhage and effects of delayed minocycline treatment. *Brain Res*. 2007;1180(1):140-154. doi:10.1016/j.brainres.2007.08.058
196. Malhotra K, Chang JJ, Khunger A, et al. Minocycline for acute stroke treatment: a systematic review and meta-analysis of randomized clinical trials. *J Neurol*. 2018;265(8):1871-1879. doi:10.1007/s00415-018-8935-3
197. Moskowitz MA, Lo EH, Iadecola C. The science of stroke: Mechanisms in search of treatments. *Neuron*. 2010;67(2):181-198. doi:10.1016/j.neuron.2010.07.002
198. Balami JS, Buchan AM. Complications of intracerebral haemorrhage. *Lancet Neurol*. 2012;11(1):101-118. doi:10.1016/S1474-4422(11)70264-2
199. Klahr AC, Dickson CT, Colbourne F. Seizure Activity Occurs in the Collagenase but not the Blood Infusion Model of Striatal Hemorrhagic Stroke in Rats. *Transl Stroke Res*.

2014;6(1):29-38. doi:10.1007/s12975-014-0361-y

200. Germonpré C, Proesmans S, Bouckaert C, et al. Acute symptomatic seizures following intracerebral hemorrhage in the rat collagenase model. *Epilepsy Res*. 2020;164. doi:10.1016/j.eplepsyres.2020.106364

201. Gabor AJ, Brooks AG, Scobey RP, Parsons GH. Intracranial pressure during epileptic seizures. *Electroencephalogr Clin Neurophysiol*. 1984;57(6):497-506. doi:10.1016/0013-4694(84)90085-3

202. Vespa PM, Miller C, McArthur D, et al. Nonconvulsive electrographic seizures after traumatic brain injury result in a delayed, prolonged increase in intracranial pressure and metabolic crisis. *Crit Care Med*. 2007;35(12):2830-2836. doi:10.1097/01.CCM.0000295667.66853.BC

203. Wilkinson CM, Kung TFC, Jickling GC, Colbourne F. A translational perspective on intracranial pressure responses following intracerebral hemorrhage in animal models. *Brain Hemorrhages*. 2021;2(1):34-48. doi:10.1016/j.hest.2020.10.002

204. Freeman WD. Management of intracranial pressure. *Contin Lifelong Learn Neurol*. 2015;21(5):1299-1323. doi:10.1212/CON.0000000000000235

205. Badri S, Chen J, Barber J, et al. Mortality and long-term functional outcome associated with intracranial pressure after traumatic brain injury. *Intensive Care Med*. 2012;38(11):1800-1809. doi:10.1007/s00134-012-2655-4

206. Sykora M, Steinmacher S, Steiner T, Poli S, Diedler J. Association of intracranial pressure with outcome in comatose patients with intracerebral hemorrhage. *J Neurol Sci*. 2014;342(1-2):141-145. doi:10.1016/j.jns.2014.05.012

207. Godoy DA, Núñez-Patiño RA, Zorrilla-Vaca A, Ziai WC, Hemphill JC. Intracranial Hypertension After Spontaneous Intracerebral Hemorrhage: A Systematic Review and Meta-analysis of Prevalence and Mortality Rate. *Neurocrit Care*. 2019;31(1):176-187. doi:10.1007/s12028-018-0658-x

208. Guo T, Ren P, Li X, et al. Neural Injuries Induced by Hydrostatic Pressure Associated with Mass Effect after Intracerebral Hemorrhage. *Sci Rep*. 2018;8(1). doi:10.1038/s41598-018-27275-7

209. Liu Q, Ju WK, Crowston JG, et al. Oxidative stress is an early event in hydrostatic pressure-induced retinal ganglion cell damage. *Investig Ophthalmol Vis Sci*. 2007;48(10):4580-4589. doi:10.1167/iovs.07-0170

210. Pranevicius M, Pranevicius H, Pranevicius O. Cerebral venous steal equation for intracranial segmental perfusion pressure predicts and quantifies reversible intracranial to extracranial flow diversion. *Sci Rep*. 2021;11(1). doi:10.1038/s41598-021-85931-x

211. Bullock R, Brock-Utne J, van Dellen J, Blake G. Intracerebral hemorrhage in a primate model: Effect on regional cerebral blood flow. *Surg Neurol*. 1988;29(2):101-107. doi:10.1016/0090-3019(88)90065-1

212. Tadevosyan A, Kornbluth J. Brain Herniation and Intracranial Hypertension. *Neurol Clin*. 2021;39(2):293-318. doi:10.1016/j.ncl.2021.02.005

213. Rincon F, Mayer SA. Clinical review: Critical care management of spontaneous intracerebral hemorrhage. *Crit Care*. 2008;12(6):237. doi:10.1186/cc7092

214. Hanley DF, Thompson RE, Rosenblum M, et al. Efficacy and safety of minimally invasive surgery with thrombolysis in intracerebral haemorrhage evacuation (MISTIE III): a randomised, controlled, open-label, blinded endpoint phase 3 trial. *Lancet*. 2019;393(10175):1021-1032. doi:10.1016/S0140-6736(19)30195-3

215. Dastur CK, Yu W. Current management of spontaneous intracerebral haemorrhage. *Stroke Vasc Neurol*. 2017;2(1):21-29. doi:10.1136/svn-2016-000047

216. Kamel H, Navi BB, Nakagawa K, Hemphill JC, Ko NU. Hypertonic saline versus mannitol for the treatment of elevated intracranial pressure: A meta-analysis of randomized clinical trials. *Crit Care Med*. 2011;39(3):554-559. doi:10.1097/CCM.0b013e318206b9be

217. Bühler D, Azghandi S, Schüller K, Plesnila N. Effect of decompressive craniectomy on

outcome following subarachnoid hemorrhage in mice. *Stroke*. 2015;46(3):819-826. doi:10.1161/STROKEAHA.114.007703

218. Bothwell SW, Janigro D, Patabendige A. Cerebrospinal fluid dynamics and intracranial pressure elevation in neurological diseases. *Fluids Barriers CNS*. 2019;16(1). doi:10.1186/s12987-019-0129-6

219. Uldall M, Botfield H, Jansen-Olesen I, Sinclair A, Jensen R. Acetazolamide lowers intracranial pressure and modulates the cerebrospinal fluid secretion pathway in healthy rats. *Neurosci Lett*. 2017;645:33-39. doi:10.1016/j.neulet.2017.02.032

220. Williamson MR, Wilkinson CM, Dietrich K, Colbourne F. Acetazolamide Mitigates Intracranial Pressure Spikes Without Affecting Functional Outcome After Experimental Hemorrhagic Stroke. *Transl Stroke Res*. 2019;10(4):428-439. doi:10.1007/s12975-018-0663-6

221. Diringer MN. The evolution of the clinical use of osmotic therapy in the treatment of cerebral Edema. In: *Acta Neurochirurgica, Supplementum*. Vol 121. ; 2016:3-6. doi:10.1007/978-3-319-18497-5_1

222. Maira G, Anile C, Colosimo C, Rossi GF. Surgical treatment of primary supratentorial intracerebral hemorrhage in stuporous and comatose patients. *Neurol Res*. 2002;24(1):54-60. doi:10.1179/016164102101199549

223. Hayashi M, Kobayashi H, Kawano H, Handa Y, Hirose S. Treatment of systemic hypertension and intracranial hypertension in cases of brain hemorrhage. *Stroke*. 1988;19(3):314-321. doi:10.1161/01.STR.19.3.314

224. Roberts DJ, Hall RI, Kramer AH, Robertson HL, Gallagher CN, Zygun DA. Sedation for critically ill adults with severe traumatic brain injury: A systematic review of randomized controlled trials. *Crit Care Med*. 2011;39(12):2743-2751. doi:10.1097/CCM.0b013e318228236f

225. John RF, Colbourne F. Delayed localized hypothermia reduces intracranial pressure

following collagenase-induced intracerebral hemorrhage in rat. *Brain Res*. 2016;1633:27-36. doi:10.1016/j.brainres.2015.12.033

226. Omileke D, Azarpeykan S, Bothwell SW, et al. Short-duration hypothermia completed prior to reperfusion prevents intracranial pressure elevation following ischaemic stroke in rats. *Sci Rep*. 2021;11(1):1-9. doi:10.1038/s41598-021-01838-7
227. Kawai N, Nakamura T, Okauchi M, Nagao S. Effects of hypothermia on intracranial pressure and brain edema formation: Studies in a rat acute subdural hematoma model. *J Neurotrauma*. 2000;17(3):193-202. doi:10.1089/neu.2000.17.193
228. Kollmar R, Staykov D, Dörfler A, Schellinger PD, Schwab S, Bardutzky J. Hypothermia reduces perihemorrhagic edema after intracerebral hemorrhage. *Stroke*. 2010;41(8):1684-1689. doi:10.1161/STROKEAHA.110.587758
229. Omileke D, Pepperall D, Bothwell SW, et al. Ultra-Short Duration Hypothermia Prevents Intracranial Pressure Elevation Following Ischaemic Stroke in Rats. *Front Neurol*. 2021;12. doi:10.3389/fneur.2021.684353
230. Omileke D, Bothwell SW, Pepperall D, et al. Decreased Intracranial Pressure Elevation and Cerebrospinal Fluid Outflow Resistance: A Potential Mechanism of Hypothermia Cerebroprotection Following Experimental Stroke. *Brain Sci*. 2021;11(12):1589. doi:10.3390/brainsci11121589
231. Eide PK, Sorteberg W. Association among intracranial compliance, intracranial pulse pressure amplitude and intracranial pressure in patients with intracranial bleeds. *Neurol Res*. 2007;29(8):798-802. doi:10.1179/016164107X224132
232. Wilson MH. Monro-Kellie 2.0: The dynamic vascular and venous pathophysiological components of intracranial pressure. *J Cereb Blood Flow Metab*. 2016;36(8):1338-1350. doi:10.1177/0271678X16648711
233. Hua J, Liu P, Kim T, et al. MRI techniques to measure arterial and venous cerebral blood volume. *Neuroimage*. 2019;187:17-31. doi:10.1016/j.neuroimage.2018.02.027

234. Hopton P, Walsh TS, Lee A. Measurement of cerebral blood volume using near-infrared spectroscopy and indocyanine green elimination. *J Appl Physiol*. 1999;87(5):1981-1987. doi:10.1152/jappl.1999.87.5.1981

235. Castro P, Azevedo E, Sorond F. Cerebral Autoregulation in Stroke. *Curr Atheroscler Rep*. 2018;20(8). doi:10.1007/s11883-018-0739-5

236. LASSEN NA. Cerebral blood flow and oxygen consumption in man. *Physiol Rev*. 1959;39(2):183-238. doi:10.1152/physrev.1959.39.2.183

237. Brassard P, Labrecque L, Smirl JD, et al. Losing the dogmatic view of cerebral autoregulation. *Physiol Rep*. 2021;9(15). doi:10.14814/phy2.14982

238. Mascarenhas S, Vilela GHF, Carlotti C, et al. The new ICP minimally invasive method shows that the monro-kellie doctrine is not valid. In: *Acta Neurochirurgica, Supplementum*. ; 2012. doi:10.1007/978-3-7091-0956-4_21

239. Hiploylee C, Colbourne F. Intracranial pressure measured in freely moving rats for days after intracerebral hemorrhage. *Exp Neurol*. 2014;255:49-55. doi:10.1016/j.expneurol.2014.02.017

240. Wells AJ, Vink R, Helps SC, Knox SJ, Blumbergs PC, Turner RJ. Elevated intracranial pressure and cerebral edema following permanent MCA occlusion in an ovine model. *PLoS One*. 2015;10(6). doi:10.1371/journal.pone.0130512

241. Cohen BA, Knopp EA, Rusinek H, Liu S, Gonen O. Brain compression without global neuronal loss in meningiomas: Whole-brain proton MR spectroscopy report of 2 cases. *Am J Neuroradiol*. 2005;26(9):2178-2182.

242. Hackett MJ, Hollings A, Caine S, et al. Elemental characterisation of the pyramidal neuron layer within the rat and mouse hippocampus. *Metallomics*. 2019;11(1):151-165. doi:10.1039/c8mt00230d

243. Kahle KT, Staley KJ, Nahed B V., et al. Roles of the cation - Chloride cotransporters in neurological disease. *Nat Clin Pract Neurol*. 2008;4(9):490-503.

doi:10.1038/ncpneuro0883

244. Pasantes-Morales H, Cruz-Rangel S. Brain volume regulation: Osmolytes and aquaporin perspectives. *Neuroscience*. 2010;168(4):871-884. doi:10.1016/j.neuroscience.2009.11.074

245. Song M, Yu SP. Ionic Regulation of Cell Volume Changes and Cell Death after Ischemic Stroke. *Transl Stroke Res*. 2014;5(1):17-27. doi:10.1007/s12975-013-0314-x

246. Basavappa S, Ellory JC. The role of swelling-induced anion channels during neuronal volume regulation. *Mol Neurobiol*. 1996;13(2):137-153. doi:10.1007/BF02740638

247. Liedtke W. Molecular mechanisms of TRPV4-mediated neural signaling. In: *Annals of the New York Academy of Sciences*. ; 2008. doi:10.1196/annals.1418.012

248. Jiang H, Sun SX. Cellular pressure and volume regulation and implications for cell mechanics. *Biophys J*. 2013;105(3):609-619. doi:10.1016/j.bpj.2013.06.021

249. Fjell AM, Walhovd KB, Fennema-Notestine C, et al. One-year brain atrophy evident in healthy aging. *J Neurosci*. 2009;29(48):15223-15231. doi:10.1523/JNEUROSCI.3252-09.2009

250. Ayus JC, Achinger SG, Arieff A. Brain cell volume regulation in hyponatremia: Role of sex, age, vasopressin, and hypoxia. *Am J Physiol - Ren Physiol*. 2008;295(3). doi:10.1152/ajprenal.00502.2007

251. Trachtman H. Cell volume regulation: a review of cerebral adaptive mechanisms and implications for clinical treatment of osmolal disturbances: II. *Pediatr Nephrol*. 1992;6(1):104-112. doi:10.1007/BF00856852

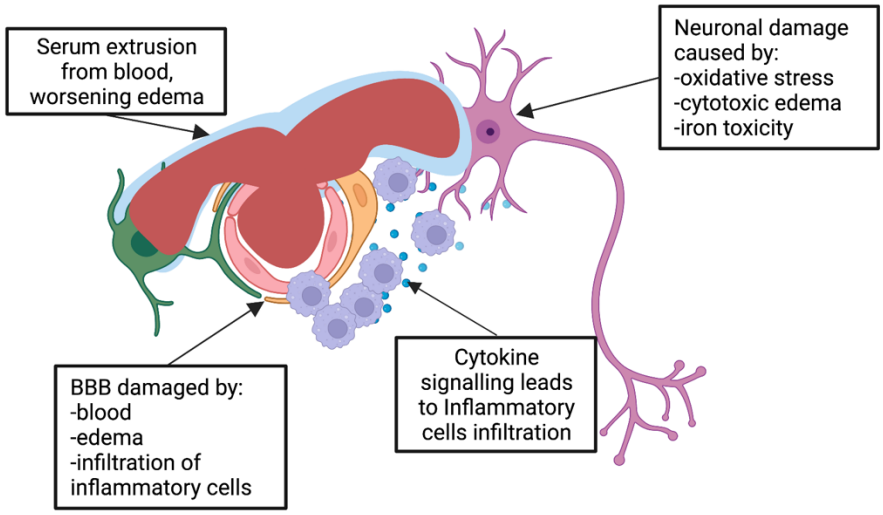

Figure 1-1. Mechanisms of damage from ICH on the neurovascular unit. As bleeding occurs, resulting damage affects the BBB, glial cells, and neurons.

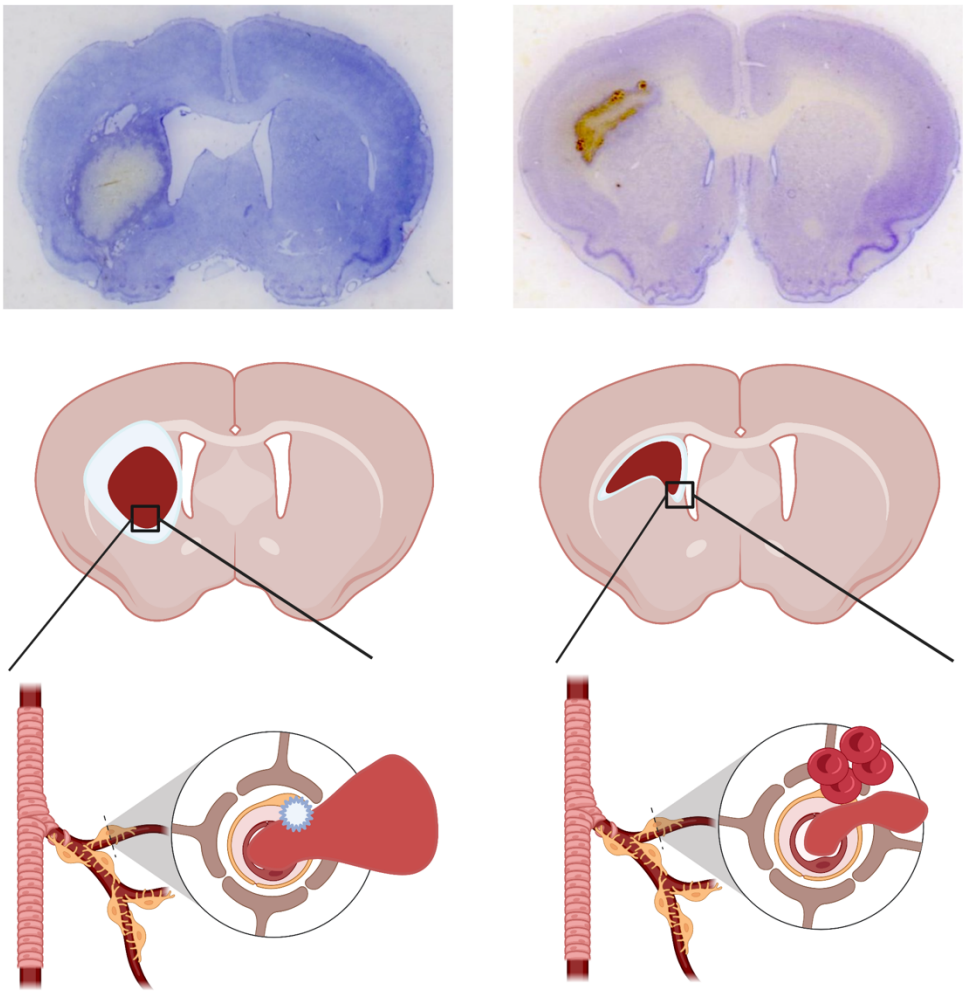

Figure 1-2. The collagenase model (left) compared to the autologous whole blood model (right). With the COL model, bacterial collagenase breaks down the basal lamina of the BBB and causes bleeding. With the AWB model, shearing forces of the injected blood can cause additional bleeding, but this occurs to a lesser extent than in the COL model. The COL model typically results in more spherical hematomas, whereas the AWB model results in more umbrella shaped hematomas.

Chapter 2: A Translational Perspective on Intracranial Pressure Responses following Intracerebral Hemorrhage in Animal Models

2.1.0 Introduction

Intracerebral hemorrhage (ICH) is a devastating stroke caused by bleeding into the brain. Approximately 40% of ICH patients die,[1] which is partly due to high intracranial pressure (ICP). Increased ICP is caused by the mass effect of the hematoma, which takes up space in the cranium. Brain edema formation after ICH can further increase ICP. Dangerously high ICP is associated with worse outcome[2,3] and can lead to decreased cerebral blood flow (CBF),[4] potentially into the ischemic range, and even death via brain herniation.[5] Treatments exist to lower critically elevated ICP, such as osmotic agents and surgical interventions, but the effectiveness of these treatments are limited and/or unclear in ICH.[6–9]

Animal models are used to assess both the pathology of ICH as well as to screen potential treatments. In this review, we summarize the harmful role of raised ICP in hemorrhagic stroke, presenting preclinical studies and how they relate to clinical findings. Here, we review ICP pathophysiology, research techniques, treatments, possible reasons for translational failures, and recommendations for improvements to strengthen future research.

2.2.0 Increased ICP due to ICH

2.1 Factors contributing to ICH-related increases in ICP

After ICH, there are many different factors that lead to increased ICP, including the mass of the hematoma, the development of edema, obstructive hydrocephalus, and other secondary complication such as seizures and fevers.[10] The hematoma occupies space in the rigid cranium, which cannot increase in volume. Increased ICP can lead to brain shifts and herniation (Figure 2-1), where the pressure causes protrusions of brain tissue through cranial foramina.[5] Additionally, the hydrostatic pressure itself can directly cause neuronal injury.[11] Hydrostatic pressure, or pressure exerted by fluid, such as blood or CSF in the cranium can upregulate apoptotic factors and lead to decreased neuronal viability and cell death. The brain can accommodate added mass in the cranium to some extent, but after severe ICH, these

compliance mechanisms are not enough to accommodate the large hematoma and subsequent swelling.

Cerebral edema, the swelling of brain tissue, contributes to mass effect and raised ICP following ICH.[12–16] Edema post-ICH is largely located in the peri-hematoma zone, and peaks at 72 hours post-ICH in rats and anywhere between 4-12 days post-ICH in humans.[14,17–20] Cytotoxic edema is the earliest form of edema, typically forming within minutes of stroke onset.[16,17] In cytotoxic edema, ion influx (Na^+, Cl^-, etc.) leads to cell swelling as water follows ions into cells from the extracellular space.[14,16,21,22] In doing so, ions are depleted from the extracellular space, creating a driving force for ionic and vasogenic edema.[16,21] Prior to BBB destruction, ions flow into the brain through channels, replenishing ions in the extracellular space and leading to water influx.[16,17,21] Following BBB injury, protein influx into the brain leads to vasogenic edema.[16,21,22] Hydrostatic pressure is one of the primary driving forces in vasogenic edema. Increased ICP affects hydrostatic pressure, and causes more vasogenic edema.[14,16] Other ICH-specific contributors to peri-hematomal edema are serum extrusion, thrombin production and red blood cells degradation. During clotting, serum is extruded from the hematoma, which adds to the water content in the brain.[18] Additionally, the protein content of serum creates an osmotic gradient, which brings in additional water. Thrombin causes edema formation, and erythrocyte breakdown processes have been linked to formation of delayed edema.[13,14,16,17] Many comprehensive reviews are available on edema formation and treatment after ICH.[16,23,24]

Beyond the mass effect of the hematoma and edema, other consequences of ICH can raise ICP. For example, ventricular bleeding can lead to obstructive hydrocephalus, which can markedly increase ICP.[25,26] Further, seizures are common after ICH, and have been associated with increases in ICP.[27,28] Seizure activity causes an increase in cerebral metabolism and elicits an increase in cerebral blood volume, which may be the reason for elevated ICP. Fever is another consequence of ICH that causes vasoconstriction, an increase in heart rate, and an

increase in blood pressure, all of which can further increase ICP.[29–31] Therefore, seizures and fever provide a therapeutic target beyond mass effect to reduced ICP.

Clinically, ICP increases within hours after ICH and can remain elevated for many days.[32] In rodents, ICP starts to elevate within hours, which matches the profile of hematoma formation in the collagenase model.[20] Similarly, ICP remains elevated for approximately three days, which follows the progression of edema resolution and the start of hematoma clearance (Figure 2-2). After the initial bleeding event, serum is extruded during clot formation and stabilization, adding to the fluid in the cranium.[33] However, subsequent hematoma clearance involves blood cell lysis and therefore, reduced cranial contents. When the only factor is mass effect, such as during microballoon inflation, ICP remains elevated while the balloon is inflated and returns to baseline minutes after deflation.[34] Thus, it is not surprising that ICP remains elevated until hematoma clearance and edema resolution begin. Hematoma and edema progression influence the choice and timing of therapies to treat ICP.

2.2.2 Brain Compliance Mechanisms

Several compliance mechanisms accommodate to mass challenges. As outlined by Monro and Kellie in 1783,[35] blood volume, particularly venous blood, as well as cerebrospinal fluid (CSF) are reduced to make space in the cranium.[36] Normally, humans have ~150 mL of CSF and ~50-75 mL of cerebral venous blood on average[37] relative to a mean brain volume of 1400 mL.[38] In contrast, rats have 90 μL of blood [39], 95 μL of CSF [40] and ~2 mL of brain volume.[41] These fluid volumes provide an absolute ceiling on the ability to accommodate mass effect, but of course not all CSF or blood can be displaced – at a certain point this leads to serious complications (e.g., ischemia). The volume required to induce a 10 mmHg increase in ICP in adults is ~ 26 mL.[41] Interestingly, people are able to accommodate a larger increase in volume if the increase occurs more slowly, suggesting that at least some compliance mechanisms happen over hours or longer.[10] A recent study found that after large ICH with

increased ICP in rats, neurons distal to the hematoma reduced their soma volume and packed closer together, likely to accommodate the mass effect.[18] This 'tissue compliance', which has yet to be evaluated directly in human stroke, may be a mechanism that would take longer to occur than blood and CSF reduction. These compliance mechanisms can be quickly overrun after large intracerebral hemorrhage, and with impaired autoregulation (local blood flow regulation) from brain injury there can be altered compliance responses.[42,43] For example, compliance is lowered in patients with higher mean ICP.[44] After traumatic brain injury (TBI), pigs with high blood pressure had higher ICP and had reduced ability to accommodate the mass effects of the injury.[45] When patients have higher arterial blood volume, ICP will increase disproportionately in response to stimuli, such as gradually increasing brain edema.[46]

Brain compliance mechanisms can prevent brain herniation and death if they are not overrun, but they can also have negative consequences. Cerebral perfusion pressure (CPP) is directly tied to ICP as explained by the equation: CPP = mean arterial pressure – ICP (Figure 2-2). Cerebral blood flow is reduced as ICP increases if blood pressure is constant, and this CBF reduction can lead to ischemic damage. Many studies find a zone of acutely reduced CBF surrounding the hematoma.[34,47,48] However, this reduced CBF does not always lead to ischemic damage.[4,47,49] Although CBF is reduced, the metabolic demands of tissue in the perihematoma zone are likely also reduced, which results in an oxygen extraction fraction that is not increased, and the result is a lack of ischemic damage.[47] This suggests that CBF reductions associated with compliance may not be as harmful as originally thought. However, this is likely dependent on the size of the stroke. Alternatively, if CSF production is reduced to make space for the mass effect, this leads to decreased waste clearance and impaired homeostasis.[50] After injuries such as ICH, waste clearance is particularly important as the hematoma is broken down and toxic blood products, including iron, are cleared from the area of injury.[51] After TBI, increased ICP in mice is associated with impaired lymphatic clearance that results in reduced toxin clearance.[52] Directly blocking lymphatic drainage resulted in a larger drop in CBF, greater increase in ICP,

and increased edema after subarachnoid hemorrhage in rats.[53] Beyond ICP increases, clotted blood in Virchow Robin spaces after ICH could block lymphatic drainage and induce downstream damage, such as focal lymphostatic encephalopathy, as seen in a rat model of ICH.[54] Clinically, glymphatic clearance can also be impaired after stroke.[55] Much of the research on the negative consequences of high ICP and compliance mechanisms focuses on reduced CBF and ischemia, but research on the downstream effects of impaired CSF after ICH is warranted. Lastly, tissue compliance can also have negative consequences. Initial evidence suggests that rapid cell shrinking leads to subcellular perturbations, such as mitochondrial damage (Kalisvaart et al., unpublished). Further research needs should determine the lasting impairments caused by tissue compliance.

2.2.3 ICP and Outcome after ICH

The relationship between ICP and outcome determines possible treatment targets and prognosis, but this relationship is not always straightforward. High ICP may limit re-bleeding and hematoma expansion, most of which occurs in the first few hours of onset,[56] due to the tamponade effect.[57] However, increased ICP can decrease CBF, potentially leading to ischemic damage. High ICP is not necessarily required for harmful effects, and mass effect is concerning, even if it does not always cause mortality.[58] Beyond mean ICP, different ICP profiles, such as increased spiking or variability, may affect outcome (see section 4.4).[2]

Larger bleeds take up more space in the skull and cause greater edema. Therefore, in general, larger hematomas lead to higher mean ICP, but this relationship is not linear.[18] When comparing different severities of AWB ICH, both peak ICP and duration of ICP elevation were not significantly different, although there were trends for differences in mean ICP. Brain compliance is one explanation for a lack of ICP differences between ICH severities. In subarachnoid hemorrhage, injury grade was a significant predictor of higher ICP.[59] In general,

smaller hematomas will have smaller increases in ICP, but when looking at a narrow range of ICH volumes, such as in preclinical studies, this effect may be harder to detect.

In animal studies, many authors do not measure the relationship between ICP and functional outcome. Thus, even if a study finds animals had increased ICP and, for example, reduced CBF, it is not clear if the reduced CBF was biologically meaningful and lead to reduced function, or if it was transient and resolved without lasting impairment. Other animal studies do not clearly report mortality, or use short survival times (e.g., less than 24 hours) that prevent the true mortality rate from being realized.[60] Finally, animal studies often do not induce a wide range of insults within a study. Those studying ICP will often pick a targeted hematoma volume that is large enough to cause increased ICP (e.g., 50 μL bleeds in the collagenase model in rat) but largely avoid spontaneous mortality, for ethical reasons. With a limited range of hematoma volumes necessarily comes a limited range of ICP responses and outcomes, and the true relationship between ICP and outcome becomes difficult to assess. Thus, researchers should consider larger ICH volumes in their models if they wish to improve clinical relevance and their ability to predict clinical responses to therapies.

Clinically, the best predictors of outcome are hematoma volume and location, as well as the level of consciousness at admission.[61] Higher ICP is related to larger hematoma volumes, which relate to poorer outcomes. The prognostic usefulness of ICP measurements alone are minor, since large hematoma volumes are already known to have poor prognosis.[62] Markers of large ICP increases, such as presence of intraventricular hemorrhage (IVH), the size of the bleed, and worsening herniation over time are likely as useful for predicting outcome and mortality as ICP after ICH. Conversely, after subarachnoid hemorrhage (SAH), rising ICP pressure predicts worse patient outcome.[59] In ICH, the lack of clear utility of ICP measurements in prognosis does not mean that ICP is not an important aspect of injury after ICH, merely that much of the variability in ICP is already explained by the size of the injury. Additionally, there is some suggestion that beyond mean ICP, other ICP measurements can be useful for predicting

outcome. For example, ICP variability and relative time spent over 20 mmHg are both associated with worse outcome in patients.[63,64]

2.3.0 ICH Models

2.3.1 ICP Across ICH Models

Preclinically, primary ICH is most-commonly induced using the collagenase and AWB models. In the first model, bacterial collagenase is injected into the brain to cause breakdown of collagen and bleeding over the course of hours.[65] The AWB model involves an infusion of blood (e.g., obtained from the tail artery) into the brain,[4,66] and results in a hematoma in the brain with less secondary injury than the collagenase model.[67] The microballoon model of ICH is used less commonly. Here a small balloon is implanted in the brain and is inflated to mimic the mass effect of the hematoma, without secondary damage caused by coagulation or blood breakdown products.[34] Similarly, injections of inert substances, such as oil, can mimic the mass effects associated with ICH while avoiding the effects of the blood.[68] Conversely, blood components can be injected to specifically examine the damage caused by certain components of the hematoma (e.g., iron).[69–72] A less commonly used model of ICH is the spontaneous ICH model, which uses stroke-prone spontaneously hypertensive rats.[73] This model leads to increased BBB permeability, edema, and likely impaired autoregulation.[73,74] No model perfectly mimics clinical ICH,[75] and all models represent certain aspects of the pathophysiology of ICH (Table 2-1). Thus, experimental ICH models should be chosen based on accurate clinical representation of the aspects of disease that researchers are interested in.

The collagenase model is associated with a significant increase in ICP. The extent of raised ICP depends on bleed size, but increases are on the order of 2-4× relative to baseline readings.[20,76] Elevated ICP begins to resolve by 3-4 days after ICH (Figure 2-3).[20,76] Despite smaller bleeds, the collagenase model causes greater and more persistent elevations in ICP

than the AWB model, likely due to more secondary injury and edema in the collagenase model.[20] This mass effect and corresponding ICP increase is similar to those seen in patients.

The AWB model, despite accurately mimicking the mass effect of a hematoma, has considerably less edema than in the collagenase model.[67] Water content in the AWB model is largely due to serum extrusion, and true peri-hematomal edema is limited.[18] This is likely a main reason that ICP increases are less dramatic after an AWB infusion. After moderate AWB-induced ICH, a mild ICP increase[18] or no ICP increase[20] have been observed, and even large infusions of blood do not lead to ICP increases as large as in the collagenase model.[18] Further, raised ICP is more transient after AWB-induced ICH, with ICP often returning to baseline levels within hours.[4] Therefore, when trying to accurately model the clinical ICP profile after ICH, we recommend the use of other animal models.

Researchers have also characterized the ICP response after microballoon inflation in rats.[34,77] Only with the largest inflation (100 μL) did they see a rise in peak ICP (16 mmHg increase) when the balloon was expanded for four hours. With lower inflation volumes (25 and 50 μL) there was no significant increase in ICP, showing that this mass could be accommodated within the cranium. This suggests that in the collagenase and AWB models, at least 50 μL of blood and edema could be accommodated. With a shorter inflation time (10 minutes), the observed ICP increase was transient, resolving completely within minutes of deflation.[34] This model does not cause significant cerebral edema, and thus, removing the mass effect by deflating the balloon resolves the ICP response. With this model, researchers can choose the duration of increased ICP with a relatively high degree of control. Although this model has less clinical relevance than others, the microballoon model is useful when researchers want to assess the mass effect of ICH without secondary injury due to blood products, such as iron-mediated toxicity and ferroptosis.[78]

Location is another important consideration when modeling ICH. Striatal bleeding is often chosen to model a bleed in the lenticulostriate arteries, which is the most common site of intracranial bleeding in people and associated with hypertension.[79] However, given that both collagenase and AWB are stereotactically injected, the location of bleeding is easy to vary. There are few studies in animal models that assess ICH in locations such as brainstem, cerebellum, or intraventricular extension.[25,80,81] However, these locations are important to consider in studies of ICP, as brainstem and cerebellar hemorrhages are more likely to lead to herniation or brainstem compression and may respond differently to surgical treatment.[82,83] Additionally, intraventricular hemorrhage can lead to hydrocephalus and further increased ICP.[84] Therefore, the location of the hemorrhage should be varied depending on the particular objectives of the experiment, and further studies are needed to characterize ICP increases associated with different ICH locations.

Side effects are common after ICH, both clinically and pre-clinically. However, in animal models, these side effects are rarely assessed and the impacts on ICP are not clear. For example, seizures increase cerebral blood flow and can increase ICP.[28] Seizure activity has been observed in the collagenase, but not the AWB model.[85,86] Fever is another side effect that can increase ICP,[29,30] but fever incidence in ICH models is not often measured. Initial data suggests that location may be an important factor in fever incidence, with hyperthermia occurring in a collagenase-induced ventricular hemorrhage model (Liddle et al., unpublished data),[87] and less so in collagenase-induced striatal ICH.[88]

2.3.2 Species Differences

Although ICP studies modelling ICH use rats most frequently,[89] ICP has been assessed in other species, including dogs,[49,90,91] cats,[92,93] rabbits,[94,95] and non-human primates.[4,96] Mice are not commonly used, likely due to technical difficulties in implanting devices onto a smaller skull. After ICH, ICP response will vary by species due to differences in the cranial

compartment, such as skull size and relative volume of brain, blood, and CSF.[20] Rodents can tolerate mass effect and increases in ICP that are comparable to other species, including humans.[18] For example, after severe middle cerebral artery occlusion, ICP increases reached up to ~30 mmHg in surviving rats.[97] This indicates an ICP tolerance that is similar to humans. However, it is difficult to compare this to other species, as most ICH studies do not induce injury that severe and life-threatening. Importantly, ICH studies usually model a small range in bleed size, typically representing a moderate ICH. As a whole, preclinical studies do not capture the full range of insults observed in patients. Additionally, preclinical studies usually use young, healthy animals that are likely better able to accommodate mass effect than older animals with co-morbidities. Clinically, ~40% of ICH patients die, and mortality is associated with higher mean ICP.[3] The number of deaths that are due to increased ICP directly are difficult to ascertain, although ~50% of deaths are due to neurological sequelae (initial bleed or re-bleeding).[98] Most deaths occur within the first few days after ICH, when ICP is elevated.[99] Animal studies directly quantifying the increase in ICP (e.g., due to mass in the cranium) that could be tolerated would be valuable, but challenging to compare and ethically difficult to conduct. The total cranial contents (brain, CSF, and blood) of rodents are ~2 mL, while humans are ~1700 mL.[38] Thus, a large hematoma of 60 μL in a rodent comprises ~3% of the cranium. A 60 mL (large)[100] hematoma in patients occupies a slightly higher 3.5% of the cranium. Thus, the relative size of a hematoma that would cause high ICP is fairly consistent between species.

Although ICH studies most commonly use murine models, larger species such as primates, cats, and dogs better represent the cerebral physiology of clinical ICH.[101] These species are evolutionarily more closely related to humans than rodents, and their brains are gyrencephalic with a similar proportion of white matter.[101] Two findings in canine studies regarding ICP dynamics after ICH have been replicated in clinical studies. The first being the finding that reducing blood pressure after ICH does not have a harmful effect on CBF or ICP and the second being that hypertonic saline is at least as effective as mannitol at controlling

ICP.[90,91,101] Therefore, larger animal species can be useful in studying cerebral physiology after ICH, but factors such as cost and ethics must be considered.

2.3.3 Animal Models in Relation to Clinical ICP

The ICP increases found in ICH models are similar to those observed in clinical settings. However, there are some key differences that must be considered when interpreting clinical studies. Baseline ICP is lower in rodents than dogs and humans, so the increase after ICH appears to be less as well. However, when looking at the increase in ICP, the relative amount across species is similar. Rodent ICP increases from about 3-4 mmHg to 9-12 mmHg, which is a 300% increase.[18,20] Similarly, after ICH in dogs, ICP almost triples, from ~9 mmHg to ~25 mmHg.[90] Baseline ICP in humans is about 10 mmHg, and after ICH, ICP typically increases to 21-40 mmHg, which is considered moderately increased and also reflects a tripling in ICP.[102] Therefore, the relative increase in ICP after ICH seems to be modelled well in animals. However, the time course of ICP resolution differs in some ways. ICP resolution is much faster in rats, resolving over the course of days rather than weeks.[20] This likely reflects the quicker resolution of edema and the start of hematoma resolution, reducing the overall mass effect (Figure 2-3). Alternatively, quicker resolution of ICP in rodents could reflect better accommodation to ICP, or fewer secondary complications related to injury (such as infection and fever), which as noted earlier is less common in rodent models of ICH.

Differences in acute care between animals and patients may affect relationships between ICP and outcome. For example, patients often receive treatments, such as osmotic therapy or surgical interventions; animal models do not use such therapies unless they are investigational. The monitoring and medical management that patients receive likely affect outcome independently of ICP. Animals are often left with no interventions other than analgesics for pain management related to the surgical induction of injury as well as the injury itself. These analgesics, such as buprenorphine, might affect ICP. Although some find buprenorphine did not

affect ICP in normal conditions,[103] opioid agonists can cause hypoventilation, inducing cerebral hypoxia and increased intracranial pressure. Therefore, differences in ICH management can lead to translational failures in ICP studies.

Demographic factors and comorbidities are widely known to be poorly represented in preclinical research.[60] Young, healthy, male animals are often used to model ICH, despite patients often being elderly, having concurrent illnesses and risk factors, and approximately half being female. In our recent literature review, only 1%, 3%, and 3% of all studies, respectively, used *either* hypertensive, female, or aged animals.[60] Age, sex, and hypertension can all affect ICP,[102,104,105] and not modeling these differences may lead to translational failures. Although age is associated with worse outcomes and greater edema,[106] ICP is more likely to be severely increased in younger people.[104] This may be because age-related atrophy could lead to increased compliance.[107,108] However, this could also be due to attrition effects, where younger patients survive or are more likely to receive intensive care to survive larger bleeds. Still, older patients may respond differently to ICP therapies than younger patients. Like age, sex has the potential to impact ICP increases. Although there are no sex differences in normal ICP,[109] sex hormones can influence injury progression and possibly ICP after ICH. Estrogen and progesterone can be neuroprotective, and in particular, can decrease bleeding and edema after brain injury.[105,110–112] This should in theory equate to less mass effect and lower ICP in females. One study found that treatment with estrogen and progesterone resulted in decreased ICP after traumatic brain injury (TBI).[105] However, this effect has not been widely documented pre-clinically or clinically after ICH. Such an effect could be missed, perhaps because the majority of female ICH occurs post-menopause where estrogen levels are decreased, and because many female rodent ICH studies use ovariectomy to control for cycling hormone levels. On the other hand, testosterone can worsen injury, but largely does not affect bleeding or edema,[112] and likely does not affect ICP to a biologically meaningful extent.

Beyond demographic factors, hypertension is one of the most prevalent risk factors for ICH.[113] Acute hypertension might serve as a protective factor in a setting of raised ICP, as it may increase CPP. However, chronic hypertension causes vascular impairments that lead to decreased compliance and increased ICP.[102] Clinically, hypertension is associated with impaired cerebral autoregulation.[114] Pre-clinically, stroke-prone spontaneously hypertensive rats lose their ability to regulate cerebral blood flow as they age.[115] This likely impairs auto-regulation of ICP as well. Therefore, work should be done to compare ICP pathology and compliance in normotensive and spontaneously hypertensive rats.

In summary, animal model choice is a key factor in accurate prediction of clinical outcomes for work examining progression of injury and response to treatment. In Table 2-1, we provide a list of suggestions for choosing an accurate model for ICP research. Beyond model choice, there are many other important considerations in experimental design that can impact the likelihood of translation, such as randomization, replication, power analyses, etc. A comprehensive review of these topics can be referenced in translational stroke research guidelines.[116,117]

2.4.0 Measuring ICP

2.4.1 ICP Measurement Location

In animals, ICP is commonly measured from the lateral ventricle,[4,34,109,118–124] the parenchyma,[20,96,125] the cisterna magna,[49,90,91,126] and the epidural space (Figure 2-4).[20,76,97,109,127,128] In patients, ICP gradients occur after ICH, such that ICP is highest near the hematoma, and lower further away from the hematoma, such as in the epidural space.[125,129] Likely, these pressure gradients are smaller in rodents due to smaller brains and less resistance from an intraparenchymal mass. After subdural hematoma in rats, ventricular and parenchymal recordings showed a large spike in ICP, while recordings in the cisterna magna only showed a slight and gradual increase from baseline.[126] However, recordings in the epidural space can

reliably detect ICP increases related to mass effect in rodents,[20,97,127] and Hiploylee and Colbourne found that epidural recordings did not differ from intraventricular recordings adjacent to the hematoma. However, these studies were not powered to detect more modest differences in recording values (e.g., <25%) that may exist. Although intraparenchymal recordings in or near the hematoma may be more accurate in some cases, invasive recordings like these can cause substantial damage to the small rodent brain.[126] Specifically, having a cannula inserted after a large injury such as ICH with significant midline shift can lead to additional damage to brain tissue and can lead to the cannula measuring from a different area, depending on the amount of tissue displacement. Further, Uldall et al. found that ventricular recordings, when compared with epidural recordings, had very similar measurements (r = 0.9-1.0), and ventricular recordings caused a much higher rate of premature euthanasia due to complications such as hydrocephalus.[109] Therefore, although epidural measurements could be slightly less accurate, the lower complication rate makes epidural recordings preferable in some settings, such as after ICH.

2.4.2 ICP Measurements in Anesthetized Animals

In preclinical studies, ICP is often measured while the animal is under anesthetic. In some studies, measurements were taken continually for hours[34] while the animal is under anesthetic, while in other studies, animals are repeatedly anesthetized for multiple measurement sessions.[109] Either approach is problematic, as ICP can be affected by volatile anesthetics such as isoflurane and sevoflurane[130] and injectable anesthetics such as ketamine.[131,132] Anesthetics can affect blood pressure, cerebral metabolism, temperature, blood gases, and autoregulation, all which can alter ICP.[133] Importantly, anesthetic effects can differ depending on pathophysiology, with small effects seen in normal conditions[122] and larger effects seen after injury, such as TBI.[130] For example, after diffuse brain injury in rats, ICP was significantly increased in animals anesthetized with sevoflurane compared to isoflurane.[130]

Similarly, halothane, another volatile anesthetic, increased ICP in patients with space-occupying lesions.[134] On the other hand, in naïve rats, ICP was lower under anesthetic than when animals were awake.[122] Anesthetics effects on ICP also differ depending on the anesthetic used, and while volatile anesthetics can increase ICP, other anesthetics, such as ketamine, propofol, and barbiturates, can lead to decreases in ICP.[131,132,135] Therefore, experiment findings should be interpreted based on which anesthetic they used as well as the dose and duration of that anesthetic.

2.4.3 ICP Measurements in Conscious Animals

Methods of anesthetized ICP measurement can be adapted for use in conscious animals.[122,123,136] For example, pressure can be measured through a cannula inserted into the lateral ventricle. While the cannula is implanted during an anesthetized procedure, measurements can be taken in non-anesthetized animals after recovery, using light restraint. This method can be used for repeated measurements, but is not optimal for continuous ICP monitoring. Efforts should be taken to minimize handling and restraint, as this can cause stress-induced alterations in physiology, such as an increase in blood pressure,[137] temperature,[138] and ICP.[139] Alternatively, connecting an inserted catheter through a fluid swivel allows for longer measurement periods, such as hours to days at a time.[123,136]

Continuous measurements of ICP can also be obtained in freely moving animals using telemetry.[20,96,97,118–121,127,128] This is advantageous, as it avoids anesthetic confounds and allows researchers to record ICP for days at a time. A pressure transducer can be used to measure ICP by inserting the catheter into the epidural space,[97,128] the lateral ventricle,[118–121] or the brain parenchyma.[96] In the case of ICP measurements after ICH induction, the catheter can be inserted into the burr hole used to induce the hemorrhage, simplifying the surgical procedure. Using telemetry, mean ICP in uninjured rodents is 3.4-6 mmHg.[18,97,119,128] Another advantage of telemetry is that dual pressure telemeters can be used to simultaneously measure ICP and

arterial blood pressure. This allows researchers to continuously measure cerebral perfusion pressure as well as dynamic changes in ICP, blood pressure, and cerebral perfusion pressure that occur in response to ICH.

When using telemetry, movement artefacts and their effect on ICP measurements must be considered. For example, dual pressure telemeters are often implanted in the abdominal cavity to allow measurements of both ICP and blood pressure simultaneously.[128] In this case, movement can create artefact,[97] both through bending the tube, causing fluid compression, and by changing the relative position of the tip of the catheter to the sensor (e.g., during rearing). Therefore, a head assembly is advantageous, as it maintains the position of the telemeter relative to the tip of the catheter, and bends in the line are rare.[97] In cases where the telemeter is implanted abdominally, using video recordings may be beneficial, as researchers could exclude data artefacts that are due to animal movement.

One disadvantage of telemetry is the relatively low accuracy of pressure telemeters. For example, Data Sciences International lists the accuracy of their PA-C10 pressure telemeter as ± 3 mmHg. When measuring a larger number, such as mean arterial pressure (~100 mmHg), this potential measurement error is less problematic than when measuring a smaller number, such as normal ICP in rodents. This accuracy range makes it difficult to discern where the true baseline ICP is in rodents, and gives researchers less confidence in detecting small but meaningful changes in ICP between experiments, such as a doubling from 4 mmHg to 8 mmHg. However, measuring offsets prior to and following probe implantation can help circumvent these accuracy problems. Further, researchers should consider changes over time and not just absolute values.

2.4.4 Analysis of ICP Measurements

One consideration when dealing with continuous measurements, sometimes hours to days' worth, is how to meaningfully express and analyze this data. Most preclinical studies

express measurements as mean ICP over time, however, it is unclear if that is the best predictor of mortality or functional outcome or if other parameters, such as peak ICP or ICP variability, have a stronger relationship. For example, one clinical study found that high ICP variability was an independent predictor of mortality and was strongly correlated with cerebral perfusion pressure.[63] Another clinical study found that there was no relationship between mean ICP or initial ICP and outcome.[32] In other work, mean ICP, ICP variability, and amount of time spent with ICP >20 mmHg were all associated with mortality and poor outcome, whereas peak ICP was not.[3] In the previous study, mean ICP was the strongest predictor of mortality, while time spent with ICP >20 mmHg was the strongest predictor of poor functional outcome.

In animals, a study testing the efficacy of acetazolamide to lower ICP found that the treatment did not affect mean ICP, but did reduce peak ICP and lower the amount of disproportionate increases in ICP, defined as sudden ICP increases greater than 10 mmHg above preceding data points (Figure 2-5), which are indicative of poor intracranial compliance.[127] However, reducing these events did not lead to improved functional outcome or decreased lesion volume. This is supported by findings that show a weak relationship between peak ICP and lesion volume in rats, but a significant relationship between mean ICP on day 3 post-ICH and edema.[20] Taken together, mean ICP, time spent > 20 mmHg, and ICP variability tend to be the strongest predictors of outcome after ICH. However, further research should be done to determine how different aspects of ICP relate to injury after ICH so future treatments can target those parameters.

2.4.5 Clinical ICP Measurement

Measuring ICP is invasive, and therefore, not all patients undergo ICP monitoring. A retrospective analysis has shown ~20% of patients have ICP monitored.[140] Commonly, ICP is monitored after hematoma evacuation or in patients with an external ventricular drain, as there is already surgical access to monitor ICP.[141,142] Intraparenchymal and epidural monitors can also

be used if a ventricular catheter is not already in place.[26,142] Ventricular monitors have a higher rate of infections compared to subdural catheters,[143,144] but are still considered the "gold standard" method for assessing elevations in global ICP.[5]

With complications related to ICP monitoring, such as increased risk of infection, some have tried to determine whether the benefits outweigh the risks. In comatose patients, ICP can be used to predict mortality and poor outcome.[64] In ICH patients overall, the relationship between ICP monitoring and outcome is less robust than in other injury types, such as TBI.[26] Still, when ICH patients were randomly assigned to receive ICP monitoring, those that had ICP monitored had lower rates of secondary brain herniation and better neurological outcome, despite similar hematoma volumes and rates of hematoma expansion.[5]

There is an interest in developing a less invasive measure of ICP, including using ultrasound to measure optic nerve diameter.[145] With this method, authors found the cut-off point of optic nerve diameter that is associated with ICP > 20 mmHg. Although this does not provide a direct measure of ICP, it is a non-invasive method that can determine which patients have elevated ICP and might benefit from treatment.

2.5.0 ICP Treatments

2.5.1 Medical Management

Although not all ICH patients undergo ICP monitoring, all patients should frequently undergo neurological examinations to detect signs of increased ICP or brainstem herniation.[146] Monitoring ICP is recommended in patients with decreased consciousness, signs of brain herniation, ventricular hemorrhage, or hydrocephalus.[129] Typically, treatment is recommended when ICP becomes elevated above 20 mmHg.[83]

Currently, treatments for elevated ICP recommended by ICH management guidelines[129] include: CSF drainage (class 2a, level B), hyperosmolar therapy (class 2a, level C), sedation (class 2a, level C), decompressive craniectomy (class 2b, level C), barbiturates (class 2b, level

C), hypothermia (class 2b, level C), and surgical evaluation of the hematoma (class 2b, level C). Draining CSF is recommended in patients with IVH and hydrocephalus, particularly when there are signs of herniation.[9,129,146] Decompressive craniectomy can be done in cases where patients have a large ICH with significant midline shift or elevated ICP persisting beyond medical management. However, while decompressive craniectomy can be lifesaving, it does not always improve outcome.[6,147,148] Hematoma evacuation is still under investigation, and the current consensus is that cerebellar hemorrhages should undergo surgical removal of the hematoma and some superficial hematomas, but the usefulness of surgery is not well established in supratentorial ICH.[83,129] Hyperosmolar therapy is commonly recommended, with both mannitol and hypertonic saline being used.[9,83] Sedation with agents such as fentanyl or propofol is recommended to minimize pain and agitation, which can worsen ICP through straining and increased cerebral oxygen metabolism.[9] Agents with a short half-life are recommended so that intermittent neurological assessments can still be performed. Similarly, barbiturates, such as pentobarbital can control ICP by decreasing cerebral metabolic activity. When using barbiturates, electroencephalography can be monitored to ensure the dose produces burst-suppression and avoids excessive sedation.[9] Hyperventilation can also be used to lower ICP, but its effects typically only last a few hours, and are best when combined with hyperosmotic therapy. Finally, moderate to mild therapeutic hypothermia has been suggested for lowering ICP, but is a strategy employed less frequently due to the many complications associated with cooling (e.g., pneumonia).[76,149] Current guidelines caution against using corticosteroids to treat elevated ICP, as they are associated with increased complications, such as infections, after ICH.[129] For further in depth review on ICP management and treatment, numerous reviews are available.[99,129,150]

Many different treatments have been and are currently being investigated to lower ICP in the acute phase of ICH. These treatments range from surgical to pharmacological, and have achieved mixed success. One issue these studies face is patient selection. Clinical trials can be

negative overall, with subgroups of patients seeing significant benefit. The heterogeneity of many clinical trials allows for this sub-analysis, which provides hypothesis-generating findings for future trials. For example, the surgical trial in ICH (STICH II) trial was negative, but patients with superficial lobar hemorrhages appeared to benefit.[151] This has led to follow up trials investigating specific subgroups that might benefit from surgical removal of the hematoma, such as lobar hemorrhages and comatose patients, and results show that endoscopic approaches in patients with lobar hematomas are most effective at improving outcome.[152,153] Animal studies are more homogenous, and while this should afford greater statistical power and fewer confounds, effects may be missed due to inappropriate model selection and the preponderance of small-N studies (statistically underpowered and unreliable). For example, studies inducing moderate-sized AWB hemorrhages may not see an effect of an ICP lowering drug, but the same drug may be effective when treating a larger hemorrhage induced by collagenase that has substantial and sustained ICP increases. Thus, results of preclinical treatment studies should always be viewed in the context of that given study, with modelling limitations considered. Further, many preclinical studies of potential ICP treatments (such as osmotic agents) rely on edema as a measurement of the treatment effect and do not measure ICP, likely owing to expense or technical challenges. Indeed, ~58% of ICH neuroprotection studies in rodents use edema as their endpoint, but do not measure ICP.[60] In rats, ICP and edema do not always directly correlate,[18] and thus, using only edema as an endpoint does not demonstrate whether the therapy would have a clinically meaningful effect on ICP. For example, therapeutic hypothermia in ICH rats can reduce mean ICP without reducing edema.[76]

Various studies illustrate the importance of considering ICP and edema in tandem when planning and implementing treatment strategies. For instance, decompressive craniectomy and other ICP lowering treatments may exacerbate edema formation.[21] It is recommended that dehydration therapies such as hypertonic saline and mannitol are tailored to each case depending on the patient's state of edema, ICP, and likelihood of re-bleeding.[14,23] Recent

guidelines on managing intracerebral hemorrhage from 2015 do not directly recommend monitoring edema when treating ICP, and vice versa.[129] However, it is acknowledged that edema contributes to raised ICP. Future experiments investigating edema and ICP treatments should monitor the treatments effect on both edema as well as ICP.

2.5.2 Osmotic Therapies

Currently, one of the first line methods for controlling ICP is osmotic therapy. Hyperosmotic agents, such as mannitol or hypertonic saline (HTS), are used to osmotically draw out fluid due to differences in osmolarity between the blood and the parenchyma. This lowers intracranial pressure by reducing the amount of fluid in edematous regions but likely other areas as well in the cranial compartment,[154] transiently reducing ICP and allowing time for further treatments, such as craniectomy or hematoma evacuation. The specific impact of osmotic therapies on CSF levels, intracellular fluid volume, and blood volume is not yet known. Additionally, these therapies can have side effects that must also be considered.[129] Historically, mannitol has been the first-choice therapy for treating edema and ICP rises, but evidence suggests that HTS may be more effective.[155,156]

2.5.2.1 Animal Data

In dogs, the differential effect of HTS and mannitol on ICP after ICH have been assessed. A single dose of HTS reduced ICP, and this effect lasted longest when low dose HTS was injected.[90,157] In a lethal intracranial model where ICP was raised to 50 mmHg with a microballoon, the animals treated with HTS and mannitol survived longer than the control group, and animals given HTS had the lowest ICP.[158] After collagenase ICH in rats, both mannitol and HTS reduced both inflammatory markers and midline shift, although no difference was detected between the two treatments.[159] In a rat model of TBI, although HTS and mannitol had equal

effects on edema, HTS had a larger effect on ICP reduction and the duration of ICP reduction.[155]

These studies used a variety of models (AWB, collagenase, and microballoon inflation), which improves confidence in these findings. The sample sizes ranged from 4-8, which is slightly below average for the field.[60] In canine studies, both sexes were used, but effects were not analyzed by sex. In studies with rabbits and rats, only males were used. Therefore, it is unknown whether the effects of osmotic therapies differ by sex. In all ICH studies, animals had no comorbidities (aged, hypertensive, diabetic, etc.).[90,157–159]

2.5.2.2 Clinical Data

This animal data is supported by a meta-analysis of randomized clinical trials comparing iso-osmolar doses of mannitol and HTS, which found that HTS was more effective than mannitol at lowering ICP.[156] Still, the data show that osmotic therapy is only effective for a limited time, and is not a feasible solution for lowering ICP for long durations, such as hours to days.[7] Additionally, it is unclear if this reduction in ICP results in a better functional outcome or reductions in secondary damage.[7,156]

2.5.3 Surgical Interventions

Multiple surgical approaches have been explored that attempt to reduce mass effect, lower ICP, and improve outcomes after ICH. Decompressive craniectomy has been tried as a method to reduce ICP by giving the brain room to swell. Ventricular catheters are an option to drain CSF from the ventricle and lower ICP.[160] Surgical removal of the hematoma has also been investigated as a treatment. Methods of hematoma evacuation include clot removal via craniotomy, minimally invasive endoscopic surgery, minimally invasive parafascicular surgery, and using tissue plasminogen activator to breakdown the clot prior to removal.[129,161] Animal data shows that the optimal time window for surgery is between 6-12 hours after ICH.[162] Preclinically,

there is a paucity of research on surgical interventions after ICH.[34,77,147] This is likely due to the difficulty performing such procedures in small animals and a lack of surgical expertise to the extent found clinically. However, preclinical studies can help determine optimal parameters for treatment, such as treatment timing[162] and which patient subgroups are likely to receive benefit. Of course, the caveat being that in order to best guide clinical studies, animal studies must be of sufficient quality (including randomization, blinding, replication, etc.), and it is clear that this is often not the case.[60] Additionally, given the differences between rodents and humans in timing of injury, parameters would likely be most beneficial as relative to injury rather than absolute timing (e.g., treatment at the time of maximum edema).

2.5.3.1 Animal Data

Craniectomies can be performed to allow for decompression and decreases in ICP. One study looked specifically at the timing of decompressive craniectomy after striatal ICH in rats, and they used the AWB model.[147] Craniectomy was performed at either 1, 6, or 24 hours after ICH. All groups had lower mortality, better neurological scores, and reduced cell death compared to the control group. Effect sizes were dependent on time, with earlier having better outcomes. There was a wide treatment window for decompressive craniectomy, with earlier intervention having more favourable results. Decompressive craniectomy can reduce ICP effectively, but the side effects, such as increased rebleeding,[148] must be characterized further and taken into account.

Ventricular shunting is commonly used to drain CSF and lower ICP in cases of hydrocephalus. Animal studies of ventricular shunting are largely done using models of hydrocephalus, rather than intracerebral hemorrhage. In a dog model of chronic hydrocephalus, ventriculoperitoneal shunts lowered ICP and improved tissue oxygen saturation.[163] After CSF shunting in rats with hydrocephalus, the inflammatory response was reduced.[164] However,

clinically, infection is a common risk associated with shunt implantation, and this risk may outweigh any benefits from minor reductions in inflammation.

Hematoma evacuation is a surgical intervention that can both lower ICP and reduce secondary injury caused by blood products in the hematoma. As with other surgical interventions, patient selection is key, as surgical risks and side effects can outweigh the benefits of hematoma removal. Preclinically, the microballoon model has been used to mimic hematoma evacuation by deflating and removing the balloon. These studies show that earlier removal is better[165] and that removing the mass can improve CBF, reduce ischemic damage, and improve neurological outcome.[166] However, removal of a microballon is much easier than removing a hematoma, which likely leads to less damage to surrounding brain regions. Others have examined hematoma evacuation by aspiration in the AWB model. After AWB-induced ICH in dogs, hematoma evacuation improved neurological deficits with earlier evacuation (within 12 hours of injury) being most effective. Pre-clinically, many studies have investigated using a thrombolytic agent, such as tissue plasminogen activator or urokinase to dissolve the hematoma prior to aspiration. This surgical procedure has been shown to improve CBF and reduce ischemic damage,[167] reduce edema and inflammation,[168] and reduce blood-brain barrier permeability.[169] However, this procedure can also lead to increased edema.[170] Unfortunately, no research to date examines the effect of hematoma evacuation on ICP after AWB or collagenase ICH. There may be additional benefit of ICP lowering in patients with severe ICH, and this should be studied.

2.5.3.2 Clinical Data

The current ICH management guidelines state that decompressive craniectomy might reduce mortality in patients who have severely elevated ICP or are comatose, but this evidence is weak (level of evidence C), highlighting the need for further study.[129] Ventricular drainage is recommended as a treatment for ICH patients that develop hydrocephalus, commonly as a

result of intraventricular hemorrhage.[129] Clinical evidence shows that patients with cerebellar hemorrhage or patients with brainstem compression/hydrocephalus benefit most from hematoma removal.[129] Minimally invasive surgical methods are being developed to decrease surgical damage, but the effectiveness of these methods are uncertain.[8,129]

2.5.4 Pharmacological Therapies

Pharmacological agents exist that can lower ICP, but few have been assessed in preclinical ICH models. Much of the research on these agents has been done after TBI, both clinically and pre-clinically. Reducing CSF production is one method of lowering ICP. Drugs such as acetazolamide, topiramate, and furosemide are some examples of agents that reduce CSF production to lower ICP.

2.5.4.1 Animal Data

In the collagenase model of ICH, acetazolamide reduced ICP variability by reducing both the magnitude and occurrence of ICP spiking, but did not reduce mean ICP.[127] This ICP variability reduction did not lead to improved functional outcome. In a dog model of hydrocephalus, acetazolamide did not reduce clinical symptoms as compared to ventricular shunting, and this study did not measure the effect of acetazolamide on ICP.[171] However, in healthy rats, acetazolamide reduced mean ICP by over 50%.[172] This suggests that the effects of acetazolamide may be dependent on the injury model. However, this study used 2-3× the scaled maximum dose recommended clinically, and thus predictive clinical value is questionable. Furosemide, a diuretic that reduces CSF secretion, has been studied in an epidural microballoon inflation model in dogs. Furosemide caused small, slow, and variable reductions in ICP, and worked beset when combined with mannitol.[173,174] In uninjured female rats, topiramate is the most effective ICP lowering agent when compared to acetazolamide and furosemide.[175] Additional studies should be done to determine the effect of topiramate after ICH.

Sedatives and anesthetics are another category of pharmacological therapies that can reduce ICP by slowing down CNS metabolism, which reduces CBF and cerebral blood volume. Pentobarbital and propofol are a few such agents that have been assessed pre-clinically. Propofol, when combined with therapeutic hypothermia, decreased ICP after diffuse TBI in rats.[176] Propofol also caused increases in CPP, which would likely aid in preventing ischemia, but might lead to increased bleeding after ICH. Conversely, pentobarbital reduced ICP and CPP after epidural microballoon inflation in dogs.[177] This also lead to profound hypotension, which increases the risk of ischemic damage but lowers risk of re-bleeding. Others have found that intraparenchymal injection of pentobarbital lowers ICP after subarachnoid hemorrhage in dogs, but only when injected into the medulla, not the hypothalamus or the pontine reticular formation.[178] The off-target effects of sedatives and anesthetics are potentially harmful, and therefore make other pharmacological therapies a more promising option.

2.5.4.2 Clinical Data

Currently, there have been no studies that have examined the efficacy of CSF reducing agents, including acetazolamide, topiramate, or furosemide for treatment of ICP after ICH. Before large-scale clinical trials are started, more animal work is warranted to evaluate the efficacy of these treatments in animals with appropriate co-morbidities.

The effects of anesthetics and sedatives on ICP have been evaluated in patients with brain injuries. In patients with subarachnoid hemorrhage, isoflurane increased CBF without increasing ICP.[179] After brain trauma, propofol can lead to decreased ICP and CPP.[133] Interestingly, although sedatives can reduce ICP, a systematic review of randomized controlled trials found that no one sedative agent is more efficacious than others at improving patient outcomes or lowering ICP after traumatic brain injury.[180]

2.5.5 Hypothermia

Therapeutic hypothermia is a neuroprotective therapy that slows down metabolism, which can be beneficial after stroke, among many other mechanisms of action.[181,182] Decreasing metabolic demands can decrease blood pressure, which can in turn lower ICP.

2.5.5.1 Animal Data

After collagenase ICH in rats, local cooling of the skull to 33°C reduced both mean ICP and peak ICP.[76] In this study, hypothermia treatment did not reduce hematoma size or edema, and therefore the decrease in ICP was not due to reducing the mass effect. After subdural hematoma in rats, neither mild (34°C) nor moderate (32°C) therapeutic hypothermia affected ICP or CPP, but it did reduce cortical edema.[183] However, this study had a much earlier survival time of four hours and animals were under anesthetic for the duration of the study, whereas John et al. began treatment 24 hours after ICH, and ICP was decreased from 48-96 hours post-ICH. Therefore, hypothermia may only reduce ICP with a longer duration of treatment. However, the most beneficial cooling method may differ depending on brain injury, and there are striking differences in the extent of protection obtained in ischemic and hemorrhagic stroke models. The side effects of cooling include increased bleeding when animals are cooled during active hemorrhaging,[184] and possibly at the time of rewarming.[185] Increasing mass effect could end up worsening ICP.

Hypothermia has also been shown to reduce ICP after ischemic stroke, but in these studies, a much shorter duration of only a few hours was used to reduce complications.[186,187] Therefore, further research should be done in ICH models to determine the optimal hypothermia parameters for lowering ICP while mitigating risks of side effects and complications of cooling.[149] Overall, evidence on the efficacy of hypothermia is mixed, because complications can outweigh benefits.[149,188] However, subgroup analysis should be done in patients with elevated ICP to determine if this group may benefit more from hypothermia than those with mild ICP increases.

2.5.5.2 Clinical Data

After ICH, hypothermia can reduce edema,[189,190] which may in turn lead to reduced ICP. However, this is level C evidence, and no randomized controlled clinical trials have looked at how hypothermia affects ICP after ICH, so further studies are warranted.

2.5.6 Future Directions

The majority of ongoing research on ICP after ICH is taking place clinically, rather than pre-clinically. Current areas of interest on ICP measurement and control after ICH include: the importance of measuring ICP, less invasive methods of ICP measurements, and ICP lowering treatments. The SynapseICU trial is currently investigating variation in the practice of ICP monitoring in ICH and how this relates to outcome.[191] This large-scale trial aims to recruit over 5000 patients, and will hopefully provide insights into the role ICP monitoring may play in outcome. Other clinical trials are comparing new ICP monitoring techniques to conventional methods. For example, one study examined the use of cochlear microphonic potential and found it is closely correlated to ICP and could be a good alternative measurement.[192] A clinical trial explored the use of near infrared spectroscopy to measure ICP, temperature, and cerebral blood flow after SAH.[193] However, this spectroscopy probe still requires an invasive implantation procedure, and therefore would likely not reduce complications or risks associated with routine ICP monitoring. Finally, an ongoing clinical trial validating the use of ultrasound measurements of optic nerve sheath diameter as a marker of increased ICP.

Other trials have focused on interventions to reduce ICP. Lima et al. measured the impact of positive end-expiratory pressure levels on both CPP and ICP. They found that this did not impact CPP, BP or ICP. A recent trial comparing anesthetics used during subdural hematoma evacuation found that ICP was significantly lower in patients given propofol instead

of isoflurane.[194] Thus far, no trial has found a therapy that significantly reduces ICP for an extended period of time without substantial complications, thus further research is needed.

Pharmacological therapies that may have secondary impacts on ICP are being explored. For example, ion channel antagonists such as glibenclamide and bumetanide are being investigated as anti-edema agents, with mixed results in ICH.[88,195,196] If these drugs lower edema, this may in turn lead to reduced ICP. Additionally, sulforaphane may increase hematoma clearance, reducing mass effect and ICP more quickly.[197,198]

2.6.0 Conclusion

In conclusion, ICP is an understudied endpoint in preclinical ICH research, likely owing to technical challenges and expenses associated with ICP measurement, especially compared to the trivial simplicity and cost of measuring cerebral edema. In efficacy studies of ICH therapies, researchers are more likely to use edema as a surrogate marker of benefit in the absence of ICP measurements, despite a lack of consistent relationship between both edema and ICP and between edema and outcome.[60] Additionally, edema measurements do not separate serum extrusion from true peri-hematomal edema.[18] Even without intervention for increased ICP, compliance mechanisms exist to accommodate ICP increases, however these compliance mechanisms are not fully understood. Mechanisms that increase ICP compliance are thought to be beneficial, but many have potential side effects (such as ischemia) that are not well studied. A better understanding of brain compliance, and particularly tissue compliance,[18] could lead to new ICP treatments to either augment compliance or reduce side effects associated with compliance. Current ICP reducing therapies have modest effects, and many have associated side effects, such as increased risk of infection. A need exists for a more robust therapy with fewer complications. New ICP lowering treatments are being investigated, and preclinical research can help with mechanistic studies, optimal dosing and timing, and determining which subgroups will benefit most from a given treatment, but only if studies are of

high translational quality (otherwise they often fail to translate[199]). Although it is difficult to measure ICP in animals, the need for this research in the ICH field is ever-present.

2.7 References

1. Feigin VL, Lawes CM, Bennett DA, Barker-Collo SL, Parag V. Worldwide stroke incidence and early case fatality reported in 56 population-based studies: a systematic review. *Lancet Neurol*. 2009;8(4):355-369. doi:10.1016/S1474-4422(09)70025-0
2. Tian Y, Wang Z, Jia Y, et al. Intracranial pressure variability predicts short-term outcome after intracerebral hemorrhage: A retrospective study. *J Neurol Sci*. 2013;330(1-2):38-44. doi:10.1016/j.jns.2013.04.001
3. Sykora M, Steinmacher S, Steiner T, Poli S, Diedler J. Association of intracranial pressure with outcome in comatose patients with intracerebral hemorrhage. *J Neurol Sci*. 2014;342(1-2):141-145. doi:10.1016/j.jns.2014.05.012
4. Bullock R, Brock-Utne J, van Dellen J, Blake G. Intracerebral hemorrhage in a primate model: Effect on regional cerebral blood flow. *Surg Neurol*. 1988;29(2):101-107. doi:10.1016/0090-3019(88)90065-1
5. Zeng J, Zheng P, Tong W, Fang W. Decreased risk of secondary brain herniation with intracranial pressure monitoring in patients with haemorrhagic stroke. *BMC Anesthesiol*. 2014;14:2-5. doi:10.1186/1471-2253-14-19
6. Cooper DJ, Rosenfeld J V, Murray L, et al. Patient outcomes at twelve months after early decompressive craniectomy for diffuse traumatic brain injury in the Randomized DECRA Clinical Trial. *J Neurotrauma*. 2020;816:810-816. doi:10.1089/neu.2019.6869
7. Misra UK, Kalita J, Ranjan P, Mandal SK. Mannitol in intracerebral hemorrhage: A randomized controlled study. *J Neurol Sci*. 2005;234(1-2):41-45. doi:10.1016/j.jns.2005.03.038
8. Hanley DF, Thompson RE, Rosenblum M, et al. Efficacy and safety of minimally invasive surgery with thrombolysis in intracerebral haemorrhage evacuation (MISTIE III): a randomised, controlled, open-label, blinded endpoint phase 3 trial. *Lancet*.

2019;393(10175):1021-1032. doi:10.1016/S0140-6736(19)30195-3

9. Rincon F, Mayer SA. Clinical review: Critical care management of spontaneous intracerebral hemorrhage. *Crit Care*. 2008;12(6):237. doi:10.1186/cc7092

10. Diringer MN. Intracerebral hemorrhage: Pathophysiology and management. *Crit Care Med*. 1993;21(10):1591-1603. doi:10.1097/00003246-199310000-00032

11. Guo T, Ren P, Li X, et al. Neural Injuries Induced by Hydrostatic Pressure Associated with Mass Effect after Intracerebral Hemorrhage. *Sci Rep*. 2018;8(1). doi:10.1038/s41598-018-27275-7

12. Gebel JM, Jauch EC, Brott TG, et al. Relative edema volume is a predictor of outcome in patients with hyperacute spontaneous intracerebral hemorrhage. *Stroke*. 2002;33(11):2636-2641. doi:10.1161/01.STR.0000035283.34109.EA

13. Lee KR, Colon GP, Betz AL, Keep RF, Kim S, Hoff JT. Edema from intracerebral hemorrhage: The role of thrombin. *J Neurosurg*. 1996;84(1):91-96. doi:10.3171/jns.1996.84.1.0091

14. Zheng H, Chen C, Zhang J, Hu Z. Mechanism and therapy of brain edema after intracerebral hemorrhage. *Cerebrovasc Dis*. 2016;42(3-4):155-169. doi:10.1159/000445170

15. Urday S, Beslow LA, Dai F, et al. Rate of Perihematomal Edema Expansion Predicts Outcome after Intracerebral Hemorrhage. *Crit Care Med*. 2016;44(4):790-797. doi:10.1097/CCM.0000000000001553

16. Stokum JA, Gerzanich V, Simard JM. Molecular pathophysiology of cerebral edema. *J Cereb Blood Flow Metab*. 2016;36(3):513-538. doi:10.1177/0271678X15617172

17. Ironside N, Chen CJ, Ding D, Mayer SA, Connolly ES. Perihematomal Edema after Spontaneous Intracerebral Hemorrhage. *Stroke*. 2019;50(6):1626-1633. doi:10.1161/STROKEAHA.119.024965

18. Williamson MR, Colbourne F. Evidence for Decreased Brain Parenchymal Volume After

Large Intracerebral Hemorrhages: a Potential Mechanism Limiting Intracranial Pressure Rises. *Transl Stroke Res*. 2017;8(4):386-396. doi:10.1007/s12975-017-0530-x

19. Volbers B, Giede-Jeppe A, Gerner ST, et al. Peak perihemorrhagic edema correlates with functional outcome in intracerebral hemorrhage. *Neurology*. 2018;90(12):e1005-e1012. doi:10.1212/WNL.0000000000005167
20. Hiploylee C, Colbourne F. Intracranial pressure measured in freely moving rats for days after intracerebral hemorrhage. *Exp Neurol*. 2014;255:49-55. doi:10.1016/j.expneurol.2014.02.017
21. Simard JM, Kent TA, Chen M, Tarasov K V., Gerzanich V. Brain oedema in focal ischaemia: molecular pathophysiology and theoretical implications. *Lancet Neurol*. 2007;6(3):258-268. doi:10.1016/S1474-4422(07)70055-8
22. Keep RF, Andjelkovic A V., Xi G. Cytotoxic and Vasogenic Brain Edema. In: *Primer on Cerebrovascular Diseases: Second Edition*. ; 2017. doi:10.1016/B978-0-12-803058-5.00029-1
23. Thiex R, Tsirka SE. Brain edema after intracerebral hemorrhage: mechanisms, treatment options, management strategies, and operative indications. *Neurosurg Focus*. 2007;22(5):E6. doi:10.3171/foc.2007.22.5.7
24. Walcott BP, Kahle KT, Simard JM. Novel Treatment Targets for Cerebral Edema. *Neurotherapeutics*. 2012;9(1):65-72. doi:10.1007/s13311-011-0087-4
25. Lodhia KR, Shakui P, Keep RF. Hydrocephalus in a rat model of intraventricular hemorrhage. *Acta Neurochir Suppl*. 2006;(96):207-211. doi:10.1007/3-211-30714-1_45
26. Helbok R, Olson DW, Le Roux P, et al. Intracranial Pressure and Cerebral Perfusion Pressure Monitoring in Non-TBI Patients: Special Considerations. *Neurocrit Care*. 2014;21(2):85-94. doi:10.1007/s12028-014-0040-6
27. Vespa PM, Miller C, McArthur D, et al. Nonconvulsive electrographic seizures after traumatic brain injury result in a delayed, prolonged increase in intracranial pressure and

metabolic crisis. *Crit Care Med*. 2007;35(12):2830-2836. doi:10.1097/01.CCM.0000295667.66853.BC

28. Gabor AJ, Brooks AG, Scobey RP, Parsons GH. Intracranial pressure during epileptic seizures. *Electroencephalogr Clin Neurophysiol*. 1984;57(6):497-506. doi:10.1016/0013-4694(84)90085-3
29. Malkinson TJ, Veale WL, Cooper KE. Fever and intracranial pressures. *Brain Res Bull*. 1985;15(3):315-319. doi:10.1016/0361-9230(85)90157-1
30. Puccio AM, Fischer MR, Jankowitz BT, Yonas H, Darby JM, Okonkwo DO. Induced normothermia attenuates intracranial hypertension and reduces fever burden after severe traumatic brain injury. *Neurocrit Care*. 2009;11(1):82-87. doi:10.1007/s12028-009-9213-0
31. Scaravilli V, Tinchero G, Citerio G. Fever management in SAH. *Neurocrit Care*. 2011;15(2):287-294. doi:10.1007/s12028-011-9588-6
32. Papo I, Janny P, Caruselli G, Colnet G, Luongo A. Intracranial pressure time course in primary intracerebral hemorrhage. *Neurosurgery*. 1979;4(6):504-511. doi:10.1227/00006123-197906000-00002
33. Juvela S, Kase CS. Advances in intracerebral hemorrhage management. *Stroke*. 2006;37(2):301-304. doi:10.1161/01.STR.0000200445.68303.25
34. Sinar EJ, Mendelow AD, Graham DI, Teasdale GM. Experimental intracerebral hemorrhage: Effects of a temporary mass lesion. *J Neurosurg*. 1987;66(4):568-576. doi:10.3171/jns.1987.66.4.0568
35. Monro A. Observations on structure and functions of the ner- vous system. *Edinburgh: Creech and Johnson*. Published online 1783.
36. Mokri B. The Monro-Kellie hypothesis: Applications in CSF volume depletion. *Neurology*. 2001;56(12):1746-1748. doi:10.1212/WNL.56.12.1746
37. Reinstrup P, Ryding E, Ohlsson T, Dahm PL, Uski T. Cerebral blood volume (CBV) in humans during normo- and hypocapnia: Influence of nitrous oxide (N2O).

Anesthesiology. 2001;95(5):1079-1082. doi:10.1097/00000542-200111000-00009

38. Bolon B, Butt MT. Appendix 6: Comparative and Correlative Neurobiological Parameters for the Brain. In: *Fundamental Neuropathology for Pathologists and Toxicologists*. ; 2011:551-554. doi:10.1002/9780470939956.app6

39. Pardridge WM. Drug transport in brain via the cerebrospinal fluid. *Fluids Barriers CNS*. 2011;8(1). doi:10.1186/2045-8118-8-7

40. Sandor P, Cox-van Put J, de Jong W, de Wied D. Continuous measurement of cerebral blood volume in rats with the photoelectric technique: Effect of morphine and naloxone. *Life Sci*. 1986;39(18):1657-1665. doi:10.1016/0024-3205(86)90163-3

41. Sakka L, Coll G, Chazal J. Anatomy and physiology of cerebrospinal fluid. *Eur Ann Otorhinolaryngol Head Neck Dis*. 2011;128(6):309-316. doi:10.1016/j.anorl.2011.03.002

42. Beard DJ, Murtha LA, McLeod DD, Spratt NJ. Intracranial Pressure and Collateral Blood Flow. *Stroke*. 2016;47(6):1695-1700. doi:10.1161/STROKEAHA.115.011147

43. Dunn LT. Raised intracranial pressure. *Neurol Pract*. 2002;73(1). doi:10.1136/jnnp.73.suppl_1.i23

44. Eide PK, Sorteberg W. Association among intracranial compliance, intracranial pulse pressure amplitude and intracranial pressure in patients with intracranial bleeds. *Neurol Res*. 2007;29(8):798-802. doi:10.1179/016164107X224132

45. Hariri RJ, Firlick AD, Shepard SR, et al. Traumatic brain injury, hemorrhagic shock, and fluid resuscitation: Effects on intracranial pressure and brain compliance. *J Neurosurg*. 1993;79(3):421-427. doi:10.3171/jns.1993.79.3.0421

46. Fan JY, Kirkness C, Vicini P, Burr R, Mitchell P. Intracranial pressure waveform morphology and intracranial adaptive capacity. *Am J Crit Care*. 2008;17(6):545-554. doi:10.4037/ajcc2008.17.6.545

47. Zazulia AR, Diringer MN, Videen TO, et al. Hypoperfusion without ischemia surrounding acute intracerebral hemorrhage. *J Cereb Blood Flow Metab*. 2001;21(7):804-810.

doi:10.1097/00004647-200107000-00005

48. Nath FP, Jenkins A, Mendelow AD, Graham DI, Teasdale GM. Early hemodynamic changes in experimental intracerebral hemorrhage. *J Neurosurg*. 1986;65(5):697-703. doi:10.3171/jns.1986.65.5.0697

49. Qureshi AI, Wilson DA, Hanley DF, Traystman RJ. No evidence for an ischemic penumbra in massive experimental intracerebral hemorrhage. *Neurology*. 1999;52(2):266-272. doi:10.1212/wnl.52.2.266

50. Bothwell SW, Janigro D, Patabendige A. Cerebrospinal fluid dynamics and intracranial pressure elevation in neurological diseases. *Fluids Barriers CNS*. 2019;16(1). doi:10.1186/s12987-019-0129-6

51. Wan S, Hua Y, Keep RF, Hoff JT, Xi G. Deferoxamine reduces CSF free iron levels following intracerebral hemorrhage. *Acta Neurochir Suppl*. 2006;(96):199-202. doi:10.1007/3-211-30714-1_43

52. Bolte AC, Dutta AB, Hurt ME, et al. Meningeal lymphatic dysfunction exacerbates traumatic brain injury pathogenesis. *Nat Commun*. 2020;11(1). doi:10.1038/s41467-020-18113-4

53. Sun BL, Xia ZL, Wang JR, et al. Effects of blockade of cerebral lymphatic drainage on regional cerebral blood flow and brain edema after subarachnoid hemorrhage. In: *Clinical Hemorheology and Microcirculation*. ; 2006.

54. He G, Lü T, Lu B, et al. Perivascular and perineural extension of formed and soluble blood elements in an intracerebral hemorrhage rat model. *Brain Res*. 2012;1451:10-18. doi:10.1016/j.brainres.2012.02.069

55. Gaberel T, Gakuba C, Goulay R, et al. Impaired glymphatic perfusion after strokes revealed by contrast-enhanced MRI: A new target for fibrinolysis? *Stroke*. 2014;45(10):3092-3096. doi:10.1161/STROKEAHA.114.006617

56. Schlunk F, Greenberg SM. The Pathophysiology of Intracerebral Hemorrhage Formation

and Expansion. *Transl Stroke Res*. 2015;6(4):257-263. doi:10.1007/s12975-015-0410-1

57. Davis SM, Broderick J, Hennerici M, et al. Hematoma growth is a determinant of mortality and poor outcome after intracerebral hemorrhage. *Neurology*. 2006;66(8):1175-1181. doi:10.1212/01.wnl.0000208408.98482.99

58. Zazulia AR, Diringer MN, Derdeyn CP, Powers WJ. Progression of mass effect after intracerebral hemorrhage. *Stroke*. 1999;30(6):1167-1173. doi:10.1161/01.STR.30.6.1167

59. Heuer GG, Smith MJ, Elliott JP, Winn HR, Leroux PD. Relationship between intracranial pressure and other clinical variables in patients with aneurysmal subarachnoid hemorrhage. *J Neurosurg*. 2004;101(3):408-416. doi:10.3171/jns.2004.101.3.0408

60. Liddle LJ, Ralhan S, Ward DL, Colbourne F. Translational Intracerebral Hemorrhage Research: Has Current Neuroprotection Research ARRIVEd at a Standard for Experimental Design and Reporting? *Transl Stroke Res*. 2020;11(6):1203-1213. doi:10.1007/s12975-020-00824-x

61. Lampl Y, Gilad R, Eshel Y, Sarova-Pinhas I. Neurological and functional outcome in patients with supratentorial hemorrhages; a prospective study. *Stroke*. 1995;26(12):2249-2253. doi:10.1161/01.STR.26.12.2249

62. Butcher K, Laidlaw J. Current intracerebral haemorrhage management. *J Clin Neurosci*. 2003;10(2):158-167. doi:10.1016/S0967-5868(02)00324-7

63. Tian Y, Wang Z, Jia Y, et al. Intracranial pressure variability predicts short-term outcome after intracerebral hemorrhage: A retrospective study. *J Neurol Sci*. 2013;330(1-2):38-44. doi:10.1016/j.jns.2013.04.001

64. Sykora M, Steinmacher S, Steiner T, Poli S, Diedler J. Association of intracranial pressure with outcome in comatose patients with intracerebral hemorrhage. *J Neurol Sci*. 2014;342(1-2):141-145. doi:10.1016/j.jns.2014.05.012

65. Rosenberg G a., Mun-Bryce S, Wesley M, Kornfeld M. Collagenase-induced intracerebral hemorrhage in rats. *Stroke*. 1990;21(5):801-807. doi:10.1161/01.STR.21.5.801

66. Ropper AH, Zervas NT. Cerebral blood flow after experimental basal ganglia hemorrhage. *Ann Neurol*. 1982;11(3):266-271. doi:10.1002/ana.410110306

67. MacLellan CL, Silasi G, Poon CC, et al. Intracerebral hemorrhage models in rat: comparing collagenase to blood infusion. *J Cereb Blood Flow Metab*. 2008;28:516-525. doi:10.1038/sj.jcbfm.9600548

68. Hanci M. Blood degradation products play a role in cerebral ischemia caused by acute subdural hematoma. *J Neurosurg Sci*. 1997;41(4):379-385.

69. Lee KR, Kawai N, Kim S, Sagher O, Hoff JT. Mechanisms of edema formation after intracerebral hemorrhage: Effects of thrombin on cerebral blood flow, blood-brain barrier permeability, and cell survival in a rat model. *J Neurosurg*. 1997;86(2):272-278. doi:10.3171/jns.1997.86.2.0272

70. Lee KR, Drury I, Vitarbo E, Hoff JT. Seizures induced by intracerebral injection of thrombin: a model of intracerebral hemorrhage. *J Neurosurg*. 1997;87(1):73-78. doi:10.3171/jns.1997.87.1.0073

71. Caliaperumal J, Ma Y, Colbourne F. Intra-parenchymal ferrous iron infusion causes neuronal atrophy, cell death and progressive tissue loss: Implications for intracerebral hemorrhage. *Exp Neurol*. 2012;237(2):363-369. doi:10.1016/j.expneurol.2012.07.001

72. Caliaperumal J, Brodie S, Ma Y, Colbourne F. Thrombin Causes Neuronal Atrophy and Acute but not Chronic Cell Death. *Can J Neurol Sci*. 2014;41(6):714-720. doi:10.1017/cjn.2014.105

73. Lee JM, Zhai G, Liu Q, et al. Vascular permeability precedes spontaneous intracerebral hemorrhage in stroke-prone spontaneously hypertensive rats. *Stroke*. 2007;38(12):3289-3291. doi:10.1161/STROKEAHA.107.491621

74. Davis LA, Smeda JS. Captopril treatment temporarily restores cerebral blood flow autoregulation in spontaneously hypertensive rats after hemorrhagic stroke. *J Cardiovasc Pharmacol*. 2010;56(3):255-262. doi:10.1097/FJC.0b013e3181e8af62

75. Kirkman MA, Allan SM, Parry-Jones AR. Experimental intracerebral hemorrhage: Avoiding pitfalls in translational research. *J Cereb Blood Flow Metab*. 2011;31(11):2135-2151. doi:10.1038/jcbfm.2011.124

76. John RF, Colbourne F. Delayed localized hypothermia reduces intracranial pressure following collagenase-induced intracerebral hemorrhage in rat. *Brain Res*. 2016;1633:27-36. doi:10.1016/j.brainres.2015.12.033

77. Kingman TA, Mendelow AD, Graham DI, Teasdale GM. Experimental intracerebral mass: Description of model, intracranial pressure changes and neuropathology. *J Neuropathol Exp Neurol*. 1988;47(2):128-137. doi:10.1097/00005072-198803000-00005

78. Karuppagounder SS, Alin L, Chen Y, et al. N-acetylcysteine targets 5 lipoxygenase-derived, toxic lipids and can synergize with prostaglandin E2 to inhibit ferroptosis and improve outcomes following hemorrhagic stroke in mice. *Ann Neurol*. 2018;84(6):854-872. doi:10.1002/ana.25356

79. Zhu W, Gao Y, Chang CF, Wan JR, Zhu SS, Wang J. Mouse models of intracerebral hemorrhage in ventricle, cortex, and hippocampus by injections of autologous blood or collagenase. *PLoS One*. 2014;9(5). doi:10.1371/journal.pone.0097423

80. Lekic T, Tang J, Zhang JH. Rat model of intracerebellar hemorrhage. *Acta Neurochir Suppl*. 2008;(105):131-134. doi:10.1007/978-3-211-09469-3_27

81. Shrestha BK, Guo X, Ma L, et al. Rat Brainstem Hemorrhage Model: Key Points to Success in Modeling. *World Neurosurg*. 2018;117:e106-e116. doi:10.1016/j.wneu.2018.05.195

82. Sloan MA, Sila CA, Mahaffey KW, et al. Prediction of 30-day mortality among patients with thrombolysis-related intracranial hemorrhage. *Circulation*. 1998;98(14):1376-1382. doi:10.1161/01.CIR.98.14.1376

83. Dastur CK, Yu W. Current management of spontaneous intracerebral haemorrhage. *Stroke Vasc Neurol*. 2017;2(1):21-29. doi:10.1136/svn-2016-000047

84. Ziai WC, Thompson CB, Mayo S, et al. Intracranial Hypertension and Cerebral Perfusion Pressure Insults in Adult Hypertensive Intraventricular Hemorrhage: Occurrence and Associations With Outcome. *Crit Care Med*. 2019;47(8):1125-1134. doi:10.1097/CCM.0000000000003848

85. Wilkinson CM, Kalisvaart ACJ, Kung TFC, et al. The collagenase model of intracerebral hemorrhage in awake, freely moving animals: The effects of isoflurane. *Brain Res*. 2020;1728:146593. doi:10.1016/j.brainres.2019.146593

86. Klahr AC, Dickson CT, Colbourne F. Seizure Activity Occurs in the Collagenase but not the Blood Infusion Model of Striatal Hemorrhagic Stroke in Rats. *Transl Stroke Res*. 2014;6(1):29-38. doi:10.1007/s12975-014-0361-y

87. Rudy TA, Westergaard JL, Yaksh TL. Hyperthermia produced by simulated intraventricular hemorrhage in the cat. *Exp Neurol*. 1978;58(2):296-310. doi:10.1016/0014-4886(78)90142-5

88. Wilkinson CM, Fedor BA, Aziz JR, et al. Failure of bumetanide to improve outcome after intracerebral hemorrhage in rat. *PLoS One*. 2019;14(1):1-20. doi:10.1371/journal.pone.0210660

89. MacLellan CL, Paquette R, Colbourne F. A critical appraisal of experimental intracerebral hemorrhage research. *J Cereb Blood Flow Metab*. 2012;32(4):612-627. doi:10.1038/jcbfm.2012.8

90. Qureshi AI, Wilson DA, Traystman RJ. Treatment of elevated intracranial pressure in experimental intracerebral hemorrhage: Comparison between mannitol and hypertonic saline. *Neurosurgery*. 1999;44(5):1055-1064. doi:10.1097/00006123-199905000-00064

91. Qureshi AI, Wilson DA, Hanley DF, Traystman RJ. Pharmacologic reduction of mean arterial pressure does not adversely affect regional cerebral blood flow and intracranial pressure in experimental intracerebral hemorrhage. *Crit Care Med*. 1999;27(5):965-971. doi:10.1097/00003246-199905000-00036

92. Kobari M, Gotoh F, Tomita M, et al. Bilateral hemispheric reduction of cerebral blood volume and blood flow immediately after experimental cerebral hemorrhage in cats. *Stroke*. 1988;19(8):991-996. doi:10.1161/01.STR.19.8.991

93. Tomita H, Ito U, Ohno K, Hirakawa K. Chronological changes in brain edema induced by experimental intracerebral hematoma in cats. *Acta Neurochir Suppl (Wien)*. 1994;60:558-560. doi:10.1007/978-3-7091-9334-1_154

94. Scheller MS, Tateishi A, Drummond JC, Zornow MH. The effects of sevoflurane on cerebral blood flow, cerebral metabolic rate for oxygen, intracranial pressure, and the electroencephalogram are similar to those of isoflurane in the rabbit. *Anesthesiology*. 1988;68(4):548-551. doi:10.1097/00000542-198804000-00012

95. Karwacki Z, Kowiański P, Moryś J, Dziewiątkowski J, Kaczmarek E, Suchorzewska J. Effect of sevoflurane on intracranial pressure and cardiovascular function in rabbits with experimental intracerebral haematoma. *Med Sci Monit*. 2001;7(2):212-217.

96. Jasien J V., Samuels BC, Johnston JM, Downs JC. Diurnal cycle of translaminar pressure in nonhuman primates quantified with continuous wireless telemetry. *Investig Ophthalmol Vis Sci*. 2020;61(2). doi:10.1167/iovs.61.2.37

97. Silasi G, MacLellan CL, Colbourne F. Use of telemetry blood pressure transmitters to measure intracranial pressure (ICP) in freely moving rats. *Curr Neurovasc Res*. 2009;6(1):62-69. doi:10.2174/156720209787466046

98. Kim KD, Chang CH, Choi BY, Jung YJ. Mortality and real cause of death from the nonlesional intracerebral hemorrhage. *J Korean Neurosurg Soc*. 2014;55(1):1-4. doi:10.3340/jkns.2014.55.1.1

99. Magistris F, Bazak S, Martin J. Intracerebral Hemorrhage: Pathophysiology, Diagnosis and Management. *McMaster Univ Med J*. Published online 2013.

100. Broderick JP, Brott TG, Duldner JE, Tomsick T, Huster G. Volume of intracerebral hemorrhage: A powerful and easy-to-use predictor of 30-day mortality. *Stroke*.

1993;24(7):987-993. doi:10.1161/01.STR.24.7.987

101. James ML, Warner DS, Laskowitz DT. Preclinical models of intracerebral hemorrhage: A translational perspective. *Neurocrit Care*. 2008;9(1):139-152. doi:10.1007/s12028-007-9030-2

102. Hayashi M, Kobayashi H, Kawano H, Handa Y, Hirose S. Treatment of systemic hypertension and intracranial hypertension in cases of brain hemorrhage. *Stroke*. 1988;19(3):314-321. doi:10.1161/01.STR.19.3.314

103. Shintani S, Umezato M, Toba Y, et al. Pharmacological properties of buprenorphine, a new analgesic agent. Part II. *Folia Pharmacol Jpn*. 1982;79(3):173-191. doi:10.1254/fpj.79.173

104. Kamel H, Hemphill JC. Characteristics and sequelae of intracranial hypertension after intracerebral hemorrhage. *Neurocrit Care*. 2012;17(2):172-176. doi:10.1007/s12028-012-9744-7

105. Shahrokhi N, Khaksari M, Soltani Z, Mahmoodi M, Nakhaee N. Effect of sex steroid hormones on brain edema, intracranial pressure, and neurologic outcomes after traumatic brain injury. *Can J Physiol Pharmacol*. 2010;88(4):414-421. doi:10.1139/Y09-126

106. Gong Y, Hua Y, Keep RF, Hoff JT, Xi G. Intracerebral hemorrhage: Effects of aging on brain edema and neurological deficits. In: *Stroke*. ; 2004. doi:10.1161/01.STR.0000145485.67827.d0

107. Hamezah HS, Durani LW, Ibrahim NF, et al. Volumetric changes in the aging rat brain and its impact on cognitive and locomotor functions. *Exp Gerontol*. 2017;99:69-79. doi:10.1016/j.exger.2017.09.008

108. Sheth K, Elm J, Cheung K, et al. Abstracts for the 10th World Stroke Congress, 2016. *Int J Stroke*. 2016;11(3_suppl):4-288. doi:10.1177/1747493016670567

109. Uldall M, Juhler M, Skjolding AD, Kruuse C, Jansen-Olesen I, Jensen R. A novel method

for long-term monitoring of intracranial pressure in rats. *J Neurosci Methods*. 2014;227:1-9. doi:10.1016/j.jneumeth.2014.01.036

110. Wagner I, Volbers B, Kloska S, Doerfler A, Schwab S, Staykov D. Sex differences in perihemorrhagic edema evolution after spontaneous intracerebral hemorrhage. *Eur J Neurol*. 2012;19(11):1477-1481. doi:10.1111/j.1468-1331.2011.03628.x
111. Jiang C, Zuo F, Wang Y, et al. Progesterone exerts neuroprotective effects and improves long-term neurologic outcome after intracerebral hemorrhage in middle-aged mice. *Neurobiol Aging*. 2016;42:13-24. doi:10.1016/j.neurobiolaging.2016.02.029
112. Chen Z, Xi G, Mao Y, Keep RF, Hua Y. Effects of progesterone and testosterone on ICH-induced brain injury in rats. In: *Acta Neurochirurgica, Supplementum*. ; 2011. doi:10.1007/978-3-7091-0693-8_48
113. Ariesen MJ, Claus SP, Rinkel GJE, Algra A. Risk factors for intracerebral hemorrhage in the general population: A systematic review. *Stroke*. 2003;34(8):2060-2065. doi:10.1161/01.STR.0000080678.09344.8D
114. Immink R V., Van Den Born BJH, Van Montfrans GA, Koopmans RP, Karemaker JM, Van Lieshout JJ. Impaired cerebral autoregulation in patients with malignant hypertension. *Circulation*. 2004;110(15):2241-2245. doi:10.1161/01.CIR.0000144472.08647.40
115. Smeda JS, VanVliet BN, King SR. Stroke-prone spontaneously hypertensive rats lose their ability to auto-regulate cerebral blood flow prior to stroke. *J Hypertens*. 1999;17(12):1697-1705. doi:10.1097/00004872-199917120-00006
116. Lapchak PA, Zhang JH, Noble-Haeusslein LJ. RIGOR Guidelines: Escalating STAIR and STEPS for Effective Translational Research. *Transl Stroke Res*. 2013;4(3):279-285. doi:10.1007/s12975-012-0209-2
117. Fisher M, Feuerstein G, Howells DW, et al. Update of the stroke therapy academic industry roundtable preclinical recommendations. *Stroke*. 2009;40(6):2244-2250. doi:10.1161/STROKEAHA.108.541128

118. Kawoos U, Gu M, Lankasky J, McCarron RM, Chavko M. Effects of exposure to blast overpressure on intracranial pressure and blood-brain barrier permeability in a rat model. *PLoS One*. 2016;11(12). doi:10.1371/journal.pone.0167510

119. Kawoos U, Meng X, Huang SM, Rosen A, McCarron RM, Chavko M. Telemetric intracranial pressure monitoring in blast-induced traumatic brain injury. *IEEE Trans Biomed Eng*. 2014;61(3):841-847. doi:10.1109/TBME.2013.2291239

120. Kawoos U, McCarron RM, Chavko M. Protective effect of N-acetylcysteine amide on blast-induced increase in intracranial pressure in rats. *Front Neurol*. 2017;8(JUN). doi:10.3389/fneur.2017.00219

121. Lin JS, Liu JHK. Circadian variations in intracranial pressure and translaminar pressure difference in sprague-dawley rats. *Investig Ophthalmol Vis Sci*. 2010;51(11):5739-5743. doi:10.1167/iovs.10-5542

122. Jiang J, Tyssebotn I. Measurement of cerebrospinal fluid pressure in conscious rats. *Undersea Hyperb Med*. 1997;24(1):39-43.

123. Malkinson TJ, Cooper KE, Veale WL. Cerebrospinal fluid pressure in conscious rats during prostaglandin E1 fever. *Am J Physiol - Regul Integr Comp Physiol*. 1990;258(3 27-3). doi:10.1152/ajpregu.1990.258.3.r783

124. Alshuhri MS, Gallagher L, McCabe C, Holmes WM. Change in CSF Dynamics Responsible for ICP Elevation After Ischemic Stroke in Rats: a New Mechanism for Unexplained END? *Transl Stroke Res*. 2020;11(2):310-318. doi:10.1007/s12975-019-00719-6

125. Eide PK. Comparison of simultaneous continuous intracranial pressure (ICP) signals from ICP sensors placed within the brain parenchyma and the epidural space. *Med Eng Phys*. 2008;30(1):34-40. doi:10.1016/j.medengphy.2007.01.005

126. Zwienenberg M, Gong QZ, Lee LL, Berman RF, Lyeth BG. ICP monitoring in the rat: Comparison of monitoring in the ventricle, brain parenchyma, and cistema magna. *J*

Neurotrauma. 1999;16(11):1095-1102. doi:10.1089/neu.1999.16.1095

127. Williamson MR, Wilkinson CM, Dietrich K, Colbourne F. Acetazolamide Mitigates Intracranial Pressure Spikes Without Affecting Functional Outcome After Experimental Hemorrhagic Stroke. *Transl Stroke Res*. 2019;10(4):428-439. doi:10.1007/s12975-018-0663-6

128. Guild SJ, McBryde FD, Malpas SC. Recording of intracranial pressure in conscious rats via telemetry. *J Appl Physiol*. 2015;119(5):576-581. doi:10.1152/japplphysiol.00165.2015

129. Hemphill JC, Greenberg SM, Anderson CS, et al. Guidelines for the Management of Spontaneous Intracerebral Hemorrhage: A Guideline for Healthcare Professionals from the American Heart Association/American Stroke Association. *Stroke*. 2015;46(7):2032-2060. doi:10.1161/STR.0000000000000069

130. Goren S, Kahveci N, Alkan T, Goren B, Korfali E. The effects of sevoflurane and isoflurane on intracranial pressure and cerebral perfusion pressure after diffuse brain injury in rats. *J Neurosurg Anesthesiol*. 2001;13(2):113-119. doi:10.1097/00008506-200104000-00008

131. Bar-Joseph G, Guilburd Y, Tamir A, Guilburd JN. Effectiveness of ketamine in decreasing intracranial pressure in children with intracranial hypertension: Clinical article. *J Neurosurg Pediatr*. 2009;4(1):40-46. doi:10.3171/2009.1.PEDS08319

132. Albanèse J, Arnaud S, Rey M, Thomachot L, Alliez B, Martin C. Ketamine decreases intracranial pressure and electroencephalographic activity in traumatic brain injury patients during propofol sedation. *Anesthesiology*. 1997;87(6):1328-1334. doi:10.1097/00000542-199712000-00011

133. Pinaud M, Lelausque JN, Chetanneau A, Fauchoux N, Menegalli D, Souron R. Effects of propofol on cerebral hemodynamics and metabolism in patients with brain trauma. *Anesthesiology*. 1990;73(3):404-409. doi:10.1097/00000542-199009000-00007

134. Jennett WB, Barker J, Fitch W, McDowall DG. Effect of anaesthesia on intracranial

pressure in patients with space-occupying lesions. *Lancet*. 1969;1(7585):61-64. doi:10.1097/00132586-197002000-00037

135. Bazin JE. Effects of anaesthetic agents on intracranial pressure. In: *Annales Francaises d'Anesthesie et de Reanimation*. ; 1997. doi:10.1016/S0750-7658(97)81477-3

136. Starcevic VP, Morrow BA, Farner LA, Keil LC, Severs WB. Long-term recording of cerebrospinal fluid pressure in freely behaving rats. *Brain Res*. 1988;462(1):112-117. doi:10.1016/0006-8993(88)90592-6

137. Irvine RJ, White J, Chan R. The influence of restrains on blood pressure in the rat. *J Pharmacol Toxicol Methods*. 1997;38(3):157-162. doi:10.1016/S1056-8719(97)00081-6

138. Wilkinson CM, Kalisvaart ACJ, Kung TFC, et al. The collagenase model of intracerebral hemorrhage in awake, freely moving animals: The effects of isoflurane. *Brain Res*. 2020;1728:146593. doi:10.1016/j.brainres.2019.146593

139. Mandell EC, Zimmermann E. Continuous measurement of cerebrospinal fluid pressure in unrestrained rats. *Physiol Behav*. 1980;24(2):399-402. doi:10.1016/0031-9384(80)90105-5

140. Chen CJ, Ding D, Ironside N, et al. Intracranial pressure monitoring in patients with spontaneous intracerebral hemorrhage. *J Neurosurg*. 2020;132(6):1854-1864. doi:10.3171/2019.3.JNS19545

141. Subramaniam S, Hill MD. Controversies in medical management of intracerebral hemorrhage. *Can J Neurol Sci*. 2005;32 Suppl 2:S13-21. http://www.ncbi.nlm.nih.gov/pubmed/16450804

142. Maira G, Anile C, Colosimo C, Rossi GF. Surgical treatment of primary supratentorial intracerebral hemorrhage in stuporous and comatose patients. *Neurol Res*. 2002;24(1):54-60. doi:10.1179/016164102101199549

143. Aucoin PJ, Kotilainen HR, Gantz NM, Davidson R, Kellogg P, Stone B. Intracranial pressure monitors. Epidemiologic study of risk factors and infections. *Am J Med*.

1986;80(3):369-376. doi:10.1016/0002-9343(86)90708-4

144. North B, Reilly P. Comparison among three methods of intracranial pressure recording. *Neurosurgery*. 1986;18(6):730-732. doi:10.1227/00006123-198606000-00009

145. Rajajee V, Vanaman M, Fletcher JJ, Jacobs TL. Optic nerve ultrasound for the detection of raised intracranial pressure. *Neurocrit Care*. 2011;15(3):506-515. doi:10.1007/s12028-011-9606-8

146. de Oliveira Manoel AL, Goffi A, Zampieri FG, et al. The critical care management of spontaneous intracranial hemorrhage: A contemporary review. *Crit Care*. 2016;20(1). doi:10.1186/s13054-016-1432-0

147. Marinkovic I, Strbian D, Pedrono E, et al. Decompressive craniectomy for intracerebral hemorrhage. *Neurosurgery*. 2009;65(4):780-786. doi:10.1227/01.NEU.0000351775.30702.A9

148. Bühler D, Azghandi S, Schüller K, Plesnila N. Effect of decompressive craniectomy on outcome following subarachnoid hemorrhage in mice. *Stroke*. 2015;46(3):819-826. doi:10.1161/STROKEAHA.114.007703

149. Kuczynski AM, Marzoughi S, Al Sultan AS, et al. Therapeutic Hypothermia in Acute Ischemic Stroke-a Systematic Review and Meta-Analysis. *Curr Neurol Neurosci Rep*. 2020;20(5):13. doi:10.1007/s11910-020-01029-3

150. Sacco S, Marini C, Carolei A. Medical treatment of intracerebral hemorrhage. *Neurol Sci*. 2004;25(SUPPL. 1). doi:10.1007/s10072-004-0206-7

151. Mendelow AD, Gregson BA, Rowan EN, et al. Early surgery versus initial conservative treatment in patients with spontaneous supratentorial lobar intracerebral haematomas (STICH II): A randomised trial. *Lancet*. 2013;382(9890):397-408. doi:10.1016/S0140-6736(13)60986-1

152. Alberio N, Cicero S, Iacopino DG, et al. Minimally Invasive Management of Spontaneous Supratentorial Intracerebral Lobar Hemorrhages by a “Homemade” Endoscopic Strategy:

The Evangelical Doctrine of “Venite ad Me” Allied to the Legacy of King Leonida. *World Neurosurg*. 2019;122:638-647. doi:10.1016/j.wneu.2018.11.136

153. Li Y, Yang R, Li Z, et al. Surgical Evacuation of Spontaneous Supratentorial Lobar Intracerebral Hemorrhage: Comparison of Safety and Efficacy of Stereotactic Aspiration, Endoscopic Surgery, and Craniotomy. *World Neurosurg*. 2017;105:332-340. doi:10.1016/j.wneu.2017.05.134
154. Ziai WC, Toung TJK, Bhardwaj A. Hypertonic saline: First-line therapy for cerebral edema? *J Neurol Sci*. 2007;261(1-2):157-166. doi:10.1016/j.jns.2007.04.048
155. Mirski MA, Denchev DI, Schnitzer MS, Hanley DF. Comparison between hypertonic saline and mannitol in the reduction of elevated intracranial pressure in a rodent model of acute cerebral injury. *J Neurosurg Anesthesiol*. 2000;12(4):334-344. doi:10.1097/00008506-200010000-00006
156. Kamel H, Navi BB, Nakagawa K, Hemphill JC, Ko NU. Hypertonic saline versus mannitol for the treatment of elevated intracranial pressure: A meta-analysis of randomized clinical trials. *Crit Care Med*. 2011;39(3):554-559. doi:10.1097/CCM.0b013e318206b9be
157. Qureshi AI, Suri MFK, Ringer AJ, Guterman LR, Hopkins LN. Regional intraparenchymal pressure differences in experimental intracerebral hemorrhage: Effect of hypertonic saline. *Crit Care Med*. 2002;30(2):435-441. doi:10.1097/00003246-200202000-00028
158. Da Silva JC, De Melo Tavares De Lima F, Valença MM, Cirne De Azevedo Filho HR. Hypertonic saline more efficacious than mannitol in lethal intracranial hypertension model. *Neurol Res*. 2010;32(2):139-143. doi:10.1179/174313209X405119
159. Schreibman DL, Hong CM, Keledjian K, et al. Mannitol and Hypertonic Saline Reduce Swelling and Modulate Inflammatory Markers in a Rat Model of Intracerebral Hemorrhage. *Neurocrit Care*. 2018;29(2):253-263. doi:10.1007/s12028-018-0535-7
160. Glimåker M, Johansson B, Halldorsdottir H, et al. Neuro-intensive treatment targeting intracranial hypertension improves outcome in severe bacterial meningitis: An

intervention-control study. *PLoS One*. 2014;9(3). doi:10.1371/journal.pone.0091976

161. Mitchell P, Gregson BA, Vindlacheruvu RR, Mendelow AD. Surgical options in ICH including decompressive craniectomy. *J Neurol Sci*. 2007;261(1-2):89-98. doi:10.1016/j.jns.2007.04.040

162. Guo G, Guo W, Feng Y, et al. Therapeutic time window of minimally invasive surgery for intracerebral hemorrhage. *Brain Hemorrhages*. 2020;(xxxx):4-6. doi:10.1016/j.hest.2020.07.002

163. Fukuhara T, Luciano MG, Brant CL, Klauscie J. Effects of ventriculoperitoneal shunt removal on cerebral oxygenation and brain compliance in chronic obstructive hydrocephalus. *J Neurosurg*. 2001;94(4):573-581. doi:10.3171/jns.2001.94.4.0573

164. Miller JM, McAllister JP. Reduction of astrogliosis and microgliosis by cerebrospinal fluid shunting in experimental hydrocephalus. *Cerebrospinal Fluid Res*. 2007;4. doi:10.1186/1743-8454-4-5

165. Lopez Valdes E, Hernandez Lain A, Calandre L, Grau M, Cabello A, Gomez-Escalonilla C. Time window for clinical effectiveness of mass evacuation in a rat balloon model mimicking an intraparenchymatous hematoma. *J Neurol Sci*. 2000;174(1):40-46. doi:10.1016/S0022-510X(99)00288-9

166. Nehls DG, Mendelow AD, Graham DI, Teasdale GM. Experimental intracerebral hemorrhage: Early removal of a spontaneous mass lesion improves late outcome. *Neurosurgery*. 1990;27(5):674-682. doi:10.1227/00006123-199011000-00002

167. Deinsberger W, Vogel J, Fuchs C, Auer LM, Kuschinsky W, Böker DK. Fibrinolysis and aspiration of experimental intracerebral hematoma reduces the volume of ischemic brain in rats. *Neurol Res*. 1999;21(5):517-523. doi:10.1080/01616412.1999.11740968

168. Thiex R, Küker W, Jungbluth P, et al. Minor inflammation after surgical evacuation compared with fibrinolytic therapy of experimental intracerebral hemorrhages. *Neurol Res*. 2005;27(5):493-498. doi:10.1179/016164105X17369

169. Wu G, Sheng F, Wang L, Wang F. The pathophysiological time window study of performing minimally invasive procedures for the intracerebral hematoma evacuation in rabbit. *Brain Res*. 2012;1465:57-65. doi:10.1016/j.brainres.2012.04.005

170. Rohde V, Rohde I, Thiex R, et al. Fibrinolysis therapy achieved with tissue plasminogen activator and aspiration of the liquefied clot after experimental intracerebral hemorrhage: Rapid reduction in hematoma volume but intensification of delayed edema formation. *J Neurosurg*. 2002;97(4):954-962. doi:10.3171/jns.2002.97.4.0954

171. Kolecka M, Ondreka N, Moritz A, Kramer M, Schmidt MJ. Effect of acetazolamide and subsequent ventriculo-peritoneal shunting on clinical signs and ventricular volumes in dogs with internal hydrocephalus. *Acta Vet Scand*. 2015;57(1). doi:10.1186/s13028-015-0137-8

172. Uldall M, Botfield H, Jansen-Olesen I, Sinclair A, Jensen R. Acetazolamide lowers intracranial pressure and modulates the cerebrospinal fluid secretion pathway in healthy rats. *Neurosci Lett*. 2017;645:33-39. doi:10.1016/j.neulet.2017.02.032

173. Wilkinson HA, Rosenfeld S. Furosemide and mannitol in the treatment of acute experimental intracranial hypertension. *Neurosurgery*. 1983;12(4):405-410. doi:10.1227/00006123-198304000-00006

174. Miller TB, Wilkinson HA, Rosenfeld SA, Furuta T. Intracranial hypertension and cerebrospinal fluid production in dogs: Effects of furosemide. *Exp Neurol*. 1986;94(1):66-80. doi:10.1016/0014-4886(86)90272-4

175. Scotton WJ, Botfield HF, Westgate CSJ, et al. Topiramate is more effective than acetazolamide at lowering intracranial pressure. *Cephalalgia*. 2019;39(2):209-218. doi:10.1177/0333102418776455

176. Kahveci FS, Kahveci N, Alkan T, Goren B, Korfali E, Ozluk K. Propofol versus isoflurane anesthesia under hypothermic conditions: Effects on intracranial pressure and local cerebral blood flow after diffuse traumatic brain injury in the rat. *Surg Neurol*.

2001;56(3):206-214. doi:10.1016/S0090-3019(01)00555-9

177. Clubb RJ, Maxwell RE, Chou SN. Experimental brain injury in the dog. The pharmacological effects of pentobarbital and sodium nitroprusside. *J Neurosurg*. 1980;52(2):189-196. doi:10.3171/jns.1980.52.2.0189

178. Hayashi M, Kobayashi H, Kawano H, Handa Y, Kabuto M. The effects of local intraparenchymal pentobarbital on intracranial hypertension following experimental subarachnoid hemorrhage. *Anesthesiology*. 1987;66(6):758-765. doi:10.1097/00000542-198706000-00009

179. Villa F, Iacca C, Molinari AF, et al. Inhalation versus endovenous sedation in subarachnoid hemorrhage patients: Effects on regional cerebral blood flow. *Crit Care Med*. 2012;40(10):2797-2804. doi:10.1097/CCM.0b013e31825b8bc6

180. Roberts DJ, Hall RI, Kramer AH, Robertson HL, Gallagher CN, Zygun DA. Sedation for critically ill adults with severe traumatic brain injury: A systematic review of randomized controlled trials. *Crit Care Med*. 2011;39(12):2743-2751. doi:10.1097/CCM.0b013e318228236f

181. Choi HA, Badjatia N, Mayer SA. Hypothermia for acute brain injury - Mechanisms and practical aspects. *Nat Rev Neurol*. 2012;8(4):214-222. doi:10.1038/nrneurol.2012.21

182. Hemmen TM, Lyden PD. Induced hypothermia for acute stroke. In: *Stroke*. ; 2007. doi:10.1161/01.STR.0000247920.15708.fa

183. Kawai N, Nakamura T, Okauchi M, Nagao S. Effects of hypothermia on intracranial pressure and brain edema formation: Studies in a rat acute subdural hematoma model. *J Neurotrauma*. 2000;17(3):193-202. doi:10.1089/neu.2000.17.193

184. John RF, Williamson MR, Dietrich K, Colbourne F. Localized Hypothermia Aggravates Bleeding in the Collagenase Model of Intracerebral Hemorrhage. *Ther Hypothermia Temp Manag*. 2015;5(1):19-25. doi:10.1089/ther.2014.0020

185. Wowk S, Fagan KJ, Ma Y, Nichol H, Colbourne F. Examining potential side effects of

therapeutic hypothermia in experimental intracerebral hemorrhage. *J Cereb Blood Flow Metab*. 2017;37(8):2975-2986. doi:10.1177/0271678X16681312

186. Murtha LA, McLeod DD, Pepperall D, et al. Intracranial pressure elevation after ischemic stroke in rats: Cerebral edema is not the only cause, and short-duration mild hypothermia is a highly effective preventive therapy. *J Cereb Blood Flow Metab*. 2015;35(4):592-600. doi:10.1038/jcbfm.2014.230

187. Murtha LA, Mcleod DD, Mccann SK, et al. Short-duration hypothermia after ischemic stroke prevents delayed intracranial pressure rise. *Int J Stroke*. 2014;9(5):553-559. doi:10.1111/ijs.12181

188. Moore EM, Nichol AD, Bernard SA, Bellomo R. Therapeutic hypothermia: Benefits, mechanisms and potential clinical applications in neurological, cardiac and kidney injury. *Injury*. 2011;42(9):843-854. doi:10.1016/j.injury.2011.03.027

189. Kollmar R, Staykov D, Dörfler A, Schellinger PD, Schwab S, Bardutzky J. Hypothermia reduces perihemorrhagic edema after intracerebral hemorrhage. *Stroke*. 2010;41(8):1684-1689. doi:10.1161/STROKEAHA.110.587758

190. Staykov D, Wagner I, Volbers B, Doerfler A, Schwab S, Kollmar R. Mild prolonged hypothermia for large intracerebral hemorrhage. *Neurocrit Care*. 2013;18(2):178-183. doi:10.1007/s12028-012-9762-5

191. Citerio G, Prisco L, Oddo M, et al. International prospective observational study on intracranial pressure in intensive care (ICU): The SYNAPSE-ICU study protocol. *BMJ Open*. 2019;9(4). doi:10.1136/bmjopen-2018-026552

192. Giraudet F, Longeras F, Mulliez A, et al. Noninvasive detection of alarming intracranial pressure changes by auditory monitoring in early management of brain injury: A prospective invasive versus noninvasive study. *Crit Care*. 2017;21(1). doi:10.1186/s13054-017-1616-2

193. Seule M, Sikorski C, Sakowitz O, et al. Evaluation of a New Brain Tissue Probe for

Intracranial Pressure, Temperature, and Cerebral Blood Flow Monitoring in Patients with Aneurysmal Subarachnoid Hemorrhage. *Neurocrit Care*. 2016;25(2):193-200. doi:10.1007/s12028-016-0284-4

194. Preethi J, Bidkar PU, Cherian A, et al. Comparison of total intravenous anesthesia vs. inhalational anesthesia on brain relaxation, intracranial pressure, and hemodynamics in patients with acute subdural hematoma undergoing emergency craniotomy: a randomized control trial. *Eur J Trauma Emerg Surg*. 2021;47(3):831-837. doi:10.1007/s00068-019-01249-4
195. Wilkinson CM, Brar PS, Balay CJ, Colbourne F. Glibenclamide, a Sur1-Trpm4 antagonist, does not improve outcome after collagenase-induced intracerebral hemorrhage. *PLoS One*. 2019;14(5). doi:10.1371/journal.pone.0215952
196. Jiang B, Li L, Chen Q, et al. Role of Glibenclamide in Brain Injury After Intracerebral Hemorrhage. *Transl Stroke Res*. 2017;8(2):183-193. doi:10.1007/s12975-016-0506-2
197. Uddin MS, Mamun A Al, Jakaria M, et al. Emerging promise of sulforaphane-mediated Nrf2 signaling cascade against neurological disorders. *Sci Total Environ*. 2020;707. doi:10.1016/j.scitotenv.2019.135624
198. Zhao X, Sun G, Zhang J, et al. Transcription factor Nrf2 protects the brain from damage produced by intracerebral hemorrhage. *Stroke*. 2007;38(12):3280-3286. doi:10.1161/STROKEAHA.107.486506
199. Schmidt-Pogoda A, Bonberg N, Koecke MHM, et al. Why Most Acute Stroke Studies Are Positive in Animals but Not in Patients: A Systematic Comparison of Preclinical, Early Phase, and Phase 3 Clinical Trials of Neuroprotective Agents. *Ann Neurol*. 2020;87(1):40-51. doi:10.1002/ana.25643
200. Tuhrim S, Horowitz DR, Sacher M, Godbold JH. Volume of ventricular blood is an important determinant of outcome in supratentorial intracerebral hemorrhage. *Crit Care Med*. 1999;27(3):617-621. doi:10.1097/00003246-199903000-00045

201. Roman MJ, Pini R, Pickering TG, Devereux RB. Non-invasive measurements of arterial compliance in hypertensive compared with normotensive adults. *J Hypertens*. 1992;10:S115-S118. doi:10.1097/00004872-199208001-00029

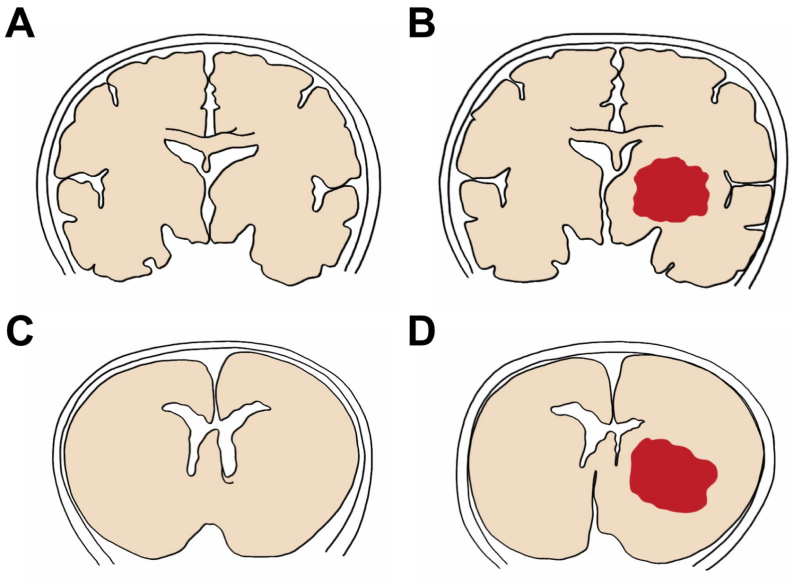

Figure 2-1. After large ICH, the mass effect of the hematoma and edema leads to midline shift, reduced CSF, compressed ventricles, and, in some cases, brain herniation. Pictured is a coronal section of a normal brain in humans (A) and the resulting brain shifts due to a hematoma (B). For comparison is a lissencephalic rat brain both before (C) and after ICH (D). In both cases, ventricular or subarachnoid extension of the hematoma can further increase ICP by obstructing CSF flow.[200]

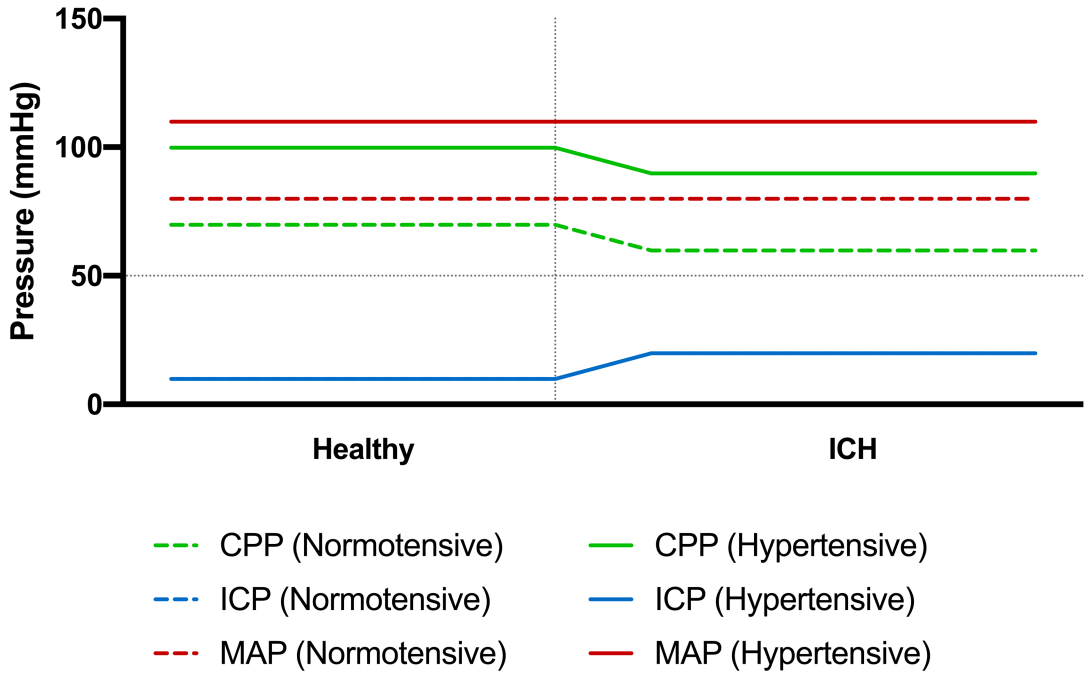

Figure 2-2. Cerebral perfusion pressure (CPP) = Mean Arterial Pressure (MAP) - Intracranial Pressure (ICP). In healthy patients, CPP is around 60 mmHg, MAP is around 70 mmHg, and ICP is around 10 mmHg.[32,201] In hypertensive patients, an increase in MAP results in a similar increase in CPP, with no change in ICP. In hypertensive patients with increased ICP, such as stroke patients, increases in MAP and ICP can result in either an increase or decrease in CPP from healthy levels. These changes are similar in rodents, albeit with lower ICP values. Following ICH, lowered CPP can lead to risk of ischemic damage.[77]

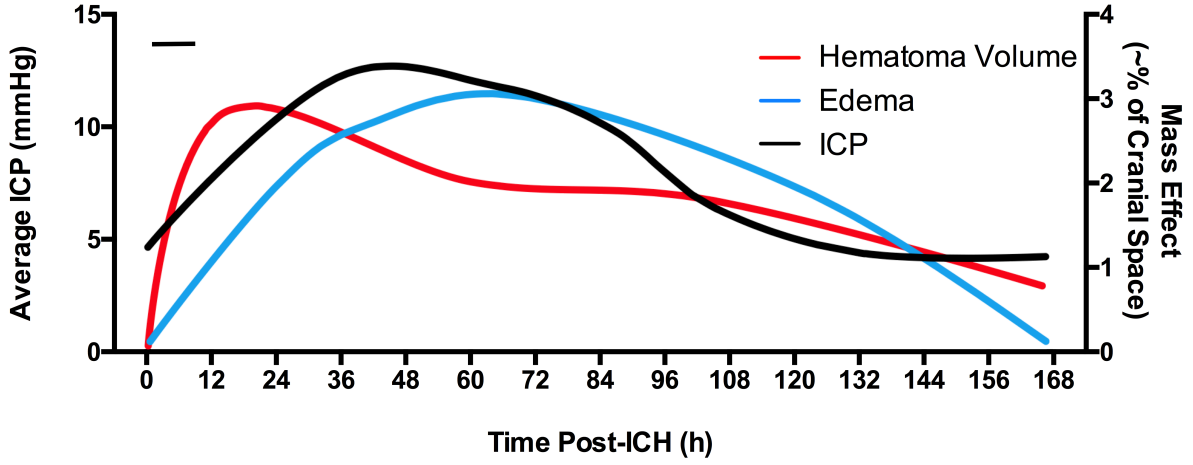

Figure 2-3. Theoretical timing of relative ICP, edema, and hematoma volume progression after collagenase ICH, assuming a maximum hematoma size of 60 μL in a rat brain. After ICH, increased hematoma volume and subsequent edema both contribute to elevated ICP. In rodents, hematoma volume increases rapidly over hours as bleeding occurs after collagenase infusion (represented by the black line). After a few days, hematoma resolution and clearance begins.[67] On the other hand, edema formation progresses over the first couple of days after ICH and peaks after about three days. Edema resolves within the first week after ICH. Consequently, ICP rises and peaks at ~ 24 hours after ICH and remains elevated for 2-3 days, and is almost resolved by a week post-ICH.[20]

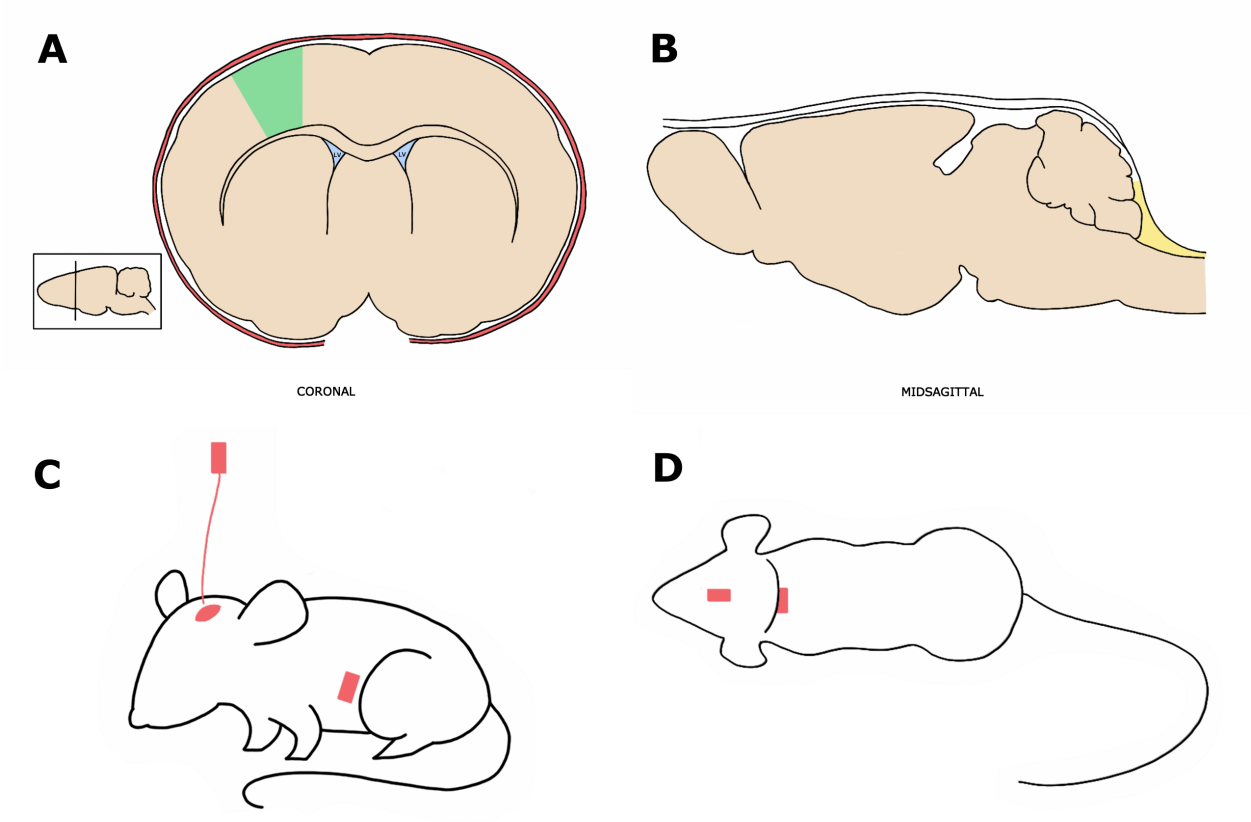

Figure 2-4. Common locations of ICP measurement are shown from a coronal (A) and midsagittal (B) view. ICP is often monitored via intraparenchymal (green), epidural (red), ventricular (blue), or intra-cisterna (yellow) methods. Using telemetry, ICP probes can be implanted either in the abdomen (C), on the head (D), or in the subcutaneous space below the neck (D). The relative size of the ICP sensor is shown. In clinical settings, ventricular catheters are the "gold standard" for monitoring ICP.

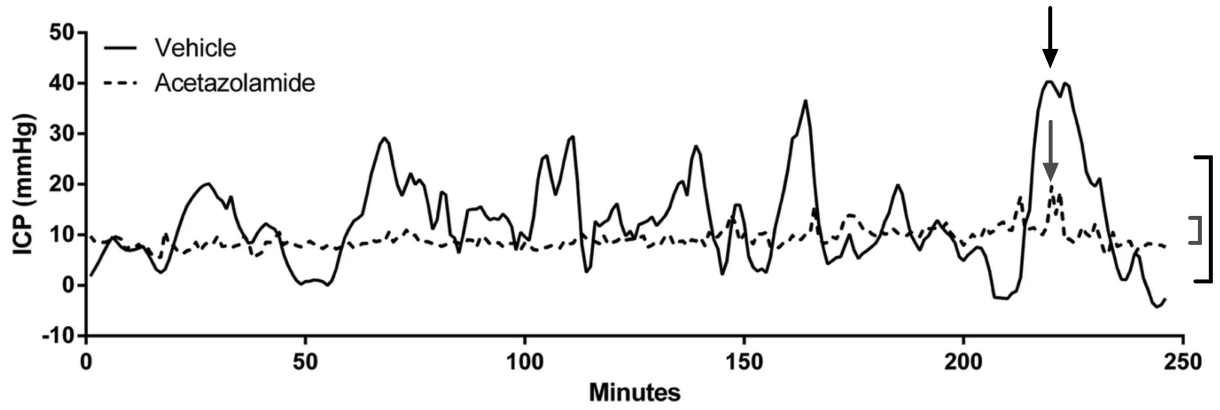

Figure 2-5. Within 72 hours after collagenase ICH (representative trace), acetazolamide treatment affected peak ICP (arrows) and ICP variability (brackets) without changing mean ICP (~10 mmHg) when compared to animals given vehicle control treatment after ICH. This graph illustrates the importance of ICP analysis and determining which parameters predict outcome, as simply looking at mean ICP over time may miss out on crucial treatment effects. Modified from Williamson et al. 2018 (http://creativecommons.org/licenses/by/4.0/).[127]

Model	Factors Contributing to ICP increase	ICP data using these models?	How well it matches clinical ICP profile
Collagenase	Hematoma, edema, seizures	Yes	Similar rise and timing
Autologous Whole Blood	Hematoma, edema (less than collagenase).	Yes	Similar rise, but shorter duration, likely due to less edema
Microballoon	Inflated microballoon	Yes	Depends on the duration of inflation and the size of the balloon. If correct inflation time and balloon size is chosen, can match clinical profile closely.
Intraventricular Hemorrhage	Hematoma, edema, obstructive hydrocephalus, fever[87]	No	Unknown
Stroke-Prone Spontaneously Hypertensive Rats	Hematoma (often multiple lesions and bleeds), edema, possibly seizures and fever (unknown), if bleeding occurs in ventricle, also obstructive hydrocephalus	No	Unknown

Table 2-1. Common models of ICH and their respective ICP responses. Studies measuring ICP

after ICH are lacking, and thus it is often unknown how well each model represents clinical ICP responses. Some models that may be representative clinically are impractical for ICP research, such as the stroke-prone models. In these models, the timing of insult is unknown, making ICP measurements unfeasible. Researchers should be mindful of side effects, such as seizures, that affect ICP and may be present in some models and not others.[86]

Considerations for Translational ICH Models	Impact on ICP
Location of ICH	Ventricular extension can raise ICP, location can affect herniation outcomes
Severity of ICH	Larger bleeds tend to cause greater ICP rises
Anesthetic effects on ICP	Mixed, depending on the type of anesthetic used
Comorbidities (e.g., hypertension)	Can lead to reduced compliance
Age	Younger age can have greater ICP responses, more data needed in animal models
Sex	Unknown effect
Amount and type of edema	More edema tends to cause to greater ICP
Model choice	Models have differing amounts of mass effect, leading to differential ICP responses
Species	May have differing compliance, different baseline ICP

Table 2-2. A summary of issues raised in this review that should be considered when planning preclinical ICH studies with respect to measuring and treating ICP. Several reviews have been done on preclinical stroke study quality,[116,117] and still guidelines are often not followed.[60] These translational issues can also be discussed in the context of their effect on ICP.

Chapter 3: The collagenase model of intracerebral hemorrhage in awake, freely moving animals: The effects of isoflurane

3.1.0 Introduction

Intracerebral hemorrhage (ICH) has a mortality rate of approximately 40%, and accounts for 10-20% of all strokes.[1] Despite improvements in patient care and risk factor management, incidence and mortality rates have not changed in the past three decades.[2] In order to enhance the chance of translational success and replicability, preclinical animal models must be as accurate to clinical presentation as possible. Currently, a major discrepancy of many preclinical ICH studies is the presence of volatile (e.g., isoflurane, sevoflurane, and desflurane) or injectable anesthetics (e.g., pentobarbital, ketamine and xylazine) shortly before or during the hemorrhage.[3] These anesthetics are often necessary, but introduce a confound not present in ICH patients. Thus, understanding the effect of anesthetics in pre-clinical stroke models is vital, especially considering their influences on physiology and neuroprotection.[4–7] For example, a photothrombosis model in awake animals has been developed to carefully examine the effects of isoflurane after focal ischemia,[8] but effects may differ after ICH.

The fluorinated ether anesthetic family, including isoflurane, sevoflurane, and desflurane, are believed to work by inhibiting pre-synaptic excitatory activity and enhancing inhibitory post-synaptic $GABA_A$ receptor activity.[9] Isoflurane, in particular, is commonly used in preclinical laboratories due to its affordability and low solubility coefficient in blood, which allows for quicker anesthetic induction and recovery.[9] Isoflurane anesthesia is commonly coupled with nitrous oxide gas as this reduces the blood solubility coefficient, increasing uptake rate.[10] Adding nitrous oxide to isoflurane also causes heightened cardiovascular activity, including increased blood pressure and heart rate compared to isoflurane alone.[11] In animals, isoflurane is eliminated from the brain according to the two-compartment model, with half-lives of 26 and 174 min.[12] Thus, while they regain consciousness quickly there is still isoflurane present at the time of the bleed.[13]

Concerningly, isoflurane alters physiology in ways that may impact stroke outcome. Notably, isoflurane can be neuroprotective when administered during pre, post, or peri-ischemic

periods.[14,15] This neuroprotective effect arises from several mechanisms including limiting excitotoxicity, pro-apoptotic signaling, and the expression of pro-inflammatory factors; improving intracellular Ca^{2+} homeostasis; and causing vasodilation, thereby improving cerebral blood flow.[14,15] Both pre- and post-conditioning with isoflurane can reduce cell death, edema, and behavioural deficits following ICH, presumably through similar mechanisms.[16,17] Therefore, the effects of anesthetics must be carefully considered in pre-clinical research, including when testing neuroprotective agents.[8]

Despite the promising neuroprotective potential of isoflurane, it may also impact stroke outcome negatively, especially during or following ICH. As mentioned, isoflurane is a potent vasodilator that increases cerebral blood flow.[18,19] During a hemorrhage, an increase in cerebral blood flow could increase the size of the hematoma; however, this may be compensated for, as isoflurane also reduces blood pressure (BP) and heart rate.[20] In addition to these hemodynamic factors, temperature is an important component in hemostasis.[21] Volatile anesthetics can lower temperature by several degrees in patients[22] and animals[23] during and transiently after surgical procedures. This is potentially problematic, as mild reductions in temperature can inhibit clotting enzymes and can cause platelet dysfunction.[21] Temperature reductions also impact other mechanisms of injury, such as inflammation and apoptosis.[24] Thus, establishing whether anesthetic influences these physiological factors in the collagenase model of ICH is vital, as the predictive accuracy of our preclinical model could be affected.

As with spontaneous ICH in patients, rats experience seizures in the days following a collagenase-induced ICH.[25,26] Electroencephalograms (EEG) show burst suppression under isoflurane anesthesia, a pattern in which cortical activity alternates between silence and high amplitude slow or sharp waves.[27] Isoflurane also disrupts cortical network connectivity,[28] a feature that has also been associated with seizures.[29] However, isoflurane also depresses glutamate transmission, which is believed to play a role in post-ICH injury.[30] How these alterations influence seizure activity following the collagenase ICH model is unclear; therefore,

establishing whether isoflurane is interacting with the injury to produce seizures, or whether this is an intrinsic property of the ICH model is important. Post-stroke seizure activity may be notably different in frequency and intensity in animals that are not given anesthetics.

Thus, in order to assess isoflurane's impact on the collagenase ICH model, we utilized a common drug delivery approach to infuse collagenase into awake animals. Rats were randomized to either the isoflurane (ISO) or the no isoflurane (NO-ISO) group. In the first experiment, we assessed the impact of isoflurane on blood glucose as well as hematoma volume, temperature, activity, and pain acutely following a small ICH (Figure 3-1). In the second experiment, based on these results, we again assessed hematoma volume, blood glucose, and pain after a large ICH to determine if our findings were dependent on ICH size. In the third experiment, we compared the historic characteristics, including rate, duration, and severity of post-stroke seizures commonly observed following an anesthetized ICH procedure with that seen here in conscious animals to determine the effect of isoflurane on seizure activity. In the fourth experiment, we compared the BP and hematoma volume observed during and following the anesthetized and conscious ICH models to investigate one mechanism by which isoflurane may be affecting hematoma volume.

3.2.0 Experimental Procedure

3.2.1 Subjects and Exclusion Criteria

All procedures were done according to the Canadian Council on Animal Care Guidelines and were approved (protocol AUP960) by the Biosciences Animal Care and Use Committee at the University of Alberta. We obtained 104 male Sprague Dawley rats (275-600 g, approximately 2-4 months old) from Charles River (Saint Constant, Quebec). Animals were kept in a temperature and humidity-controlled room with lights on from 7:00 am-7:00 pm, and all procedures were done during the light phase. Animals were single-housed during experiments with food and water provided *ad libitum.* All animals were handled for a total of 30 m over days

prior to the collagenase infusion to decrease stress during handling. Rats were handled gently including repeatedly touching the dummy cannula to give rats exposure to having the device manipulated and to increase comfort level with experimenter handling.

We established *a priori* exclusion criteria of a hematoma volume <5 μL for experiment 2, thus excluding any animals that did not receive a moderate-sized ICH.

3.2.2 Experimental Design

Animals were randomly assigned to groups using random.org and data was analyzed in a blinded manner for all experiments. Group sizes were calculated *a priori* using a power analysis for a desired 80% power to detect a 33% difference in the primary endpoint, hematoma volume. In this study, we conducted 4 experiments to examine the effect of isoflurane on collagenase-induced ICH (Figure 3-1). Animals were either randomized to receive an ICH under ISO or NO-ISO to examine the effect of isoflurane on ICH. In experiment 1, we assessed whether isoflurane affected temperature and activity for the first 24 h after a small collagenase-induced ICH. Blood glucose was measured at the beginning and end of anesthetic procedures, and pain was measured at 6 and 23 h post-ICH. Hematoma volume was assessed at 24 h post-ICH. Rats were randomized to ISO (n=12) or NO-ISO (n=12) conditions. As the variability was greater than expected, this experiment was repeated with an additional 12 animals per group to increase statistical power and the results were pooled for a total sample of n=24 per group. Temperature and activity were only assessed in the first 24 animals (n=12 per group). In experiment 2, we assessed the impact of isoflurane on blood glucose before and after surgical procedures, pain at 6 and 23 h post-ICH, and hematoma volume at 24 h after a large collagenase ICH. We used a larger insult in this experiment to test whether the effects of isoflurane were dependent on bleed size. Here, we had 18 animals per group. In experiment 3, we determined whether isoflurane influenced seizure activity after stroke. Here, we assigned 8 animals to the NO-ISO group. After 48 h of EEG activity measurements post-ICH, we collected

brain tissue for lesion volume assessment. We determined post-ICH seizure incidence rate, which is ~61.3% after anesthetized collagenase-induced ICH.[25,26] In experiment 4, we determined the effects of isoflurane and the no-anesthetic procedure on BP and hematoma volume after ICH. Animals were randomly assigned to ISO (n=6) or NO-ISO (n=6).

3.2.3 Telemetry Probe Implantation

Rats were anesthetized with isoflurane (4% induction, 2-2.25% maintenance, 60% N_2O, and remainder O_2). In animals used for core temperature measurements, a sterile calibrated probe was inserted into the peritoneal cavity (Model TA10TA-F40, Data Sciences International, St. Paul, MN, accurate to ± 0.2˚C). In animals used for EEG analysis, an EEG telemetry probe (F40EET, Data Sciences International, sampled at 500 Hz and low-pass filtered at 100 Hz) was inserted into the peritoneal cavity as previously described.[25] Leads were channeled under the skin and attached to screws (B000FMWBA0, Small Parts) and secured with dental cement. The screws were placed ipsilateral (AP -4, ML -4) and contralateral (AP-4, ML 4) to Bregma to avoid interfering with the cannula. In animals used for BP measurements, a calibrated PA-C10 probe's catheter was inserted into the left femoral artery, and the probe (Data Sciences International, accurate to ± 3 mmHg) was implanted subcutaneously, as previously described.[31]

A guide cannula was implanted (see Cannula Implantation and Collagenase Infusion) following implantation procedures. Meloxicam (0.2 mg/kg SC) and bupivacaine hydrochloride (0.5 mg/kg SC) were administered for analgesia, with the exception of EEG implantation where only bupivacaine hydrochloride was administered. This was to avoid excessive analgesia and suture removal by animals, which otherwise would be more problematic with this procedure.

Baseline measurements were taken for 24 h prior to ICH induction. Data measurements were taken every 30 s prior to and after the ICH. Post-ICH core temperature and activity readings were corrected hourly to baseline values in order to account for temperature changes due to circadian rhythm. In our past experience, we do not see spontaneous seizure activity in

this rat strain.[25,26] Nonetheless, baseline EEG data was assessed to ensure there was no pre-ICH seizure activity. For BP measurements, baseline data was taken for 3 h on the day prior to ICH and averaged. All data were corrected for probe offset readings taken prior to implantation.

3.2.4 Cannula Implantation and Collagenase Infusion

Animals were anesthetized with isoflurane and temperature was maintained at 37˚C using a rectal temperature probe and heating pad placed under the animal. Meloxicam and bupivacaine hydrochloride were administered at the start of surgical procedures as an analgesic. A hole was drilled into the skull at 0.5 mm anterior, 3.5 mm lateral (left side) to Bregma.[32] The dura mater was punctured to minimize possible pain during the non-anesthetized procedure. A guide cannula (C316G/SPC guide with 1 mm below pedestal, Invivo1, Roanoke, VA) was placed onto the hole and secured in place using 3 anchoring screws and dental cement. Dummy cannula was placed on the guide cannula to prevent pathogen entry and to maintain patency during recovery.

Rats recovered for 3 days following the cannula implant procedure. Then, an internal cannula (C316I/SPC, 5.5 mm extension from guide, invivo1, Roanoke, VA) was inserted 6.5 mm into the striatum (the depth used in previous research). Bacterial collagenase (Type IV-S, Sigma, 0.6 U/μL in sterile saline) was infused through PE tubing into the internal cannula and striatum. Either 1.0 μL (experiment 1) or 3.0 μL (experiments 2-4) of collagenase solution was infused over 2.5 m. The internal cannula was kept in place for 5 m to prevent backflow before being slowly removed, and the dummy cannula reinserted. Animals in the NO-ISO group were awake and lightly restrained by the experimenter during the cannula insertion. The experimenter used her hands to briefly hold the rat's head still for ~ 10 s. Animals were allowed to freely move within the procedure space during infusion after the infusion cannula was inserted. Animals tolerated this procedure well, with no visible signs of discomfort. This infusion technique is commonly used in neuroscience research, including research on anxiety.[33,34] Animals in the

anesthetized group were kept under isoflurane anesthesia for exactly 25 m including an ~ 2 m induction time (the duration of a typical collagenase procedure in prior research) with temperature maintained as described above. Note that the cannula implant procedure 3 days prior also took approximately 25 m of anesthetic.

3.2.5 Blood Glucose Measurements

Blood glucose was measured with a glucometer immediately after anesthetic induction and again prior to the end of surgical procedures (Contour Next One, Ascensia Diabetes Care, Mississauga, ON). A small drop of capillary blood was obtained from the tail by needle prick. Rats were free feeding prior to surgical procedures.

3.2.6 Grimace Scale

Pain was assessed using the rat grimace scale (RGS) to test whether the conscious ICH procedure caused additional pain post-ICH.[35] Animals were video recorded for 10 m at both 6 h and 23 h post-ICH. We did not assess pain during the procedure, as collagenase-induced bleeding occurs over hours.[13] Ten images were selected from each video at approximately 1 m intervals (to allow full view of face at each time). Each image was scored on orbital tightening, nose/cheek flattening, ear changes, and whisker changes by a scorer blinded to group identity. Each subscale was scored from 0-2, and the combined score was added for a total score ranging from 0-8, with zero meaning no pain. Group differences between both subscales and total scores were assessed. For ease of comparison with previous research, total scores were averaged by subscale for a score ranging from 0-2. Animal's scores were the average of the scores on the 10 images. A second rater scored a subset of animals to check for inter-rater reliability, which was high (Spearman's rho of 0.839, $P = 0.004$). Four naïve animals served as a negative control, and these animals received an average score of 0.162.

3.2.7 Hemoglobin Assay

The amount of hemoglobin in each hemisphere was determined using a spectroscopic assay based upon a standard curve.[13] Hematoma volume was calculated as ipsilateral blood volume minus contralateral blood volume. This accounts for the blood in the hemisphere that is not attributed to the hematoma (blood in the vasculature).

3.2.8 Electroencephalogram Analysis

Baseline and post-infusion EEG traces were visualized with Dataquest A.R.T software (v. 2.3, Data Sciences International). Two to five-minute long epochs of slow wave sleep (SWS) activity prior, and day 1 and 2 after collagenase injection as well as putative epileptiform traces were exported and analyzed using custom code written in MATLAB (R2012a, Mathworks, Natick, MA). We computed the root mean square (RMS), a measure of the fluctuation in the EEG signal, for all traces of interest. Baseline SWS from the day prior to collagenase injection (control) and putative epileptiform traces were exported and analyzed using custom code written in MATLAB for detection of seizures. The code identified epileptiform peaks that were above 4 standard deviations from the mean of the control traces, and considered 10 peak clusters occurring within 1 s apart as seizures.[25,26] Seizures detected by the MATLAB code were also visually verified for further accuracy, and any artefacts were excluded.

Power spectral density using Welch's averaged modified periodogram method (6 s window, 2 s overlap) as well as dual channel coherence for ipsilateral and contralateral recordings (3 s window; 1 s overlap) were computed for those epileptiform traces longer than 25 s. Power spectra and coherence were compared to non-epileptic control traces similar in duration to determine which frequencies had significant changes in power during seizure activity (i.e., outside 95% confidence interval, CI). A randomized coherence distribution based on a series of sequential time-shifted (and also time-reversed) coherence computations from these actual traces was computed to calculate the coherence significance level. During epileptiform

activity, we determine cross-hemispheric coupling changes by subtracting the coherence values of normal activity from those of epileptic traces and considered that any increases equal to or larger than the confidence limit for that trace to be significant.

3.2.9 Histological Analysis

At 48 h post-ICH, animals from the seizure experiment were injected with 100 mg/kg IP of sodium pentobarbital and perfused with 0.9% saline followed by 10% neutral-buffered formalin. Prior to cryostat sectioning, brains were placed in a 20% sucrose solution for cryoprotection. Coronal sections (20-μm thick) were taken every 200 μm and analyzed every 400 μm. Sections were stained with cresyl-violet as done previously.[36] Due to edema confounds and distortion caused by the hematoma itself at 48 h post-ICH, we were not able to calculate lesion volume by comparing hemisphere volumes.[37] Instead, the lesion was calculated as: (average area of damage x interval between sections x number of sections).

3.2.10 Statistical Analysis

Data were analyzed using GraphPad Prism (v 6.0, GraphPad Software Inc., La Jolla, CA). Data are presented as mean ± 95% CI. T-tests were used for 2 group comparisons. Welch's correction was applied when group variances were not equal, as determined with an F test to compare variances. Blood glucose levels and RMS were analyzed using a 2-way repeated measures ANOVA with Sidak's multiple comparisons test. Rat grimace scale scores were assessed using a Mann-Whitney U-test, and group contingencies were assessed using a Fisher's exact test. Relationship between variables was assessed with Pearson's correlation coefficient. All *P* values below 0.05 were considered statistically significant.

3.3.0 Results

3.3.1 Exclusions and Mortality

Five animals were completely excluded from experiment 2, and 1 additional animal was excluded from RGS analysis. One animal in the ISO group died spontaneously after ICH, and 1 animal in the NO-ISO group was not given an ICH due to a blocked cannula. In the ISO group, 1 animal was excluded from RGS analysis because he was sleeping for the duration of the video. Three animals were excluded based on insufficient hematoma volume, 2 from the ISO group and 1 from the NO-ISO group. In experiment 3, one animal spontaneously died ~42 h after ICH, but was still included in assessment. Two animals' BP data in experiment 4 were excluded from the ISO group due to blocked catheter. No animals were excluded or prematurely euthanized for animal welfare concerns.

3.3.2 Experiment 1: The effects of isoflurane after a small ICH

3.3.2.1 Blood Glucose

Blood glucose was measured before and after the telemetry probe and cannula implantation surgeries, in which both groups were exposed to isoflurane. There was a significant interaction between group and time on blood glucose after the telemetry probe and cannula implantation surgeries (Figure 3-2c, $P = 0.046$, interaction effect). There were no group differences at either time of measurement (Figure 3-2c, $P = 0.489$, before surgery; $P = 0.300$, after surgery). Blood glucose was increased by the end of the surgery in both groups (Figure 3-2c, ISO group $P < 0.001$, Cohen's d = 1.91; NO-ISO group $P < 0.001$, Cohen's d = 2.83).

3.3.2.2 Hematoma Volume

Hematoma volume was significantly larger in the NO-ISO group (Figure 3-2a, $P = 0.042$, Cohen's d = 0.60, moderate effect). Data was not distributed normally in either group (both $P < 0.05$, Shapiro-Wilk's test). When assessed with a Mann-Whitney U-test, the NO-ISO group still had a significantly larger hematoma volume than the ISO group (Figure 3-2, $P = 0.008$).

3.3.2.3 Temperature and Activity

Average core temperature during the 24 h baseline did not differ between the groups (P = 0.088, data not shown). There was a significant interaction between time and group on temperature post-ICH (Figure 3-3a, P < 0.001, interaction effect). Isoflurane significantly reduced temperature for the first 2 h after surgery (Figure 3-3a, P < 0.001 immediately post-ICH, Cohen's d = 3.62; P = 0.037 at 1 h post-ICH, Cohen's d = 1.10). Similarly, there was a significant interaction between time and group on activity (Figure 3-3b, P = 0.020, interaction effect). The NO-ISO rats were less active at 10 h post-ICH only (Figure 3-3b, P = 0.037, Cohen's d = 1.17). As this difference only occurred at one time comparison, it is likely due to chance.

3.3.2.4 Rodent Grimace Scale

There was no group difference in observed pain after ICH at either time (Figure 3-4a, P = 0.577, 6 h; P = 0.529, 23 h). Pain scores did not differ between 6 and 23 h post-ICH (Figure 3-4a, P = 0.333). There were no differences in pain between groups on any of the four subscales (all P > 0.454). The median score of 0.28 shows the majority of rats are below the proposed analgesic intervention threshold of 0.67.[38] In the ISO group, 1 rat was above this threshold, and in the NO ISO group there were 3 rats above this threshold (P = 0.609).

3.3.2.5 Weight Loss

There was no difference in weight loss between the groups (P = 0.602, interaction effect; P = 0.685, group main effect) and there was no significant weight loss after the ICH (P = 0.659, time main effect, data not shown).

3.3.3 Experiment 2: The effects of isoflurane after a large ICH

3.3.3.1 Blood Glucose

Blood glucose levels were significantly increased after cannula implantation surgery (Figure 3-2d, $P < 0.001$, time main effect, partial $\eta^2 = 0.79$). There was no difference in blood glucose between the ISO and NO-ISO groups (Figure 3-2d, $P = 0.824$, group main effect; $P = 0.807$, interaction effect), who were both given isoflurane for that procedure, which was three days prior to the ICH induction.

3.3.3.2 Hematoma Volume

Isoflurane did not significantly impact hematoma volume in this experiment (Figure 3-2b, $P = 0.169$). Animals in the NO-ISO group had significantly less variability in hematoma sizes as compared to the ISO group (Figure 3-2b, $P = 0.009$). Our conclusion did not change when analysis was redone with Welch's correction ($P = 0.183$).

3.3.3.3 Rodent Grimace Scale

There was no effect of isoflurane on rat grimace scale scores after ICH (Figure 3-4b, $P = 0.358$, 6 h; $P = 0.936$, 23 h). There were no group differences in pain on any of the four subscales (all $P > 0.151$). With the larger ICH, the median score of 0.80 is slightly higher than the analgesic intervention score of 0.67, indicating that approximately half of the animals were experiencing pain.[38] The median score seen here is similar to what is seen in other injuries, such as spinal cord injury,[39] and lower than a previous ICH study.[40] The grimace scale scores did not improve or worsen by 23 h post-ICH (Figure 3-4b, $P = 0.333$).

3.3.3.4 Weight Loss

There was significant weight loss after the ICH ($P < 0.001$, time main effect), with animals losing an average of 5.7% of their body weight. However, there was no difference in weight loss between the groups ($P = 0.991$, interaction effect; $P = 0.537$, group main effect, data not shown).

3.3.4 Experiment 3: Seizure activity after non-anesthetized ICH

3.3.4.1 Baseline EEG in the Collagenase Group

When we compared the RMS of slow wave sleep of the day prior to stroke with days 1 and 2 post-ICH in those rats with epileptiform activity, we found a significant effect for day (P = 0.013) but no interaction with hemisphere (P = 0.266, Figure 3-5a). This shows that the fluctuations in non-epileptic EEG traces after ICH were higher on day 1 compared to day 2 in both hemispheres.

3.3.4.2 Seizures after Collagenase

Five out of eight rats (62.5%) had seizures and interictal spikes (Figures 3-5 to 3-7) within the first two days after the stroke, with the earliest occurring after ~7.5 h and the latest occurring after ~45 h. Seizures ranged in duration from ~5 to 45 s (Table 3-1, Figure 3-6). All rats with seizures had extended periods of abnormal interictal activity, as seen previously.[25,26] Most seizures were bilateral, but there were instances in which seizures occurred only ipsilaterally or contralaterally (Figure 3-6). Surprisingly, in animals that spent the least amount of time in seizure activity, slow wave sleep RMS was reduced compared to baseline slow wave sleep RMS, indicating that the fluctuations had decreased in amplitude. Increases in power were seen at almost all frequencies for rat 3 (Table 3-1), which had the highest RMS and the longest seizures. However, for rat 1, which had a low RMS, it had an overall decrease in power, with those frequencies mainly affected being those below 11 Hz.

For coherence calculations, we concentrated on increases in coherence at the frequencies in which we noted a change in power. Coherence values range from 0 to 1; values

of 0 indicating that signal-specific frequencies between the two channels are completely unrelated, whereas values of 1 indicate that they are completely related. We found that for animal 1 there was an overall increase in coherence. Even though rat 3 had power increases along most frequencies of interest, the seizures showed mostly decreases in coherence in frequencies ranging from 0.12-1 Hz and 6-13 Hz, and 20-25 Hz and increases in frequencies between 40-50 Hz.

3.3.4.3 Lesion Volume

The mean lesion volume in this experiment was 47.1 mm^3 ($\pm$ 31.8 mm^3, 95% CI). We did not observe a relationship between lesion volume and number of seizures (Figure 3-5b, R^2 = 0.170, P = 0.310) or the total duration of seizures (R^2 = 0.103, P = 0.438).

3.3.5 Experiment 4: Effect of isoflurane on blood pressure and hematoma volume

3.3.5.1 Blood Pressure

During the ICH procedure, there was a significant interaction between group and time on BP (Figure 3-8a, P = 0.003), but these changes were transient. The animals in the NO-ISO group had significantly elevated BP as compared to the ISO group from minutes 2-12 of the ICH procedure (Figure 3-8a, all P < 0.007, partial η^2 = 0.43). Note that animals in the ISO group were kept under isoflurane for 25 m, but animals in the NO-ISO group were typically done their infusion procedure within 10 m (infusion plus handling time).

In the 24 h following the ICH procedure, there was no effect of group on hourly averaged BP (Figure 3-8b, P = 0.492, group main effect; P = 0.152, interaction effect). There was a significant effect of time on BP (Figure 3-8b, P < 0.001), likely a circadian rhythm effect.

3.3.5.2 Hematoma Volume

There was no group difference in hematoma volume (P = 0.955, ISO = 12.61 $\pm$ 11.7, NO-ISO = 13.05 $\pm$ 15.69, data not shown). The size of the hematoma was not predicted by peak BP (R^2 = 0.097, P = 0.382), average BP (R^2 = 0.244, P = 0.147), average BP during the ICH procedure (R^2 = 0.103, P = 0.366), or average BP during the first 6 h post-ICH (R^2 = 0.252, P = 0.139).

3.4.0 Discussion

Models of ICH typically require anesthetics that often markedly affect physiology.[6] Here, we used a cannula infusion system to investigate a model of collagenase ICH in non-anesthetized animals in order to assess the effects of isoflurane on outcome after ICH. Non-anesthetized animals had similar pain levels after ICH, indicating that this is an ethically acceptable way to induce an ICH and avoid anesthetic confounds. Isoflurane slightly decreased BP, decreased temperature, and increased blood glucose; however, these effects often resolved within minutes to hours. The effects of isoflurane lead to an increase in bleeding when the ICH was small, but did not significantly increase bleeding when the ICH was large. It is possible that other anesthetics or a longer duration of anesthetic may have greater effects, although Esposito and colleagues found that higher doses of isoflurane did not impact collagenase induced ICH.[41] This cannula infusion model is beneficial in cases where anesthetic effects are being tested and when anesthetic interactions with other therapies are a concern.

We used the RGS to determine if the lack of isoflurane during the ICH procedure resulted in additional pain. We found that the RGS scores both 6 and 23 h after ICH did not differ between groups, indicating that conducting the procedure under isoflurane anesthesia does not reduce post-ICH pain and is an ethical method of ICH induction. Further, animals experienced approximately the same weight loss in the day after ICH, providing further evidence that the non-anesthetized procedure was not causing significant additional stress. This also

suggests that most of the pain experienced after collagenase-induced ICH is due to the injury itself and not the surgical procedure. After a large ICH, the majority of rats in both groups were above the analgesic intervention threshold.[38] In this case, we did not treat the pain in these rats, as selectively treating animals with analgesics that influence injury can confound studies.[40,42] Others may choose to treat post-stroke pain, and further research is needed to determine the impact of those analgesics on bleeding and brain injury.

As hypothesized, isoflurane affected all of the physiological variables that were tested, and would have affected many others that were not tested. Despite that, we found only little differences between models, likely because these effects were short lasting or minor in nature. Temperature was decreased for the first 2 h post-surgery in the isoflurane group, and was increased above baseline in the conscious group. However, in this case, such a transient and modest temperature difference only led to a significant difference in hematoma volume after a small ICH. In the NO-ISO group, BP increased for the duration of the ICH procedure, and quickly returned to baseline once the dummy cannula was replaced. Anesthetized animals had their BP gradually drop throughout the duration of the surgical procedure, which returned to baseline within the hour. We expected that fluctuations in BP would result in large differences in hematoma volume. The lack of effect may be due to the collagenase enzyme causing prolonged bleeding over several hours,[13] whereas the observed BP changes only lasted for up to 25 m. Additionally, BP increases may not have equated to increases in cerebral perfusion pressure. Isoflurane increases intracranial pressure,[43] and after large ICH, intracranial pressure increases even further.[31,44] When intracranial pressure increases and arterial BP decreases by a similar amount, cerebral perfusion pressure stays the same,[45] which may be one possible explanation for why we only see an increase in hematoma volume after a small ICH. The observed BP changes may have effects that we did not measure. For example, stress responses, such as increased corticosterone, may also affect the course of injury and recovery.[46] Clinically, ICH patients are commonly hypertensive, and BP is often elevated during the course of injury,[47]

although this is typically for longer and to a much greater extent than what we see in rats. Therefore, the more accurate model of BP during an ICH would likely be increased BP.

Isoflurane increased blood glucose by nearly twofold by the end of the surgical procedure. We could not determine how long blood glucose was elevated for, as blood sampling required anesthetizing the animals or additional handling stress. However, previous research shows that in mice, isoflurane-induced glucose elevations resolve within an hour.[48] In patients, higher blood glucose at admission was associated with mortality.[49] In a rat model of focal ischemia, the harmful effects of hyperglycemia are well known.[50] It is possible that the non-anesthetized rats experience a stress induced hyperglycemic response during the ICH procedure, as indicated by increased blood-pressure.[51] As many preclinical ICH studies use a similar anesthetic protocol as we use here, we believe that a substantial portion of ICH research involves acute hyperglycemia at the time of the bleed.

In animals without anesthetic, we observed a post-ICH seizure rate of 5/8 (62.5%), which is similar to past work that found 61.3% of rats had seizures[25,26] but higher than the rate seen clinically and in the autologous whole blood model of ICH.[25,52] With the NO-ISO, we saw seizures limited to the contralateral hemisphere in two animals, which has not been seen in our previous work. One animal had decreased coherence during a bilateral seizure, suggesting two independent seizures were happening at the same time. Following ischemic stroke, seizure foci may be present contralaterally as well as ipsilaterally, and can propagate via the thalamus.[53] Isoflurane can affect thalamic information transfer,[54] thalamic $GABA_A$ receptors,[55] and blunt the overall thalamic response to stimuli,[56] which may be the reason we do not see contralateral seizures after animals have been exposed to isoflurane. Additionally, in most cases, the power change during seizures compared to baseline was quite small, suggesting that these seizures were not as severe as in previous work.[25] As isoflurane supresses neural activity and prevents seizures in some cases,[57,58] it is possible that after isoflurane exposure, animals may experience rebound effects, such as exacerbated seizure activity.[59] Therefore, isoflurane may

be altering the characteristics of seizures rather than the frequency that seizures occur. However, because this experiment used historical controls rather than an experimental control group, results should be interpreted with caution, and follow up experiments should be done.

This study had some additional limitations. We did not assess the long-term effects of isoflurane on injury and behaviour.[4,5,8] Future studies should examine chronic effects of isoflurane after ICH. Further, we did not assess the effects of isoflurane in the autologous whole blood model, as it would be technically difficult to do. Although the physiological impacts are likely similar, our conclusions are limited to the collagenase model of ICH. Here, we did not visually assess seizure activity, as we did not video record any of the seizure events. Therefore, we cannot provide any conclusive information regarding the behavioural manifestations of this epileptiform activity.

Our findings suggest brief use of isoflurane anesthesia is an appropriate model. However, we recommend the use of non-anesthetized ICH in specific cases. For example, isoflurane may interact with many drugs,[60,61] including analgesics (e.g., opiates) and potential neuroprotectants, especially those given before or at the time of surgery. Validating findings in a model without anesthesia would be beneficial to ensure effects are not dependent on or interacting with isoflurane. Further, avoiding anesthetic would be ideal in situations where researchers are specifically examining the effects of anesthetics. Previously, most studies looking at the effects of anesthetics on stroke simply compared anesthetics to each other, or compared different doses of the same anesthetic, without having a no anesthetic control group.[62,63] By using a non-anesthetized ICH, researchers can compare findings to a no anesthetic control group, increasing the validity of their findings. Although we only assessed isoflurane in this study, a variety of other anesthetics are commonly used in ICH research (e.g., chloral hydrate and sodium pentobarbital)[3] and should be similarly assessed for their effects on ICH outcome.

In conclusion, we demonstrate that isoflurane can have small hematoma-size-dependent impacts on the ICH model. The physiological impacts of isoflurane are often transient, but should still be considered a potential confound. The non-anesthetized ICH is an important tool for researchers when anesthetic effects are a concern, especially in neuroprotectant studies and those with a short survival time.

3.5 References

1. Feigin VL, Lawes CM, Bennett DA, Barker-Collo SL, Parag V. Worldwide stroke incidence and early case fatality reported in 56 population-based studies: a systematic review. *Lancet Neurol*. 2009;8(4):355-369. doi:10.1016/S1474-4422(09)70025-0
2. van Asch CJ, Luitse MJ, Rinkel GJ, van der Tweel I, Algra A, Klijn CJ. Incidence, case fatality, and functional outcome of intracerebral haemorrhage over time, according to age, sex, and ethnic origin: a systematic review and meta-analysis. *Lancet Neurol*. 2010;9(2):167-176. doi:10.1016/S1474-4422(09)70340-0
3. MacLellan CL, Paquette R, Colbourne F. A critical appraisal of experimental intracerebral hemorrhage research. *J Cereb Blood Flow Metab*. 2012;32(4):612-627. doi:10.1038/jcbfm.2012.8
4. Jiang M, Sun L, Feng DX, et al. Neuroprotection provided by isoflurane pre-conditioning and post-conditioning. *Med Gas Res*. 2017;7(1):48-55. doi:10.4103/2045-9912.202910
5. Statler KD, Alexander H, Vagni V, et al. Isoflurane exerts neuroprotective actions at or near the time of severe traumatic brain injury. *Brain Res*. 2006;1076(1):216-224. doi:10.1016/j.brainres.2005.12.106
6. Tsurugizawa T, Takahashi Y, Kato F. Distinct effects of isoflurane on basal BOLD signals in tissue/vascular microstructures in rats. *Sci Rep*. 2016;6. doi:10.1038/srep38977
7. Archer DP, Walker AM, McCann SK, Moser JJ, Appireddy RM. Anesthetic Neuroprotection in Experimental Stroke in Rodents: A Systematic Review and Meta-analysis. *Anesthesiology*. 2017;126(4):653-665. doi:10.1097/ALN.0000000000001534
8. Seto A, Taylor S, Trudeau D, et al. Induction of ischemic stroke in awake freely moving mice reveals that isoflurane anesthesia can mask the benefits of a neuroprotection therapy. *Front Neuroenergetics*. 2014;6(APR). doi:10.3389/fnene.2014.00001
9. Ramachandra V, Moore C, Kaur N, Carli F. Effect of halothane, enflurane and isoflurane on body temperature during and after surgery. *Br J Anaesth*. 1989;62(4):409-414.

doi:10.1093/bja/62.4.409

10. Xie GM, Lauber R, Zbinden AM. Nitrous oxide decreases solubility of isoflurane and halothane in blood. *Anesth Analg*. 1993;77(4):761-765. doi:10.1213/00000539-199310000-00018
11. Duke T, Caulkett NA, Tataryn JM. The effect of nitrous oxide on halothane, isoflurane and sevoflurane requirements in ventilated dogs undergoing ovariohysterectomy. *Vet Anaesth Analg*. 2006;33(6):343-350. doi:10.1111/j.1467-2995.2005.00274.x
12. Chen M, Olsen JI, Stolk JA, Schweizer MP, Sha M, Ueda I. An in vivo 19F NMR study of isoflurane elimination as a function of age in rat brain. *NMR Biomed*. 1992;5(3):121-126. doi:10.1002/nbm.1940050304
13. MacLellan CL, Silasi G, Poon CC, et al. Intracerebral hemorrhage models in rat: comparing collagenase to blood infusion. *J Cereb Blood Flow Metab*. 2008;28:516-525. doi:10.1038/sj.jcbfm.9600548
14. Matchett GA, Allard MW, Martin RD, Zhang JH. Neuroprotective effect of volatile anesthetic agents: Molecular mechanisms. *Neurol Res*. 2009;31(2):128-134. doi:10.1179/174313209X393546
15. Wang H, Li P, Xu N, et al. Paradigms and mechanisms of inhalational anesthetics mediated neuroprotection against cerebral ischemic stroke. *Med Gas Res*. 2016;6(4):194-205. doi:10.4103/2045-9912.196901
16. Khatibi NH, Ma Q, Rolland W, et al. Isoflurane posttreatment reduces brain injury after an intracerebral hemorrhagic stroke in mice. *Anesth Analg*. 2011;113(2):343-348. doi:10.1213/ANE.0b013e31821f9524
17. Gigante PR, Appelboom G, Hwang BY, et al. Isoflurane preconditioning affords functional neuroprotection in a murine model of intracerebral hemorrhage. In: *Acta Neurochirurgica, Supplementum*. ; 2011. doi:10.1007/978-3-7091-0693-8_23
18. Constantinides C, Murphy K. Molecular and Integrative Physiological Effects of Isoflurane

Anesthesia: The Paradigm of Cardiovascular Studies in Rodents using Magnetic Resonance Imaging. *Front Cardiovasc Med*. 2016;3. doi:10.3389/fcvm.2016.00023

19. Sicard K, Shen Q, Brevard ME, et al. Regional cerebral blood flow and BOLD responses in conscious and anesthetized rats under basal and hypercapnic conditions: Implications for functional MRI studies. *J Cereb Blood Flow Metab*. 2003;23(4):472-481. doi:10.1097/01.WCB.0000054755.93668.20
20. Yang C-F, Yu-Chih Chen M, Chen T-I, Cheng C-F. Dose-dependent effects of isoflurane on cardiovascular function in rats. *Tzu Chi Med J*. 2014;26(3):119-122. doi:10.1016/J.TCMJ.2014.07.005
21. Wolberg AS, Meng ZH, Monroe DM, Hoffman M. A systematic evaluation of the effect of temperature on coagulation enzyme activity and platelet function. *J Trauma - Inj Infect Crit Care*. 2004;56(6):1221-1228. doi:10.1097/01.TA.0000064328.97941.FC
22. Khurram S, Hayes I, Buggy D. Pharmacology of anaesthetic agents I: intravenous anaesthetic agents. *Contin Educ Anesth Crit Care Pain*. 2014;4(3):100-105. doi:10.1093/bjaceaccp/mkt039
23. Colbourne F, Nurse SM, Corbett D. Temperature changes associated with forebrain ischemia in the gerbil. *Brain Res*. 1993;602(2):264-267. doi:10.1016/0006-8993(93)90691-F
24. Moore EM, Nichol AD, Bernard SA, Bellomo R. Therapeutic hypothermia: Benefits, mechanisms and potential clinical applications in neurological, cardiac and kidney injury. *Injury*. 2011;42(9):843-854. doi:10.1016/j.injury.2011.03.027
25. Klahr AC, Dickson CT, Colbourne F. Seizure Activity Occurs in the Collagenase but not the Blood Infusion Model of Striatal Hemorrhagic Stroke in Rats. *Transl Stroke Res*. 2014;6(1):29-38. doi:10.1007/s12975-014-0361-y
26. Klahr AC, Dietrich K, Dickson CT, Colbourne F. Prolonged Localized Mild Hypothermia Does Not Affect Seizure Activity after Intracerebral Hemorrhage in Rats. *Ther*

Hypothermia Temp Manag. 2016;6(1):40-47. doi:10.1089/ther.2015.0028

27. Amzica F. Basic physiology of burst-suppression. *Epilepsia*. 2009;50:38-39. doi:10.1111/j.1528-1167.2009.02345.x

28. Hentschke H, Raz A, Krause BM, Murphy CA, Banks MI. Disruption of cortical network activity by the general anaesthetic isoflurane. *Br J Anaesth*. 2017;119(4):685-696. doi:10.1093/bja/aex199

29. Vega-Zelaya L, Pastor J, De Sola RG, Ortega GJ. Disrupted ipsilateral network connectivity in temporal lobe epilepsy. *PLoS One*. 2015;10(10). doi:10.1371/journal.pone.0140859

30. Castillo J, Dávalos A, Álvarez-Sabín J, et al. Molecular signatures of brain injury after intracerebral hemorrhage. *Neurology*. 2002;58(4):624-629. doi:10.1212/WNL.58.4.624

31. Hiploylee C, Colbourne F. Intracranial pressure measured in freely moving rats for days after intracerebral hemorrhage. *Exp Neurol*. 2014;255:49-55. doi:10.1016/j.expneurol.2014.02.017

32. Paxinos G, Watson C. Index of Structures. *Rat Brain Stereotaxic Coord*. Published online 1982:7-9. doi:10.1016/b978-0-12-547620-1.50008-4

33. Williams LR, Vahlsing HL, Lindamood T, Varon S, Gage FH, Manthorpe M. A small-gauge cannula device for continuous infusion of exogenous agents into the brain. *Exp Neurol*. 1987;95(3):743-754. doi:10.1016/0014-4886(87)90313-X

34. Shah AA, Sjovold T, Treit D. Inactivation of the medial prefrontal cortex with the GABA A receptor agonist muscimol increases open-arm activity in the elevated plus-maze and attenuates shock-probe burying in rats. *Brain Res*. 2004;1028(1):112-115. doi:10.1016/j.brainres.2004.08.061

35. Sotocinal SG, Sorge RE, Zaloum A, et al. The Rat Grimace Scale: A partially automated method for quantifying pain in the laboratory rat via facial expressions. *Mol Pain*. 2011;7. doi:10.1186/1744-8069-7-55

36. MacLellan CL, Auriat AM, McGie SC, et al. Gauging recovery after hemorrhagic stroke in rats: Implications for cytoprotection studies. *J Cereb Blood Flow Metab*. 2006;26(8):1031-1042. doi:10.1038/sj.jcbfm.9600255

37. Williamson MR, Colbourne F. Evidence for Decreased Brain Parenchymal Volume After Large Intracerebral Hemorrhages: a Potential Mechanism Limiting Intracranial Pressure Rises. *Transl Stroke Res*. 2017;8(4):386-396. doi:10.1007/s12975-017-0530-x

38. Oliver V, De Rantere D, Ritchie R, Chisholm J, Hecker KG, Pang DSJ. Psychometric assessment of the rat grimace scale and development of an analgesic intervention score. *PLoS One*. 2014;9(5). doi:10.1371/journal.pone.0097882

39. Schneider LE, Henley KY, Turner OA, Pat B, Niedzielko TL, Floyd CL. Application of the Rat Grimace Scale as a Marker of Supraspinal Pain Sensation after Cervical Spinal Cord Injury. *J Neurotrauma*. 2017;34(21):2982-2993. doi:10.1089/neu.2016.4665

40. Saine L, Hélie P, Vachon P. Effects of fentanyl on pain and motor behaviors following a collagenase-induced intracerebral hemorrhage in rats. *J Pain Res*. 2016;9:1039-1048. doi:10.2147/JPR.S121415

41. Esposito E, Mandeville ET, Lo EH. Lower doses of isoflurane treatment has no beneficial effects in a rat model of intracerebral hemorrhage. *BMC Neurosci*. 2013;14. doi:10.1186/1471-2202-14-129

42. Ferland C, Veilleux-Lemieux D, Vachon P. Effects of buprenorphine on intracerebral collagenase-induced hematoma in Sprague-Dawley rats. *J Am Assoc Lab Anim Sci*. 2007;46(3):13-16. http://www.embase.com/search/results?subaction=viewrecord&from=export&id=L47068011%5Cnhttp://limo.libis.be/resolver?&sid=EMBASE&issn=15596109&id=doi:&atitle=Effects+of+buprenorphine+on+intracerebral+collagenase-induced+hematoma+in+Sprague-Dawley+rats&stit

43. Scheller MS, Tateishi A, Drummond JC, Zornow MH. The effects of sevoflurane on

cerebral blood flow, cerebral metabolic rate for oxygen, intracranial pressure, and the electroencephalogram are similar to those of isoflurane in the rabbit. *Anesthesiology*. 1988;68(4):548-551. doi:10.1097/00000542-198804000-00012

44. Silasi G, MacLellan CL, Colbourne F. Use of telemetry blood pressure transmitters to measure intracranial pressure (ICP) in freely moving rats. *Curr Neurovasc Res*. 2009;6(1):62-69. doi:10.2174/156720209787466046

45. Su TM, Lee TH, Chen WF, Lee TC, Cheng CH. Contralateral acute epidural hematoma after decompressive surgery of acute subdural hematoma: Clinical features and outcome. *J Trauma - Inj Infect Crit Care*. 2008;65(6):1298-1302. doi:10.1097/TA.0b013e31815885d9

46. DeVries AC, Joh HD, Bernard O, et al. Social stress exacerbates stroke outcome by suppressing Bcl-2 expression. *Proc Natl Acad Sci U S A*. 2001;98(20):11824-11828. doi:10.1073/pnas.201215298

47. Shah QA, Ezzeddine MA, Qureshi AI. Acute hypertension in intracerebral hemorrhage: Pathophysiology and treatment. *J Neurol Sci*. 2007;261(1-2):74-79. doi:10.1016/j.jns.2007.04.036

48. Durand JL, Hosinking W, Jelicks LA. Time course of effects of inhalation anesthesia on blood glucose level in male and female C57BL/6 mice. *Horm Metab Res*. 2009;41(4):339-341. doi:10.1055/s-0028-1112114

49. Fogelholm R, Murros K, Rissanen A, Avikainen S. Admission blood glucose and short term survival in primary intracerebral haemorrhage: A population based study. *J Neurol Neurosurg Psychiatry*. 2005;76(3):349-353. doi:10.1136/jnnp.2003.034819

50. Kagansky N, Levy S, Knobler H. The role of hyperglycemia in acute stroke. *Arch Neurol*. 2001;58(8):1209-1212. doi:10.1001/archneur.58.8.1209

51. Márquez C, Nadal R, Armario A. The hypothalamic-pituitary-adrenal and glucose responses to daily repeated immobilisation stress in rats: Individual differences.

Neuroscience. 2004;123(3):601-612. doi:10.1016/j.neuroscience.2003.10.016

52. De Herdt V, Dumont F, Hénon H, et al. Early seizures in intracerebral hemorrhage: Incidence, associated. *Neurology*. 2011;77(20):1794-1800. doi:10.1212/WNL.0b013e31823648a6

53. Kogure K, Schwartzman RJ. Seizure Propagation and ATP Depletion in the Rat Stroke Model. *Epilepsia*. 1980;21(1):63-72. doi:10.1111/j.1528-1157.1980.tb04045.x

54. Detsch O, Vahle-Hinz C, Kochs E, Siemers M, Bromm B. Isoflurane induces dose-dependent changes of thalamic somatosensory information transfer. *Brain Res*. 1999;829(1-2):77-89. doi:10.1016/S0006-8993(99)01341-4

55. Jia F, Yue M, Chandra D, Homanics GE, Goldstein PA, Harrison NL. Isoflurane is a potent modulator of extrasynaptic GABAA receptors in the thalamus. *J Pharmacol Exp Ther*. 2008;324(3):1127-1135. doi:10.1124/jpet.107.134569

56. Vahle-Hinz C, Detsch O, Siemers M, Kochs E. Contributions of GABAergic and glutamatergic mechanisms to isoflurane-induced suppression of thalamic somatosensory information transfer. *Exp Brain Res*. 2007;176(1):159-172. doi:10.1007/s00221-006-0604-6

57. Bar-Klein G, Klee R, Brandt C, et al. Isoflurane prevents acquired epilepsy in rat models of temporal lobe epilepsy. *Ann Neurol*. 2016;80(6):896-908. doi:10.1002/ana.24804

58. Ito BM, Sato S, Kufta C V., Tran D. Effect of isoflurane and enflurane on the electrocorticogram of epileptic patients. *Neurology*. 1988;38(6):924-928. doi:10.1212/wnl.38.6.924

59. Veronesi MC, Kubek DJ, Kubek MJ. Isoflurane exacerbates electrically evoked seizures in amygdala-kindled rats during recovery. *Epilepsy Res*. 2008;82(1):15-20. doi:10.1016/j.eplepsyres.2008.06.007

60. Glass PSA, Gan TJ, Howell S, Ginsberg B. Drug interactions: Volatile anesthetics and opioids. In: *Journal of Clinical Anesthesia*. ; 1997. doi:10.1016/S0952-8180(97)00122-0

61. Wood M. Pharmacokinetic Drug Interactions in Anaesthetic Practice. *Clin Pharmacokinet*. 1991;21(4):285-307. doi:10.2165/00003088-199121040-00005

62. Bhardwaj A, Castro AF, Alkayed NJ, Hurn PD, Kirsch JR. Anesthetic choice of halothane versus propofol impact on experimental perioperative stroke. *Stroke*. 2001;32(8):1920-1925. doi:10.1161/01.STR.32.8.1920

63. Zausinger S, Baethmann A, Schmid-Elsaesser R. Anesthetic methods in rats determine outcome after experimental focal cerebral ischemia: Mechanical ventilation is required to obtain controlled experimental conditions. *Brain Res Protoc*. 2002;9(2):112-121. doi:10.1016/S1385-299X(02)00138-1

64. Colbourne F, Auer RN, Sutherland GR. Characterization of postischemic behavioral deficits in gerbils with and without hypothermic neuroprotection. *Brain Res*. 1998;803(1-2):69-78. doi:10.1016/S0006-8993(98)00612-X

Rat	Incidence	Laterality	Total duration (Mean±CI, Seconds)	Time	RMS Ratio (Mean±CI)	Frequencies affected (Hz)	Power Change (mV^2, Mean±CI)	Coherence change (Mean±CI)
1	3	2 Bilateral 1 Contralateral	60.23 (20.08±4.89)	First: 9h 12m Last: 34h 6m	0.73±0.22	↑ 11.23 - 47.00 ↓ 0.01 - 48.58	↑ 0.00030 ↓ 0.056	↑ 0.41±0.04
2	14	7 Bilateral 7 Ipsilateral	136.20 (9.73±1.03)	First: 11h 46m Last: 45h 13m	1.81±0.27	N/A	N/A	N/A
3	3	2 Bilateral 1 Contralateral	92.35 (30.78±17.63)	First: 12h 17m Last: 31h 36m	4.31±0.78	↑ 1.10-50.05 ↓ 0.12-0.98	↑0.52±0.019	↑ 0.29±0.05 ↓0.38±0.04
4	3	2 Bilateral	45.63 (15.21±7.56)	First: 7h 33m Last: 37h 37m	0.76±0.16	N/A	N/A	N/A

		1 Ipsilateral						
5	1	1 Ipsilateral	19.64	31h 19m	0.88	N/A	N/A	N/A

Table 3-1 Characteristics of seizures after NO-ISO ICH. EEG activity occurring in the first two days after collagenase injection were visualized and analyzed. For each rat, the number of seizures, laterality, total duration, and time of onset were documented. We also reported other factors to determine the variability of the traces as depicted by the RMS ratio (RMS seizure/RMS non-epileptic activity) and changes in voltage according to their frequency, as depicted by power changes and frequencies affected. Also, for those frequencies in which the power was affected, coherence was assessed as an indicator of how coupled the activity was in between both channels. Here, coherence was computed as an increase from baseline coherence. Traces shorter than 25 s were not analyzed for power and coherence.

Study	Seizure Incidence Rate	ID	Number of Seizures (Ipsilateral or Bilateral)	Total Duration (s)	Time of onset after ICH
Klahr et al. 2014	6/9 (Collagenase Group Only)	1	1	14	11 h 52 m
		2	2	102	18 h 50 m
		3	3	163	22 h 7 m
		4	4	108	16 h 25 m
		5	8	173	11 h 37 m
		6	14	616	9 h 57 m
Klahr et al. 2016	13/22 (Control Group Only)	1	34	481	6 h 27 m
		2	18	375	9 h 3 m
		3	60	1455	8 h 12 m
		4	37	607	7 h
		5	40	718	4 h 48 m
		6	27	495	6 h 36 m
		7	1	18	9 h 44 m
		8	2	31	19 h 32 m
		9	203	4138	7 h 23 m
		10	2	21	17 h 18 m
		11	21	537	3 h 53 m
		12	124	1831	11 h 32 m
		13	3	45	8 h 58 m

Table 3-2 Characteristics of seizures from our previous studies (Klahr et al., 2014, 2016). In both studies, the lesion volume averaged 33 mm^3. Note that survival times were longer in these studies, ranging from 11-66 d. However, first seizure incidence was always within the first 24 h.

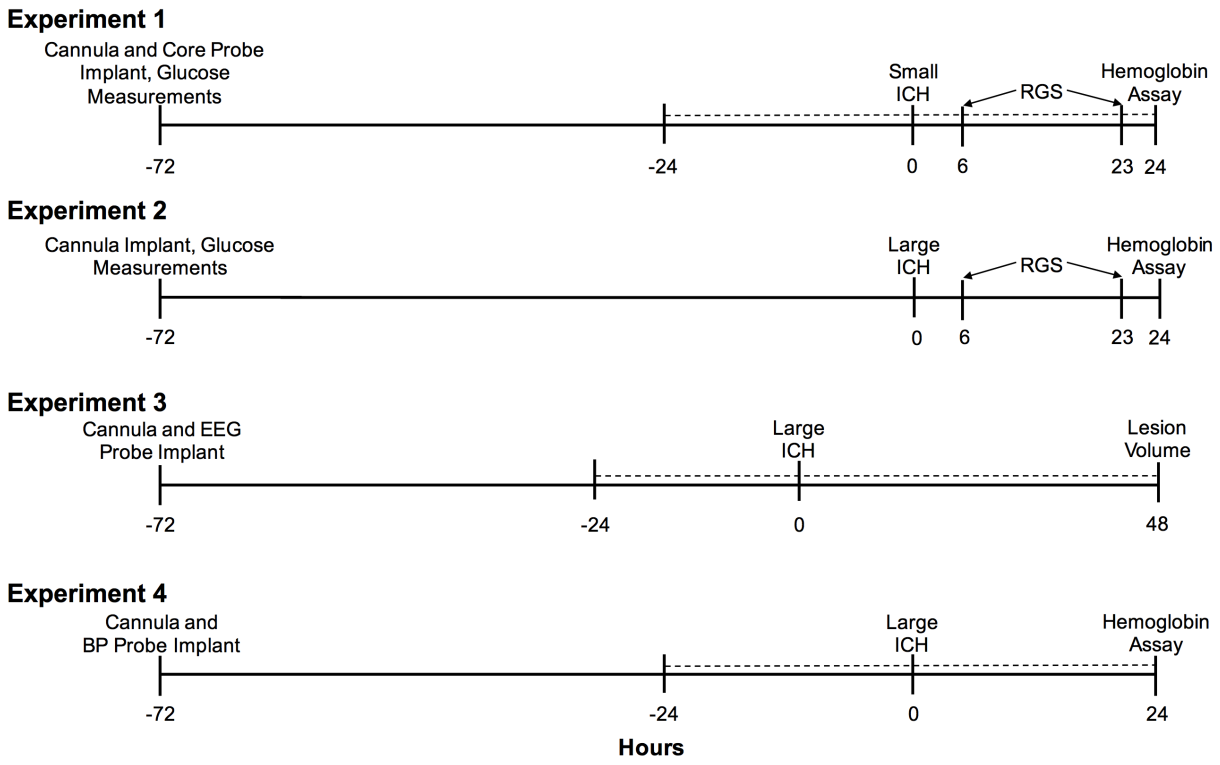

Figure 3-1 Timeline of Experiments. Dashed line denotes telemetry recordings. Sample sizes were 24 per group in experiment 1, 18 per group in experiment 2, 8 in experiment 3, and 6 per group in experiment 4. Figure not to scale.

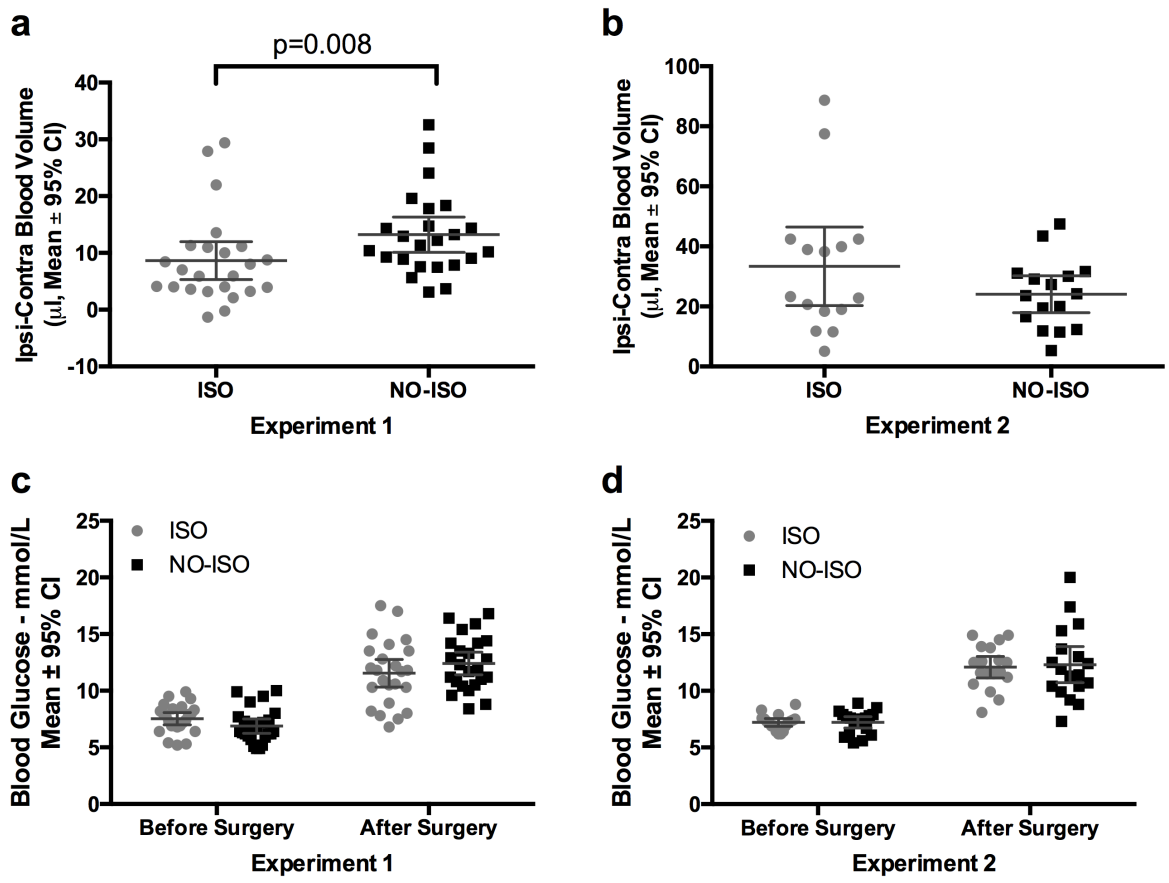

Figure 3-2 Experiment 1. Blood glucose was assessed immediately before and after surgery, while hematoma volume was assessed 24 h after small ICH induction. Awake ICH rats had significantly larger (a) hematoma volumes (µL; $P = 0.0082$), and both groups had higher (c) post-surgical blood glucose levels (mmol/L; $P < 0.001$) compared to pre-surgery, with significant group by time interactions ($P = 0.046$). One rat in this experiment had a negative hematoma volume, likely due to variability in vasculature blood between hemispheres, as hematoma volume is calculated as ipsilateral – contralateral blood volume. **Experiment 2**. After a larger ICH, isoflurane had no effect on (b) hematoma volume ($P = 0.183$), though awake rats had less variability in hematoma size observed ($P = 0.009$). Both groups had (d) elevated blood glucose levels following cannula surgery ($P < 0.001$) and were not significantly different ($P = 0.824$). Sample sizes were n=24 per group in experiment 1 and n=18 per group in experiment 2.

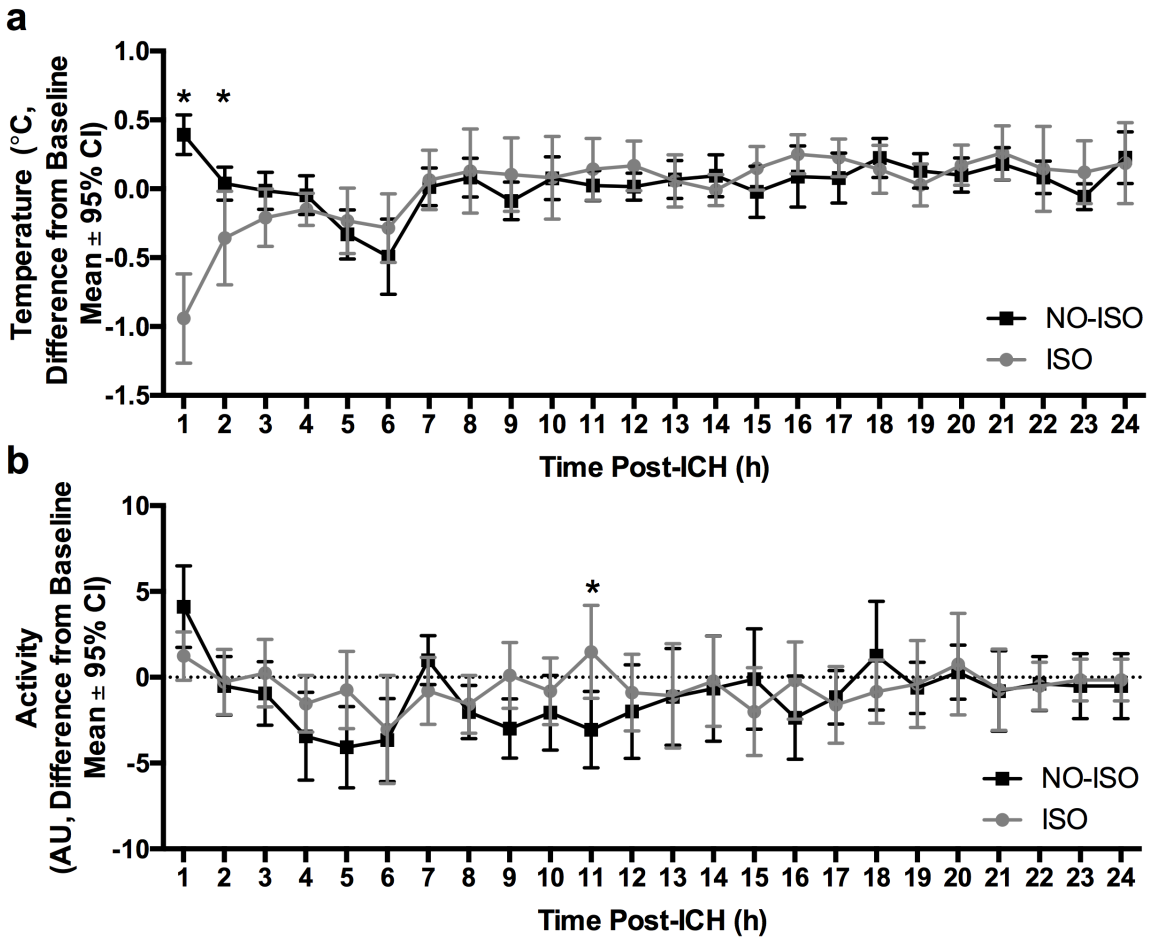

Figure 3-3 Experiment 1. Isoflurane rats had (a) significantly lower core temperature (°C) for 2 h ($P < 0.001$ at 1 h, p=0.0373 at 2 h) and (b) lower activity at 11h (AU = arbitrary units of animal movement detected and recorded by a telemetry receiver placed under the animal [64], p=0.0372) post-ICH induction. There was a group by time interaction for both (a) temperature ($P < 0.01$) and (b) activity ($P = 0.020$). Sample size was 12 per group.

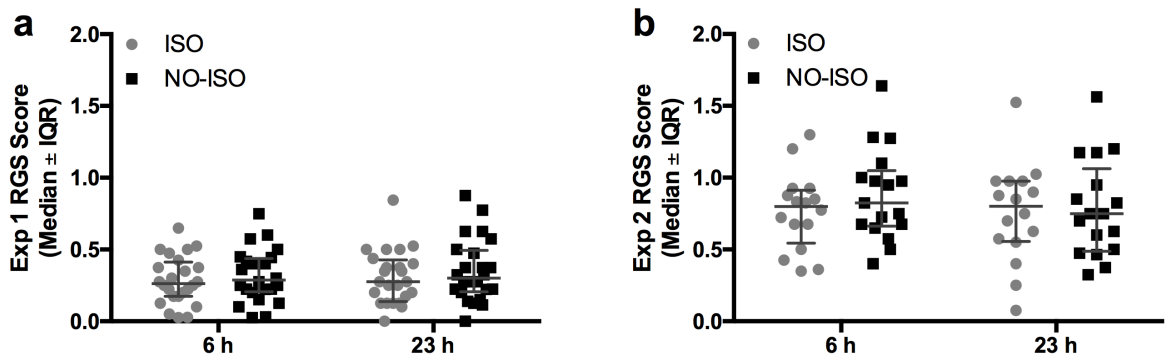

Figure 3-4 Experiments 1 and 2. Rat grimace scale (RGS) scores were not significantly different between isoflurane and awake rats after (a) a small ICH (P = 0.369) or (b) a moderate to large ICH (P = 0.402). Pain was also not significantly different between 6 and 23 h post-ICH induction for either experiment 1 (P = 0.421) or experiment 2 (P = 0.435). Sample size was 24 per group for experiment 1 and 18 per group for experiment 2.

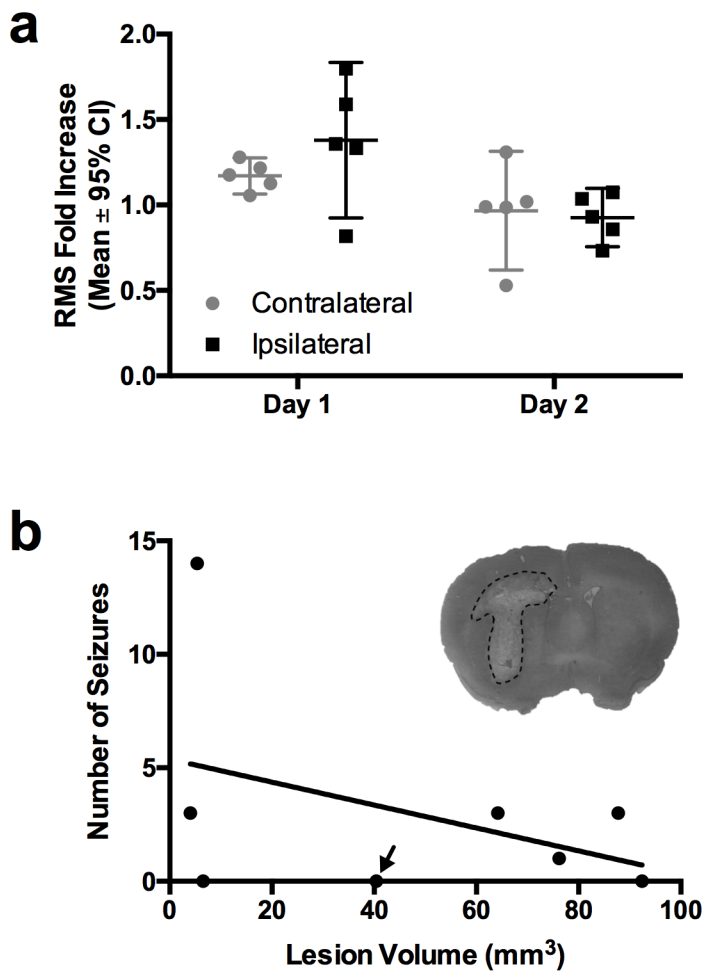

Figure 3-5 Experiment 3. (a) RMS in the rats that had seizures was higher for day 1 compared to day 2 in SWS EEG after collagenase-induced ICH. This indicates that there were more fluctuations in the EEG traces during the first day after the stroke. There were no statistical differences between sides. Sample size was n=5. There was no relationship between the number of seizures and the lesion volume in all rats (b, N=8, *P* = 0.310). Representative section from the middle of the lesion is embedded into graph b (lesion volume = 40.40 mm^3 as indicated by arrow).

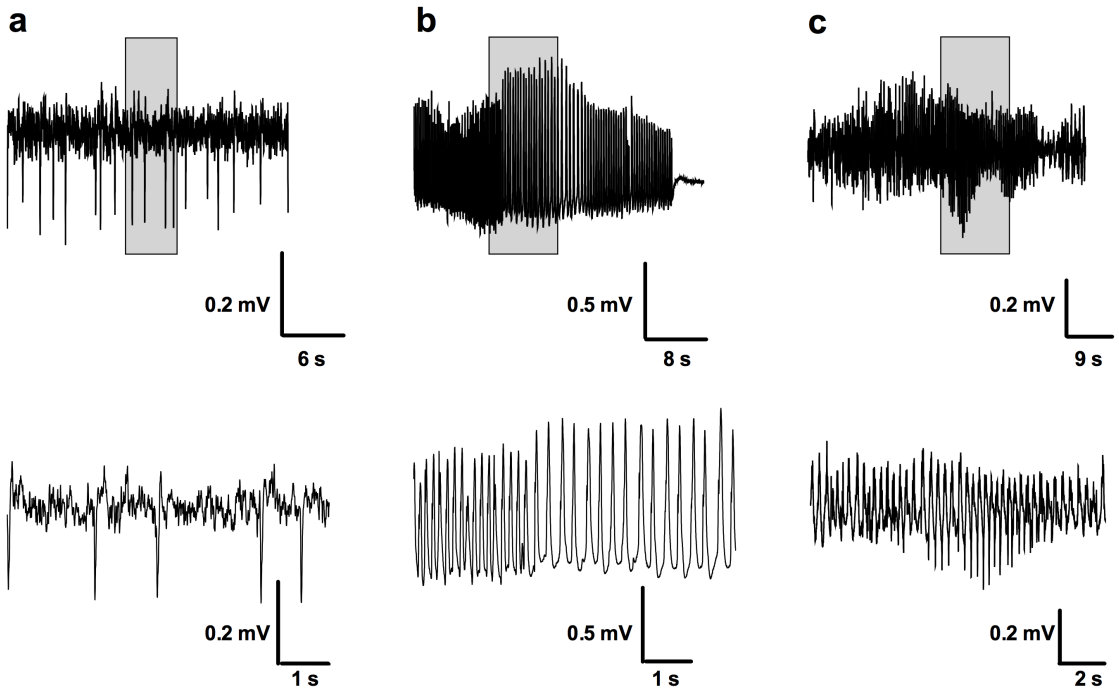

Figure 3-6 Experiment 3. Seizures occurring after NO-ISO collagenase-induced ICH. (a) Seizure of rat 1, which had a decreased RMS and power compared to baseline SWS (see Table 3-1). (b) Seizure of rat 3 with high power and high RMS. (c) Example of contralateral seizure occurring in rat 1.

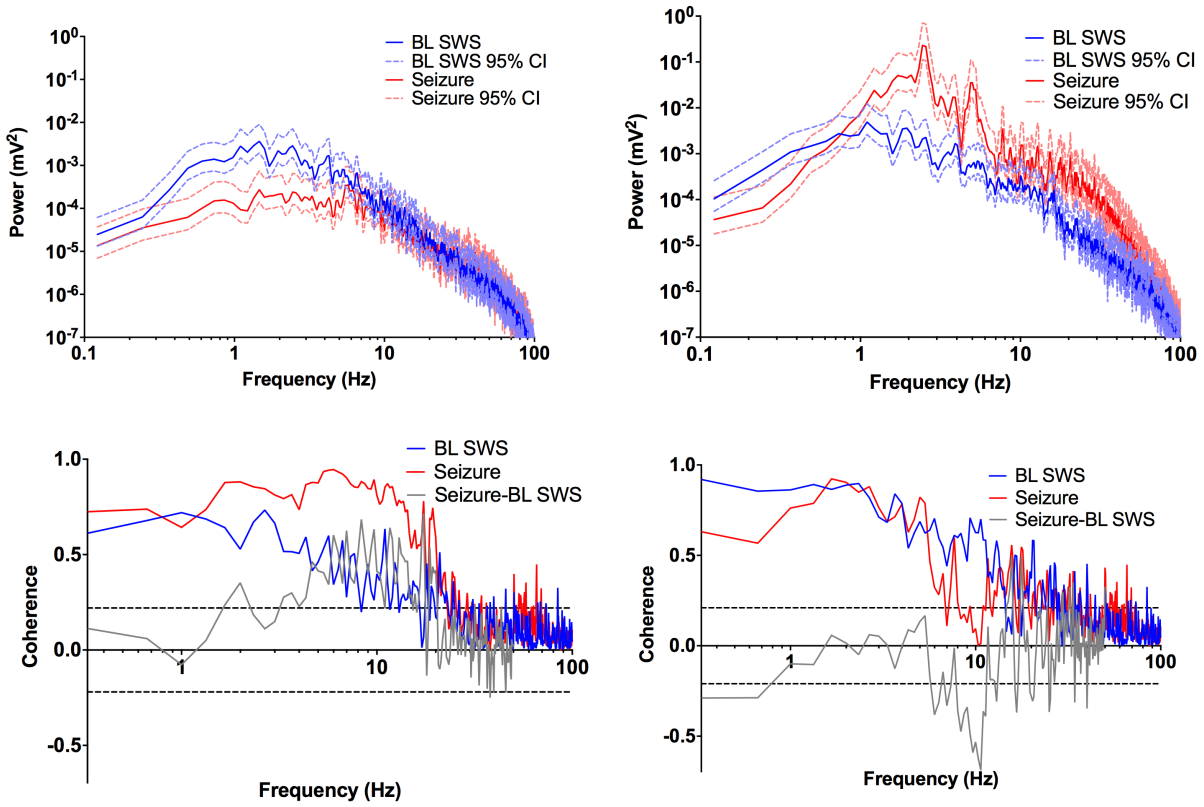

Figure 3-7 Experiment 3. Left- Example of seizure in rat 1 displayed lower power (top) but increased coherence (bottom) than baseline SWS EEG, meaning the magnitude of brain activity was decreased while coordination between hemispheres was increased. Right- Example of seizure in rat 3 displayed higher power and lower coherence, signifying that the magnitude of epileptiform activity was increased, and activity in each hemisphere was less coordinated than baseline levels. The dashed lines in the power spectrum represent the 95 % CI and the solid lines represent the mean values for the spectrum. For the coherence, any increase in coherence above the CI limit of the difference between seizure and baseline SWS was significant.

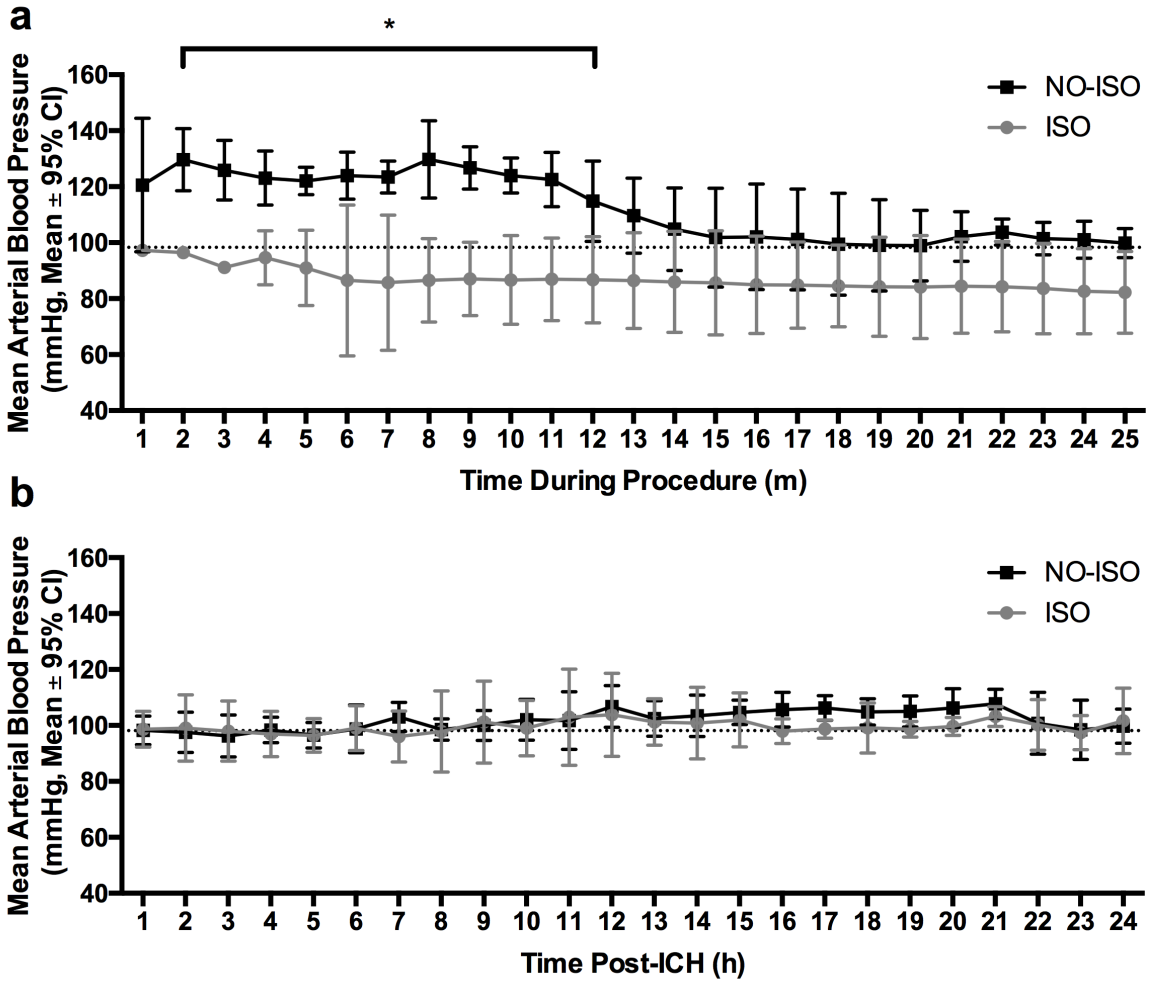

Figure 3-8 Experiment 4. There was a significant group by time interaction on (a) BP during the ICH procedure (mmHg; averaged per minute, $P = 0.003$). Awake animals had significantly higher BP from 2-12 m of the ICH surgery when compared to the isoflurane group ($P < 0.007$). Isoflurane animals were kept under anesthesia for 25 m, while the infusion procedure was typically completed in awake animals within 10 m. No group effect was found on (b) BP 24 h after ICH induction (mmHg; averaged hourly, $P = 0.4924$, interaction effect $P = 0.1524$). There was a significant effect of time on BP ($P < 0.001$). Sample size was 4 in the isoflurane group and 6 in the awake group.

Chapter 4: Glibenclamide, a Sur1-Trpm4 antagonist, does not improve outcome after collagenase-induced intracerebral hemorrhage

4.1 Introduction

Intracerebral hemorrhage (ICH) is a devastating stroke with a 40% mortality rate.[1] In the hours after an ICH, ionic homeostasis becomes disrupted, and this dyshomeostasis can persist for weeks.[2–4] Sodium (Na) and chloride (Cl) concentration increase whereas potassium (K) concentration declines. These ionic perturbations are greatest near the hematoma, but extend well into the perihematoma zone.[4] Perhaps because of this, and other factors, there is considerable cellular injury (e.g., loss of dendrites[5]) and death in the perihematoma region.[6] Likely these ionic perturbations also directly impair neural function, and could lead to seizures, commonly seen in preclinical work and in patients.[7,8] Further indirect support comes from our research that shows that rehabilitation normalizes Cl levels in the peri-hematoma zone after experimental ICH,[3] which might underlie how rehabilitation improves behavioral recovery. These data also suggest that pharmacological therapies to restore ion homeostasis may improve outcome after ICH.

Sulfonylurea receptor 1 (Sur 1) is constitutively expressed, but the transient receptor potential melastatin 4 (Trpm4) is not normally present in brain tissue. Sur 1 and Trpm4 are both upregulated and co-expressed as the heteromeric Sur1-Trpm4 channel after brain injury, such as ICH, ischemic stroke, and traumatic brain injury.[9,10] These channels allow for Na entry into cells, thereby contributing to cytotoxic edema[11] and likely persistent ionic dyshomeostasis. Glibenclamide, a Sur1 receptor antagonist, is being explored as a treatment to reduce edema after brain injuries, such as ischemic stroke, traumatic brain injury, and subarachnoid hemorrhage.[12–15] Higher doses of glibenclamide are used as a hypoglycemic agent to treat diabetes, as glibenclamide inhibits Sur1 receptors on pancreatic β cells, stimulating insulin release.[13] In ICH, lower doses of glibenclamide are a promising therapeutic due to the role Sur1-Trpm4 channels may play in edema formation and ion dyshomeostasis.

Two studies have explored whether glibenclamide improves outcome in a preclinical ICH model. Jiang et al. used the autologous whole blood model of ICH in Sprague Dawley rats, and

found glibenclamide reduced edema, protected blood-brain barrier (BBB) integrity, and improved long-term neurological deficits.[16] Another study, using the collagenase model of ICH in rats, reported that glibenclamide reduced oxidative stress, inhibited apoptosis, and improved neurological deficits.[17] Neither of these studies measured ion concentrations nor did they assess lesion size. As lesion volume is a key predictor in patient and rodent outcomes, it makes sense to determine the impact of potential therapies on total lesion size,[18,19] which is the definitive method to measure neuroprotection. Glibenclamide after ICH has been investigated clinically as well. Ghasami et al. compared the use of glibenclamide and insulin when given to diabetic hemorrhagic stroke patients.[20] The glibenclamide group had no benefit as compared to the insulin group. However, we note that this was a small, non-randomized, non-placebo-controlled trial, and further clinical work in hemorrhage would be needed.

In our study, we rigorously tested the effectiveness of glibenclamide after ICH in rats. We produced ICH using an intra-striatal injection of collagenase. As we were investigating the ability of glibenclamide to reduce edema and improve BBB integrity, we used the collagenase model that causes more extensive edema and BBB damage than the autologous whole blood model.[21] Arguably, the collagenase model may better represent the amount of edema and BBB injury seen in many ICH patients.[4,21–23] Further, ion dyshomeostasis persists for at least 14 days in the collagenase model,[3] likely far longer than what occurs in the standard autologous whole blood model of ICH.[2] The effect of glibenclamide on edema, BBB integrity, and ion homeostasis have only been assessed using the autologous whole blood model of ICH. The use of multiple models is a recommended step in pre-clinical translational research,[21,24,25] as ICH patients have heterogeneous injuries that are not reproduced by any one model. First, we assessed the safety of glibenclamide by measuring its effects on bleeding, blood glucose, core temperature, and activity. Glibenclamide affects vasodilation and could potentially impact bleeding after ICH.[26] These cardiovascular actions further support investigation using the collagenase model, which directly causes bleeding. Additionally, the effect of glibenclamide on core temperature has not

been assessed, although it is needed, as core temperature can confound preclinical research and affect outcome in diverse ways.[25,27] Next, we determined the acute effects of glibenclamide on edema, BBB integrity, ion concentrations, neurological deficits, and blood glucose to directly assess the intended effects of blocking Sur1-Trpm4. Finally, we evaluated the long-term effects of glibenclamide on skilled reaching, walking ability, blood glucose, and lesion volume.

4.2 Materials and Methods

4.2.1 Subjects

Procedures were in accordance with the Canadian Council on Animal Care Guidelines and were approved by the Biosciences Animal Care and Use Committee at the University of Alberta. All surgical procedures were performed under isoflurane anesthesia, and bupivacaine hydrochloride was used as an analgesic to minimize suffering.

We used 80 male Sprague Dawley rats (275-450 g, ~2-4 months old) from Charles River (Saint Constant, Quebec). Purina rodent chow and water were provided *ad libitum*, except when behavioural testing required food deprivation. Animals were single-housed, except during experiment 3, where they were housed in groups of 4, in a temperature- and humidity-controlled room with a 12-hour light cycle. All procedures were done in the light phase.

4.2.2 Experimental Design

In all experiments, animals were randomly assigned to groups and data was collected and analysed while blinded to group assignment. A complete experimental plan, including power calculations and statistical analyses were made prior to the start of the experiment and is available at https://www.ualberta.ca/science/about-us/contact-us/faculty-directory/fred-colbourne. *A priori* power analyses were based on expected effect size and variability of the primary endpoint.

In experiment 1, we assessed the impact of glibenclamide on bleeding, blood glucose, core temperature, and activity at 24 hours post-ICH to determine the safety of glibenclamide with the collagenase ICH model. Rats were randomized to either glibenclamide (n=6) or vehicle (n=6), which was estimated to give 80% power to detect a 40% change in hematoma volume. Note that this experiment was stopped after a blinded and planned interim analysis (at a N of 6 per group, set at α=0.1) showed no change in hematoma volume.

In experiment 2, we determined the effect of glibenclamide on edema, BBB permeability, ion concentrations, blood glucose, and neurological deficits at 3 days post-ICH. With 16 animals per group (glibenclamide vs. vehicle), we anticipated having 80% power to detect a 1% increase or decrease in edema. This effect size was based on Jiang et al, who saw a 1.04% decrease in edema after treatment with glibenclamide.[16]

In experiment 3, we assessed whether glibenclamide provided long-term efficacy by assessing skilled reaching, walking ability, and lesion volume at 28 days post-ICH. Animals were randomized to either glibenclamide (n=18) or vehicle (n=18), which was expected to give 80% power to detect a 30% increase or decrease in lesion volume. This effect size is detectable,[19] and should be biologically meaningful.

4.2.3 Telemetry Probe Implantation (Exp. 1)

Animals were anesthetized with isoflurane (4% induction, 2–2.5% maintenance, 60% N_2O, and remainder O_2).[28–30] A sterile probe was inserted into the abdomen (Model TA10TA-F40, Data Sciences International, St. Paul, MN, accurate to $\pm$0.1°C). Meloxicam (0.2 mg/kg SC) and bupivacaine hydrochloride (0.5 mg/kg SC) were administered for analgesia. Baseline data was recorded for 24 hours prior to ICH induction. Core temperature and activity were recorded every 30 seconds and averaged every hour. Post-ICH core temperature and activity readings were corrected hourly to baseline values in order to control for circadian rhythm effects.

According to our typical analysis methods, extreme data points (e.g., above 42°C), which occasionally occur due to signal noise, were excluded.

4.2.4 Blood Glucose Measurement

Blood glucose was measured using a glucometer (Contour Next One, Ascensia Diabetes Care, Mississauga, ON) in free feeding rats.[29] Immediately following induction of isoflurane anesthesia, the tail was pricked with a needle to obtain a small drop of capillary blood for analysis.

4.2.5 Intracerebral Hemorrhage

Rats were anesthetized (isoflurane) and rectal temperature was maintained at 37°C using a rectal thermometer probe and a heating pad. Bacterial collagenase (Type IV-S, Sigma, 1.0 µL of 0.6 U/µL in saline) was infused into the striatum at 0.5 mm anterior, 3.5 mm lateral, and 6.5 mm down from Bregma over 5 minutes.[28,31,32] The needle was left in place over 5 minutes to prevent backflow. A screw was used to close the needle hole and the incision was stapled shut. Bupivacaine hydrochloride was applied subcutaneously for analgesia. Collagenase was injected into the left striatum, except in experiment 3, where collagenase was injected into the side contralateral to the preferred paw, as assessed by the skilled reaching task baseline. Animals were randomly assigned following the ICH procedure.

4.2.6 Glibenclamide Administration

Glibenclamide (Abcam, Product # ab120267, Toronto, ON) was prepared using dimethylsufoxide, sodium hydroxide, and saline as described.[12] Glibenclamide was infused subcutaneously starting at 2 hours post-ICH using a mini-osmotic pump (Alzet osmotic pumps, 2001, 1.0 µl/h) to give a dose of 200ng/hour. This was the same dose used in previous work in

hemorrhage and ischemia.[12,16,33] Mini-osmotic pumps were primed overnight before implant. At the time of pump insertion, a loading dose of 10 µg/kg was given intraperitoneally. Drug delivery was verified by measuring amount of solution remaining after pump removal.

4.2.7 Hemoglobin Assay (Exp. 1)

A spectroscopic hemoglobin assay was used to determine the amount of hemoglobin in each hemisphere based upon a standard curve.[21] Hematoma volume, our primary endpoint in experiment 1, was calculated as: ipsilateral blood volume minus contralateral blood volume, which accounts for blood in the vasculature not attributed to the hematoma.

4.2.8 Behavioural Assessment

The Montoya staircase task was used to measure skilled reaching ability and was the primary behavioural endpoint.[19] During training and testing, animals were maintained at 90% of their free feeding weight to encourage reaching. Rats were trained twice daily, 5 days per week for 4 weeks prior to ICH. The average number of pellets retrieved by the dominant paw over the last 3 training days was used as the baseline score. Animals were excluded if they failed to retrieve at least 9 out of 21 pellets (45 mg reward pellets, Bio-Serv Flemington, NJ). Excluded animals still underwent behavioural testing and were otherwise included in the experiment. Testing took place on days 8-10 and 25-27 post-ICH.

Walking ability was assessed using the horizontal-ladder walking test.[19] Rats were videotaped while crossing a horizontal ladder with variable spaced rungs (3-5 cm apart). On each testing day, 2 crosses were recorded. The number of slips for each limb was recorded and averaged across the trials. Animals were assessed at baseline (pre-ICH), and days 7 and 28 post-ICH. Rats were excluded from only the ladder test if they failed to cross the apparatus twice in the baseline testing session.

Neurological deficits were assessed using the Neurological Deficit Scale (NDS), which is comprised of beam walking, spontaneous circling, forelimb flexion, bilateral forelimb grasping, and contralateral hind limb retraction.[19] Scores ranged from 0-14, with 0 indicating no impairments.

4.2.9 Brain Water and Ion Determination (Exp. 2)

Rats were briefly anesthetized (isoflurane) and received an injection of Magnevist (gadopentetate dimeglumine; 2.5 mL/kg; Bayer, Mississauga, ON) into the tail vein.[4] Gadolinium (Gd) does not normally cross an intact BBB, and thus high Gd content in the brain is an indicator of BBB permeability.[34] Magnevist has a short half-life, so rats were decapitated 10 minutes after injection. Brains were rapidly removed and assessed for water and element content.

Brain water content was measured using the wet weight-dry weight method, our primary endpoint in experiment 2.[35] Here, we define edema as increased water content, and note that this can include serum extrusion as well as edema. Brains were blocked from 2 mm anterior to 4 mm posterior to the collagenase needle tract, and tissue was separated into striatal and cortical tissue for each hemisphere. The cerebellum was taken as a control. The tissue wet weight was measured, and the dry weight was determined after tissue samples were baked at 100°C for 24 hours.

After measurement of brain water content, the dried tissue was digested in high purity nitric acid (Sigma-Aldrich) for 1 week, as done previously.[4,28] Na, K, iron (Fe), and Gd were measured using inductively coupled plasma mass spectrometry (ICP-MS; Thermo Scientific ICAP-Q quadrupole ICP-MS, Canadian Centre for Isotopic Microanalysis, University of Alberta). Fe was used as a rough indicator of hematoma volume. Other elements measured but not presented include magnesium, phosphorus, calcium, manganese, copper, zinc, and selenium (data available in S1 dataset).

4.2.10 Histology (Exp. 3)

On day 28 post-ICH, animals were injected with 100 mg/kg IP of Na pentobarbital and perfused with 0.9% saline followed by 10% neutral-buffered formalin. Brains were cryoprotected with 20% sucrose prior to cryostat sectioning. Coronal sections were 40-μm thick and taken every 200 μm from before, through, and beyond the lesion. Tissue was stained with cresyl violet and lesion volume was analyzed, as previously done.[19] The volume of a hemisphere was calculated as: (average area of complete coronal section of hemisphere-area of damage-area of ventricle) x interval between sections x number of sections). The volume of tissue lost, which includes observable tissue loss, atrophy and ventriculomegaly, was calculated as the contralateral hemisphere minus the ipsilateral hemisphere volume. Total tissue loss was the primary endpoint of experiment 3, and *a priori*, what we considered to be our most important endpoint overall.

4.2.11 Statistical Analysis

Data were analyzed using GraphPad Prism (v. 6.0, GraphPad Software Inc., La Jolla, CA) or RStudio (v. 1.1.456, RStudio, Boston, MA). All data are presented as mean ± 95% confidence interval, except non-parametric data, which are expressed as median ± interquartile range. Two group comparisons were made using a t-test, except NDS, which was compared with a Mann-Whitney U-test. Repeated measures data was assessed using a 2-way ANOVA with Sidak's multiple comparisons test. Level of statistical significance was set at α=0.05.

4.3 Results

4.3.1 Mortality and Exclusions

In experiment 1, 1 rat died due to surgical complications during the telemetry probe implantation, prior to group assignment. In experiment 2, 1 animal in the glibenclamide group and 3 animals in the vehicle group were excluded from Gd analysis due to technical problems with the Gd injection. These animals were excluded at the time of the procedure, prior to data analysis. In experiment 3, 1 animal from the vehicle group was excluded from skilled reaching analysis due to failure to meet baseline criteria. One glucose reading in the vehicle group was excluded due to failure to obtain sufficient blood sample. Five individual ladder videos were excluded from analysis due to experimenter error.

4.3.2 Experiment 1

4.3.2.1 Temperature and Activity

Core temperature varied over time after ICH, with core temperatures above baseline until approximately 12 hours post-ICH, indicating mild post-stroke hyperthermia (Figure 4-1A, $P < 0.001$, time main effect, partial η^2=0.901, large effect). Core temperature was not significantly impacted by glibenclamide (Figure 4-1A, $P = 0.187$, group main effect; $P = 0.323$, interaction effect). The ICH reduced spontaneous activity, (Figure 4-1B, $P = 0.001$, time main effect, partial η^2=0.683, large effect), but glibenclamide did not impact activity levels (Figure 4-1B, $P = 0.876$, group main effect, $P = 0.240$, interaction effect).

4.3.2.2 Hematoma Volume

Glibenclamide did not significantly alter hematoma volume measured 24 hours post-ICH (Figure 4-1C, $P = 0.991$).

4.3.2.3 Blood Glucose

Blood glucose values dropped at 24 hours after ICH (Figure 4-1D, $P = 0.040$, time main effect, partial $\eta^2=0.795$, large effect). This was likely due to rats eating less. Glibenclamide did not significantly impact blood glucose (Figure 4-1D, $P = 0.763$, group main effect; $P = 0.475$, interaction effect).

4.3.3 Experiment 2

4.3.3.1 Brain Water Content

Water content was increased in the ipsilateral striatum after ICH (Figure 4-2A, $P < 0.001$, region main effect; Cohen's d=2.68). Glibenclamide did not affect the amount of water in the brain (Figure 4-2A, $P = 0.629$, group main effect; $P = 0.897$, interaction effect). There was no effect of ICH or glibenclamide on water content in the contralateral hemisphere (see S1 dataset).

4.3.3.2 NDS

The ICH led to significant neurological deficits (Figure 4-2B, $P < 0.001$, Cohen's d = 2.31, large effect), which were unaffected by glibenclamide (Figure 4-2B, $P = 0.743$).

4.3.3.3 Blood Glucose

There was no effect of glibenclamide or time on blood glucose concentration (Figure 4-2C, $P = 0.506$, time main effect; $P = 0.531$, group main effect; $P = 0.823$).

4.3.3.4 Ion Concentrations

The integrity of the BBB was measured using tissue Gd content (ICP-MS). While ICH increased Gd levels significantly (Figure 4-3C, $P < 0.001$, Cohen's $d = 0.50$, medium effect), glibenclamide did not significantly affect Gd concentration (Figure 4-3C, $P = 0.288$). Glibenclamide did impact the distribution of Gd concentrations in the ipsilateral striatum (P =

0.031, Shapiro-Wilk normality test), as data in the glibenclamide group was positively skewed (skewness = 1.47), indicating fewer values with higher levels of Gd. Thus, we also analyzed the Gd data using a non-parametric test which also indicated glibenclamide did not affect BBB permeability (P = 0.777). After ICH, Na concentration was significantly increased (Figure 4-3A P < 0.001, Cohen's d = 1.94, large effect), but not significantly altered by glibenclamide (Figure 4-3A, P = 0.798). K was lowered after ICH (Figure 4-3B, P < 0.001, Cohen's d = 2.11, large effect), but glibenclamide did not attenuate this decrease (Figure 4-3B, P = 0.979). ICH increased Fe concentration in the ipsilateral hemisphere (Figure 4-3D, P = 0.025, region main effect). Glibenclamide did not significantly change Fe concentration (Figure 4-3D, P = 0.317, group main effect; P = 0.735, interaction effect), which indicates that the hematoma size and resolution did not differ between groups.

Ion concentrations (Na, K, and Gd) and edema levels were closely related. Increased Na was associated with increased edema (Figure 4-4A, R^2 = 0.47, P < 0.001), and decreased K was associated with increased edema (Figure 4-4B, R^2 = 0.60, P < 0.001). Similarly, a larger increase in Na predicted a greater amount of BBB permeability (Figure 4-4C, R^2 = 0.37, P = 0.001), as did a larger decrease in K (Figure 4-4D, R^2 = 0.23, P = 0.009). A greater extent of BBB permeability, as assessed with Gd, predicted increases in edema (Figure 4-4E, R^2 = 0.22, P = 0.012).

4.3.4 Experiment 3

4.3.4.1 Lesion Volume

Collagenase infusion caused significant damage to the striatum and ventriculomegaly (Figure 4-5B). Glibenclamide increased tissue lost by 23% at 28 days post-ICH, but this was not statistically significant. (Figure 4-5A, P=0.089, Cohen's d=0.56).

4.3.4.2 Behaviour

As expected, ICH caused reaching impairments in the staircase test. (Figure 4-5C, P < 0.001, time main effect, partial η^2=0.994, large effect). Glibenclamide did not attenuate the impairments in reaching ability (Figure 4-5C, P = 0.927, group main effect; P = 0.798, interaction effect). Similarly, ICH caused significant impairments in walking ability in both forelimbs and the impaired hindlimb (Figure 4-6, all P<0.020, all partial η^2 between 0.850 and 0.881, large effect). After ICH, the unimpaired hindlimb was not significantly affected (P=0.638, partial η^2=0.472). There was no effect of glibenclamide on walking ability with any limb (Figure 4-6, all P>0.110). Unimpaired limb data is not shown but is available in S1 dataset.

4.3.4.3 Blood Glucose

Glucose levels dropped post-ICH (Figure 4-5D, P = 0.035, time main effect, partial η^2=0.829, large effect), and glibenclamide had no effect (Figure 4-5D, P = 0.419, group main effect; P = 0.086, interaction effect).

4.4 Discussion

Glibenclamide, a Sur1-Trpm4 inhibitor and hypoglycemic agent, has shown promising results in ischemic and hemorrhagic stroke, including reducing edema, mortality, and functional deficits.[12,14,16,17] In contrast to those findings, glibenclamide did not reduce BBB permeability, element concentration alterations, edema, behavioral impairment or brain injury after collagenase-induced ICH in our experiments. The failure to affect BBB injury, ion alterations and edema suggests that glibenclamide will not mitigate brain swelling or intracranial pressure rises after ICH in patients. Furthermore, the lack of benefit against functional impairments and brain injury after ICH does not support the clinical use of glibenclamide as a neuroprotectant after ICH. Fortunately, physiological measures, including core temperature, activity, and blood glucose were not affected by glibenclamide. As well, hematoma volume was not affected.

Many preclinical glibenclamide studies, which support clinical investigation, have focused on reductions in edema.[10,13,36,37] Similarly, a previous study in ICH found that the same dose of glibenclamide used here significantly reduced brain water content,[16] while we report no change in water content. This discrepancy is hard to reconcile because Zhou et al. used the autologous whole blood model, where most of the increase in brain water content arises from serum extrusion and not true edema.[21,35] Conversely, the collagenase model causes considerably more extensive BBB damage and edema,[21] which one would think would be more amenable to glibenclamide. Perhaps the extent of injury after even moderate collagenase ICH is still too devastating, and despite blocking an important transporter, ions and water can still move freely through the damaged BBB. The autologous whole blood model has considerably less BBB damage,[21] and thus simply blocking the Sur1-Trpm4 transporter may be an effective strategy in this environment. The magnitude of injury and edema observed in our study was modest, and caused no mortality. In a model of traumatic brain injury, which is also characterized by extensive BBB damage,[38] it was also shown that a similar dose of glibenclamide did not attenuate increases in edema.[39] Therefore, the failure of glibenclamide was likely not because the ICH was exceptionally severe, but more so that the nature of damage in collagenase ICH is too disruptive or complex.

While our findings suggest that glibenclamide is safe to use, further testing should be conducted. Glibenclamide is currently being clinically investigated for use after large hemispheric infarction, an injury associated with hemorrhagic transformation.[40] Given the likelihood of hemorrhagic transformation in this population, the safety of glibenclamide after hemorrhage must be considered. Our data suggests no increase in bleeding, edema, or BBB damage. However, we saw a non-significant increase in lesion volume (Cohen's d=0.56, moderate effect size) in the glibenclamide group. This suggests that glibenclamide may worsen injury after hemorrhage, but we note that we are underpowered to detect an effect of this size. Unfortunately, other ICH studies did not examine lesion size, so we do not know if this effect

occurred in those experiments.[16,17] No other study of glibenclamide in ICH has quantified lesion volume or total tissue loss (including cell death, cavity formation, and ventriculomegaly), which is an accepted and unambiguous measure of neuroprotection.[25]

With our novel approach, we were able to measure edema, BBB permeability, and ion concentrations in the same tissue. ICP-MS enables precise determination of element concentrations, including Gd, Na, K, Fe, and others, excluding chloride. This measure of BBB permeability has translational relevance, as Magnevist is used in clinical imaging.[34] Further, assessing Gd extravasation in dried tissue assesses the amount of BBB permeability while excluding edema confounds, which would affect element concentrations on a per gram of wet weight basis.[41] Other common BBB permeability assays, such as Evans Blue, are unable to control for edema, and have been heavily criticized.[42–44] We are the first to directly assess the relationship between Na, K, edema, and BBB permeability in an experimental ICH model. The extent of Na increases and K decreases are closely related to increased edema, as reported by others.[45,46] Additionally, the amount BBB permeability correlates with both Na and K dyshomeostasis as well as edema. This data provides further support that edema, BBB permeability, and ionic dyshomeostasis have closely related mechanisms of damage after ICH,[4,11,47] but the sometimes weak correlations suggest that other key mechanisms are involved. Interestingly, data using x-ray fluorescence imaging, a method used to precisely and spatially image elements in tissue, shows that BBB permeability and ionic dyshomeostasis are not spatially correlated.[4]

The current study has limitations. We only assessed one dose of glibenclamide, and perhaps other doses may have conferred benefit. However, the dose we used is most commonly used after brain injury,[12,16,33,48] as it selectively inhibits Sur1-Trpm4 and does not impact blood glucose levels. Additionally, the mini-osmotic pumps maintain a steady plasma concentration of glibenclamide, reducing risk of fluctuations in blood glucose. Further, we did not assess brain levels of glibenclamide to ensure the drug was biologically available in brain

tissue. However, effects in the brain (e.g., lowering brain water content) with the same dose in other studies certainly suggests that this dose is bioavailable in the brain after injury.[12,16,17,33] We did not directly assess expression of Sur1 or Trpm4 in the collagenase model of ICH. However, Sur1-Trpm4 expression is reproducibly increased after ICH in rats, clinical ICH, and other preclinical models of injury such as ischemia and subarachnoid hemorrhage.[9,10,13,16,36,37] Glibenclamide may only work in large insults, and data in ischemia suggests glibenclamide can lower mortality by reducing life-threatening edema.[14] Our study had no mortality, and thus we may be unable to detect such benefits in ICH. However, we did see large (~4%), non-life-threatening increases in edema that glibenclamide did not reduce. We acknowledge that glibenclamide may reduce mortality in cases where edema is increased to a greater extent. Similarly, glibenclamide may improve outcome when using different models, such as comorbidities (e.g., diabetes, hypertension), aged animals, or female animals, which we did not test. While our studies were designed to have adequate power to detect biologically meaningful effects, it is possible as in all negative studies, that smaller effects went undetected.

We have taken steps to reduce bias and improve translation quality in this study. *A priori* experimental design with power calculations,[49] statistical plan, and exclusion criteria reduce the potential for unconscious bias to interfere with the course of the study. *Post hoc* changes to experimental design or statistical analysis increase the rate of type I errors in a given study.[50–52] In this study, there were no *post hoc* changes made. Translational quality is another strength of this study. We used blinding, randomization, multiple endpoints, short- and long-term assessment, multiple measures of functional outcome, and a direct, unambiguous measure of neuroprotection.[24]

In conclusion, this study demonstrates that glibenclamide is not beneficial after ICH. Glibenclamide failed to attenuate injury and improve outcome in every endpoint measured, including both the acute and chronic phases after ICH. Despite benefits seen in other pre-

clinical ICH studies, we did not find any reduction in edema, BBB permeability, bleeding, or lesion volume after treatment with glibenclamide.

4.5 References

1. Ikram MA, Wieberdink RG, Koudstaal PJ. International epidemiology of intracerebral hemorrhage. *Curr Atheroscler Rep*. 2012;14(4):300-306. doi:10.1007/s11883-012-0252-1
2. Patel TR, Schielke GP, Hoff JT, Keep RF, Lorris Betz A. Comparison of cerebral blood flow and injury following intracerebral and subdural hematoma in the rat. *Brain Res*. 1999;829(1-2):125-133. doi:10.1016/S0006-8993(99)01378-5
3. Williamson MR, Dietrich K, Hackett MJ, et al. Rehabilitation Augments Hematoma Clearance and Attenuates Oxidative Injury and Ion Dyshomeostasis After Brain Hemorrhage. *Stroke*. 2016;48(1):195-203. doi:10.1161/STROKEAHA.116.015404
4. Nadeau CA, Dietrich K, Wilkinson CM, et al. Prolonged Blood-Brain Barrier Injury Occurs After Experimental Intracerebral Hemorrhage and Is Not Acutely Associated with Additional Bleeding. *Transl Stroke Res*. 2019;10(3):287-297. doi:10.1007/s12975-018-0636-9
5. Nguyen AP, Huynh HD, Sjovold SB, Colbourne F. Progressive brain damage and alterations in dendritic arborization after collagenase-induced intracerebral hemorrhage in rats. *Curr Neurovasc Res*. 2008;5(3):171-177. doi:10.2174/156720208785425710
6. Del Bigio MR, Yan HJ, Buist R, Peeling J. Experimental intracerebral hemorrhage in rats: Magnetic resonance imaging and histopathological correlates. *Stroke*. 1996;27(12):2312-2320. doi:10.1161/01.str.27.12.2312
7. Klahr AC, Dickson CT, Colbourne F. Seizure Activity Occurs in the Collagenase but not the Blood Infusion Model of Striatal Hemorrhagic Stroke in Rats. *Transl Stroke Res*. 2014;6(1):29-38. doi:10.1007/s12975-014-0361-y
8. Merkler AE, Gialdini G, Lerario MP, et al. Population-Based Assessment of the Long-Term Risk of Seizures in Survivors of Stroke. *Stroke*. 2018;49(6):1319-1324. doi:10.1161/STROKEAHA.117.020178
9. Mehta RI, Tosun C, Ivanova S, et al. Sur1-Trpm4 Cation Channel Expression in Human

Cerebral Infarcts. *J Neuropathol Exp Neurol*. 2015;74(8):835-849. doi:10.1097/NEN.0000000000000223

10. Simard JM, Woo SK, Schwartzbauer GT, Gerzanich V. Sulfonylurea Receptor 1 in Central Nervous System Injury: A Focused Review. *J Cereb Blood Flow Metab*. 2012;32(9):1699-1717. doi:10.1038/jcbfm.2012.91
11. Simard JM, Kahle KT, Gerzanich V. Molecular mechanisms of microvascular failure in central nervous system injury—synergistic roles of NKCC1 and SUR1/TRPM4. *J Neurosurg*. 2010;113(3):622-629. doi:10.3171/2009.11.JNS081052
12. Simard JM, Geng Z, Kyoon Woo S, et al. Glibenclamide reduces inflammation, vasogenic edema, and caspase-3 activation after subarachnoid hemorrhage. *J Cereb Blood Flow Metab*. 2009;29(2):317-330. doi:10.1038/jcbfm.2008.120
13. Simard JM, Sheth KN, Kimberly WT, et al. Glibenclamide in cerebral ischemia and stroke. *Neurocrit Care*. 2014;20(2):319-333. doi:10.1007/s12028-013-9923-1
14. Sheth K, Elm J, Cheung K, et al. Abstracts for the 10th World Stroke Congress, 2016. *Int J Stroke*. 2016;11(3_suppl):4-288. doi:10.1177/1747493016670567
15. DeWitt D, Hawkins BE, Dixon CE, et al. Preclinical Testing of Therapies for Traumatic Brain Injury. *J Neurotrauma*. 2018;2754:neu.2018.5778. doi:10.1089/neu.2018.5778
16. Jiang B, Li L, Chen Q, et al. Role of Glibenclamide in Brain Injury After Intracerebral Hemorrhage. *Transl Stroke Res*. 2017;8(2):183-193. doi:10.1007/s12975-016-0506-2
17. Zhou F, Liu Y, Yang B, Hu Z. Neuroprotective potential of glibenclamide is mediated by antioxidant and anti-apoptotic pathways in intracerebral hemorrhage. *Brain Res Bull*. 2018;142(June):18-24. doi:10.1016/j.brainresbull.2018.06.006
18. Rathor MY, Rani MFA, Jamalludin AR, Amran M, Shahrin TCA, Shah A. Prediction of functional outcome in patients with primary intracerebral hemorrhage by clinical-computed tomographic correlations. *J Res Med Sci*. 2012;17(11):1056-1062.
19. MacLellan CL, Auriat AM, McGie SC, et al. Gauging recovery after hemorrhagic stroke in

rats: Implications for cytoprotection studies. *J Cereb Blood Flow Metab*. 2006;26(8):1031-1042. doi:10.1038/sj.jcbfm.9600255

20. Ghasami K, Rezvanfar MR, Faraji F, Mohammadbeigi A, Rafiee M. Impact of glibenclamide versus Insulin on neurological and functional outcomes of hemorrhagic stroke in diabetic patients. *Neurol Asia*. 2013;18(2):137-141.
21. MacLellan CL, Silasi G, Poon CC, et al. Intracerebral hemorrhage models in rat: comparing collagenase to blood infusion. *J Cereb Blood Flow Metab*. 2008;28:516-525. doi:10.1038/sj.jcbfm.9600548
22. Rosenberg GA, Estrada E, Kelley RO, Kornfeld M. Bacterial collagenase disrupts extracellular matrix and opens blood-brain barrier in rat. *Neurosci Lett*. 1993;160(1):117-119. doi:10.1016/0304-3940(93)90927-D
23. Keep RF, Zhou N, Xiang J, Andjelkovic A V., Hua Y, Xi G. Vascular disruption and blood-brain barrier dysfunction in intracerebral hemorrhage. *Fluids Barriers CNS*. 2014;11(1). doi:10.1186/2045-8118-11-18
24. Corbett D, Carmichael ST, Murphy TH, et al. Enhancing the Alignment of the Preclinical and Clinical Stroke Recovery Research Pipeline: Consensus-Based Core Recommendations from the Stroke Recovery and Rehabilitation Roundtable Translational Working Group ∗. *Neurorehabil Neural Repair*. 2017;31(8):699-707. doi:10.1177/1545968317724285
25. MacLellan CL, Paquette R, Colbourne F. A critical appraisal of experimental intracerebral hemorrhage research. *J Cereb Blood Flow Metab*. 2012;32(4):612-627. doi:10.1038/jcbfm.2012.8
26. Smits P, Bijlstra PJ, Russel FGM, Lutterman JA, Thien T. Cardiovascular effects of sulphonylurea derivatives. In: *Diabetes Research and Clinical Practice*. Vol 31. ; 1996. doi:10.1016/0168-8227(96)01230-2
27. DeBow SB, Clark DL, MacLellan CL, Colbourne F. Incomplete Assessment of

Experimental Cytoprotectants in Rodent Ischemia Studies. *Can J Neurol Sci*. 2003;30(4):368-374. doi:10.1017/S0317167100003097

28. Wilkinson CM, Fedor BA, Aziz JR, et al. Failure of bumetanide to improve outcome after intracerebral hemorrhage in rat. *PLoS One*. 2019;14(1):1-20. doi:10.1371/journal.pone.0210660
29. Williamson MR, Wilkinson CM, Dietrich K, Colbourne F. Acetazolamide Mitigates Intracranial Pressure Spikes Without Affecting Functional Outcome After Experimental Hemorrhagic Stroke. *Transl Stroke Res*. 2019;10(4):428-439. doi:10.1007/s12975-018-0663-6
30. Klahr AC, Nadeau CA, Colbourne F. Temperature Control in Rodent Neuroprotection Studies: Methods and Challenges. *Ther Hypothermia Temp Manag*. 2017;7(1):42-49. doi:10.1089/ther.2016.0018
31. Rosenberg G a., Mun-Bryce S, Wesley M, Kornfeld M. Collagenase-induced intracerebral hemorrhage in rats. *Stroke*. 1990;21(5):801-807. doi:10.1161/01.STR.21.5.801
32. Paxinos G, Watson C. Index of Structures. *Rat Brain Stereotaxic Coord*. Published online 1982:7-9. doi:10.1016/b978-0-12-547620-1.50008-4
33. Simard JM, Woo SK, Tsymbalyuk N, et al. Glibenclamide-10-h Treatment Window in a Clinically Relevant Model of Stroke. *Transl Stroke Res*. 2012;3(2):286-295. doi:10.1007/s12975-012-0149-x
34. Elster AD, Moody DM. Early cerebral infarction: Gadopentetate dimeglumine enhancement. *Radiology*. 1990;177(3):627-632. doi:10.1148/radiology.177.3.2243961
35. Williamson MR, Colbourne F. Evidence for Decreased Brain Parenchymal Volume After Large Intracerebral Hemorrhages: a Potential Mechanism Limiting Intracranial Pressure Rises. *Transl Stroke Res*. 2017;8(4):386-396. doi:10.1007/s12975-017-0530-x
36. Caffes N, Kurland DB, Gerzanich V, Marc Simard J. Glibenclamide for the treatment of ischemic and hemorrhagic stroke. *Int J Mol Sci*. 2015;16(3):4973-4984.

doi:10.3390/ijms16034973

37. Walcott BP, Kahle KT, Simard JM. Novel Treatment Targets for Cerebral Edema. *Neurotherapeutics*. 2012;9(1):65-72. doi:10.1007/s13311-011-0087-4

38. Chodobski A, Zink BJ, Szmydynger-Chodobska J. Blood-Brain Barrier Pathophysiology in Traumatic Brain Injury. *Transl Stroke Res*. 2011;2(4):492-516. doi:10.1007/s12975-011-0125-x

39. Jha RM, Molyneaux BJ, Jackson TC, et al. Glibenclamide produces region-dependent effects on cerebral edema in a combined injury model of traumatic brain injury and hemorrhagic shock in mice. *J Neurotrauma*. 2018;35(17):2125-2135. doi:10.1089/neu.2016.4696

40. Zhang J, Yang Y, Sun H, Xing Y. Hemorrhagic transformation after cerebral infarction: Current concepts and challenges. *Ann Transl Med*. 2014;2(8). doi:10.3978/j.issn.2305-5839.2014.08.08

41. Katzman R, Clasen R, Klatzo I, Meyer J., Pappius H., Waltz A. Report of Joint Committee for Stroke Resources. IV. Brain edema in stroke. *Stroke*. 1977;8(4):512-540. doi:10.1161/01.STR.8.4.512

42. Yao L, Xue X, Yu P, Ni Y, Chen F. Evans Blue Dye: A Revisit of Its Applications in Biomedicine. *Contrast Media Mol Imaging*. 2018;2018. doi:10.1155/2018/7628037

43. Saunders NR, Dziegielewska KM, Møllgård K, Habgood MD. Markers for blood-brain barrier integrity: How appropriate is Evans blue in the twenty-first century and what are the alternatives? *Front Neurosci*. 2015;9(OCT). doi:10.3389/fnins.2015.00385

44. Wang HL, Lai TW. Optimization of Evans blue quantitation in limited rat tissue samples. *Sci Rep*. 2014;4. doi:10.1038/srep06588

45. Yang GY, Chen SF, Kinouchi H, Chan PH, Weinstein PR. Edema, cation content, and atpase activity after middle cerebral artery occlusion in rats. *Stroke*. 1992;23(9):1331-1336. doi:10.1161/01.STR.23.9.1331

46. Rungta RL, Choi HB, Tyson JR, et al. The cellular mechanisms of neuronal swelling underlying cytotoxic edema. *Cell*. 2015;161(3):610-621. doi:10.1016/j.cell.2015.03.029

47. Hladky SB, Barrand MA. *Fluid and Ion Transfer across the Blood–Brain and Blood–Cerebrospinal Fluid Barriers; a Comparative Account of Mechanisms and Roles*. Vol 13.; 2016. doi:10.1186/s12987-016-0040-3

48. Simard JM, Tsymbalyuk N, Tsymbalyuk O, Ivanova S, Yurovsky V, Gerzanich V. Glibenclamide Is Superior to Decompressive Craniectomy in a Rat Model of Malignant Stroke. *Stroke*. 2010;41(3):531-537. doi:10.1161/STROKEAHA.109.572644

49. Button KS, Ioannidis JPA, Mokrysz C, et al. Power failure: Why small sample size undermines the reliability of neuroscience. *Nat Rev Neurosci*. 2013;14(5):365-376. doi:10.1038/nrn3475

50. Begley CG, Ioannidis JPA. Reproducibility in science: Improving the standard for basic and preclinical research. *Circ Res*. 2015;116(1):116-126. doi:10.1161/CIRCRESAHA.114.303819

51. Ioannidis JPA. Why most published research findings are false. *Get to Good Res Integr Biomed Sci*. Published online 2018:2-8. doi:10.1371/journal.pmed.0020124

52. Macleod MR, Fisher M, O'collins V, et al. Good laboratory practice: Preventing introduction of bias at the bench. *Int J Stroke*. 2009;4(1):3-5. doi:10.1111/j.1747-4949.2009.00241.x

53. Colbourne F, Auer RN, Sutherland GR. Characterization of postischemic behavioral deficits in gerbils with and without hypothermic neuroprotection. *Brain Res*. 1998;803(1-2):69-78. doi:10.1016/S0006-8993(98)00612-X

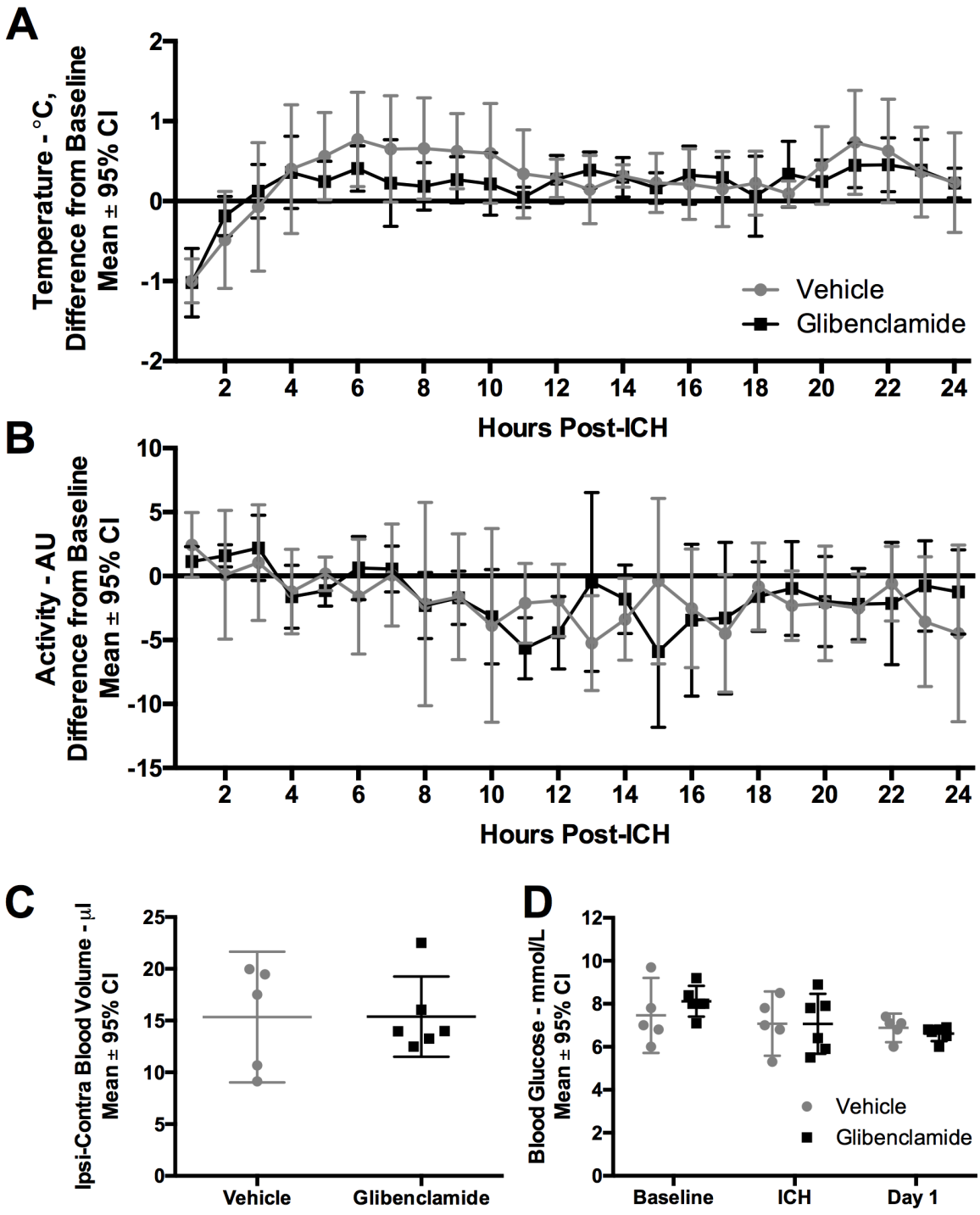

Figure 4-1. Experiment 1.

Glibenclamide did not significantly impact (A) core temperature (P = 0.187, group main effect) or (B) activity (P = 0.876, group main effect) after ICH (n=6 in glibenclamide group, n=5 in control

group). Core temperature (°C) and activity (AU, arbitrary units – detections of animal movement over a receiver[53]) data were taken using implanted telemetry devices. Core temperature and activity averages for each hour were corrected for the same hour of baseline data to account for time of day effects (e.g., circadian rhythms). Rectal temperature was regulated during surgical procedures; the core temperature drop within the first few hours was due to rapid but mild cooling after post-surgical anaesthesia. (C) Bleeding (P = 0.991) and (D) blood glucose (P = 0.763, group main effect) were both unaffected by glibenclamide. Sample sizes were n=6 in glibenclamide group, n=5 in control group.

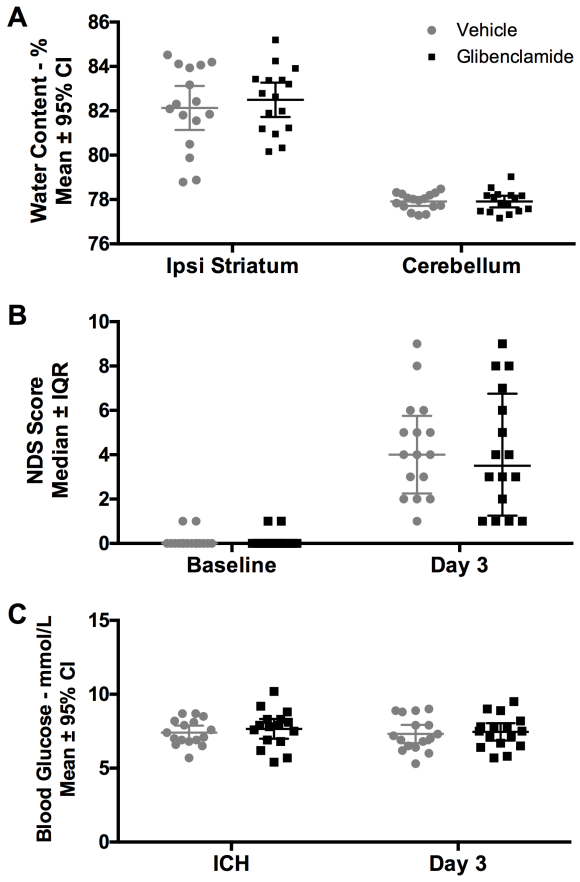

Figure 4-2. Experiment 2

Glibenclamide did not reduce (A) brain water content (P = 0.629, group main effect) or (B) neurological deficits (P = 0.743). (C) Blood glucose was not impacted by glibenclamide (P = 0.531, group main effect). Sample size was n=16/group.

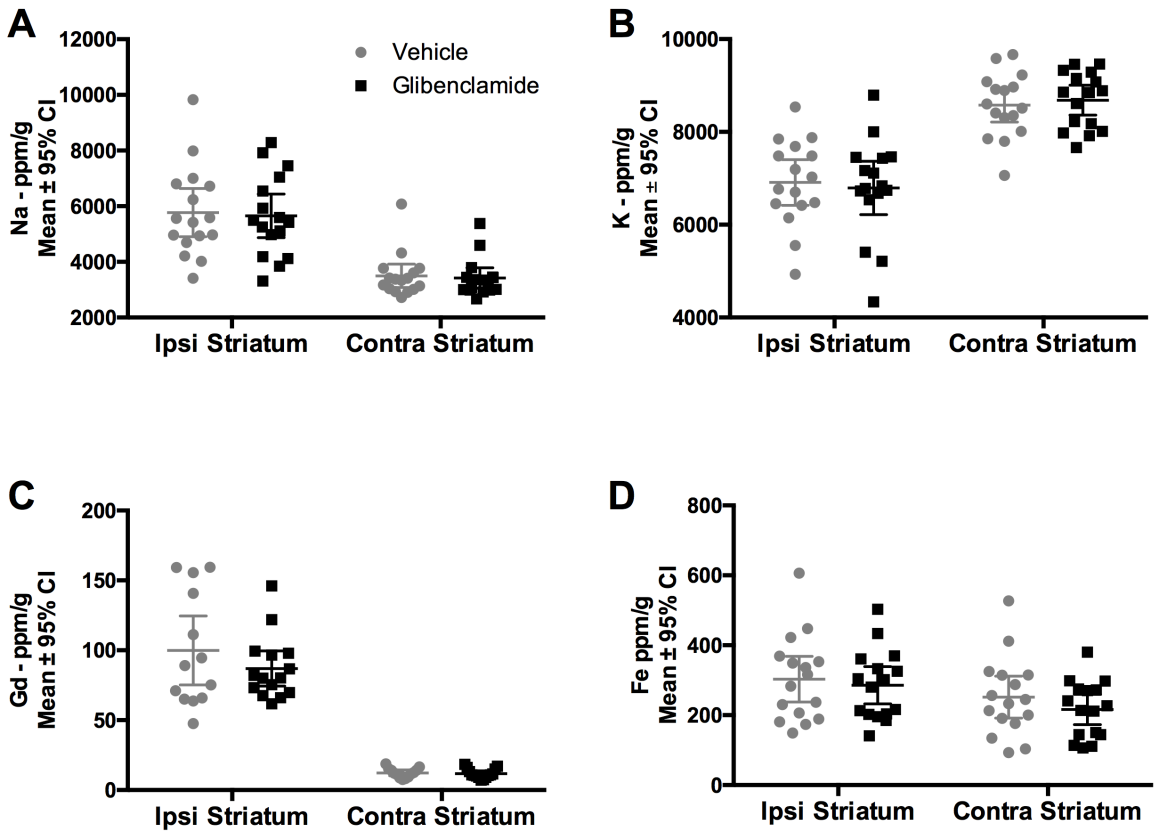

Figure 4-3. Experiment 2-Ion Concentrations

(A) Na (*P* = 0.798) and (B) K (*P* = 0.979) concentrations were not affected by glibenclamide, despite being increased in the hemisphere ipsilateral to the ICH. Treatment with glibenclamide did not impact (C) BBB permeability, as assessed with Gd (*P* = 0.288), or amount of Fe in the striatum (*P* = 0.317, group main effect). Sample size was n=16/group.

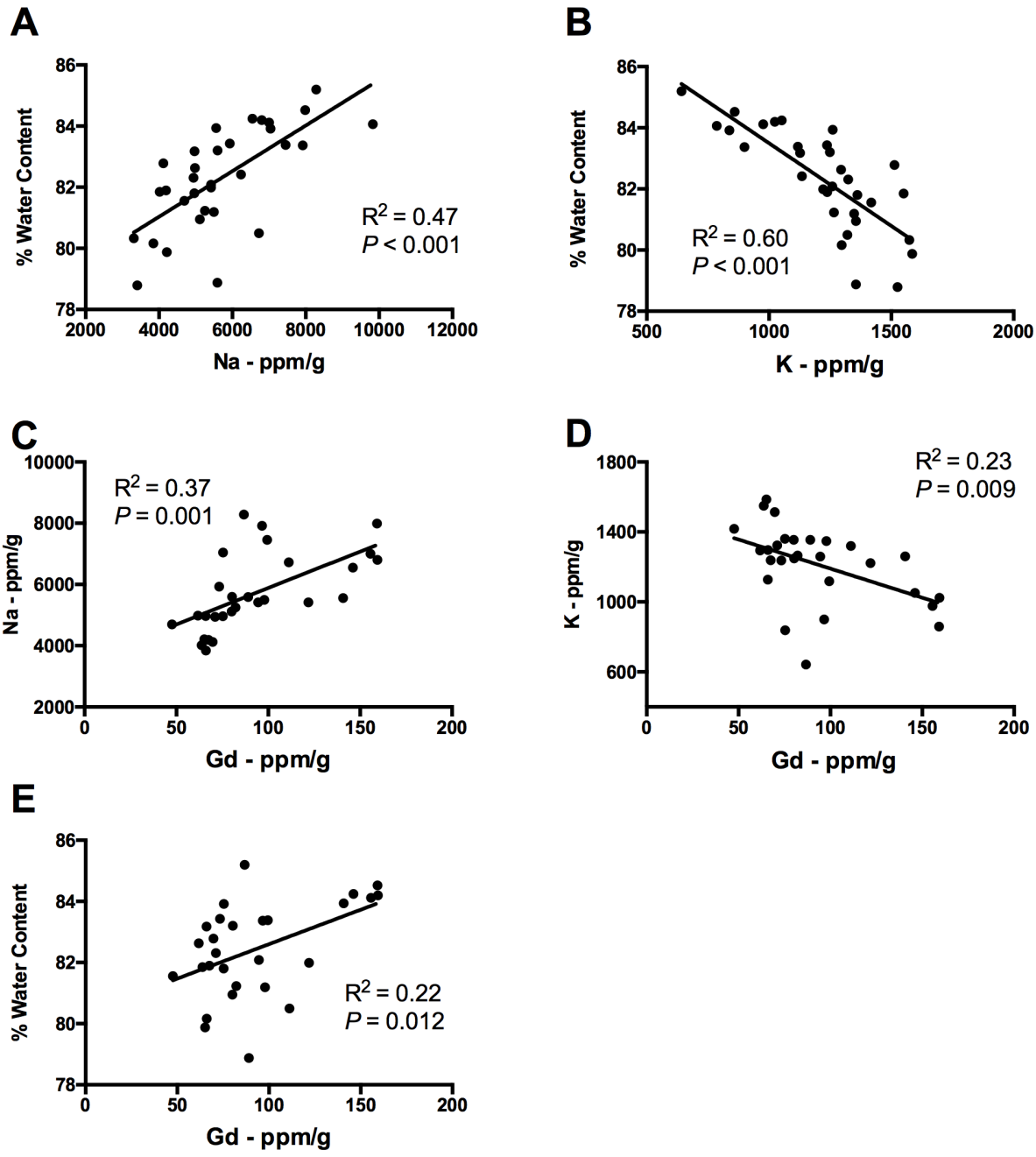

Figure 4-4. Experiment 2-Correlations

(A) Increased Na (R^2 = 0.47, P < 0.001), and (B) decreased K (R^2 = 0.60, P < 0.001) was associated with increased ipsilateral striatum edema. (C) More Na (R^2 = 0.37, P = 0.001) and (D) less K (R^2 = 0.23, P = 0.009) was related to greater amount of Gd extravasation. (E) More Gd extravasation was positively associated with edema (R^2 = 0.22, P = 0.012).

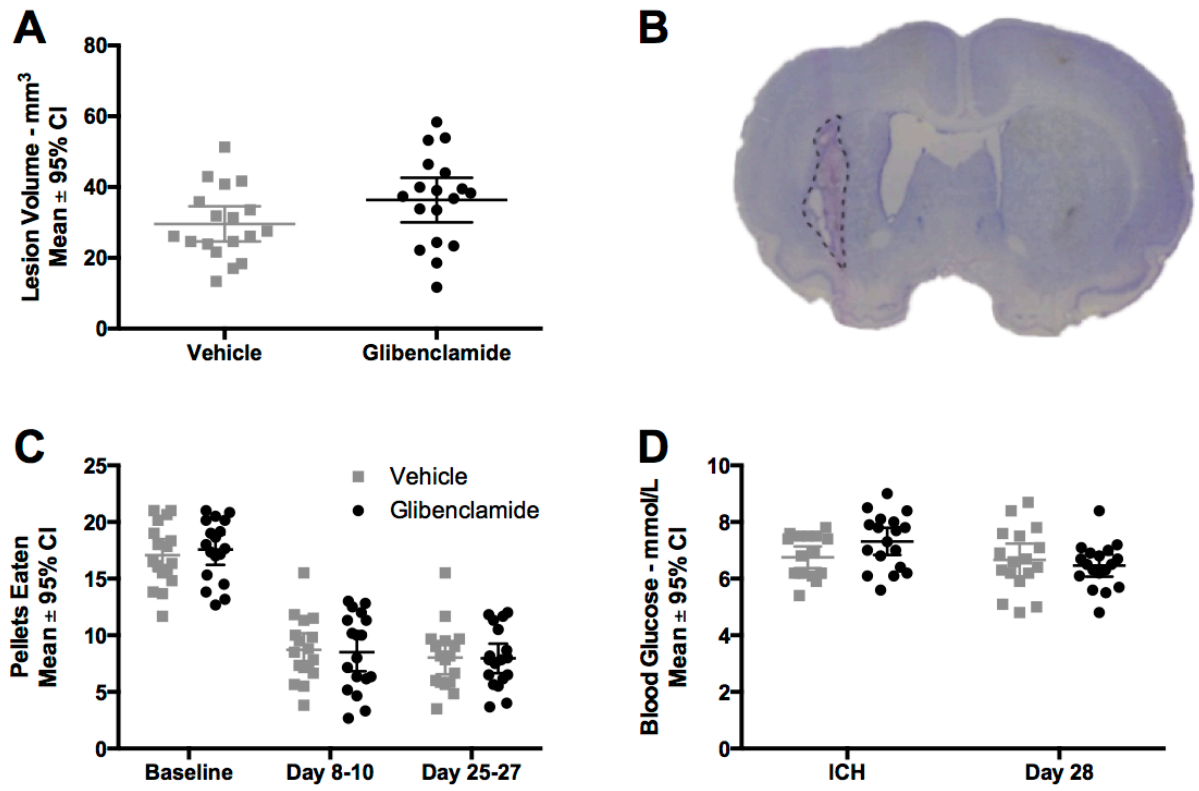

Figure 4-5. Experiment 3

Glibenclamide did not affect (A) tissue lost at a 28-day survival (*P*=0.089). (B) Representative lesion volume image (vehicle group) demonstrating area of dead tissue (dashed line) and ventriculomegaly (larger ventricle). (C) reaching ability (*P* = 0.927, group main effect) and (D) blood glucose (*P* = 0.419, group main effect) we not impacted by glibenclamide. Sample size was n=18/group.

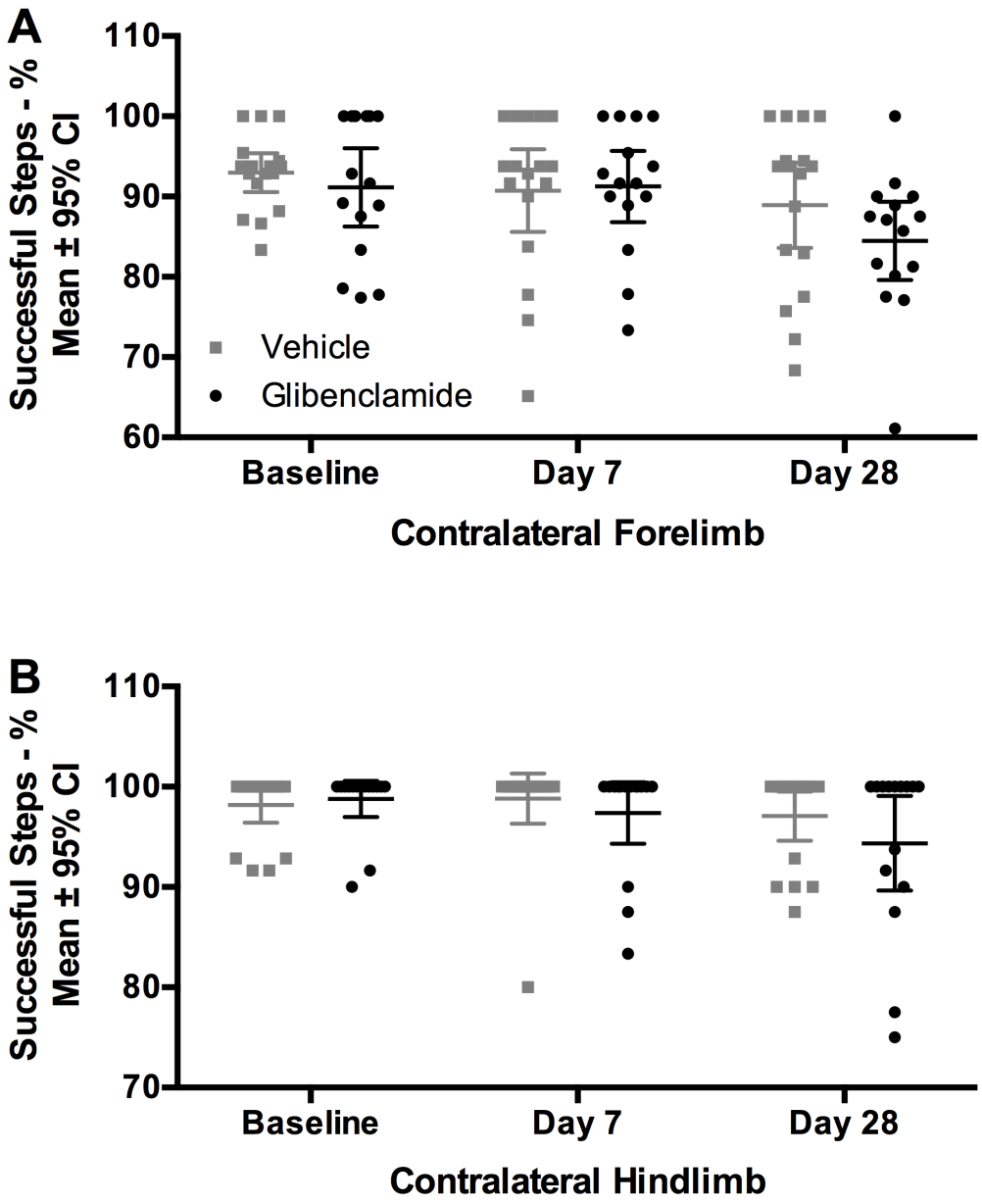

Figure 4-6. Experiment 3-Ladder.

After ICH, walking ability was impaired (all $P<0.020$). Glibenclamide did not impact walking ability in (A) the contralateral forelimb or (B) the contralateral hindlimb (all $P>0.11$). Sample size was n=18/group.

Chapter 5: The effects of chronic hypertension on tissue compliance after severe collagenase-induced intracerebral hemorrhage

5.1 Introduction

Primary intracerebral hemorrhage (ICH) has two main causes: amyloid angiopathy and hypertension.[1,2] Hypertension is defined as blood pressure over 140/90 mmHg.[3] Approximately 60% of ICHs are considered hypertensive ICH.[4,5] With continued improved medical management of hypertension, many countries have seen decreased rates of hypertensive ICH, including Japan and the United Kingdom.[6] Chronic hypertension leads to smooth muscle cell death and replacement with collagen, making vasculature more brittle and increasing the likelihood of bleeding.[4] Additionally, when intracranial bleeding does occur, the hematoma is expected to be larger than in normotensive patients due to increased blood pressure. However, the rate of hematoma growth is more closely related to the size of the initial blood vessel, the size of the opening in that vessel, and the ability to stop the bleed (coagulation), meaning blood pressure plays a relatively smaller role in hematoma growth.[4] Although acute control and reduction of blood pressure has been of great interest as a method of reducing hematoma expansion, chronic hypertension itself is not a reliable predictor of hematoma expansion.[7] This could be due to a variety of factors, including the tamponade effect countering bleeding.[8]

Spontaneously hypertensive rats (SHR) are commonly used as an animal model of chronic hypertension.[9,10] This strain was made by selectively breeding Wistar Kyoto rats with high blood pressure. These rats have a systolic blood pressure of ~170 mmHg as compared to ~130 mmHg in normotensive rats.[11] This equates to a mean arterial pressure (MAP) of ~150 mmHg compared to ~100 mmHg in normotensive rats.[12] Research using SHRs to model ICH has shown SHRs have more severe neurological deficits and increased neuronal death after both the whole blood and collagenase models of ICH.[13] However, hematoma volume and brain edema remained consistent between SHRs and WKRs.[13] Despite higher blood pressure, SHRs do not demonstrate hypercoagulation or increased blood viscosity compared to WKRs.[14] There are many animal models of hypertension beyond phenotype-driven genetic strains, including surgical (e.g., constricting renal arteries) and pharmacological (e.g., administration of

glucocorticoids) models.[15,16] Like essential hypertension in humans (~90% of hypertension cases[17]), hypertension in the SHRs is caused by a variety of genes contributing to the hypertensive phenotype. Surgical, pharmacological, and dietary approaches do not mimic the underlying genetic contributors in many clinical cases of hypertension, and thus are more reflective of secondary hypertension. Indeed, 60% of contributing factors to developing hypertension are genetic, while 40% are environmental.[18] While the SHR model has limitations, such as genetic variation and limited environmental contribution, it is still a useful model of chronic essential hypertension.

After large ICHs, the hematoma occupies limited space in the skull, causing intracranial pressure (ICP) to rise. The Monro-Kellie doctrine states that in order to maintain a constant combined volume of brain, blood, and cerebrospinal fluid (CSF) within the skull and maintain normal ICP, blood and CSF will be diverted (e.g., drained into sinuses to exit the cranium via jugular veins).[19,20] Once blood and CSF can no longer be diverted, ICP will increase. We have documented an additional form of ICP compliance in Sprague Dawley rats (SDR) that we call tissue compliance. After mass effect producing injuries, including ICH and middle cerebral artery occlusion, neurons and astrocytes in uninjured brain regions reduce their volume and pack closer together.[21,22] This effect is transient, and cells return to their normal volume after ~7 days, without signs of cell death. By reducing neuronal volume, and therefore, brain volume, additional space is made in the skull to accommodate the hematoma and resultant edema.

However, tissue compliance has only been documented in young and healthy SDRs, and it is possible this effect may be impaired in animals with chronic hypertension. There are numerous parenchymal changes that happen with chronic hypertension in otherwise naïve rats that can affect stroke outcome, including increased inflammation, impaired neurogenesis, and astrocyte dysfunction.[23] Notably, SHRs experience decreased cortical volume, reduced neuronal density, and decreased dendritic density across brain regions.[24–26] Compared to WKRs, SHRs hippocampal volume was reduced by 21% by 6 months of age.[27] Neurogenesis

continues to decline by 12 months of age, and the extent of atrophy in aged SHRs is unclear.[28] Therefore, due to atrophy (enlarged cerebral ventricles[26]), SHRs may be able to divert more CSF, increasing ICP compliance and reducing the need for tissue compliance. Conversely, due to an unfavourable parenchymal environment (impaired vasculature, dysfunctional glial cells that could impair glymphatic drainage), SHRs may be less capable of rapidly reducing neuron volumes. Differing growth trajectories in SHRs may also affect skull size, reducing the cranial compartment, however, this has not been investigated to our knowledge.

In this experiment, we investigated the effect of chronic hypertension on ICH outcomes and tissue compliance using the SHR strain. Our primary objective was to determine differences in brain morphology as well as the extent of tissue compliance across each strain. Our secondary objective was to compare ICH outcomes across rodent strains, including neurological deficits and hematoma volume. We hypothesize that SHRs will have larger hematoma volumes and will exhibit more limited tissue compliance than control strains. We used two control strains, the SDR strain, as we have previously observed tissue compliance in this strain,[21,22] and the Wistar Kyoto rat (WKR) strain, which the SHR strain was bred from.[29] We used the collagenase model of ICH,[30,31] as it reflects ongoing bleeding, and hypertensive ICH could play a role in increasing ongoing bleeding. We compared an ICH to a sham group across each of the three strains. After ICH was induced, we assessed neurological deficits, feeding and drinking behaviour, weight, and hydration. A subset of animals had telemetry probes implanted to assess strain differences in temperature and activity after ICH. Animals were perfused and tissue was formalin fixed to measure histological measures of outcome, including hematoma volume, brain volume, cortical thickness, and stereological measures of tissue compliance.

5.2 Methods

5.2.1 Subjects

All procedures were done in accordance with the Canadian Council on Animal Care Guidelines and approved by the University of Alberta Biosciences Animal Care and Use Committee (Protocol 960). We used a total of 72 male rats (20 Sprague Dawley, 20 Wistar Kyoto, 20 SHRs, plus an additional 12 SHRs for pilot work) obtained from Charles River Canada (Saint Constant, QC), with weights ranging from 250-450g (~3-5 months old). Animals were housed in a temperature- and humidity-controlled environment with the lights on from 7:00 am-7:00 pm, and all procedures were done during the "lights on" period. Strains were housed in separate rooms. During experiments, animals were single-housed with *ad libitum* access to food (Purina Rodent Chow) and water.

5.2.2 Telemetry Probe Implantation Surgery

Four days prior to ICH induction, a subset of animals (n = 4/group) underwent surgery to implant a core temperature telemetry probe. Animals were anesthetized with isoflurane (4% induction, 2-2.25% maintenance in 60% N_2O and remainder O_2). The abdominal area was shaved, with betadine used for sterility. A sterile calibrated probe was inserted in the peritoneal cavity (Model TA10TA-F40, Data Sciences International, St. Paul, MN, accurate to ± 0.2 °C) as previously described.[32] Animals were treated with meloxicam (0.2 mg/kg SC, Boehringer Ingelheim, Burlington, ON) and bupivacaine hydrochloride (0.5 mg/kg SC, SteriMax, Oakville, ON) as a surgical analgesic. Animals recovered quickly and were not displaying signs of ongoing pain,[33] and thus, no top up analgesic doses were administered. Baseline temperature and activity measurements were taken for 24 hrs prior to ICH induction. Data was recorded every 30 s prior to and after ICH. Post-ICH temperature and activity measurements were normalized using hourly-averaged baseline readings taken the day before ICH induction in order to correct for fluctuations due to circadian rhythms. Activity was quantified by measuring variations in signal strength, which occurred with movement of the probe across the receiver, using arbitrary units.[34]

5.2.3 Collagenase Intracerebral Hemorrhage

Animals were anesthetized with isoflurane, as done above, and temperature was maintained at 37°C using a rectal temperature probe and heating pad. The ICH was induced as previously described.[30,35] Briefly, a hole was drilled into the skull at 3.5 mm to the left and 0.5 mm anterior to bregma (Paxinos and Watson, 7th edition).[36] Bacterial collagenase (Type IV-S, Sigma, 2.1 µL of 0.6 U/µL) was injected over 5 mins at 6.5 mm depth from the skull. This amount was chosen as pilot work demonstrated it caused severe hemorrhages (mean = 100.53 µL) at 24 hrs post-ICH with low (<15%) mortality in SHRs (n = 6/group). The burr hole was then sealed with a metal screw. Animals were injected with 5 mL saline (0.9%, SC) to maintain hydration and 0.5 mg/kg bupivacaine hydrochloride (SC) as a local anesthetic. For sham procedures, animals were kept under anesthetic for approximately the same duration (25 mins). An incision was made, but no hole was drilled to maintain normal ICP, and no collagenase was injected.

5.2.4 Neurological Deficits

Rats were assessed for severity of neurological deficits using the neurological deficit scale (NDS) at 24 hrs post-ICH, just prior to euthanasia. The NDS scale is scored from 0-14, with 0 meaning no deficits and 14 meaning maximal deficits, and is the total score of 5 subscales.[37] Subscales include spontaneous circling, beam walking, bilateral forelimb grasping, contralateral hind limb retraction, and contralateral forelimb flexion.

5.2.5 Feeding and Hydration Assessment

Animals were weighed daily, and had food and water weighed daily to track eating/drinking behaviour throughout the experiment. After animals were euthanized (see 5.2.6), small sections of abdominal muscle were taken and assessed for water content as a measure of

hydration.[22] Muscle samples were dried in the oven at 100°C for 24 hrs. Water content was measured as: ((wet weight-dry weight)/wet weight)×100.

5.2.6 Histology

At 24 hrs post-ICH, animals were anesthetized with sodium pentobarbital (100 mg/kg IP, Bimeda, Cambridge, ON) and transcardially perfused with 0.9% saline followed by 10% neutral buffered formalin. Brains were vibratome sectioned at 80 μM with sections taken every 240 μM. Sections were taken as far anterior and poster as possible (~4.5 mm anterior and -5.5 mm posterior to Bregma)[36]. The cerebellum and brain stem were also sectioned. Sections were stained with cresyl violet, as done previously.[37]

Stained sections were analyzed to assess brain volume, ventricle volume, cortical thickness, and hematoma volume. Using Image J (v. 1.52A, NIH), the contralateral hemisphere was measured in all sections from 3.9 mm anterior to Bregma up to 5 mm posterior to Bregma. Average section area was multiplied by the number of sections and the section interval to get a partial contralateral hemisphere volume. The ipsilateral hemisphere was not measured to avoid edema confounds. Similarly, the contralateral lateral ventricle volume was assessed in all animals. Cortical thickness was assessed by measuring the average distance between the dorsal aspect of the cingulum to cortical layer II in 5 sections from +1.89 to -2.0 from Bregma, as done previously.[22] Hematoma volume was measured by calculating the area of the hematoma on all sections where blood was present. The average hematoma area was then multiplied by the section interval and number of sections. Due to the early survival time, true lesion volume could not be assessed, as edema skews the volume of the ipsilateral hemisphere as a whole.[32]

5.2.8 Stereological Analysis

Neuron soma volume and neuronal density were assessed in the CA1 region of the contralateral hippocampus, the contralateral S1 region, and in lobe VI of the cerebellum. Cell volume was assessed using the nucleator probe method in conjunction with the physical dissector, and cellular density was assessed using the physical and optical dissector probes, as we have previously done.[22,38] Briefly, volume was calculated by measuring the area of 15 randomly selected neurons per animal at 5 different levels within the z-axis. The average radius for each cell was assessed and used to calculate volume.

5.2.9 Statistical Analysis

Power analyses and effect sizes (Cohen's f) were calculated using GPower (v 3.1.9.6). Using a sample size of 9 per group would give ~80% power to detect an 35% percent change in cell volume, based on a standard deviation of 443 μm^3 and a mean cell volume of 1791 μm^3 in SHRs (pilot data). We increased this to 10/group in anticipation of mortality and exclusions. Data were analyzed using GraphPad Prism (v 9.0, GraphPad Software Inc., La Jolla, CA) and are presented as mean $\pm$ 95% confidence interval (CI), with the exception of NDS scores (presented as median and interquartile range). Neurological deficit scores were assessed using a Kruskal Wallis test for comparisons between strains and a Mann-Whitney U-test for comparisons between ICH and sham groups within a strain. Data collected at a single timepoint (e.g., brain volume, muscle water content) was assessed using a 2-way between subjects ANOVA with Sidak's multiple comparisons test to assess effects of strain and injury (ICH vs sham). Data collected at multiple timepoints (e.g., food consumption, body weight) was assessed using a mixed effects analysis with Tukey's multiple comparisons test. Time was considered a within-subjects factor, while strain and injury were between subjects' factors. Mortality rate between groups was assessed using Pearson's chi-squared test. A P-value of <0.05 was considered statistically significant.

5.3 Results

5.3.1 Exclusions/Mortality

Three SHRs in the ICH group died prematurely and were excluded from NDS testing and muscle water content measurements. Brains were immersion fixed in formalin for histological analysis. As no other animals died prematurely, there was a statistically significant effect of strain on mortality (chi-squared $p = 0.036$). One SDR in the sham group and one WKR in the ICH group were excluded from all histological analysis due to technical error in tissue processing. An additional 2 SDRs in the ICH group, 1 WKR in each of the sham and ICH groups, and 1 SHRs in the sham group were excluded from stereological analysis in the S1 region due to histological artefact. For CA1 analysis, 1 SDR in the ICH group, 1 SHR in the sham group, and 2 SHRs in the ICH group were excluded from stereological analysis due to histological artefact.

5.3.2 Neurological Deficit Score

All ICH groups regardless of strain demonstrated increased deficits when compared to the sham group of the same strain (Figure 5-1, all $p < 0.001$). However, there were no significant differences when comparing the 3 strains in each of the surgical groups (Figure 5-1, $p = 0.417$ among sham groups and $p = 0.095$ between ICH groups). In both sham and ICH groups, the WKRs tended to perform worse than the other strains, but this was not statistically significant.

5.3.3 Feeding, Drinking, and Body Weight

There was a significant interaction effect between injury type and time on food and water consumption (Figure 5-2, interaction effect $p = 0.001$), indicating that overall, ICH rats decreased consumption after the surgery regardless of strain. The SDRs drank (Figure 5-2A, strain main effect $p < 0.001$) and ate (Figure 5-2B, strain main effect $p < 0.001$) more on

average than the other strains, regardless of whether they had an ICH or not. In addition, all animals in the ICH groups had a decrease in feeding (ICH main effect $p < 0.001$) and drinking (ICH main effect $p = 0.011$) behaviour post-ICH. The differences noted in feeding behaviour for SDRs is also demonstrated in rodent weight (average SDR pre-surgery weight = 371 g, average WKR/SHR pre-surgery weight = 284 g) but not the amount of weight loss after ICH induction as a percent of baseline weight. All strains assigned to sham groups maintained a consistent weight, while all strains in the ICH experienced weight loss after the injury (Figure 5-2C, ICH main effect $p < 0.001$, strain effect $p = 0.807$ interaction effect $p = 0.521$).

5.3.4 Muscle Water

There was a significant interaction between group and strain (Figure 5-2D, $p = 0.021$), however, there were no significant subgroup comparisons when using Sidak's multiple comparisons test (all $p > 0.361$). The significant interaction was likely driven by slightly increased water content in the SDR sham group, which could be reflective of the trend towards increased water consumption in this group. The lack of a significant group effect or main effect suggests that the ICH groups were not significantly dehydrated, and that any strain differences in hydration were minor.

5.3.5 Temperature and Activity

There was no effect of strain on temperature (Figure 5-3A, strain main effect $p = 0.164$, interaction effect $p = 0.072$) or activity (Figure 5-3B, strain main effect $p = 0.217$, interaction effect $p = 0.273$). There was a significant effect of ICH on temperature (Figure 5-3A, ICH main effect $p = 0.011$), where ICH animals had increased temperature from 0-12 hrs post-ICH, potentially indicating mild post-ICH fever.[39] There was a significant effect of time on temperature (time main effect $p < 0.001$), and activity (time main effect $p < 0.001$) likely due to anesthetic

causing a temporary (<1 hr) drop in temperature and activity in all groups after surgical procedures.

5.3.6 Hematoma Volume

The SHR ICH group and the WKR ICH group had a larger hematoma volume on average when compared to the SDR ICH group. However, there was no statistically significant effect of strain on hematoma volume (Figure 5-1, $p = 0.122$). Additionally, the SHR group had a significantly higher rate of ventricular extension, defined as extension of the hematoma from the parenchyma into the ventricles (100% in SHRs, vs 30% in SDRs and 60% in WKRs, chi-squared $p = 0.005$ among groups).

5.3.7 Histological Brain Quantification

There were significant main effects of ICH (Figure 5-4A, $p < 0.001$) and strain ($p = 0.008$) on right hemisphere volume (interaction effect, $p = 0.551$). In the ICH groups, contralateral hemisphere volume was decreased compared to the sham groups. Within the sham groups, WKRs had significantly larger hemisphere volumes than SHRs ($p = 0.027$). Within the ICH groups, SDRs had significantly smaller hemisphere volumes than WKRs ($p = 0.044$). Contralateral lateral ventricle volume significantly differed across strains and injury type (Figure 5-4B, interaction effect $p < 0.001$). Specifically, the SHR sham group had a significantly increased ventricle volume compared to both other strains ($p < 0.001$). The cortex of ICH animals across groups was thinner than the sham groups (Figure 5-4C, ICH main effect $p = 0.006$, interaction effect $p = 0.300$). Additionally, cortical thickness varied by strain, with SDRs having the largest cortex and SHRs having the smallest cortex, on average (Figure 5-4C, strain main effect $p = 0.001$).

As an exploratory (unplanned) analysis, we performed a linear regression to compare hematoma volume and brain volume, to see if ICH volume was related to the amount of tissue

compliance overall and if this differed among strains. We found that hematoma volume was not significantly correlated with brain volume in any strain (all $p > 0.316$), and that the slopes of the regression were not significantly different across the three strains ($p = 0.339$).

5.3.8 Stereology

The animals in the ICH group had significantly decreased cell volume in the CA1 region compared to sham animals in all strains (Figure 5-5A, ICH main effect $p < 0.001$, interaction effect $p = 0.181$), and the lack of interaction indicates this effect was consistent among strains. There was a significant effect of strain on CA1 volume (strain main effect $p = 0.003$), with SDRs having larger cell volumes than both WKRs ($p = 0.037$) and SHRs ($p < 0.001$) in both the sham and ICH groups. There was a significant effect of ICH (Figure 5-5B, ICH main effect $p = 0.025$, interaction effect $p = 0.924$) and strain (strain main effect $p = 0.016$) on cell density in CA1.

In the contralateral S1 region, there was a significant effect of ICH on cell volume (Figure 5-5C, ICH main effect $p < 0.001$). There was a significant interaction effect, indicating a differential effect of ICH by strain ($p = 0.042$). Still, all strains had a significantly decreased cell volume in the ICH group compared to the respective sham group (all $p < 0.001$). There was a significant effect of ICH on cell density in S1, where ICH animals had increased cell density (Figure 5-5D, ICH main effect $p = 0.044$, strain main effect $p = 0.781$, interaction effect $p = 0.475$).

In the cerebellum region, there was no effect of ICH (ICH main effect $p = 0.522$, interaction effect $p = 0.891$) or strain (strain main effect $p = 0.354$) on cell volume. Similarly, there was no effect of ICH (ICH main effect $p = 0.458$, interaction effect $p = 0.986$) or strain (strain main effect $p = 0.246$) on cell density (data not shown).

5.4 Discussion

Compared to SDRs and WKRs, SHRs have an increased mortality rate after severe, collagenase-induced intracerebral hemorrhage. Contrary to our hypothesis, SHRs did not have significantly larger hematoma volumes, and demonstrated approximately the same decrease in brain volume and cell volume after ICH. Despite not having significantly larger bleed volumes, SHRs did have an increased rate of ventricular extension (100% of animals) compared to the other strains (30% in SDRs and 60% in WKRs). We found significantly decreased contralateral hemisphere volume, decreased cell volume, and increased cell density in the ICH group compared to the sham group, indicating intact tissue compliance mechanisms in all strains. We observed strain specific differences in brain morphology, including contralateral brain volume, cortical thickness, and ventricle volumes between the sham groups. Despite these differences in brain morphology, surviving SHRs did not display significantly increased functional deficits compared to SDRs and WKRs, which may be in part due to the lack of sensitivity in the NDS scale.[37]

Despite slight and statistically insignificant differences in hematoma volumes, SHRs also underwent tissue compliance, however, they experienced the smallest effect of ICH on overall brain volume (SHR Cohen's d = 2.73 – 7.8% reduction, SDR Cohen's d = 3.29 – 9.8% reduction, WKR Cohen's d = 2.91 – 8.2% reduction). Chronic hypertension does not appear to impede the ability of uninjured neurons to shrink in volume, pack closer together, and make space to accommodate a large bleed. Across strains, we found the cerebellum did not undergo tissue compliance, making this region a potential internal negative control for future studies. Interestingly, we found that the sham SHRs had increased lateral ventricle volumes compared to all other groups. This could be indicative of tissue atrophy commonly seen in SHRs by this age.[26] Indeed, we observed decreased brain volume (~2.5%) in sham SHR animals compared to sham WKR animals, similar to decreases that have been previously observed.[26] However, after ICH, the ventricle volume decreases in SHRs, possible reflecting increased CSF outflow as an ICP compliance mechanism. This would suggest that compared to SDRs and WKRs, SHRs

have a greater CSF volume, and likely a greater capacity to comply with ICP challenges by redirecting CSF.[40] However, other studies have shown glymphatic clearance is supressed in SHRs compared to WKR rats, which could be due to vascular wall remodelling.[41] Therefore, although there is more CSF to drain in SHRs, the drainage may be less efficient.

We observed a greater mortality rate in SHRs compared to the WKR and SDR groups. This leads to possible attrition confounds when examining behavioural data, as well as other endpoints. For example, although we did not see a significant difference in NDS scores between strains, it is possible that the animals that died had more severe deficits. If that is the case, then not testing these animals may have obscured the true effect of strain on NDS score. Therefore, it is difficult to make conclusions about the functional outcomes in SHRs vs. other strains at the insult severity we used here.

The stereology findings indicate that sham SHRs have decreased cell volume compared to sham SDRs, replicating previous findings of hippocampal and cortical atrophy in SHRs by 4 months of age.[23] Interestingly, the WKR animals have cell volumes in between that of the SDRs and SHRs, which follows the trends observed in overall cortical thickness and indicates neuronal differences between our two control strains. Previous research shows SHRs have increased neurogenesis in the dentate gyrus compared to SDRs,[28] but there are limited studies directly comparing brain morphology (e.g., neuron volumes, size of brain regions) of SHRs, SDRs, and WKRs. Our results suggest that experimental findings in WKRs should not be directly compared with SDRs, as these strains have baseline differences in brain morphology.

There were some notable differences between the strains that could impact ICH experiments. When performing behavioural tests, SHRs exhibited high energy and increased movement compared to SDRs and WKRs. The SHRs tend to be hyperactive and harder to train on certain tasks, such as skilled reaching, in our experience.[42] However, the SHRs were easier to train on tasks that required movement, such as the beam walking component of NDS. Conversely, the WKRs had decreased movement and increased apprehension when training on

the NDS tasks. Some WKRs refused to walk across the beam, or had limited movement in the circling task. These motor differences have been noted previously, and the SHR model has been used as a model of attention-deficit hyperactivity disorder.[43,44] These baseline differences make it difficult to discern how much of the observed "deficits" are due to the ICH and how many are due to inherent strain differences, especially given that many post-ICH functional tests examine motor deficits. Despite the anecdotal differences during behavioural testing, baseline measurements of activity did not differ by group, indicating that these behavioural differences may only appear in certain settings (e.g., stress caused by behavioural testing). Therefore, a sham group is recommended when comparing functional outcomes between strains. Additionally, the weight trajectories differed between strains. The SDRs ate/drank more, and gained weight more quickly than the SHR and WKRs. Therefore, it is important to consider rodent age in experiments, rather than relying on weight as a proxy measure for age.

There are limitations with this study that should be noted. Due to the slight but statistically insignificant differences in hematoma volume by strain, we must compare the extent of tissue compliance with caution. For example, animals with greater injury have larger mass effect (e.g., hematoma and edema volume) and could be undergoing relatively greater tissue compliance to accommodate this added mass. Additionally, in order to determine the upper limit of tissue compliance capabilities in each strain, we would need to use greater hematoma volumes, resulting in greater mortality, which is ethically difficult to complete. In this study, hematoma volume may not be accurate in all animals due to our inability to accurately quantify ventricular bleeding in vibratome sections. Thus, animals with ventricular bleeding likely had a larger hematoma volume in total, but blood was likely lost during the sectioning process. Further, this study only looked at the effect of chronic hypertension in young animals. Future studies should examine the effects of acute hypertension (e.g., pharmacologically induced) and chronic hypertension in aged animals on tissue compliance. Finally, studies examining how

tissue compliance is affected when hypertension is treated would be warranted, however, it is unlikely that acute treatment would reverse the tissue atrophy noted in the SHR strain.[23]

In conclusion, given an equal volume of collagenase, SHRs experience greater mortality but not significantly increased hematoma volume or functional deficits compared to WKRs and SDRs. After a large ICH that would lead to increased intracranial pressure, all strains were able to reduce total brain volume, likely in order to accommodate the bleed. Despite evidence of atrophy in sham SHR animals, they were still able to reduce ventricle volume and contralateral hemisphere volume. This indicates that chronic hypertension does not prevent tissue compliance responses to a large ICP challenge.

5.5 References

1. Sutherland GR, Auer RN. Primary intracerebral hemorrhage. *J Clin Neurosci*. 2006;13(5):511-517. doi:10.1016/j.jocn.2004.12.012
2. Qureshi AI, Tuhrim S, Broderick JP, Batjer HH, Hondo H, Hanley DF. Spontaneous Intracerebral Hemorrhage. *N Engl J Med*. 2001;344(19):1450-1460. doi:10.1056/NEJM200105103441907
3. Kaplan NM, Victor RG. *Kaplan's Clinical Hypertension: Eleventh Edition*.; 2014.
4. Schlunk F, Greenberg SM. The Pathophysiology of Intracerebral Hemorrhage Formation and Expansion. *Transl Stroke Res*. 2015;6(4):257-263. doi:10.1007/s12975-015-0410-1
5. Mirsen T. Acute treatment of hypertensive intracerebral hemorrhage. *Curr Treat Options Neurol*. 2010;12(6):504-517. doi:10.1007/s11940-010-0096-3
6. An SJ, Kim TJ, Yoon BW. Epidemiology, risk factors, and clinical features of intracerebral hemorrhage: An update. *J Stroke*. 2017;19(1):3-10. doi:10.5853/jos.2016.00864
7. Brouwers HB, Chang Y, Falcone GJ, et al. Predicting hematoma expansion after primary intracerebral hemorrhage. *JAMA Neurol*. 2014;71(2):158-164. doi:10.1001/jamaneurol.2013.5433

8. Kroin JS, Nagalla SKS, Buvanendran A, McCarthy RJ, Tuman KJ, Ivankovich AD. The mechanisms of intracranial pressure modulation by epidural blood and other injectates in a postdural puncture rat model. *Anesth Analg*. 2002;95(2):423-429. doi:10.1213/00000539-200208000-00035

9. Ganten U, Rascher W, Lang RE, et al. Development of a new strain of spontaneously hypertensive rats homozygous for hypothalamic diabetes insipidus. *Hypertension*. 1983;5(2):I-119-I-128. doi:10.1161/01.hyp.5.2_pt_2.i119

10. Pravenec M, Křen V, Landa V, et al. Recent progress in the genetics of spontaneously hypertensive rats. *Physiol Res*. 2014;63(SUPPL.). doi:10.33549/physiolres.932622

11. Okamoto K, Aoki K. Development of a strain of spontaneously hypertensive rats. *Jpn Circ J*. 1963;27(3):282-293. doi:10.1253/jcj.27.282

12. Duale H, Waki H, Howorth P, Kasparov S, Teschemacher AG, Paton JFR. Restraining influence of A2 neurons in chronic control of arterial pressure in spontaneously hypertensive rats. *Cardiovasc Res*. 2007;76(1):184-193. doi:10.1016/j.cardiores.2007.06.018

13. Wu G, Bao X, Xi G, Keep RF, Thompson BG, Hua Y. Brain injury after intracerebral hemorrhage in spontaneously hypertensive rats: Laboratory investigation. *J Neurosurg*. 2011;114(6):1805-1811. doi:10.3171/2011.1.JNS101530

14. Nagaoka A, Sudo K, Orita S, Kikuchi K, Aramaki Y. Hematological studies on the spontaneously hypertensive rats with special reference to the development of thrombosis. *Jpn Circ J*. 1971;35(11):1379-1390. doi:10.1253/jcj.35.11_1379

15. Lerman LO, Chade AR, Sica V, Napoli C. Animal models of hypertension: An overview. *J Lab Clin Med*. 2005;146(3):160-173. doi:10.1016/j.lab.2005.05.005

16. Sarikonda K V., Watson RE, Opara OC, DiPette DJ. Experimental animal models of hypertension. *J Am Soc Hypertens*. 2009;3(3):158-165. doi:10.1016/j.jash.2009.02.003

17. Budd JT. Adult secondary hypertension: What the primary care provider needs to know.

Consultant. 2021;61(3):E1-E8. doi:10.25270/con.2020.09.00006

18. Saxena T, Ali AO, Saxena M. Pathophysiology of essential hypertension: an update. *Expert Rev Cardiovasc Ther*. 2018;16(12):879-887. doi:10.1080/14779072.2018.1540301

19. Mokri B. The Monro-Kellie hypothesis: Applications in CSF volume depletion. *Neurology*. 2001;56(12):1746-1748. doi:10.1212/WNL.56.12.1746

20. Wilson MH. Monro-Kellie 2.0: The dynamic vascular and venous pathophysiological components of intracranial pressure. *J Cereb Blood Flow Metab*. 2016;36(8):1338-1350. doi:10.1177/0271678X16648711

21. Williamson MR, Colbourne F. Evidence for Decreased Brain Parenchymal Volume After Large Intracerebral Hemorrhages: a Potential Mechanism Limiting Intracranial Pressure Rises. *Transl Stroke Res*. 2017;8(4):386-396. doi:10.1007/s12975-017-0530-x

22. Kalisvaart ACJ, Wilkinson CM, Gu S, et al. An update to the Monro–Kellie doctrine to reflect tissue compliance after severe ischemic and hemorrhagic stroke. *Sci Rep*. 2020;10(1). doi:10.1038/s41598-020-78880-4

23. Maiër B, Kubis N. Hypertension and Its Impact on Stroke Recovery: From a Vascular to a Parenchymal Overview. *Neural Plast*. 2019;2019. doi:10.1155/2019/6843895

24. Nelson DO, Boulant JA. Altered CNS neuroanatomical organization of spontaneously hypertensive (SHR) rats. *Brain Res*. 1981;226(1-2):119-130. doi:10.1016/0006-8993(81)91087-8

25. Lehr RP, Browning RA, Myers JH. Gross morphological brain differences between wistar-Kyoto and spontaneously hypertensive rats. *Clin Exp Hypertens*. 1980;2(1):123-127. doi:10.3109/10641968009038555

26. Tajima A, Hans FJ, Livingstone D, et al. Smaller local brain volumes and cerebral atrophy in spontaneously hypertensive rats. *Hypertension*. 1993;21(1):105-111. doi:10.1161/01.HYP.21.1.105

27. Sabbatini M, Strocchi P, Vitaioli L, Amenta F. The hippocampus in spontaneously hypertensive rats: A quantitative microanatomical study. *Neuroscience*. 2000;100(2):251-258. doi:10.1016/S0306-4522(00)00297-9

28. Hwang IK, Yoon YS, Choi JH, et al. Doublecortin-immunoreactive neuronal precursors in the dentate gyrus of spontaneously hypertensive rats at various age stages: Comparison with Sprague-Dawley rats. *J Vet Med Sci*. 2008;70(4):373-377. doi:10.1292/jvms.70.373

29. Kurtz TW, Curtis Morris R. Biological variability in wistar-kyoto rats implications for research with the spontaneously hypertensive rat. *Hypertension*. 1987;10(1):127-131. doi:10.1161/01.HYP.10.1.127

30. Rosenberg G a., Mun-Bryce S, Wesley M, Kornfeld M. Collagenase-induced intracerebral hemorrhage in rats. *Stroke*. 1990;21(5):801-807. doi:10.1161/01.STR.21.5.801

31. MacLellan CL, Silasi G, Poon CC, et al. Intracerebral hemorrhage models in rat: comparing collagenase to blood infusion. *J Cereb Blood Flow Metab*. 2008;28:516-525. doi:10.1038/sj.jcbfm.9600548

32. Wilkinson CM, Kalisvaart ACJ, Kung TFC, et al. The collagenase model of intracerebral hemorrhage in awake, freely moving animals: The effects of isoflurane. *Brain Res*. 2020;1728:146593. doi:10.1016/j.brainres.2019.146593

33. Sotocinal SG, Sorge RE, Zaloum A, et al. The Rat Grimace Scale: A partially automated method for quantifying pain in the laboratory rat via facial expressions. *Mol Pain*. 2011;7. doi:10.1186/1744-8069-7-55

34. Colbourne F, Auer RN, Sutherland GR. Characterization of postischemic behavioral deficits in gerbils with and without hypothermic neuroprotection. *Brain Res*. 1998;803(1-2):69-78. doi:10.1016/S0006-8993(98)00612-X

35. Wilkinson CM, Fedor BA, Aziz JR, et al. Failure of bumetanide to improve outcome after intracerebral hemorrhage in rat. *PLoS One*. 2019;14(1):1-20. doi:10.1371/journal.pone.0210660

36. Paxinos G, Watson C. Index of Structures. *Rat Brain Stereotaxic Coord*. Published online 1982:7-9. doi:10.1016/b978-0-12-547620-1.50008-4

37. MacLellan CL, Auriat AM, McGie SC, et al. Gauging recovery after hemorrhagic stroke in rats: Implications for cytoprotection studies. *J Cereb Blood Flow Metab*. 2006;26(8):1031-1042. doi:10.1038/sj.jcbfm.9600255

38. Napper RMA. Total number is important: Using the disector method in design-based stereology to understand the structure of the rodent brain. *Front Neuroanat*. 2018;12. doi:10.3389/fnana.2018.00016

39. Liddle LJ, Dirks CA, Almekhlafi M, Colbourne F. An Ambiguous Role for Fever in Worsening Outcome After Intracerebral Hemorrhage. *Transl Stroke Res*. 2022;(0123456789). doi:10.1007/s12975-022-01010-x

40. Bedussi B, Naessens DMP, De Vos J, et al. Enhanced interstitial fluid drainage in the hippocampus of spontaneously hypertensive rats. *Sci Rep*. 2017;7(1). doi:10.1038/s41598-017-00861-x

41. Mortensen KN, Sanggaard S, Mestre H, et al. Impaired glymphatic transport in spontaneously hypertensive rats. *J Neurosci*. 2019;39(32):6365-6377. doi:10.1523/JNEUROSCI.1974-18.2019

42. Colbourne F, Corbett D, Zhao Z, Yang J, Buchan AM. Prolonged but delayed postischemic hypothermia: A long-term outcome study in the rat middle cerebral artery occlusion model. *J Cereb Blood Flow Metab*. 2000;20(12):1702-1708. doi:10.1097/00004647-200012000-00009

43. Fasmer OB, Johansen EB. Patterns of motor activity in spontaneously hypertensive rats compared to Wistar Kyoto rats. *Behav Brain Funct*. 2016;12(1). doi:10.1186/s12993-016-0117-9

44. Kim P, Choi I, dela Pena IC, et al. A simple behavioral paradigm to measure impulsive behavior in an animal model of attention deficit hyperactivity disorder (ADHD) of the

spontaneously hypertensive rats. *Biomol Ther*. 2012;20(1):125-131. doi:10.4062/biomolther.2012.20.1.125

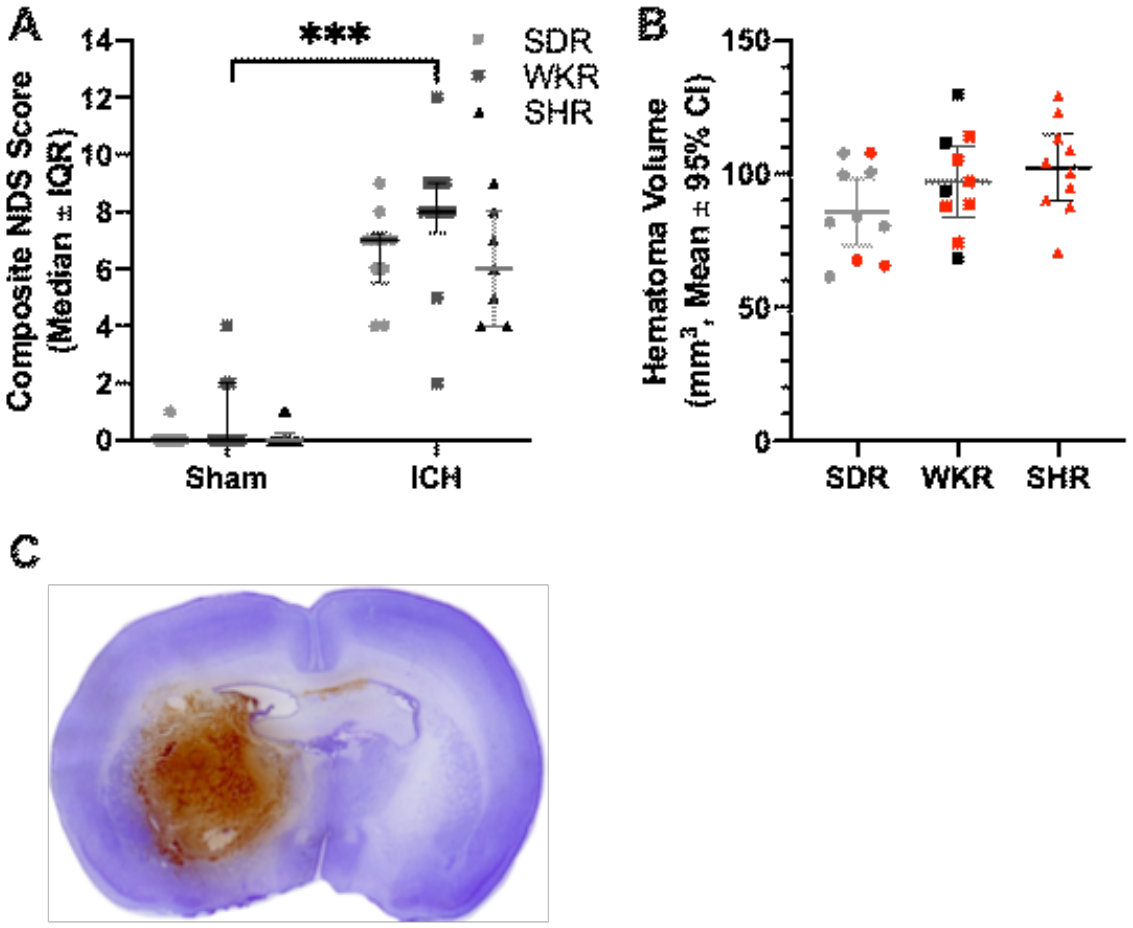

Figure 5-1. A. Neurological deficits were increased in the ICH groups compared to the sham groups ($p < 0.001$), however, there were no significant strain differences in NDS score ($p = 0.417$ between strains in the sham groups and $p = 0.095$ between strains in the ICH groups). B. Hematoma volume did not significantly differ between strains ($p = 0.122$). However, rate of ventricular extension of the bleed did differ between groups ($p = 0.005$), denoted by red symbols. C. Representative image of an SHR animal with a hematoma volume of 100.4 mm^3. *** denotes $p < 0.001$.

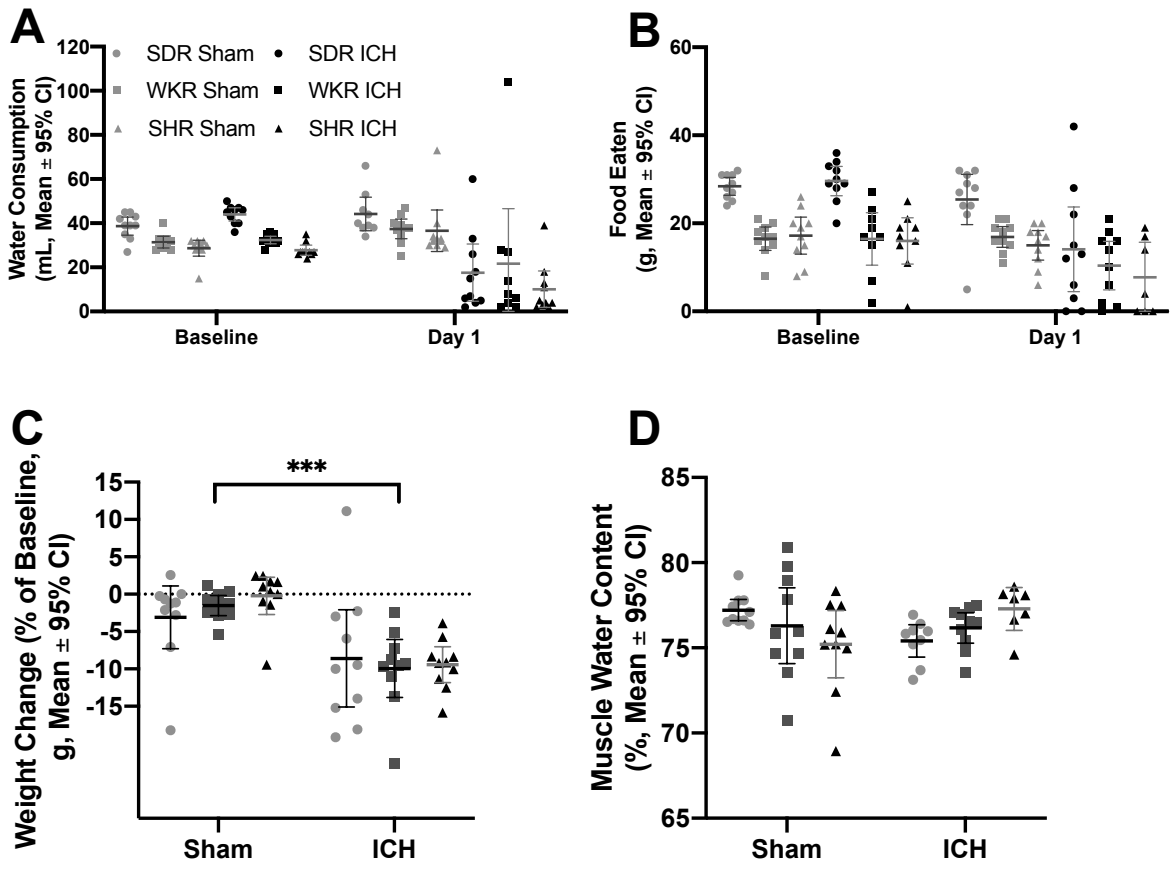

Figure 5-2. A. Water consumption decreased after ICH in all strains (ICH by time interaction effect p = 0.001). B. All ICH animals had a decrease in feeding behaviour post ICH (ICH by time interaction effect p < 0.001). Additionally, the SDRs ate more than the other strains in both the sham and the ICH group (p < 0.001). C. Animals in the ICH group experienced significantly more weight loss than the sham animals, and this effect was consistent across strains (ICH main effect p < 0.001, interaction effect p = 0.521). D. There was a significant interaction between group and strain on muscle water content (interaction effect p = 0.021), however, there were no significant strain differences within the ICH and sham groups. *** denotes p < 0.001.

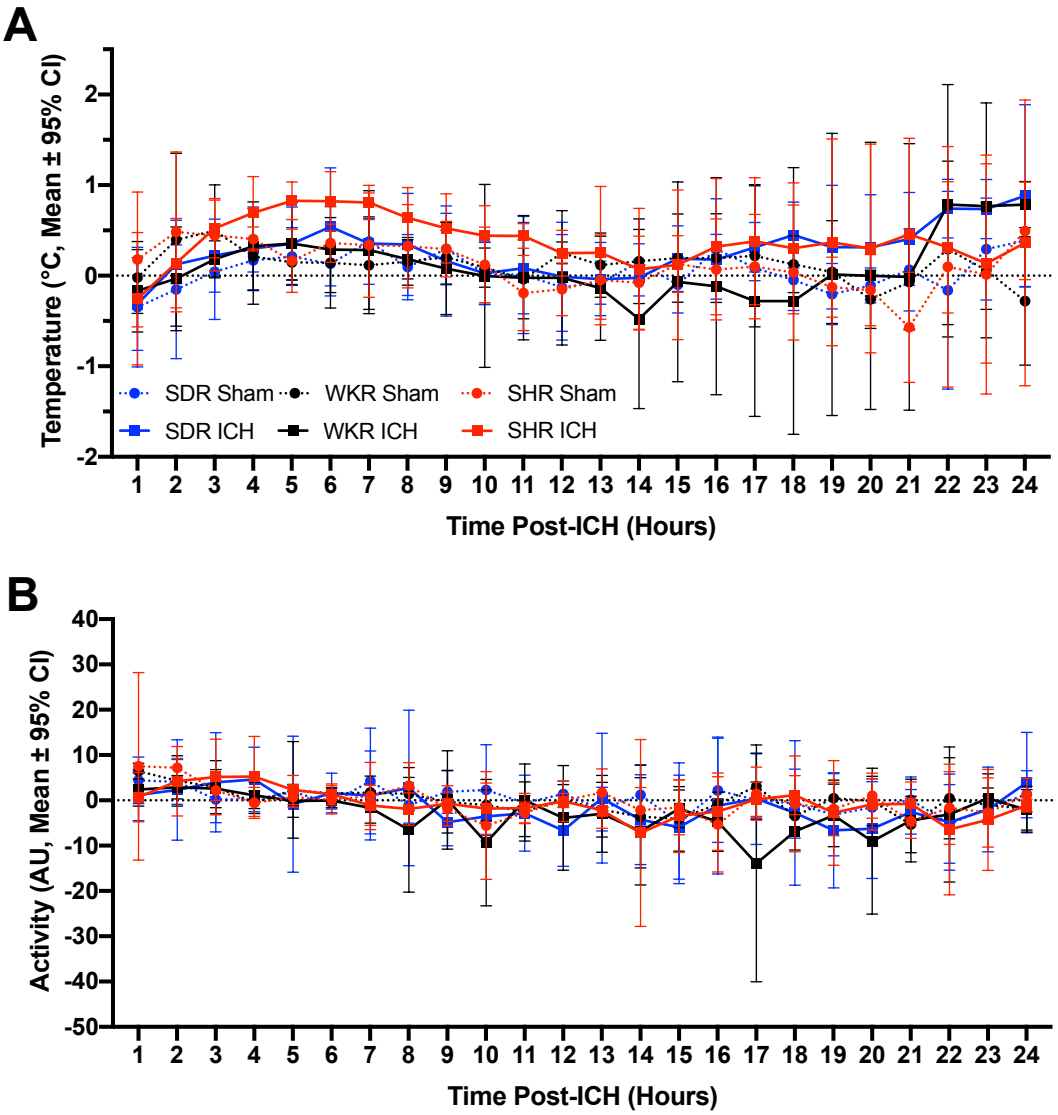

Figure 5-3. There was a significant main effect of time on temperature and activity (time main effect $p < 0.001$, 3-way interaction term $p = 0.122$). There was a significant main effect of ICH on temperature, indicative of a mild fever in these groups (ICH main effect $p = 0.011$). There was no effect of strain on temperature or activity levels (strain main effect $p = 0.327$ and $p = 0.217$, respectively). Temperature and activity measurements were averaged hourly and subtracted from hourly baseline measurements to correct for circadian effects. AU = arbitrary units, measured variations in signal strength as the probe moves over the receiver.[34] Both

temperature and activity are corrected for baseline hourly to account for circadian rhythm effects.

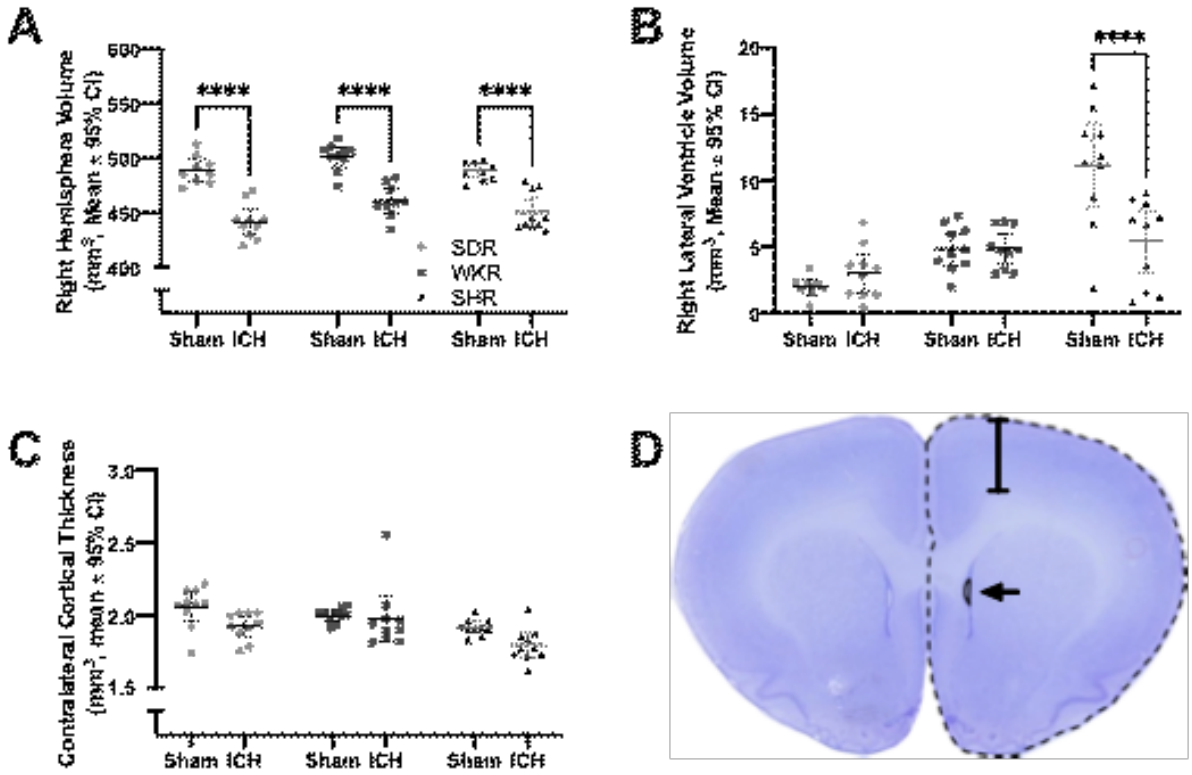

Figure 5-4. A. Contralateral hemisphere volume was reduced in animals in the ICH group regardless of strain (ICH main effect $p < 0.001$, interaction main effect $p = 0.551$). There was also a significant effect of strain on contralateral hemisphere volume (strain main effect $p = 0.008$). B. The volume of the contralateral (right) lateral ventricle was significantly increased in the sham SHR group (interaction effect $p < 0.001$). C. Cortical thickness varied significantly by strain (strain main effect $p = 0.001$, interaction effect $p = 0.300$), and was also decreased in the ICH group compared to the sham group (ICH main effect $p = 0.006$). D. Representative image of a brain from the sham WKR group. Dotted lines represent the contralateral hemisphere measurement, arrow and solid line denote the ventricle measurement, and the capped line represents the cortical thickness. **** denotes $p < 0.0001$.

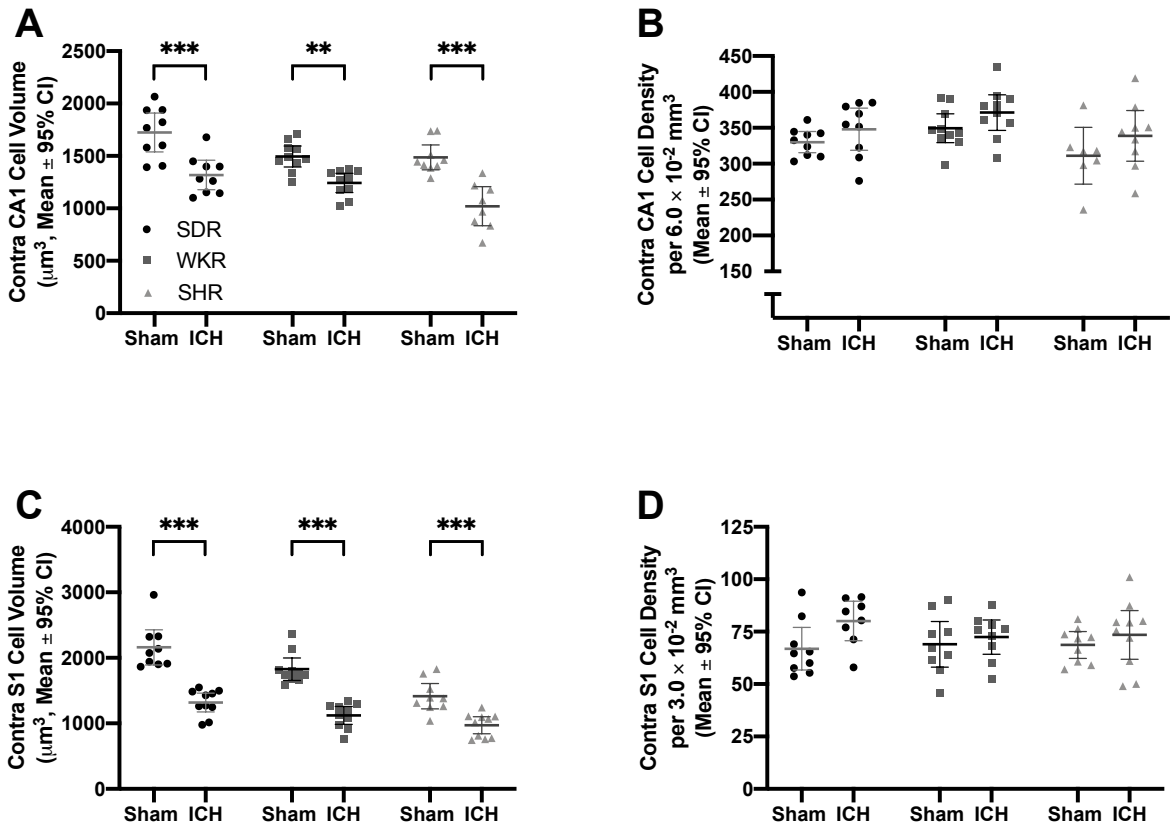

Figure 5-5. There was a significant effect of ICH on contralateral CA1 cell volume across all strains (A, ICH main effect $p < 0.001$, interaction effect $p = 0.181$), and there was an effect of strain on CA1 volume (strain main effect $p < 0.001$), where sham SDRs had the largest cell volume. Animals in the ICH groups had significantly higher cell densities than animals in the sham groups (B, ICH main effect $p = 0.025$, interaction effect $p = 0.924$). Cell volume in contralateral S1 was significantly decreased in ICH animals compared to sham animals, and this effect was smallest in the SHRs (C, ICH main effect $p < 0.001$, interaction effect $p = 0.042$). There was a significant effect of ICH on cell density in S1 (D, ICH main effect $p = 0.044$, interaction effect $p = 0.475$). ** denotes $p < 0.01$, *** denotes $p < 0.001$.

Chapter 6: Tissue Compliance and Intracranial Pressure Responses to Large Intracerebral Hemorrhage in Young and Aged Hypertensive Rats.

6.1 Introduction

Intracerebral hemorrhage (ICH) is a subtype of stroke characterized by bleeding within the parenchyma. Primary ICH is a hemorrhage caused by hypertension or amyloid angiopathy, both of which are increasingly common with advanced age.[1,2] Indeed, age is the most important risk factor for ICH,[3] as about 35% of ICH patients are older than 80 years old.[4] The incidence rate of ICH increases by approximately 30 times in those aged 75-94 compared to individuals aged 35-54 years.[4] Despite this, ~95% of preclinical ICH studies use young, healthy rodents to represent this patient population.[5]

In cases of large hemorrhage, the mass effect of the hematoma takes up space in the skull and drives up intracranial pressure (ICP).[6] Increased ICP can lead to decreased consciousness, herniation, and in some cases, death.[6,7] Intracranial pressure monitoring is not standard after ICH, so there is limited data on how ICP differs in different patient populations.[8,9] However, mean ICP tends to decrease with age.[10] For instance, in people, ICP decreases 0.7 mmHg per decade on average.[10] In rats, mean ICP is typically ~5 mmHg, which can vary widely among studies depending on methodology.[6] Aged spontaneously hypertensive rats (SHRs) exhibited a 40% decrease in mean ICP compared to young SHRs.[11] This ICP decrease could be due to age-related changes in ICP regulation mechanisms (e.g., vascular changes affecting filtration rate), or it could be related to brain atrophy (decrease in number of neurons,[12] neuron volume,[13] dendritic arborization,[14] etc.) that is seen with normal aging. It is unclear if ICP is decreased in older patients after ICH, as there is little research on how age impacts ICP responses after stroke.[6,15] For example, if older patients with brain atrophy had relatively larger amounts of CSF, they may be able to accommodate an ICP challenge more easily. Indeed, evidence has suggested that there is more CSF and larger ventricle volumes in patients with more extensive atrophy.[16,17] Conversely, if their ICP regulation mechanisms were affected by age-related vascular changes, then they may have a more difficult time responding to ICP challenges. Additionally, many ICH patients are also hypertensive. This may contribute to impairments in

ICP regulation. After head trauma, increased age was associated with lower ICP, and this was related to deteriorations in cerebral blood flow (CBF) autoregulation and pressure reactivity.[18,19] Therefore, although it appears aged animals may have reduced ICP regulation with age, lower baseline ICP and brain atrophy may both result in lower ICP after a mass effect-inducing injury such as ICH. Additionally, it is important to think beyond just mean ICP and consider the entire ICP waveform.[6] For example, younger animals may be able to compensate for larger ICP spikes than aged animals.

When ICP is drastically increased, compliance mechanisms act to reduce ICP and prevent ischemic damage and brain herniation.[6] According to the centuries-old Monro-Kellie Doctrine, blood and CSF volumes will decrease (e.g., reduced production and increased CSF drainage) in order to accommodate a mass. Recently, we have shown that neurons and astrocytes also participate in ICP compliance.[20,21] Neurons will reduce their volume (e.g., by up to 50%) and increase packing density, resulting in a reduced brain volume overall. This effect has been documented in young, healthy animals[20,21] as well as chronically hypertensive rats (Chapter 5). However, it is unclear how this tissue compliance may change with age. If ICP is lower in aged animals, they may have less need for tissue compliance as they can accommodate ICP by simply reducing CSF and blood. Conversely, aged animals may be unable to rapidly decrease cell volumes if the mechanism to do so loses efficacy or efficiency over time.

Despite the vast majority of stroke patients having one or more comorbid conditions, such as diabetes and hypertension, most preclinical stroke studies use young, healthy animals.[22,23] For example, approximately 77% of patients experiencing their first stroke had hypertension (either treated or untreated).[23] A recent systematic review found that only 3.1% of ICH neuroprotection studies used animals older than 12 months, and only 5% used animals with comorbid conditions.[5] Of the studies that use hypertensive animals, the SHR model is the most common (see Chapter 5 for further information on models of hypertension). To our knowledge, no study has assessed collagenase-induced intracerebral hemorrhage in aged spontaneously

hypertensive rats. One study measured ICP in aged SHRs, but these animals did not undergo any model of brain injury.[11] Another study assessed brain injury using the collagenase and whole blood models in SHRs, but these animals were only 12 weeks of age.[24]

In this study, we compared ICH injury and ICP responses in aged (20–24-month-old) and young-adult SHRs using the collagenase model. Our primary objective was to compare tissue compliance and ICP responses in young vs. aged SHRs, and our secondary objective was to compare the course of injury (functional deficits, edema). We used the collagenase model of ICH as it is associated with substantial edema and a larger ICP response when compared to the autologous whole blood model and leads to a larger ICP response,[6,25] which makes this model ideal for assessing tissue compliance. In our first experiment, we measured hematoma volume, neurological deficits, and tissue compliance after collagenase-induced ICH or a sham surgery in aged and young SHRs. In our second experiment, we compared neurological deficits, ICP responses, and edema between aged and young SHRs.

6.2 Methods

6.2.1 Subjects

In total, 88 animals were used in these experiments, including 44 young SHRs (265-341 g) and 44 aged SHRs (342-415 g). The rats in the young SHR group were aged 3-4 months, while rats in the aged SHR group were aged 20-24 months. Animals were housed in a temperature- and humidity-controlled room with a 12-hour light cycle. Experiments were done during the light cycle. All experiments and procedures were approved by the Biosciences Animal Care and Use Committee at the University of Alberta and were in accordance with the Canadian Council on Animal Care guidelines.

6.2.2 Surgical Procedures

Rats were anesthetized using isoflurane (4% induction, 2-2.5% maintenance, in 60% N_2O and remainder O_2) with temperature maintained at 37°C using a heating pad and rectal thermocouple probe. Intracerebral hemorrhage was induced as described previously.[26,27] Briefly, 2.1 μL of bacterial collagenase (Type IV-S, Sigma, 0.6 U/μL in saline) was infused 0.5 mm anterior, 3.5 mm lateral, and 6.5 mm down from Bregma.[28] Collagenase was infused into the left striatum over 5 mins, and the needle was withdrawn after an additional 5 mins to prevent backflow. For the sham procedure, animals had the burr hole drilled and were kept under isoflurane for an equivalent amount of time, but had no collagenase injected. The burr hole was sealed with a screw and the incision was closed using staples.

For experiment 2, ICP probes were implanted immediately after ICH induction.[20,29] Animals had four metal anchoring screws inserted into the skull surrounding the injection site. The catheter of a PA-C10 probe (Data Sciences Int., St. Paul, MN) was inserted into the injection site and glued in place using vet bond (n-butyl cyanoacrylate, 3M, Saint Paul, Minnesota). The probe was placed on the skull encased in plastic tubing, which was kept in place using dental cement (Jet Denture Repair Fast Curing Acrylic Resin, Lang Dental Mfg. Co., Wheeling, IL).

In both experiments, bupivacaine hydrochloride (0.5 mg/kg SC, SteriMax, Oakville, ON) was used as a post-operative analgesic at the end of surgery and animals were injected with 5 mL of 0.9% saline (SC) once at the end of surgery to ensure hydration.

6.2.3 Neurological Deficit Assessment

Neurological deficits were scored at 23 hrs post-ICH in experiment 1 and 71 hrs post-ICH in experiment 2. Animals were scored on five subtests: spontaneous circling, forelimb flexion, beam-walking, bilateral forelimb grasp, and contralateral hindlimb retraction, for a total score ranging from 0-14 where 0 indicates no deficits.[30]

6.2.4 Euthanasia and Histology

For experiment 1, at 24 hrs after ICH, rats were anesthetized with sodium pentobarbital (100 mg/kg IP, Bimeda, Cambridge, ON) and transcardially perfused with 0.9% saline and 10% neutral-buffered formalin.[21] Brains were coronally sectioned at 80 μm using a vibratome (Leica VT1000S, Leica Biosystems, Concord, ON). Sections were stained using cresyl violet. Hematoma volume was calculated as: average area of damage × interval between sections × number of sections.[31] Total contralateral hemisphere volume was assessed from 3.9 mm anterior to 5 mm posterior to Bregma.[28] Contralateral lateral ventricle volume was calculated as: average area of ventricle × interval between sections × number of sections containing lateral ventricle.

Neuron soma volume and neuronal density were quantified in the CA1 region of the hippocampus and the S1 region in the contralateral hemisphere as both are regions known to be involved in tissue compliance[21] and would be distal to the hematoma. The nucleator probe method in conjunction with the physical dissector were used to quantify cell volume, and the physical and optical dissector probes were used to assess cell density, as done previously.[21,32]

6.2.5 Brain Water Content Determination

For experiment 2, animals were anesthetized with isoflurane, as described above, at 72 hrs post-ICH. A blood sample was taken from the femoral artery and analyzed for blood gases and electrolytes (ABL80, Radiometer Canada). Animals were immediately decapitated and brains were extracted and dissected using a brain matrix. A 6 mm section of brain was taken from 2 mm anterior to 4 mm posterior to the injection site. This section was divided into ipsilateral and contralateral hemisphere. Brains were dried for 24 hrs at 100°C, and brain water content was determined using the wet weight-dry weight method.[26]

6.2.6 Statistical Analysis

Power analyses were calculated using GPower (v 3.1.9.6). A sample size of 9 per group gave us 80% power to detect an 35% percent change in cell volume, based on a standard deviation of 443 μm^3 and a mean cell volume of 1791 μm^3 in SHRs (pilot data). This was increased to 12/group to mitigate data loss due to mortality or exclusions. For experiment 2, we used a sample of 10/group in order to have 80% power to detect a 1% increase or decrease in edema. Data were analyzed using a two-way ANOVA, with age and injury as the independent variables. Data assessed at multiple times were assessed using a three-way ANOVA, with time as a within-subjects variable. Neurological deficit scores were analyzed using a Kruskal-Wallis test, as there is no nonparametric equivalent to a two-way ANOVA. Anaconda Spyder3 (v2021.05, Anaconda Inc, Austin, TX) was used to process ICP data, and GraphPad Prism (v9.0, San Diego, California) was used to analyze and graph all other data. Data are presented as mean $\pm$ 95% confidence interval, with the exception of NDS, which is presented as median $\pm$ interquartile range. All p-values less than 0.05 were considered statistically significant.

6.3 Results

6.3.1 Exclusions and Mortality

In experiment 1, one animal died prematurely, from the young ICH group. An additional 4 aged sham animals were excluded from S1 analysis due to histological processing error. For the CA1 analysis, 1 young sham, 1 aged sham, and 4 aged ICH animals were excluded from stereological analysis due to a histological processing error.

In experiment 2, 5 animals died prematurely, all from the young ICH group (Chi squared $p = 0.001$). On average, these animals died ~6 hrs after ICH, thus, initial ICP data was included for analysis of mean and peak hourly ICP, but all other endpoints were not collected.

6.3.2 Experiment 1

6.3.2.1 Neurological Deficits

There was a significant effect of group on neurological deficits (Figure 6-1A, $p < 0.001$). However, these differences were between the sham and ICH groups, and there was no significant difference between the aged ICH and young ICH group ($p > 0.999$) or the aged sham and young sham group ($p > 0.999$). The young sham animals all scored 0 on NDS (no deficits) whereas 7/10 aged sham animals scored 1 or more, indicating that there were minor deficits present, but we were not powered to detect these differences. Deficits present in the aged sham group were largely on beam walking (minor slips), potentially indicating decreased coordination.[33]

6.3.2.2 Histology

The hematoma volume in the young ICH group was significantly larger than in the aged ICH group (Figure 6-1B, $p = 0.002$), despite using the same amount of collagenase. Despite these differences, both ICH groups displayed a similar decrease in contralateral brain hemisphere volume (Figure 6-1C, interaction effect $p = 0.523$, ICH main effect $p = 0.003$, age main effect $p = 0.145$). There was an effect of age on ventricle volume, with aged animals having increased lateral ventricle volume (Figure 6-1D, interaction effect $p = 0.390$, ICH main effect $p = 0.116$, age main effect $p = 0.002$).

6.3.2.3 Stereology

In contralateral CA1, we found a significant effect of ICH on cell volume (Figure 6-2A, ICH main effect $p < 0.001$, interaction effect $p = 0.652$), where CA1 volume decreased after ICH compared to sham. There was no effect of age on CA1 volume (age main effect $p = 0.173$). After ICH, animals had significantly increased neuronal density in CA1 (ICH main effect $p <$

0.001, interaction effect p = 0.642). Aged animals had slightly lower neuronal density than young animals, but this was not statistically significant (age main effect p = 0.064).

In contralateral S1, we similarly found the ICH groups had decreased cell volume compared to sham groups (Figure 6-2C, ICH main effect p = 0.001, interaction effect p = 0.176). There was a significant effect of age on cell volume, with aged SHRs having decreased cell volume compared to young SHRs, regardless of injury type (age main effect p = 0.013). There was no effect of ICH on neuronal density in S1 (Figure 6-2D, ICH main effect p = 0.291, interaction effect p = 0.232). The aged animals had significantly greater cell density in S1 than the young animals (age main effect p = 0.032).

6.3.3 Experiment 2

6.3.3.1 Neurological Deficits

There was a significant difference between post-ICH NDS scores (Figure 6-3A, p < 0.001). However, there were no significant differences between young and aged ICH or young and aged sham (all p > 0.999). Pooled NDS scores from experiments 1 and 2 (to afford more statistical power) still found no significant differences in deficits between aged and young animals (p = 0.539).

6.3.3.2 Intracranial Pressure

The surviving young ICH rats displayed greater hourly mean ICP than the aged ICH rats (Figure 6-3B, ICH by age by time interaction p < 0.001). Both ICH groups had significantly increased ICP compared to the sham groups (ICH effect p < 0.001). There were no significant differences in hourly mean ICP between the two sham groups (all p > 0.05). Peak ICP was significantly increased in the ICH groups compared to the sham groups (ICH main effect p < 0.001, interaction effect p = 0.260), however, there were no significant effects of age on peak ICP (age main effect p = 0.455).

6.3.3.3 Blood Gases

Sodium was significantly increased in ICH animals regardless of age (Figure 6-4A, age main effect $p = 0.786$, interaction effect $p = 0.204$, ICH main effect $p = 0.012$). Sodium levels increased as functional deficits increased, but this was not statistically significant ($p = 0.070$, $R^2 = 0.230$). There were no group differences in blood oxygen or blood carbon dioxide (Figure 6-4B-C, all $p > 0.141$).

6.3.3.4 Edema

There was a significant interaction of region and group on edema (Figure 6-5, $p < 0.001$), as well as a significant region main effect ($p < 0.001$), age main effect ($p = 0.005$), and ICH main effect ($p < 0.001$). The aged animals had reduced brain water content when compared to the young animals in their respective groups regardless of region, indicating age-related decreases in brain water content. When correcting for contralateral brain water content (e.g., ipsilateral – contralateral) to control for pre-existing water content differences, we see a significant effect of ICH (ICH main effect $p < 0.001$, interaction effect $p = 0.906$) with a statistically non-significant trend towards higher brain water content in young animals (age main effect $p = 0.052$).

6.4 Discussion

In this study, we found evidence of tissue compliance in both aged and young SHRs after collagenase-induced ICH. After ICH, animals had increased neuronal density in CA1, decreased neuron volumes in CA1 and S1, and overall brain volume was decreased. Despite significantly reduced hematoma volumes in aged animals, we did not see any differences in functional outcomes at 24- or 72-hours post-ICH. Both mean ICP and edema were lower in

aged animals than young animals after ICH. Additionally, aged SHRs had a significantly lower mortality rate than young SHRs. Aged animals had, comparatively, as severe outcomes (edema and functional deficits) despite the fact that their hematoma volume was considerably smaller. The smaller mass effect in aged rats still raised ICP, although to a lesser extent, and resulted in a comparable tissue compliance response. Despite the confound caused by differing hematoma volumes, our results show that aged animals still demonstrate considerable tissue compliance, and likely their outcomes would have been far worse had hematoma volumes been the same as the younger SHRs.

Despite having reduced hematoma volumes and edema (e.g., less mass effect), likely contributing to reduced ICP, the aged SHR group still exhibited decreases in contralateral brain volume compared to the sham group, indicating a tissue compliance response. For example, the young SHRs had an average hematoma volume of 129.15 μL and an average contralateral hemisphere volume decrease of 16.91 mm^3 compared to sham, which is a decrease of 0.131 μL/μL of hematoma. The aged SHRs had an average hematoma volume of 67.22 μL and an average contralateral hemisphere volume decrease of 25.48 mm^3 compared to sham, giving them a decrease of 0.379 μL/μL of hematoma, nearly triple that of the young SHRs. Therefore, although age-related atrophy may theoretically reduce the need for tissue compliance, age-related changes do not prevent animals from being able to reduce tissue volume in order to accommodate mass effect. Additionally, our stereological data provides further evidence of tissue compliance. We see that the neurons in CA1 reduce volume and pack closer together, and this effect is consistent in both aged and young animals. In S1, we see a reduction in neuron volume after ICH, but did not see an effect on neuron density. This may be due to increased variability in this region, as seen previously,[21] or it is possible that cells have reduced volume but haven't packed closer together in this region yet. For example, if SHRs are slower at

clearing cellular contents from the extracellular space, this may lead to delays in packing density changes in some cases, and a reduced effect size at 24 hrs as a result.

There are physiological changes across the lifespan that can impact stroke outcomes and are important to study. Intracranial pressure typically decreases with age in people,[10] yet here we found that in sham animals, ICP was consistent between the young and aged hypertensive animals. Although blood pressure increases with age in humans,[34] the evidence in SHRs is not clear. In people, blood pressure increases are partially attributable to lifestyle factors, including high sodium diets and reduced physical activity over the lifespan.[34] In this study, although animals were fed *ad libitum* and were sedentary, they were fed a standard laboratory animal diet and were consistently sedentary through their lifespan. Blood pressure in SHRs remains constant from 15-60 weeks of age,[35,36] and beyond 60 weeks is unknown. Brain water content decreases with age in both rats and people, which can reflect changes in myelination and aquaporin channels (particularly during development).[37–39] After correcting for baseline differences in water content, studies have found aged animals to have worse edema post-ICH than younger animals.[40] Unlike our study, that study used the autologous whole blood model, and had a consistent hematoma volume between the groups. However, clinically, results are less clear, with age not consistently predicting edema.[41,42] Brain volume is also known to decrease with age, as individuals experience neuronal atrophy.[17] In SHRs specifically, atrophy is noted from a much younger age (~6 months old).[43,44] Here, we did not see an age effect on contralateral hemisphere volume. This may be due to earlier cortical atrophy in SHRs, and future studies should compare aged and young SHRs to aged and young Sprague Dawley rats to quantify cortical atrophy. Therefore, there are many ways by which age-related physiological changes can affect the outcome after ICH, and it is important to use aged animal models when possible.

To our knowledge, this is both the first study measuring ICP in aged SHRs after ICH as well as the first study comparing young and aged SHRs after collagenase-induced ICH. A

previous study assessed the collagenase model of ICH in SHRs, but these animals were 12 weeks old.[24] In addition, it is unclear if the ICH size would have been large enough to cause substantial increases in ICP. As studies measuring ICP after ICH are limited,[6] it is important to use a clinically relevant animal model to help guide future clinical studies. As demonstrated here, aged animals have significantly different ICP responses than young animals to a large hemorrhage, likely due to the differing amounts of mass effect. The young animals had an ICP increase of 19.5 mmHg relative to sham animals after a hematoma volume of ~129.2mm^3 (0.15 $mmHg/mm^3$), whereas aged animals had an increase of 15.1 mmHg relative to sham animals after a hematoma volume of ~67.2 mm^3 (0.22 $mmHg/mm^3$). Although this doesn't take total mass effect into account, it does indicate that the aged animals had a higher ICP response relative to bleed volume.

Since age is the most important risk factor for ICH, it is important to know how outcomes may differ with age. Yet, the majority of research still relies on young, healthy animals,[5] even when investigating basic physiological responses.[45] The lifespan of SHRs has been documented as between 1.5-1.8 years on average, which is shorter than the 2.5-3 year lifespan of Wistar Kyoto rats.[46] Therefore, the rats used in this study can be considered elderly, at 1.5-2 years of age. However, the difficulty and expense of working with aged animals should not be minimized. Most vendors do not carry aged animals, and therefore, researchers need to plan these experiments years in advance. The additional cost of specific strains as well as the logistics and cost of housing animals for years needs to be taken into consideration. An advantage of working with aged SHRs vs. aged Sprague Dawley rats are that the SHRs maintain a consistent body weight (~350 g) across the lifespan, and therefore, are readily handled and can easily fit into behavioural apparatuses at most ages. This is contrasted with Sprague Dawley rats, which reach an average weight of 700 g within 6 months.[47]

Our blood sodium data indicates that some animals were hypernatremic (blood sodium levels above 145 mEq/L, normal range = 135-145 mEq/L) after ICH. Hypernatremia occurs in

approximately 12% of ICH patients and is associated with mortality and worse outcomes.[48,49] The sodium levels in this study could be related to the severity of ICH, and there was a trend for rodents with higher sodium to have a higher NDS score. Alternatively, the high blood sodium levels could induce changes in cellular volume (e.g., osmotic volume decreases)[50,51]. However, the fact that we see normal water content in the contralateral hemisphere suggests that the uninjured tissue, despite having reduced volumes, is osmotically "normal." Additionally, we see cell volume reductions up to 7 days post-ICH, at which time feeding and drinking have returned to pre-ICH levels.[20,21] The more likely explanation may be that cells are osmotically reducing their volume when undergoing tissue compliance, and this leads to the extrusion of sodium, along with other osmolytes (e.g., K^+, taurine), detected as higher sodium levels in the blood. This may be normalized quickly in young, healthy animals (where pilot work indicates normal sodium levels at 24 hrs post-ICH), but could pose a problem for hypertensive rats, known to have kidney abnormalities.[52] Of note, some of our sham SHRs were also in the hypernatremic range. As our data is correlational, future studies should investigate the effect of hypernatremia on tissue compliance, either by inducing hypernatremia (e.g., reduced drinking water) or by treating hypernatremia (e.g., with 5% dextrose or hydration).[53]

A major limitation in this study is the difference in hematoma volume, leading to differences in injury and edema progression. Here, we wanted to see how outcomes would differ between ages when given the same insult. However, the same amount of collagenase resulted in a different bleed volume. Although this needs to be taken into account when interpreting the results, these experiments still provide insight into the age differences when subjected to the same amount of collagenase, and presumably, the same amount of initial basal lamina damage. Similarly, another limitation in this study is the mortality difference. This affects how we can interpret the NDS scores. For example, the animals that died presumably had more severe neurological deficits, so in this case, the median score of the young ICH animals is not reflective of where the median would be if all animals were able to be tested. If this were the

case, we may have seen a difference between the young ICH and aged ICH groups, where the aged ICH groups may have had significantly fewer neurological deficits. If the aged SHRs had a hematoma volume that matched that of the young SHR group, we expect mortality would have been far higher, and thus, future studies should consider using a relatively smaller hematoma volume (e.g., 75 vs. 100 mm^3). Finally, this study focused on age differences within hypertensive animals. Without a normotensive or treated hypertensive control group, we are unable to directly assess the effects of hypertension vs. the effects of age in these experiments, and how hypertension and age may interact. For example, if hypertension was treated (e.g., with a diuretic or angiotensin converting enzyme inhibitor),[54] this may have impacted hematoma volume and resulting edema and ICP values. However, previous experiments (Chapter 5) have examined young hypertensive vs. normotensive animals, which can give insights into the effects of hypertension on ICP and tissue compliance.

In conclusion, we provide clear evidence of tissue compliance in aged, hypertensive animals. However, even with a more modest ICP response and a smaller bleed volume, aged animals still had an equally poor neurological outcome. Future studies should investigate ICH outcomes in young vs. aged SHRs where the injury volume is consistent between groups. This study highlights the importance of using aged animals as well as animals with comorbidities in ICH research rather than relying on young, healthy animals, as the effects of age and comorbidities on various endpoints are not always intuitive.

6.5 References

1. Kearney PM, Whelton M, Reynolds K, Whelton PK, He J. Worldwide prevalence of hypertension: A systematic review. *J Hypertens*. 2004;22(1):11-19. doi:10.1097/00004872-200401000-00003
2. Keage HAD, Carare RO, Friedland RP, et al. Population studies of sporadic cerebral amyloid angiopathy and dementia: A systematic review. *BMC Neurol*. 2009;9. doi:10.1186/1471-2377-9-3
3. Camacho E, Lopresti MA, Bruce S, et al. The role of age in intracerebral hemorrhages. *J Clin Neurosci*. 2015;22(12):1867-1870. doi:10.1016/j.jocn.2015.04.020
4. An SJ, Kim TJ, Yoon BW. Epidemiology, risk factors, and clinical features of intracerebral hemorrhage: An update. *J Stroke*. 2017;19(1):3-10. doi:10.5853/jos.2016.00864
5. Liddle LJ, Ralhan S, Ward DL, Colbourne F. Translational Intracerebral Hemorrhage Research: Has Current Neuroprotection Research ARRIVEd at a Standard for Experimental Design and Reporting? *Transl Stroke Res*. 2020;11(6):1203-1213. doi:10.1007/s12975-020-00824-x
6. Wilkinson CM, Kung TFC, Jickling GC, Colbourne F. A translational perspective on intracranial pressure responses following intracerebral hemorrhage in animal models. *Brain Hemorrhages*. 2021;2(1):34-48. doi:10.1016/j.hest.2020.10.002
7. Kamel H, Hemphill JC. Characteristics and sequelae of intracranial hypertension after intracerebral hemorrhage. *Neurocrit Care*. 2012;17(2):172-176. doi:10.1007/s12028-012-9744-7
8. Rincon F, Mayer SA. Clinical review: Critical care management of spontaneous intracerebral hemorrhage. *Crit Care*. 2008;12(6):237. doi:10.1186/cc7092
9. Chen CJ, Ding D, Ironside N, et al. Intracranial pressure monitoring in patients with spontaneous intracerebral hemorrhage. *J Neurosurg*. 2020;132(6):1854-1864. doi:10.3171/2019.3.JNS19545

10. Pedersen SH, Lilja-Cyron A, Andresen M, Juhler M. The Relationship Between Intracranial Pressure and Age—Chasing Age-Related Reference Values. *World Neurosurg*. 2018;110:e119-e123. doi:10.1016/j.wneu.2017.10.086
11. Murtha LA, Yang Q, Parsons MW, et al. Cerebrospinal fluid is drained primarily via the spinal canal and olfactory route in young and aged spontaneously hypertensive rats. *Fluids Barriers CNS*. 2014;11(1). doi:10.1186/2045-8118-11-12
12. Pakkenberg B, Gundersen HJG. Neocortical neuron number in humans: Effect of sex and age. *J Comp Neurol*. 1997;384(2):312-320. doi:10.1002/(SICI)1096-9861(19970728)384:2<312::AID-CNE10>3.0.CO;2-K
13. Finch CE. Neuron atrophy during aging: programmed or sporadic? *Trends Neurosci*. 1993;16(3):104-110. doi:10.1016/0166-2236(93)90134-8
14. Pakkenberg B, Pelvig D, Marner L, et al. Aging and the human neocortex. In: *Experimental Gerontology*. Vol 38. ; 2003:95-99. doi:10.1016/S0531-5565(02)00151-1
15. Schwab S, Aschoff A, Spranger M, Albert F, Hacke W. The value of intracranial pressure monitoring in acute hemispheric stroke. *Neurology*. 1996;47(2):393-398. doi:10.1212/WNL.47.2.393
16. De Vis JB, Zwanenburg JJ, van der Kleij LA, et al. Cerebrospinal fluid volumetric MRI mapping as a simple measurement for evaluating brain atrophy. *Eur Radiol*. 2016;26(5):1254-1262. doi:10.1007/s00330-015-3932-8
17. Erten-Lyons D, Dodge HH, Woltjer R, et al. Neuropathologic basis of age-associated brain atrophy. *JAMA Neurol*. 2013;70(5):616-622. doi:10.1001/jamaneurol.2013.1957
18. Czosnyka M, Balestreri M, Steiner L, et al. Age, intracranial pressure, autoregulation, and outcome after brain trauma. *J Neurosurg*. 2005;102(3):450-454. doi:10.3171/jns.2005.102.3.0450
19. Czosnyka M, Hutchinson PJ, Balestreri M, Hiler M, Smielewski P, Pickard JD. Monitoring and interpretation of intracranial pressure after head injury. *Acta Neurochir Suppl*.

2006;(96):114-118. doi:10.1007/3-211-30714-1_26

20. Williamson MR, Colbourne F. Evidence for Decreased Brain Parenchymal Volume After Large Intracerebral Hemorrhages: a Potential Mechanism Limiting Intracranial Pressure Rises. *Transl Stroke Res*. 2017;8(4):386-396. doi:10.1007/s12975-017-0530-x
21. Kalisvaart ACJ, Wilkinson CM, Gu S, et al. An update to the Monro–Kellie doctrine to reflect tissue compliance after severe ischemic and hemorrhagic stroke. *Sci Rep*. 2020;10(1). doi:10.1038/s41598-020-78880-4
22. Ankolekar S, Rewell S, Howells DW, Bath PMW. The influence of stroke risk factors and comorbidities on assessment of stroke therapies in humans and animals. *Int J Stroke*. 2012;7(5):386-397. doi:10.1111/j.1747-4949.2012.00802.x
23. Ergul A, Hafez S, Fouda A, Fagan SC. Impact of Comorbidities on Acute Injury and Recovery in Preclinical Stroke Research: Focus on Hypertension and Diabetes. *Transl Stroke Res*. 2016;7(4):248-260. doi:10.1007/s12975-016-0464-8
24. Wu G, Bao X, Xi G, Keep RF, Thompson BG, Hua Y. Brain injury after intracerebral hemorrhage in spontaneously hypertensive rats: Laboratory investigation. *J Neurosurg*. 2011;114(6):1805-1811. doi:10.3171/2011.1.JNS101530
25. MacLellan CL, Silasi G, Poon CC, et al. Intracerebral hemorrhage models in rat: comparing collagenase to blood infusion. *J Cereb Blood Flow Metab*. 2008;28:516-525. doi:10.1038/sj.jcbfm.9600548
26. Wilkinson CM, Brar PS, Balay CJ, Colbourne F. Glibenclamide, a Sur1-Trpm4 antagonist, does not improve outcome after collagenase-induced intracerebral hemorrhage. *PLoS One*. 2019;14(5). doi:10.1371/journal.pone.0215952
27. Rosenberg G a., Mun-Bryce S, Wesley M, Kornfeld M. Collagenase-induced intracerebral hemorrhage in rats. *Stroke*. 1990;21(5):801-807. doi:10.1161/01.STR.21.5.801
28. Paxinos G, Watson C. Index of Structures. *Rat Brain Stereotaxic Coord*. Published online 1982:7-9. doi:10.1016/b978-0-12-547620-1.50008-4

29. Williamson MR, Wilkinson CM, Dietrich K, Colbourne F. Acetazolamide Mitigates Intracranial Pressure Spikes Without Affecting Functional Outcome After Experimental Hemorrhagic Stroke. *Transl Stroke Res*. 2019;10(4):428-439. doi:10.1007/s12975-018-0663-6

30. Wilkinson CM, Fedor BA, Aziz JR, et al. Failure of bumetanide to improve outcome after intracerebral hemorrhage in rat. *PLoS One*. 2019;14(1):1-20. doi:10.1371/journal.pone.0210660

31. Wilkinson CM, Kalisvaart ACJ, Kung TFC, et al. The collagenase model of intracerebral hemorrhage in awake, freely moving animals: The effects of isoflurane. *Brain Res*. 2020;1728:146593. doi:10.1016/j.brainres.2019.146593

32. Napper RMA. Total number is important: Using the disector method in design-based stereology to understand the structure of the rodent brain. *Front Neuroanat*. 2018;12. doi:10.3389/fnana.2018.00016

33. Emerich DF, Mcdermott P, Krueger P, et al. Locomotion of aged rats: Relationship to neurochemical but not morphological changes in nigrostriatal dopaminergic neurons. *Brain Res Bull*. 1993;32(5):477-486. doi:10.1016/0361-9230(93)90294-L

34. Pinto E. Blood pressure and ageing. *Postgrad Med J*. 2007;83(976):109-114. doi:10.1136/pgmj.2006.048371

35. Harrap SB, Van Der Merwe WM, Griffin SA, Macpherson F, Lever AF. Brief angiotensin converting enzyme inhibitor treatment in young spontaneously hypertensive rats reduces blood pressure long-term. *Hypertension*. 1990;16(6):603-614. doi:10.1161/01.HYP.16.6.603

36. Okamoto K, Aoki K. Development of a strain of spontaneously hypertensive rats. *Jpn Circ J*. 1963;27(3):282-293. doi:10.1253/jcj.27.282

37. Gottschalk A, Scafidi S, Toung TJK. Brain water as a function of age and weight in normal rats. *PLoS One*. 2021;16(9 September). doi:10.1371/journal.pone.0249384

38. Zelenina M, Zelenin S, Aperia A. Water Channels (Aquaporins) and Their Role for Postnatal Adaptation. *Pediatr Res*. 2005;57(5):47R-53R. doi:10.1203/01.PDR.0000159572.79074.0B

39. Wozniak JR, Lim KO. Advances in white matter imaging: A review of in vivo magnetic resonance methodologies and their applicability to the study of development and aging. *Neurosci Biobehav Rev*. 2006;30(6):762-774. doi:10.1016/j.neubiorev.2006.06.003

40. Gong Y, Hua Y, Keep RF, Hoff JT, Xi G. Intracerebral hemorrhage: Effects of aging on brain edema and neurological deficits. In: *Stroke*. ; 2004. doi:10.1161/01.STR.0000145485.67827.d0

41. Staykov D, Wagner I, Volbers B, et al. Natural course of perihemorrhagic edema after intracerebral hemorrhage. *Stroke*. 2011;42(9):2625-2629. doi:10.1161/STROKEAHA.111.618611

42. Volbers B, Willfarth W, Kuramatsu JB, et al. Impact of Perihemorrhagic Edema on Short-Term Outcome After Intracerebral Hemorrhage. *Neurocrit Care*. 2016;24(3):404-412. doi:10.1007/s12028-015-0185-y

43. Tajima A, Hans FJ, Livingstone D, et al. Smaller local brain volumes and cerebral atrophy in spontaneously hypertensive rats. *Hypertension*. 1993;21(1):105-111. doi:10.1161/01.HYP.21.1.105

44. Maiër B, Kubis N. Hypertension and Its Impact on Stroke Recovery: From a Vascular to a Parenchymal Overview. *Neural Plast*. 2019;2019. doi:10.1155/2019/6843895

45. Becker KJ. Strain-Related Differences in the Immune Response: Relevance to Human Stroke. *Transl Stroke Res*. 2016;7(4):303-312. doi:10.1007/s12975-016-0455-9

46. Lee RMKW, Delaney KH, Lu M. Perindopril treatment prolonged the lifespan of spontaneously hypertensive rats. *J Hypertens*. 1995;13(4):471-476. doi:10.1097/00004872-199504000-00013

47. Brower M, Grace M, Kotz CM, Koya V. Comparative analysis of growth characteristics of

Sprague Dawley rats obtained from different sources. *Lab Anim Res*. 2015;31(4):166. doi:10.5625/lar.2015.31.4.166

48. Boland T, Henderson G V., Gibbons FK, et al. Hypernatremia at Hospital Discharge and Out of Hospital Mortality Following Primary Intracerebral Hemorrhage. *Neurocrit Care*. 2016;25(1):110-116. doi:10.1007/s12028-015-0234-6
49. Liu J, Li Q, Ren J, Liang X, Zhang Q, Han Y. Association of Sex with Serum Potassium, Sodium, and Calcium Disorders after Hypertensive Intracerebral Hemorrhage. *World Neurosurg*. 2020;141:e367-e373. doi:10.1016/j.wneu.2020.05.137
50. Vereninov IA, Yurinskaya VE, Vereninov AA. Commentary: How Cells Can Control Their Size by Pumping Ions. *Front Cell Dev Biol*. 2017;5(May):1-14. doi:10.3389/fcell.2017.00072
51. Pasantes-Morales H, Cruz-Rangel S. Brain volume regulation: Osmolytes and aquaporin perspectives. *Neuroscience*. 2010;168(4):871-884. doi:10.1016/j.neuroscience.2009.11.074
52. Hultström M. Development of structural kidney damage in spontaneously hypertensive rats. *J Hypertens*. 2012;30(6):1087-1091. doi:10.1097/HJH.0b013e328352b89a
53. Kim SW. Hypernatemia: Successful treatment. *Electrolyte Blood Press*. 2006;4(2):66-71. doi:10.5049/EBP.2006.4.2.66
54. Oparil S, Schmieder RE. New Approaches in the Treatment of Hypertension. *Circ Res*. 2015;116(6):1074-1095. doi:10.1161/CIRCRESAHA.116.303603

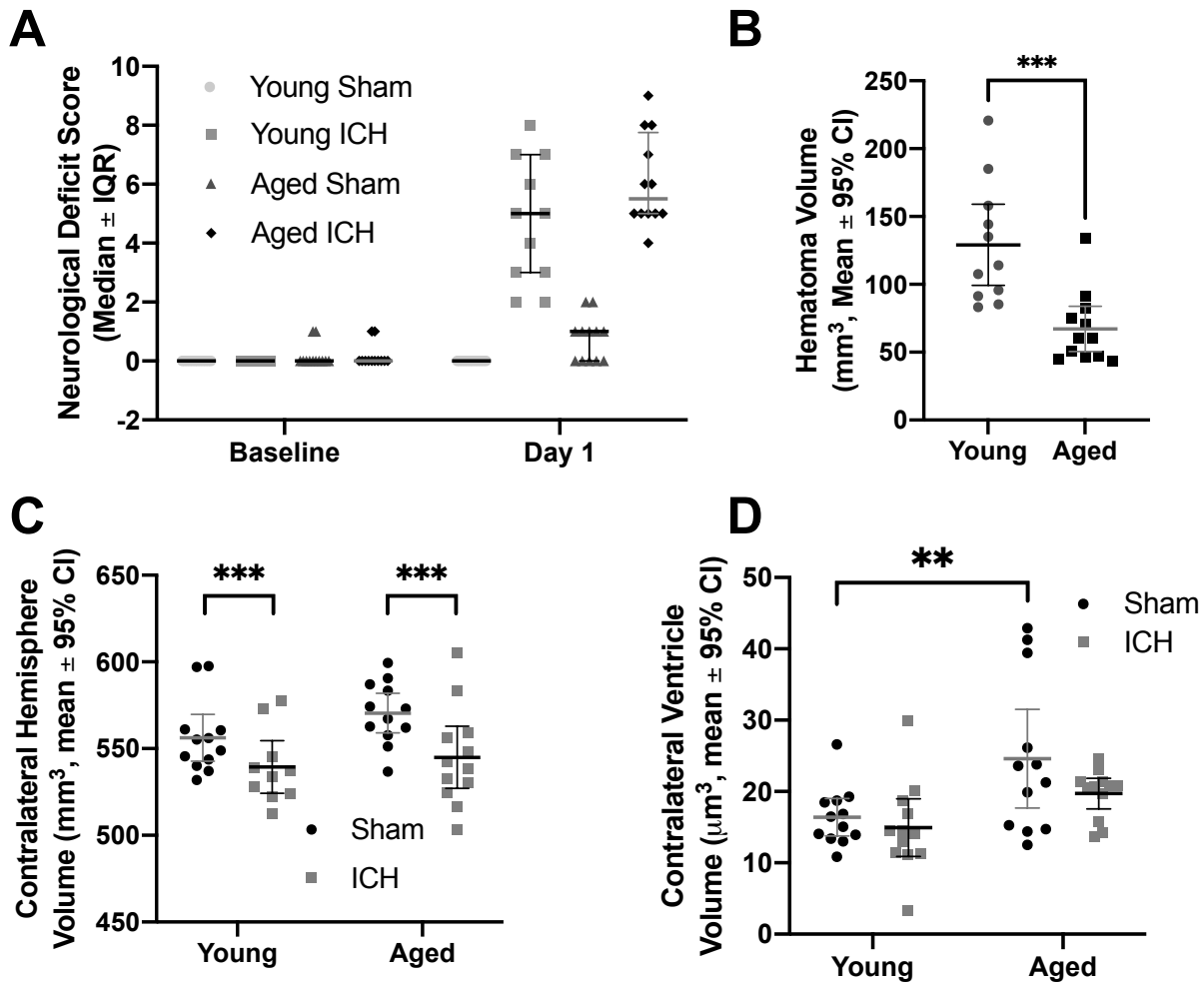

Figure 6-1 Neurological Deficits and Histology. There were no significant differences in neurological deficits by age (A, $p > 0.999$). The young animals had a substantially larger hematoma volume compared to the aged animals when given the same amount of collagenase into the striatum (B, $p = 0.002$). Despite large differences in hematoma size, the volume of the contralateral hemisphere was reduced in both ICH groups (C, ICH main effect, $p = 0.003$, interaction effect $p = 0.523$, age main effect $p = 0.145$). Aged animals in the sham group had significantly larger ventricle volumes (D, interaction effect $p = 0.390$, ICH main effect $p = 0.116$, age main effect $p = 0.002$), perhaps indicating greater atrophy (n=12/group, except for the young ICH group where n=11). ** denotes $p < 0.01$, *** denotes $p < 0.001$.

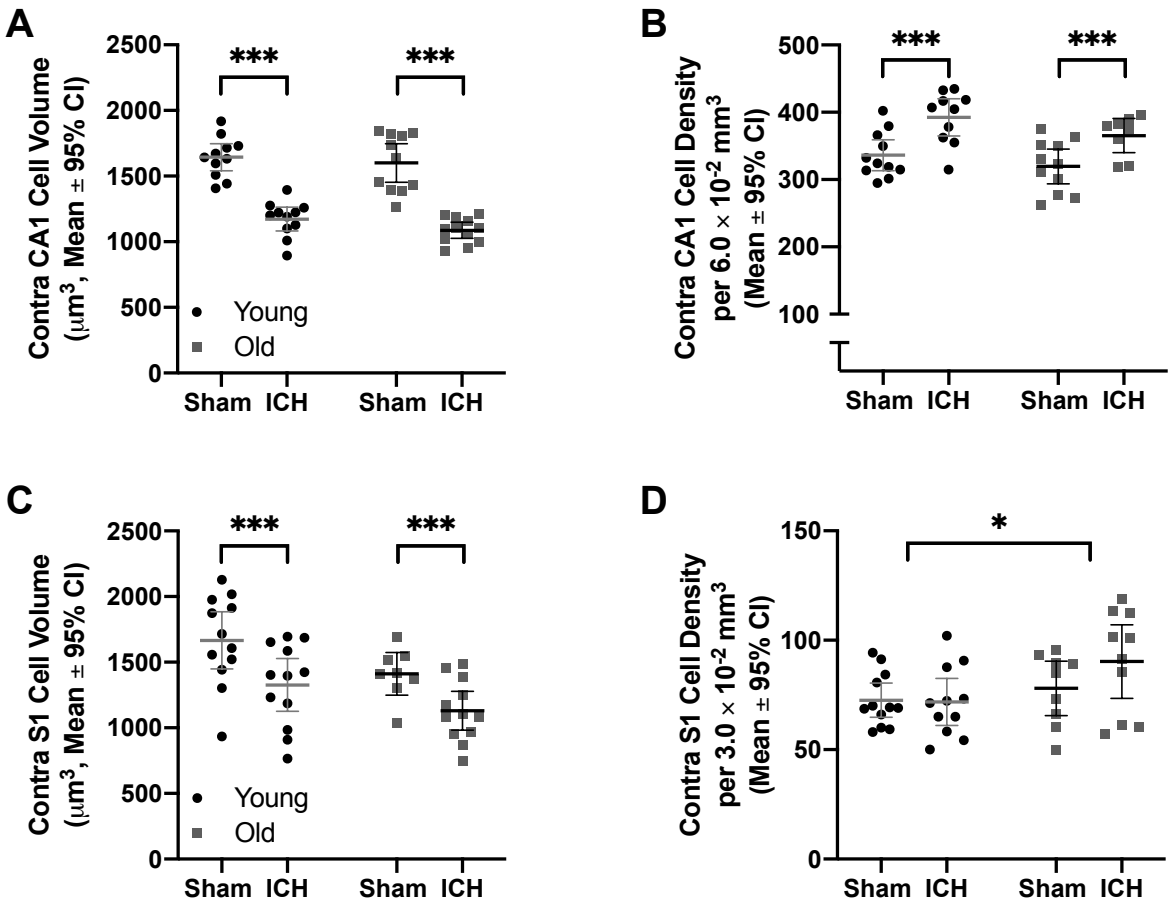

Figure 6-2 Stereology. Cell volume was significantly reduced in CA1 after a striatal ICH (A, ICH main effect $p < 0.001$, age main effect $p = 0.173$, interaction effect $p = 0.652$). Neuron density in CA1 was significantly increased after ICH (B, ICH main effect $p < 0.001$, age main effect $p = 0.064$, interaction effect $p = 0.642$). In contralateral S1, neuron volumes were significantly decreased after ICH (C, ICH main effect $p = 0.001$, interaction effect $p = 0.176$) and aged animals had smaller neuron volumes on average (age main effect $p = 0.013$). There was no effect of ICH on S1 cell density (D, ICH main effect $p = 0.291$, age main effect $p = 0.153$, interaction effect $p = 0.232$), but there was an age effect in which aged animals had higher cell density (age main effect $p = 0.032$). * denotes $p < 0.05$, *** denotes $p < 0.001$.

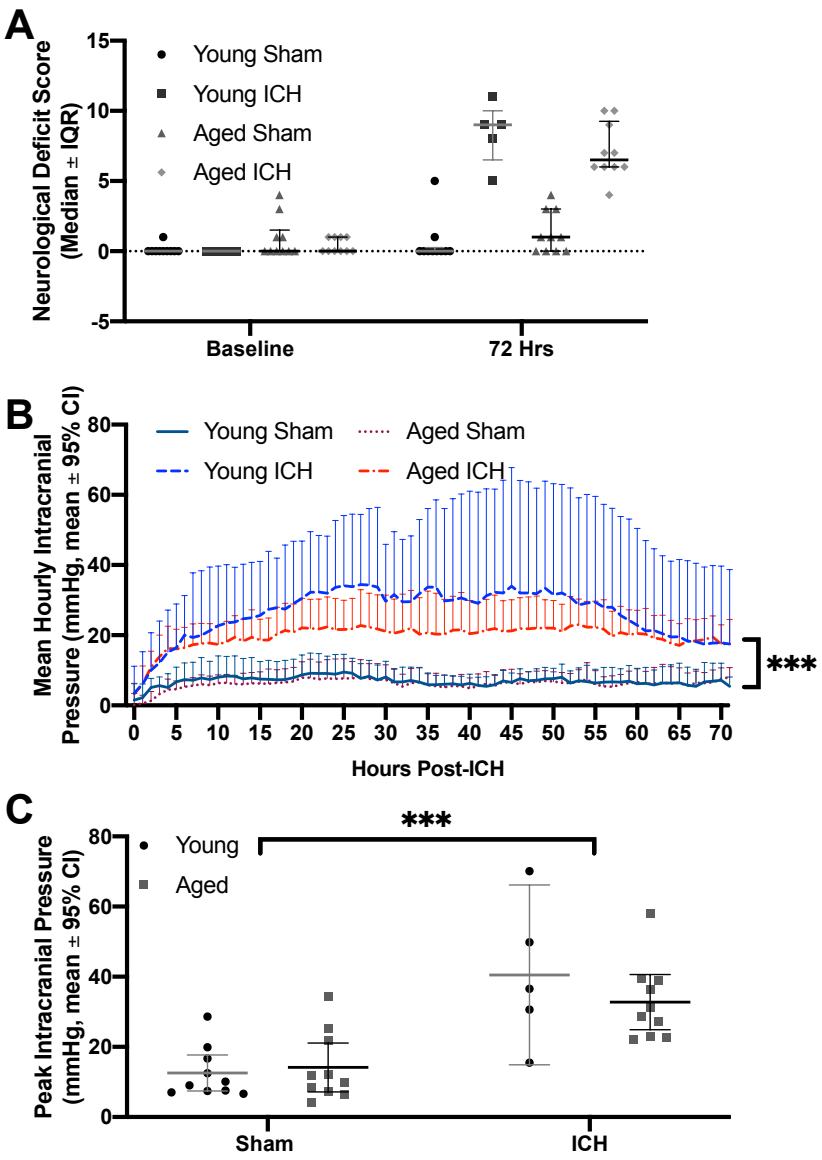

Figure 6-3 Neurological Deficits and Intracranial Pressure. A. Although the ICH surgery led to significant neurological deficits ($p < 0.001$), there was no effect of age on neurological deficits ($p > 0.999$). B. Young ICH animals had higher ICP (epidural space) compared to the aged ICH animals ($p < 0.001$), and by day 3 the mean ICP was similar across ages (n=10/group except for young ICH, where n=5). The sham animals of both ages did not have significantly different ICP (all $p > 0.05$). C. Peak ICP was significantly higher in ICH groups compared to sham groups (ICH main effect $p < 0.001$, interaction effect $p = 0.260$), however, there were no effects of age on peak ICP (age main effect $p = 0.455$). *** denotes $p < 0.001$.

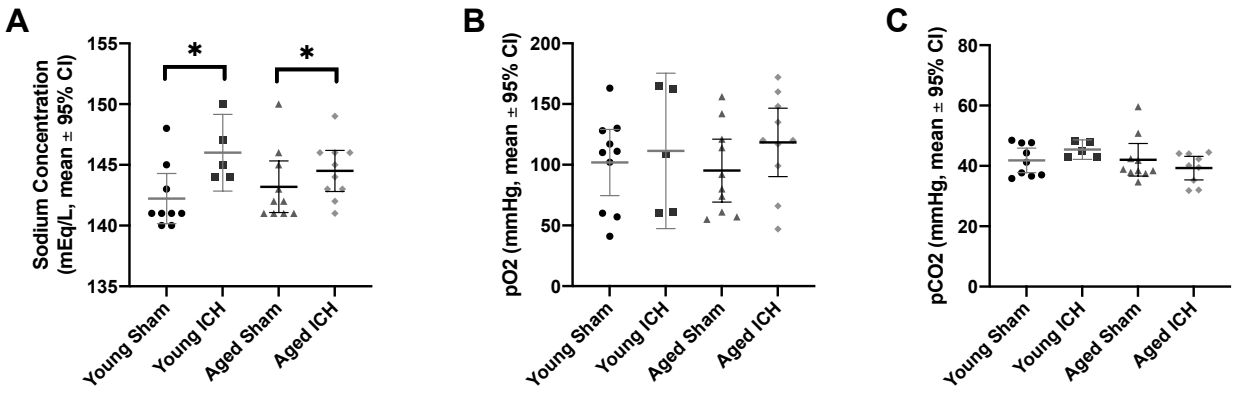

Figure 6-4 Arterial Blood Gases and Sodium. Sodium was modestly but statistically significantly increased after ICH in both young and old animals (A, age main effect p = 0.786, interaction effect p = 0.204, ICH main effect p = 0.012). There was no effect of age or ICH on blood gases (B-C, all p > 0.141). n=10/group for aged animals, n=5 for young ICH, n=9 for young sham. * denotes p < 0.05.

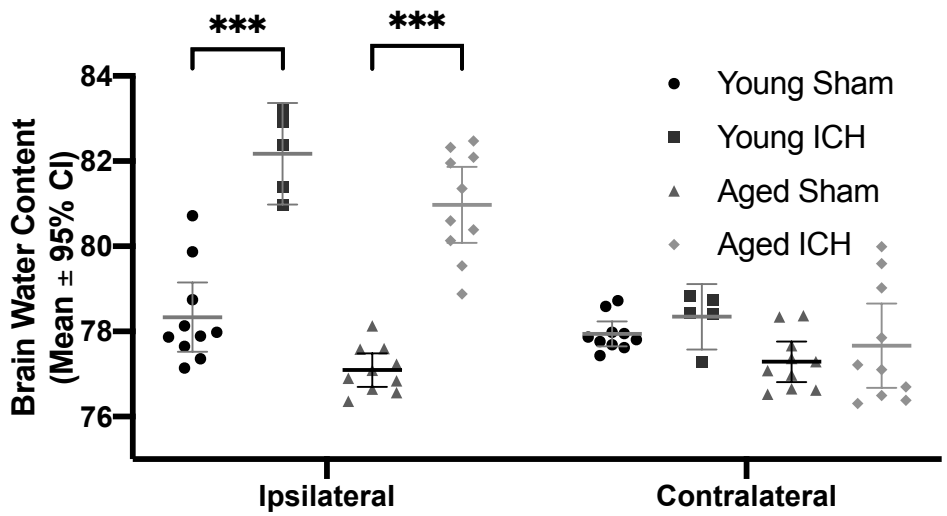

Figure 6-5 Brain Water Content. There was a significant interaction between region and injury on brain water levels ($p < 0.001$). Aged animals had lower brain water content across regions and injury types. (n=10/group, except for the young ICH group where n=5). ICH animals had higher brain water content in the ipsilateral (left) hemisphere, which is indicative of serum extrusion and brain edema. The effect is comparable in young and old rats despite the difference in hematoma volume. There was no difference in the contralateral (right) hemisphere, as expected. *** denotes $p < 0.001$.

Chapter 7:

Quantitative analysis of ICH patient CT images to assess tissue compliance

7.1 Introduction

Intracerebral hemorrhage (ICH) is a devastating type of stroke, with a mortality rate of approximately 40%.[1] One contribution to this mortality rate is the drastically elevated intracranial pressure (ICP).[2] After a large ICH, the added mass of the hematoma along with the resulting edema within the skull causes ICP to rise. When ICP is increased to a dangerous level, this can lead to decreases in cerebral blood flow, ischemic damage, and brain herniation.[3] According to the Monro-Kellie doctrine, blood and cerebrospinal fluid (CSF) volumes will be reduced (e.g., decreased production and increased clearance) in order to make space to accommodate for the added mass.[4,5] Recent data suggests that in rats, there is an additional compliance mechanism to counter ICP elevations after large mass effect (hematoma + edema), known as tissue compliance.[4,6] Neurons in regions distal to the injury will reduce their volume and pack together more densely, effectively reducing the volume of the brain parenchyma itself. This effect is transient, and resolves within a week without death to neurons.

We have observed and replicated this tissue compliance effect in many post-stroke settings in rodents using experimental models. For instance, tissue compliance has been noted after both the collagenase and autologous whole blood models of ICH, as well as the intraluminal suture occlusion model of middle cerebral artery occlusion.[4,6] Although this was first documented in young, healthy animals, we have recently confirmed that both aged and hypertensive animals, which are much closer to the clinical ICH population,[7] also undergo tissue compliance after a large, collagenase-induced ICH. Although it is encouraging that aged animals with comorbidities are still capable of tissue compliance, it is possible that this compliance mechanism may be specific to rodents, or not present in humans. For example, humans have relatively greater amounts of white matter than rodents,[8] and white matter may be less capable of rapid volume reduction than grey matter is.[9]

While tissue compliance can be measured in rodents using histological techniques (e.g., stereological assessment of neuron volume), these methods require specific tissue processing

to minimize artefact. For example, animals are perfused with 0.9% saline followed by 10% neutral buffered formalin to preserve tissue and minimize the interval between death and tissue fixation. In people, this level of precision and rapid tissue preservation is much more difficult, with even rapid autopsies occurring ~12-24 hrs after death[10–12] (compared to fixation procedure as the cause of death in rodent models). However, since bodies are typically maintained in cold storage prior to autopsy, the effect of post-mortem interval on stereological quantification of cells is unclear. We have tested the effect of delayed fixation in rodents and were still able to observe tissue compliance (Kalisvaart et al., unpublished data), but this was only tested at a 6-hr delay, which is still faster than the average autopsy in people. Additionally, using these techniques in a clinical setting would require post-mortem tissue. Thus, assessing cell volume using stereology is subject to mortality confounds. For example, it is possible that the ICH patients that die are those that have the least effective tissue compliance, thus biasing the sample. However, in animals, we have also demonstrated tissue compliance using whole hemisphere quantification. When regions undergo tissue compliance, the reductions in cell volume and increases in cell density can be seen on a macro level as decreases in overall contralateral hemisphere volume. Therefore, we may be able to assess tissue compliance in a clinical setting using computed tomography (CT) or magnetic resonance imaging (MRI) to quantify brain volume, thus avoiding mortality confounds. Using CT images to quantify brain volume has been done routinely, including studies of dementia,[13] brain development,[14] and atrophy after stroke.[15]

In this study, our goals were to determine the feasibility of using CT to quantify tissue compliance, identify potential variables of concern for future studies (e.g., sex effects), and obtain an estimate of variability in brain volume to better determine statistical power needed for future studies. We quantified the contralateral hemisphere volume using computed tomography (CT) images of ICH patients from the Intracerebral Hemorrhage Acutely Decreasing Arterial Pressure Trial (ICH ADAPT I) trial[16,17] that were stratified into two groups, large ICH and small ICH. These groups were further divided by sex, resulting in four groups: male large ICH, female

large ICH, male small ICH, and female small ICH. We hypothesized that the patients with smaller hematomas would not experience high ICP elevations, and thus would not need to undergo tissue compliance and would have higher hemisphere volumes on average.

7.2 Methods

7.2.1 CT Image Datasets

Retrospective data and CT images were collected from 50 patients enrolled in the ICH ADAPT I trial from the University of Alberta hospital arm of the study. The ICH ADAPT I trial was approved by the Research Ethics Board at the University of Alberta. All patients underwent non-contrast CT imaging at 24 (range = 21-27 hrs) after ICH in order to assess hematoma expansion.[17] Images consisted of 5 mm slices through the entire brain (18-20 slices).

We selected the 30 patients with the largest hematoma volume (average = 47.6 ± 12.14 cm^3) and 20 patients with the smallest hematoma volume (average = 5.21 ± 0.81 cm^3). Two patients from the small ICH group were excluded based on incomplete imaging at 24 hrs, leaving 18 patients in the small ICH group.

Our exclusion criteria initially excluded patients with lobar ICH, due to potential atrophy associated with amyloid angiopathy; age greater than 70 years, due to age related atrophy; and any additional mass within the brain, such as a tumor, that may contribute to elevated ICP. However, this left us with a very small sample size (< 30) and disproportionately excluded many of the large ICHs that would cause increased ICP. Therefore, we ran our analysis with patients of all ages and ICH locations in an exploratory manner.

7.2.2 CT Image Analysis

Images were gantry tilt corrected in MRIcroGL (v2.1.59-0, University of South Carolina, USA) to ensure accuracy and consistency, as gantry angle can vary between CT scanners.[18]

Images were then manually segmented in ITK-SNAP (itksnap.org, v3.6.0, University of Pennsylvania, USA)[19] for volume quantification (Figure 7-1). ITK-SNAP is a software regularly used for accurately segmenting brain regions from three-dimensional images, either manually or automatically.[20–22] Hematoma volume, contralateral parenchyma volume, and cerebrospinal fluid volumes were quantified using voxel counts of segmented regions of interest using all image slices.

7.2.3 Statistical Analysis

As this is a pilot study, we used a convenience sample size of 48 based on the images that were available to us. Variables between groups were compared using a two-way ANOVA to assess the main effects and interaction of ICH size and sex. Significant main effects were further analyzed with Sidak's multiple comparison's test. Relationships between variables (e.g., hematoma volume and contralateral hemisphere volume) were assessed using least squares multiple linear regression, with contralateral hemisphere plus CSF volume as the predicted variable. As hematoma volume had already been assessed in this study, the inter-rater reliability of this measurement was assessed using Pearson's correlation coefficient. All data were analyzed using GraphPad Prism (v9.0, San Diego, California). The threshold for statistical significance was set at α=0.05.

7.3 Results

7.3.1 Patient Characteristics

Baseline information between the two groups is included in Table 7-1. There were no significant group differences in prevalence of hypertension (p = 0.289), rate of IVH (p = 0.141) or original group assignment (intensive vs. standard blood pressure lowering, p = 0.849). The male large ICH group had a significantly higher mortality rate than any other group (p = 0.006). There was a significant difference in age, with the male small ICH group being younger on

average than the two female groups ($p = 0.026$), but not significantly different than the male large ICH group ($p = 0.513$). We had 40% power to detect the observed effect size (Cohen's $d = 0.66$) in our male sample and 12% observed power to detect this effect in our female sample.

7.3.2 Contralateral Parenchyma and CSF Volume

There was a significant main effect of ICH size on the sum of contralateral parenchyma and CSF volume (Figure 7-2A, ICH main effect $p = 0.046$, interaction effect $p = 0.757$). However, this main effect was not significant when looking at contralateral parenchymal volume alone (Figure 7-2B, ICH main effect $p = 0.092$, interaction effect $p = 0.728$). For both contralateral parenchymal volume and combined contralateral parenchymal and CSF volume, the large ICH group displayed a 5% reduction in volume. However, in the case of contralateral parenchymal volume alone, there was increased variability that reduced statistical power. There was a significant main effect of sex on both contralateral parenchymal volume (Figure 7-2B, sex main effect $p < 0.001$) as well as contralateral parenchymal and CSF volume (Figure 7-2A, sex main effect $p < 0.001$). There was no effect of ICH size (Figure 7-2C, ICH main effect $p = 0.657$, interaction effect $p = 0.948$) or sex (sex main effect $p = 0.342$) on contralateral CSF volume.

7.3.3 Simple Correlations

The hematoma volume measurements between the two raters were significantly correlated ($R^2 = 0.976$, $p < 0.001$), indicating high inter-rater reliability in segmentations.

The variables mass effect (hematoma volume and edema volume) and contralateral parenchyma and CSF volume were not significantly correlated (Figure 7-3, $R^2 = 0.056$, $p = 0.104$). These variables had a negative relationship, where those with increased mass effect tended to have decreased contralateral parenchyma and CSF volume. Within the male large ICH group alone, there was a significant relationship between these two variables ($R^2 = 0.23$ $p = 0.030$), but this was not true in any of the other groups ($R^2 < 0.064$ $p > 0.583$).

7.3.4 Multiple Linear Regression

We performed a linear regression to predict contralateral parenchyma and CSF volume using a model that took the following variables into account: sex, age, race, hypertension, blood pressure management group (intensive or standard, the independent variable in the ICH-ADAPT trial), intraventricular hemorrhage, and mass effect (hematoma + edema volume). This model significantly predicted combined contralateral parenchyma and CSF volume ($R^2 = 0.742$, $p < 0.001$). In this model, sex ($p < 0.001$), south Asian race ($p = 0.006$), and mass effect (hematoma + edema volume, $p = 0.028$) were significant predictors of contralateral parenchyma and CSF volume.

7.4 Discussion

In this pilot study, we demonstrated that existing CT images can be used to accurately and reliably quantify contralateral hemisphere volume as a potential indicator of tissue compliance. Here, we found a significant effect of ICH size on contralateral parenchyma and CSF volume when patients were stratified into “large” and “small” ICH groups. We used this approach based on the assumption that mass effect load would be a factor and that small ICHs would have little mass effect and be similar to controls. For instance, we have showed that moderate-sized ICHs did not result in tissue compliance, whereas very large ICHs did in rats.[4] Due to the large natural variability in brain volume, this study was statistically underpowered, and thus, results should be interpreted with caution and future studies examining tissue compliance in humans should use larger sample sizes. We estimate that a sample size of 30 males and 30 females per group is needed to detect a 5% reduction in contralateral parenchyma volume, as seen here. We recommend that future studies measure both sex and body size to statistically account for some of the variability in brain volume between people.

Additionally, we recommend enrolling patients with both small and severe ICH, to ensure the sample includes patients with large mass effect and significantly elevated ICP.

In this study, we quantified both contralateral parenchyma volume and contralateral CSF volume. Using the sum of these two measurements may be more useful than either alone when assessing tissue compliance. When comparing the contralateral parenchymal volumes to the combined contralateral parenchymal and CSF volumes, we see a similar effect size (~5% reduction), but additional variability in the parenchyma measurement reduces our statistical power. Both cortical atrophy and natural individual variability can lead to pre-existing differences in brain volume.[23,24] Typically, those with more extensive atrophy may have additional CSF in order to maintain normal ICP prior to ICH.[25,26] Indeed, MRI measurements of CSF volume have been used as a marker of cerebral atrophy.[25] Conversely, after ICH, patients with large ICH and increased ICP (the most likely group to experience tissue compliance) are likely diverting CSF from the cranium as per the Monro-Kellie doctrine.[4,5,27] In cases of mass effect, CT scans can show sulcal effacement, where adjacent gyri are pushed together, displacing CSF from the sulci.[28–30] Thus, combining both CSF volume and parenchyma volume may be a method to distinguish patients that have smaller hemisphere volumes due to atrophy from those who have smaller hemisphere volumes due to tissue compliance. If we only saw significant differences in CSF volumes between groups, this may indicate that only classic ICP compliance mechanisms are at play. We are primarily interested in the contralateral parenchyma volume measurement alone, to look at the effect of just one compliance mechanism. Therefore, it is important that future studies are adequately powered for the variability in brain volume.

These results demonstrate preliminary evidence that tissue compliance may be occurring in people after ICH, and may not be limited to rodents. This evidence provides justification for future studies to characterize tissue compliance in people. Although we were underpowered for correlational analyses, we did see that mass effect significantly predicted contralateral parenchyma and CSF volume after accounting for variability due to sex and other

variables. However, the true relationship between these variables may be non-linear. For example, there may be a threshold of mass effect wherein initial compliance mechanisms (e.g., CSF diversion) are exhausted and tissue compliance begins. Studies with greater statistical power and a large range of ICH volumes should assess the relationship between mass effect and tissue compliance using nonlinear (e.g., asymptotic) or cluster analysis. We did not see an interaction between sex and ICH size, suggesting that the effect of tissue compliance is consistent in males and females.

Here, we found that sex most strongly predicted brain volume. It has been well documented that females have smaller brain volumes than males,[31,32] which is related in part to differences in body size.[33,34] Additionally, we found that those of South Asian descent were more likely to have a smaller brain volume. This is likely due to our limited sample size, since in this case, all South Asian patients were female. Interestingly, there was no significant effect of age on our measures of brain volume. This may be due to the relatively older age of our sample (average age = 69.13, range = 45-91) and ICH patients in general, and we may have seen a stronger relationship between age and brain volume if we had a wider range of ages in our sample. Another variable that likely relates to pre-ICH brain volume is body size.[33,34] In this study, we did not have any information collected on patient body size (e.g., weight, height, body mass index). Future studies should measure body size in order to possibly account for more variability in brain volume.

There are several limitations in this pilot study. For example, there was a large range in contralateral hemisphere volumes, which left us underpowered to detect smaller effects. Additionally, the ICH volumes of the patients in this study were relatively small (mean = 31.7 cm^3, or ~2.6% of average brain volume)[35,36]. In rodents that demonstrate parenchyma compliance, we typically induce a severe ICH (hematoma volume of ~ 60-100 μL, or ~4-7% of average brain volume).[4,6] We use a larger hematoma volume, as this will lead to substantial swelling and severe increases in ICP that need to be compensated for. A previous clinical study

found patients with elevated ICP (mean ICP = ~27 mmHg) had a hematoma volume of 66.81 mL on average.[37] Of note, in the autologous whole blood model, a blood injection of 130μL (lesion volume of ~40 mm^3, or ~2.5% of brain volume) did not cause compensatory changes in neuron volumes.[6] This model also has less edema than the collagenase model,[38] and thus there was less mass effect in this setting. Therefore, it is possible that the ICH volumes here were not large enough to cause persistent ICP elevations in all patients in the large ICH group. In addition to the potentially lower ICP, there were a variety of ages and ICH types in this study. Cortical atrophy is common in older patients, as well as those with amyloid angiopathy.[23,39] This atrophy can both contribute to variability in both groups as well as reduce the need for tissue compliance, if more space is available to accommodate the bleed (e.g., greater baseline CSF volumes, which can then be reduced). Additionally, this study did not have a true control group, as all patients in this study had an ICH. This study would be strengthened by having an age-matched healthy control group to compare to. Finally, this study is susceptible to bias due to a lack of blinding and randomization. Although quantification was done while blinded to group (small vs. large and male vs. female) identity, the hematoma was visible and ICH size could be approximated. The lack of randomization to group prevents any causal claims from being made, and this is an unfortunate limitation of comparing ICH vs. controls clinically. This study is correlational in nature, and it is possible that a third variable can explain the difference observed here. For example, patients with larger brains may have decreased hematoma volumes (e.g., due to the tamponade effect).[2,40]

This pilot study provides valuable information for future studies using brain imaging to assess tissue compliance in patients. Routine CT imaging can be used to quantify brain volumes. Studies should ensure demographic variables are collected that may be able to account for the large variability in brain volumes (e.g., sex, body size). Future studies should explore the use of MRI in order to quantify volumes of specific structures/regions known to exhibit tissue compliance (e.g., the hippocampus).[4,6] Additionally, future studies could employ a

within subjects' design by quantifying brain volume before and after hematoma expansion or hematoma removal. This would afford greater statistical power and account for between subjects' variability in brain volume. In this study, all CT scans were done at 24 hrs, which corresponds to a time of high compliance in rats. However, injury and edema formation progress more slowly in people than rodents (e.g., edema peaks around day 3 in rodents[41] and day 7-10 in people)[42], and therefore, 24 hrs may not represent the peak of tissue compliance in people. Repeated imaging would give insight into the timing of tissue compliance in relation to hematoma growth.

In conclusion, it is feasible to quantify brain volume using CT images. Initial pilot evidence indicates that brain volume may be decreased after large ICH, but this should be replicated using a larger sample size with additional measures (e.g., body size) to account for variability in brain volume.

7.5 References

1. An SJ, Kim TJ, Yoon BW. Epidemiology, risk factors, and clinical features of intracerebral hemorrhage: An update. *J Stroke*. 2017;19(1):3-10. doi:10.5853/jos.2016.00864
2. Wilkinson CM, Kung TFC, Jickling GC, Colbourne F. A translational perspective on intracranial pressure responses following intracerebral hemorrhage in animal models. *Brain Hemorrhages*. 2021;2(1):34-48. doi:10.1016/j.hest.2020.10.002
3. Tadevosyan A, Kornbluth J. Brain Herniation and Intracranial Hypertension. *Neurol Clin*. 2021;39(2):293-318. doi:10.1016/j.ncl.2021.02.005
4. Kalisvaart ACJ, Wilkinson CM, Gu S, et al. An update to the Monro–Kellie doctrine to reflect tissue compliance after severe ischemic and hemorrhagic stroke. *Sci Rep*. 2020;10(1). doi:10.1038/s41598-020-78880-4
5. Wilson MH. Monro-Kellie 2.0: The dynamic vascular and venous pathophysiological components of intracranial pressure. *J Cereb Blood Flow Metab*. 2016;36(8):1338-1350. doi:10.1177/0271678X16648711
6. Williamson MR, Colbourne F. Evidence for Decreased Brain Parenchymal Volume After Large Intracerebral Hemorrhages: a Potential Mechanism Limiting Intracranial Pressure Rises. *Transl Stroke Res*. 2017;8(4):386-396. doi:10.1007/s12975-017-0530-x
7. Juvela S, Hillbom M, Palomaki H. Risk factors for spontaneous intracerebral hemorrhage. *Stroke*. 1995;26(9):1558-1564. doi:10.1161/01.STR.26.9.1558
8. Ventura-Antunes L, Mota B, Herculano-Houzel S. Different scaling of white matter volume, cortical connectivity, and gyrification across rodent and primate brains. *Front Neuroanat*. 2013;(MARCH). doi:10.3389/fnana.2013.00003
9. Dóczi T. Volume regulation of the brain tissue-a survey. *Acta Neurochir (Wien)*. 1993;121(1-2):1-8. doi:10.1007/BF01405174
10. Mitsios N, Gaffney J, Krupinski J, et al. Expression of signaling molecules associated with apoptosis in human ischemic stroke tissue. *Cell Biochem Biophys*. 2007;47(1):73-85.

doi:10.1385/CBB:47:1:73

11. Frugier T, Taylor JM, McLean C, et al. Evidence for the recruitment of autophagic vesicles in human brain after stroke. *Neurochem Int*. 2016;96:62-68. doi:10.1016/j.neuint.2016.02.016
12. White K, Yang P, Li L, Farshori A, Medina AE, Zielke HR. Effect of Postmortem Interval and Years in Storage on RNA Quality of Tissue at a Repository of the NIH NeuroBioBank. *Biopreserv Biobank*. 2018;16(2):148-157. doi:10.1089/bio.2017.0099
13. Yerby MS, Sundsten JW, Larson EB, Wu SA, Sumi SM. A new method of measuring brain atrophy: The effect of aging in its application for diagnosing dementia. *Neurology*. 1985;35(9):1316-1320. doi:10.1212/wnl.35.9.1316
14. Hamano K, Iwasaki N, Kawashima K, Takita H. Volumetric quantification of brain volume in children using sequential CT scans. *Neuroradiology*. 1990;32(4):300-303. doi:10.1007/BF00593049
15. Kim JH, Kim YS, Kim SH, et al. Contralateral Hemispheric Brain Atrophy After Primary Intracerebral Hemorrhage. *World Neurosurg*. 2017;102:56-64. doi:10.1016/j.wneu.2017.02.105
16. Butcher KS, Jeerakathil T, Hill M, et al. The intracerebral hemorrhage acutely decreasing arterial pressure trial. *Stroke*. 2013;44(3):620-626. doi:10.1161/STROKEAHA.111.000188
17. Butcher K, Jeerakathil T, Emery D, et al. The Intracerebral Haemorrhage Acutely Decreasing Arterial Pressure Trial: ICH ADAPT. *Int J Stroke*. 2010;5(3):227-233. doi:10.1111/j.1747-4949.2010.00431.x
18. Li X, Morgan PS, Ashburner J, Smith J, Rorden C. The first step for neuroimaging data analysis: DICOM to NIfTI conversion. *J Neurosci Methods*. 2016;264:47-56. doi:10.1016/j.jneumeth.2016.03.001
19. Yushkevich PA, Piven J, Hazlett HC, et al. User-guided 3D active contour segmentation

of anatomical structures: Significantly improved efficiency and reliability. *Neuroimage*. 2006;31(3):1116-1128. doi:10.1016/j.neuroimage.2006.01.015

20. Gomes AF, Brasil DM, Silva AIV, Freitas DQ, Haiter-Neto F, Groppo FC. Accuracy of ITK-SNAP software for 3D analysis of a non-regular topography structure. *Oral Radiol*. 2020;36(2):183-189. doi:10.1007/s11282-019-00397-y
21. Yushkevich PA, Gerig G. ITK-SNAP: An Intractive Medical Image Segmentation Tool to Meet the Need for Expert-Guided Segmentation of Complex Medical Images. *IEEE Pulse*. 2017;8(4):54-57. doi:10.1109/MPUL.2017.2701493
22. Yuan Q, Rubic M, Seah J, et al. Do maternal opioids reduce neonatal regional brain volumes: A pilot study. *J Perinatol*. 2014;34(12):909-913. doi:10.1038/jp.2014.111
23. Fjell AM, Walhovd KB, Fennema-Notestine C, et al. One-year brain atrophy evident in healthy aging. *J Neurosci*. 2009;29(48):15223-15231. doi:10.1523/JNEUROSCI.3252-09.2009
24. Sele S, Liem F, Mérillat S, Jäncke L. Age-related decline in the brain: a longitudinal study on inter-individual variability of cortical thickness, area, volume, and cognition. *Neuroimage*. 2021;240. doi:10.1016/j.neuroimage.2021.118370
25. De Vis JB, Zwanenburg JJ, van der Kleij LA, et al. Cerebrospinal fluid volumetric MRI mapping as a simple measurement for evaluating brain atrophy. *Eur Radiol*. 2016;26(5):1254-1262. doi:10.1007/s00330-015-3932-8
26. Czosnyka M, Czosnyka Z, Momjian S, Pickard JD. Cerebrospinal fluid dynamics. *Physiol Meas*. 2004;25(5). doi:10.1088/0967-3334/25/5/R01
27. Mokri B. The Monro-Kellie hypothesis: Applications in CSF volume depletion. *Neurology*. 2001;56(12):1746-1748. doi:10.1212/WNL.56.12.1746
28. Zazulia AR, Diringer MN, Derdeyn CP, Powers WJ. Progression of mass effect after intracerebral hemorrhage. *Stroke*. 1999;30(6):1167-1173. doi:10.1161/01.STR.30.6.1167
29. de Lucas EM, Sánchez E, Gutiérrez A, et al. CT protocol for acute stroke: Tips and tricks

for general radiologists. *Radiographics*. 2008;28(6):1673-1687. doi:10.1148/rg.286085502

30. Park KY, Kim JH, Chung CS, et al. Cortical sulcal effacement on brain CT associated with cerebral hyperperfusion after carotid artery stenting. *J Neurol Sci*. 2007;260(1-2):83-86. doi:10.1016/j.jns.2007.04.010
31. Steinmetz H, Staiger JF, Schlaug G, Huang Y, Jäncke L. Corpus callosum and brain volume in women and men. *Neuroreport*. 1995;6(7):1002-1004. doi:10.1097/00001756-199505090-00013
32. Allen JS, Damasio H, Grabowski TJ. Normal neuroanatomical variation in the human brain: An MRI-volumetric study. *Am J Phys Anthropol*. 2002;118(4):341-358. doi:10.1002/ajpa.10092
33. Ward MA, Carlsson CM, Trivedi MA, Sager MA, Johnson SC. The effect of body mass index on global brain volume in middle-aged adults: A cross sectional study. *BMC Neurol*. 2005;5. doi:10.1186/1471-2377-5-23
34. Bayat PD, Ghanbari A, Sohouli P, Amiri S, Sari PA. Correlación del tamaño craneal y volumen del cerebro, con edad, peso, talla e índice de masa corporal de los estudiantes de Ciencias Médicas de Arak. *Int J Morphol*. 2012;30(1):157-161. doi:10.4067/S0717-95022012000100028
35. Adeoye O, Broderick JP. Advances in the management of intracerebral hemorrhage. *Nat Rev Neurol*. 2010;6(11):593-601. doi:10.1038/nrneurol.2010.146
36. Ziai WC, Carhuapoma JR. Intracerebral Hemorrhage. *Contin Lifelong Learn Neurol*. 2018;24(6):1603-1622. doi:10.1212/CON.0000000000000672
37. Tian Y, Wang Z, Jia Y, et al. Intracranial pressure variability predicts short-term outcome after intracerebral hemorrhage: A retrospective study. *J Neurol Sci*. 2013;330(1-2):38-44. doi:10.1016/j.jns.2013.04.001
38. MacLellan CL, Silasi G, Poon CC, et al. Intracerebral hemorrhage models in rat:

comparing collagenase to blood infusion. *J Cereb Blood Flow Metab*. 2008;28:516-525. doi:10.1038/sj.jcbfm.9600548

39. Erten-Lyons D, Dodge HH, Woltjer R, et al. Neuropathologic basis of age-associated brain atrophy. *JAMA Neurol*. 2013;70(5):616-622. doi:10.1001/jamaneurol.2013.1957
40. Kroin JS, Nagalla SKS, Buvanendran A, McCarthy RJ, Tuman KJ, Ivankovich AD. The mechanisms of intracranial pressure modulation by epidural blood and other injectates in a postdural puncture rat model. *Anesth Analg*. 2002;95(2):423-429. doi:10.1213/00000539-200208000-00035
41. Yang GY, Betz AL, Chenevert TL, Brunberg JA, Hoff JT. Experimental intracerebral hemorrhage: Relationship between brain edema, blood flow, and blood-brain barrier permeability in rats. *J Neurosurg*. 1994;81(1):93-102. doi:10.3171/jns.1994.81.1.0093
42. Staykov D, Wagner I, Volbers B, et al. Natural course of perihemorrhagic edema after intracerebral hemorrhage. *Stroke*. 2011;42(9):2625-2629. doi:10.1161/STROKEAHA.111.618611

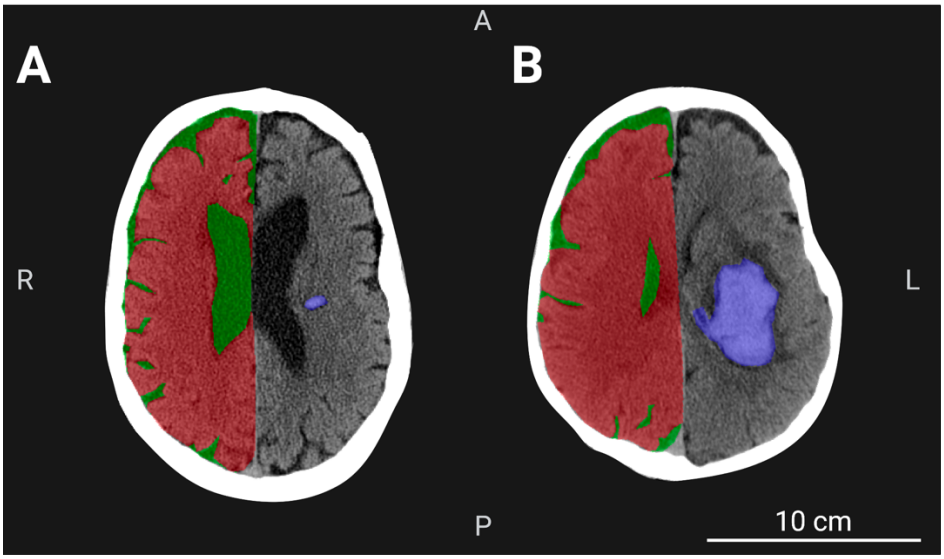

Figure 7-1. Representative images of segmentations from the small ICH group (A) and the large ICH group (B). Blue tracing represents the hematoma measurement, red tracing represents the contralateral brain parenchyma measurement, and green tracing represents the contralateral CSF measurement. Small letters represent axes (right, left, anterior, posterior).

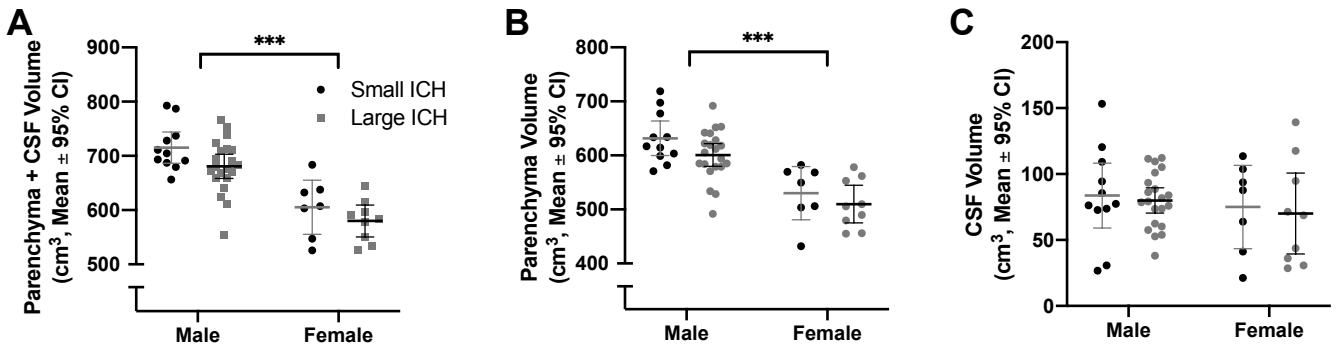

Figure 7-2. Both the combination of contralateral parenchyma and CSF volume as well as contralateral parenchyma volume were significantly lower in females, regardless of ICH size (both $p < 0.001$). There was a significant main effect of ICH size on contralateral parenchyma + CSF volume ($p = 0.046$), which was not seen when analyzing contralateral parenchyma volume alone ($p = 0.092$). There was no effect of sex or ICH size on contralateral CSF volumes (sex main effect $p = 0.342$, ICH main effect $p = 0.657$). *** denotes $p < 0.001$.

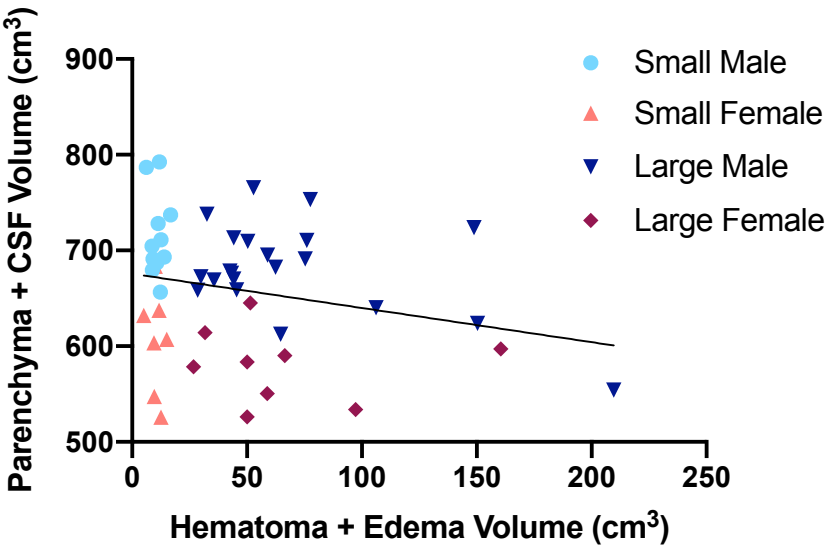

Figure 7-3. Relationship between mass effect (hematoma and edema volume) and contralateral parenchyma +CSF volume across groups (R^2 = 0.056 p = 0.104). Within groups, there was a significant correlation between these variables in the large male group (R^2 = 0.23 p = 0.030), but the other three groups were not significantly correlated (R^2 < 0.064 p > 0.583).

Variable	Male Large ICH	Female Large ICH	Male Small ICH	Female Small ICH
n	21	9	11	7
Age (mean 95% ± CI)	67.38 ± 4.14	76.11 ± 7.59	61.73 ± 6.50	77.00 ± 9.98
% Hypertensive	52% (11/21)	78% (7/9)	73% (8/11)	86% (6/7)
% Intensive BP lowering group	57% (12/21)	44% (4/9)	36% (4/11)	43% (3/7)
Mortality	38% (8/21)	0% (0/9)	0% (0/11)	0% (0/7)
% IVH	52% (11/21)	33% (3/9)	18% (2/11)	14% (1/7)
Hematoma Volume (mean 95% ± CI)	48.71 ± 15.35	44.99 ± 20.02	5.12 ± 0.99	5.37 ± 1.66
Edema Volume (mean 95% ± CI)	21.73 ± 6.01	20.91 ± 7.64	5.99 ± 1.09	5.14 ± 0.90

Table 7-1. Patient characteristics by group.

Chapter 8:
General Discussion

8.1 Summary of Findings

The main objective of this thesis was to characterize the effects of ICP elevation after ICH in translationally relevant models, with a focus on the tissue compliance response to raised ICP. Preclinical experiments studying ICP after ICH are limited, and often confounded (e.g., anesthetic confounds[1–3]). Here, we summarize these ICP studies, connect them to the clinical literature, and propose suggestions for improving ICP studies and ensuring translational relevance (Chapter 2). We found that tissue compliance occurs in multiple healthy rodent strains, in chronically hypertensive rodents, and in aged hypertensive rodents. This gives additional confidence in the relevance of this phenomenon to ICH patients, who are commonly hypertensive and elderly.

To test potential anesthetic confounds in our own research, we investigated the effect of isoflurane on collagenase-induced ICH by infusing collagenase in awake animals (Chapter 3).[3] Previous research has shown that isoflurane has many physiological effects[4] and can mask neuroprotective effects in stroke studies.[5] These results suggest that although isoflurane has many physiological effects, such as decreasing blood pressure and temperature, these effects are transient and resolve quickly. Despite isoflurane leading to decreased bleed volume in smaller ICHs, we did not see a significant effect of isoflurane after more severe ICH. Thus, these findings ensure that our subsequent experiments, which used a model with a large ICH, were not notably impacted by anesthesia.

Our study of glibenclamide (Chapter 4)[6] aimed to reduce mass effect by reducing edema formation after ICH. However, we did not find an effect of glibenclamide on edema, BBB permeability, or functional outcomes post-ICH. Blocking the Sur1-Trpm4 receptor is theorized to prevent cytotoxic edema, but is likely unable to prevent the serum-derived water influx after clot retraction.[7] This could be one explanation for the success of glibenclamide treatment after ischemic stroke, which causes vasogenic edema (vs. significant edema from clot retraction after an ICH[8]). Additionally, the new factor of tissue compliance raises the issue of treatment effects

beyond the area of injury. For example, if Sur1-Trpm4 is involved in cell volume regulation after large ICH,[9,10] then treatment with glibenclamide, despite being meant to reduce edema in the ipsilateral hemisphere, could have unintended effects on the contralateral hemisphere. Following the publication of Chapter 4, we assessed glibenclamide treatment following a more severe ICH model, since our current study resulted in a small hematoma with limited edema.[11] We found that glibenclamide still did not reduce edema, limit lesion volume, or improve behavioural outcomes. These results conflict with other studies of glibenclamide treatment after ICH,[12–14] and thus, a meta-analysis of these studies is warranted and currently underway (PROSPERO #CRD42021283614).

Chapters 5 and 6 aimed to determine if tissue compliance was possible in both young and aged chronically hypertensive rats. We found that young and aged SHRs were both capable of significantly decreasing contralateral brain volume in response to a large bleed. The contralateral hemisphere volume decreased by 5.3% in aged animals and 3.5% in young animals, on average. We found decreased cell volume and increased cell density in two regions, CA1 and S1, after ICH in three strains (SDRs, SHRs, and WKRs) and in young and aged SHRs. This indicates that tissue compliance is not limited to healthy animals, and that chronic hypertension does not prevent tissue compliance. Interestingly, when looking at the contralateral hemisphere, we found a reduction of approximately 50 mm^3 after an average hematoma volume of 100 μL plus associated edema. Thus, the contralateral hemisphere reductions alone account for 50% of the mass effect caused by the hematoma. As there is also tissue compliance in distal structures in the ipsilateral hemisphere, tissue compliance may account for a large portion of ICP compliance relative to CSF and blood reductions. Additionally, this experiment aimed to characterize differences in responses to collagenase-induced ICH, including functional deficits and hematoma volume. Interestingly, given the same amount of collagenase, aged rodents had significantly smaller bleed volumes, a lower mortality rate, but similar functional deficits as young rodents. This has implications for future studies, which

should be aware of these potential differences in the collagenase model with age. Future research should ensure a consistent hematoma volume between groups of different ages, either by adjusting the dose of collagenase or by using the whole blood model.

Our retrospective image analysis was a pilot study to determine the feasibility of using CT images to quantify brain volume in ICH patients. Unsurprisingly, we found large variability in brain volume, likely due to age-related atrophy, sex differences, and possibly disease related atrophy (e.g., amyloid angiopathy).[15,16] Despite this variability, we saw a significant decrease in total contralateral brain and CSF volume in patients with a large ICH vs a small ICH that was unlikely to raise ICP. This analysis was underpowered and missing many key variables (e.g., ICP measurements, a non-ICH control group), but is still a promising sign that tissue compliance may be occurring in people and that future studies may be able to assess tissue compliance in stroke studies using common imaging tools.

This thesis addresses multiple translational issues common in stroke research as well as gaps in the preclinical stroke literature. For example, the experiments in chapter 4 (involving pre-clinical tests of efficacy) were completely pre-planned in order to avoid bias in both experimental design and statistical analysis of results. We employed power calculations and used a large sample size (e.g., 16-18/group compared to the average sample size of 6/group[17]). We ensured the dose we used was clinically relevant, using osmotic pumps to ensure a consistent plasma level of glibenclamide, rather than intermittent injections. For the published Chapters (2-4), all data are published with the manuscripts for added transparency and to aid in reproducibility and future meta-analyses. Additional publications will similarly publish all data. In all of the preclinical studies, we used telemetry, a precise method of continuously measuring physiological parameters such as temperature, EEG, and ICP. This avoids the acute effects of anesthetic that are problematic in all surgical methods of measuring physiology. Most studies measuring ICP after stroke do so in anesthetized animals, which can confound the readings. Additionally, continuous measurements allow us to look at trends over the whole course of the

experiment, rather than relying on a few point measurements at pre-determined times (e.g., 1 hr period once post-ICH). Finally, we used both aged and hypertensive animals to better mimic the stroke patient population. This is rare in the stroke field, and there have not been any studies to date using both aged and hypertensive animals after ICH in the collagenase model. Although we could not address all translational issues in these chapters (e.g., replicating experiments across labs, investigating different ICH locations, etc.), we made many efforts to minimize bias (e.g., blinding and randomization when possible) and improve the odds of translation.[18]

8.2 Limitations

Despite the strengths of these studies, there are also limitations to consider. The major limitations that will be discussed include only investigating one co-morbidity, only using male rodents, not having a consistent ICH volume between the young and aged SHRs, and limited stereological analysis of cells. Although we examined the effect of chronic hypertension, a common co-morbidity in ICH patients, there are many other common co-morbidities that we did not assess. For example, many ICH patients are diabetic, which can influence stroke outcomes. Diabetic patients are more likely to both have an ICH and have a poor prognosis, including mortality, after ICH.[19,20] This may relate to impacts of diabetes on cell death (e.g., hyperglycemia worsening acidosis)[21] or the course of injury (e.g., impairing post-stroke plasticity,[22] augmenting blood flow dynamics[23]), and has the potential to affect tissue compliance. For example, diabetes is associated with increased plasma osmolality and sorbitol, a polyol osmolyte, is synthesized from glucose.[24] Hyperglycemia stimulates sorbitol absorption, resulting in cell swelling.[25,26] Therefore, it is possible that diabetic patients would have impairments in tissue compliance. Although it is not possible for any one study to address all co-morbidities and their interactions, due to the cost and experimental complexity, studies should still strive to include relevant co-morbidities where possible, and where findings in young,

healthy animals justify follow up experiments. Future studies should examine how other co-morbidities, such as diabetes, can influence ICP responses and tissue compliance.

Another limitation of these studies is that they only used male rodents. Despite the fact that approximately half of stroke patients are female, many preclinical studies rely on male subjects to test treatments. One reason for this is the actions of estrogen on clotting and neuroprotection, which can influence the collagenase model.[27–29] Further, in female rodents, estrogen levels fluctuate over the four-day cycle, adding to variability and potential confounds in experiments.[30,31] It is possible that tissue compliance may be affected by estrogen,[32,33] and therefore vary across the female cycle and across the lifespan. For instance, estrogen impacts osmotic cell volume regulation via receptor inhibition.[32] This could have implications for how ICP is managed across the lifespan in females, and thus, should be investigated. Regardless, these hormone effects and potential variability are clinically relevant, and should not prevent the inclusion of females in preclinical research. Researchers can control for the effects of estrogen by inducing ICH at the same point in the cycle, by using ovariectomized females (which better model post-menopausal stroke patients), or by measuring estrogen levels and statistically controlling for them. Our lab is currently studying tissue compliance responses in female rats and determining the effect of estrogen levels on outcomes in settings of high ICP after ICH.

Another major weakness of this work is the difference in lesion volume between the aged and young SHRs, which make age-based comparisons difficult. As discussed in Chapter 6, differences in outcomes can be attributed either to age or to differences in initial hematoma volume. Despite having a smaller hematoma volume, the aged animals experienced a greater decrease in brain volume (nearly 3× on a $mm^3/\mu L$ basis) relative to sham controls. Instead of giving the same amount of collagenase, as we did here, future studies might consider adjusting the collagenase dose in order to achieve consistent hematoma volumes between the groups. However, this comes with its own limitations and considerations. For example, differing amounts of collagenase could differentially affect inflammation in a dose-dependent manner. Additionally,

the number of animals needed to confidently determine the necessary dose is large, and minor differences in hematoma volume could go undetected if statistical power is low. Especially when using expensive and time-sensitive aged animal models, undergoing such extensive pilot work is difficult. Alternatively, researchers could assess outcomes using the whole blood model, which has little ongoing bleeding and thus is less likely to result in a hematoma volume difference. Indeed, this hematoma volume difference should be further explored to ascertain why aged animals experience less bleeding after the same dose of collagenase. It is possible that vasculature collagen levels differ,[34] leading to differential bleed volumes, or perhaps reduced collateral circulation in aged animals limits the ongoing bleeding.[35,36]

In this thesis, we only quantified the volume and density of neurons in two regions. This is a limitation, as tissue compliance has also been observed in astrocytes.[37] Neurons make up just 40% of brain cells,[38] and thus, only looking at neurons does not provide a full picture of what is happening within the parenchyma after ICH. Additionally, by only looking at two regions, we may be missing out on other regional differences. Although we assessed the contralateral hemisphere volume as an overall measurement of combined regional effects, it is still important to assess cell changes across regions. For example, in very distal regions, such as the cerebellum, or in smaller neuron populations, it is unknown if tissue compliance is occurring. Finding a region where tissue compliance does not occur could serve as a powerful internal control.

8.3 Future directions

Demonstrating that tissue compliance occurs in aged and hypertensive animals has helped lay the groundwork for clinical studies. As most ICH patients are hypertensive and elderly, these findings suggest that we will find evidence of tissue compliance in this population. Of course, there is still the possibility that tissue compliance is species specific to rodents. Thus, future studies should determine if tissue compliance is evident in patients after severe ICH. We

will be starting a small, prospective clinical trial to answer this research question. In this trial, we will enroll patients with large, life-threatening hematomas. Those that pass away from the ICH will undergo rapid autopsy, where brain samples will be processed from key regions of tissue compliance. Tissue will be processed for multiple endpoints, including electron microscopy, RNA analysis, and stereological analysis to assess tissue compliance. Brain images (CT or MRI) will be analyzed to determine brain hemisphere volume, and, where possible, quantify sub-region (e.g., hippocampus, caudate nucleus, etc.) volumes. Beyond this, we will also plan a larger retrospective study to expand on the findings in Chapter 7. Using existing ICH trial databases (e.g., VISTA-ICH), we can analyze large datasets with CT or MR imaging. Finding a marker of tissue compliance that can be assessed in ICH survivors is a key step in this research, as post-mortem studies are subject to mortality confounds. If we do not see tissue compliance in patients, this may indicate this phenomenon is specific to rodents. This would highlight a key weakness of the models that should be considered in studies of large ICH. However, it also highlights a potential area of treatment, and if this mechanism could be induced in patients, it may be a way to combat life threatening increases in ICP.

Discovering the mechanisms behind tissue compliance is another key research direction. We hypothesize that these rapid reductions in cell volume involve extrusion of ions (e.g., sodium and potassium) and osmolytes (e.g., taurine, methylamines, and polyols).[39–41] However, there are many channels involved in cell volume regulation, including volume-regulated anion channels, Na^+/H^+ exchangers, and Na^+ and Cl^- dependent taurine transporters.[42,43] Future studies will use x-ray fluorescence microscopy[44] to spatially quantify element concentrations while cells are reducing volume (6 hrs post-ICH) and when they are at their minimum volume (24 hrs post-ICH). Quantifying elements, such as K^+ and Cl^-, through the course of tissue compliance will help determine if cells are reducing their volume using osmotic mechanisms, and may also identify a contributor to the modest hypernatremia we observed in rodents. However, this hypernatremia has not been seen in pilot work in young, healthy animals,

and further research is needed on which rodent populations are hypernatremic after ICH and how this may relate to tissue compliance. Molecular analyses, such as microarray analysis of mRNA changes during tissue compliance, can help identify genes that are upregulated or downregulated and implicated in cell volume regulations in this setting. If we can identify a pharmacological intervention that either induces or blocks cell volume reductions, this would be a powerful tool to help study the short- and long-term effects of tissue compliance. Additionally, mechanistic knowledge can help find a treatment to either augment tissue compliance to reduce ICP, or to reduce potential harmful side effects of tissue compliance.

Another important future research direction is to determine the impact of tissue compliance on short- and long-term cellular function. Such drastic changes in neuronal volume over a short period of time may result in impairments to neuronal function, even after the volume has returned to normal. For example, a previous study has documented sub-lethal cellular damage, including mitochondrial damage.[37] Additionally, osmotic cell volume regulation mechanisms[24,45] may result in ion dyshomeostasis[44,46] and impaired ion concentration gradients, which may affect neuronal signalling. Potential movement or disconnection of synapses may also affect neuronal communication. Hypernatremia also results in neurological symptoms, including seizures and altered sensorium.[41] However, in ICH patients with hypernatremia, it is difficult to discern the effects of high blood sodium from the effects of the ICH.

Neurological impairments, including decreased consciousness and gaze abnormalities are well documented in acute severe ICH.[47,48] Many neurological impairments are believed to be due to diaschisis as well as distortion and physical damage of brain regions caused by increased intracranial pressure.[47] However, it is possible that drastic volume changes at the cellular level are also contributing to these neurological impairments, or underlying other stroke sequalae such as seizures[49] and diaschisis.[50] In surviving ICH patients, as edema decreases and ICP normalizes, many of these acute neurological symptoms resolve. This would also align with the documented timing of cell volume changes, where volume returns to normal levels by 7

days post-ICH. Interestingly, when neurological score is first measured at 3 hrs, before the peak ICP and edema would be expected, approximately half of patients will display neurological deterioration. This indicates an ongoing process, such as edema progression and tissue compliance, that may be contributing to acute neurological outcomes.[51] Additionally, late neurological deterioration has been associated with cases of late edema progression.[47,52] In these cases, it is likely that ICP would remain elevated, and cells may maintain a smaller volume until the mass effect is resolved. Therefore, the neurological deficits that are largely attributed to edema may also be caused in part by drastic cell volume changes. Future studies should assess the functional impact of tissue compliance, as it may be contributing to short- or long-term functional impairments. However, it is difficult to disentangle the functional impairments of ICH from those that may be caused by tissue compliance. For example, most cognitive assessments of hippocampal function are confounded by motor deficits after striatal ICH. We are currently planning studies to assess the feasibility of using motor evoked potentials as a measure of neuron function in primary motor cortex, which we can then assess before and after tissue compliance.

Although using aged and hypertensive animals is a good first step, further work is needed to fully model the clinical patient population. Beyond demographic variables that affect translation, animal models should be used to investigate the effects of medical management and current ICP treatments on tissue compliance. For example, hypertensive ICH patients may receive blood pressure lowering medications to prevent rebleeding,[53] whereas our hypertensive rats went untreated. Patients with elevated ICP may undergo surgical procedures (decompressive craniectomy or ventricular CSF drainage)[54–56] that might prevent tissue compliance or reduce the extent of cell volume reductions. Additionally, ICH patients with elevated ICP may receive treatment with mannitol or hypertonic saline,[57,58] which could exacerbate cell volume decreases in the contralateral hemisphere, potentially worsening any associated side effects (e.g., subcellular damage, functional impairments). If tissue compliance

is found in patients, it would be important to know how current treatments could be impacting this compliance mechanism. Currently, our lab is investigating how treatment with hypertonic saline after ICH affects ICP and tissue compliance in rodents.

8.4 Conclusions

These studies address a novel compliance mechanism in the setting of raised ICP. It challenges a centuries-old doctrine that states tissue volume is incompressible. The brain has many imperfect ways to compensate for the increased ICP, and one of these methods is tissue compliance, where neuron and astrocyte volumes are reduced and cells pack closer together, reducing the overall volume of the healthy brain hemisphere. Here, we review the causes and consequences of raised intracranial pressure, as well as the current treatment options. We investigated glibenclamide, one possible treatment to reduce edema, and found it was unsuccessful at reducing edema and mass effect after ICH. We then found that both hypertensive and aged animals were capable of tissue compliance, and had reduced contralateral hemisphere volumes after severe ICH. After the same dose of collagenase, young hypertensive animals had larger hematoma volumes, significantly higher ICP, and higher mortality rates. Further research should explore the potential side effects of tissue compliance, including impaired neuronal function, and explore tissue compliance in a clinical setting. Exploring tissue compliance may help improve our understanding of impairments in consciousness, limitations of existing treatment options, and help discover novel treatment options (e.g., pharmacologically augmenting or inhibiting ion channels involved in volume regulation).[59] Although this research is focused on severe ICH, this research has applications in other disease-states with mass effect and raised ICP, including ischemic stroke, brain tumours, and traumatic brain injury.

8.5 References

1. Gigante PR, Appelboom G, Hwang BY, et al. Isoflurane preconditioning affords functional neuroprotection in a murine model of intracerebral hemorrhage. In: *Acta Neurochirurgica, Supplementum*. ; 2011. doi:10.1007/978-3-7091-0693-8_23
2. Bar-Klein G, Klee R, Brandt C, et al. Isoflurane prevents acquired epilepsy in rat models of temporal lobe epilepsy. *Ann Neurol*. 2016;80(6):896-908. doi:10.1002/ana.24804
3. Wilkinson CM, Kalisvaart ACJ, Kung TFC, et al. The collagenase model of intracerebral hemorrhage in awake, freely moving animals: The effects of isoflurane. *Brain Res*. 2020;1728:146593. doi:10.1016/j.brainres.2019.146593
4. Yang CF, Yu-Chih Chen M, Chen TI, Cheng CF. Dose-dependent effects of isoflurane on cardiovascular function in rats. *Tzu Chi Med J*. Published online 2014. doi:10.1016/j.tcmj.2014.07.005
5. Seto A, Taylor S, Trudeau D, et al. Induction of ischemic stroke in awake freely moving mice reveals that isoflurane anesthesia can mask the benefits of a neuroprotection therapy. *Front Neuroenergetics*. 2014;6(APR). doi:10.3389/fnene.2014.00001
6. Wilkinson CM, Brar PS, Balay CJ, Colbourne F. Glibenclamide, a Sur1-Trpm4 antagonist, does not improve outcome after collagenase-induced intracerebral hemorrhage. *PLoS One*. 2019;14(5). doi:10.1371/journal.pone.0215952
7. Hoff JT, Xi G. Brain edema from intracerebral hemorrhage. *Acta Neurochir Suppl*. 2003;(86):11-15. doi:10.1007/978-3-7091-0651-8_3
8. Simard JM, Sheth KN, Kimberly WT, et al. Glibenclamide in cerebral ischemia and stroke. *Neurocrit Care*. 2014;20(2):319-333. doi:10.1007/s12028-013-9923-1
9. Stokum JA, Kwon MS, Woo SK, et al. SUR1-TRPM4 and AQP4 form a heteromultimeric complex that amplifies ion/water osmotic coupling and drives astrocyte swelling. *Glia*. 2018;66(1):108-125. doi:10.1002/glia.23231
10. Woo SK, Tsymbalyuk N, Tsymbalyuk O, Ivanova S, Gerzanich V, Simard JM. SUR1-

TRPM4 channels, not KATP, mediate brain swelling following cerebral ischemia. *Neurosci Lett*. 2020;718. doi:10.1016/j.neulet.2019.134729

11. Kung TFC, Wilkinson CM, Dirks CA, Jickling GC, Colbourne F. Glibenclamide does not improve outcome following severe collagenase-induced intracerebral hemorrhage in rats. *PLoS One*. 2021;16(6 June). doi:10.1371/journal.pone.0252584
12. Jiang B, Li L, Chen Q, et al. Role of Glibenclamide in Brain Injury After Intracerebral Hemorrhage. *Transl Stroke Res*. 2017;8(2):183-193. doi:10.1007/s12975-016-0506-2
13. Jiang B, Zhang Y, Wang Y, et al. Glibenclamide Attenuates Neuroinflammation and Promotes Neurological Recovery After Intracerebral Hemorrhage in Aged Rats. *Front Aging Neurosci*. 2021;13. doi:10.3389/fnagi.2021.729652
14. Xu F, Shen G, Su Z, He Z, Yuan L. Glibenclamide ameliorates the disrupted blood–brain barrier in experimental intracerebral hemorrhage by inhibiting the activation of NLRP3 inflammasome. *Brain Behav*. 2019;9(4). doi:10.1002/brb3.1254
15. Akiyama H, Meyer JS, Mortel KF, Yasuo Terayama, Thornby JI, Konno S. Normal human aging: Factors contributing to cerebral atrophy. *J Neurol Sci*. 1997;152(1):39-49. doi:10.1016/S0022-510X(97)00141-X
16. Gur RC, Gunning-Dixon FM, Turetsky BI, Bilker WB, Gur RE. Brain region and sex differences in age association with brain volume: A quantitative MRI study of healthy young adults. *Am J Geriatr Psychiatry*. 2002;10(1):72-80. doi:10.1097/00019442-200201000-00009
17. Liddle LJ, Ralhan S, Ward DL, Colbourne F. Translational Intracerebral Hemorrhage Research: Has Current Neuroprotection Research ARRIVEd at a Standard for Experimental Design and Reporting? *Transl Stroke Res*. 2020;11(6):1203-1213. doi:10.1007/s12975-020-00824-x
18. Seyhan AA. Lost in translation: the valley of death across preclinical and clinical divide – identification of problems and overcoming obstacles. *Transl Med Commun*. 2019;4(1).

doi:10.1186/s41231-019-0050-7

19. Boulanger M, Poon MTC, Wild SH, Al-Shahi Salman R. Association between diabetes mellitus and the occurrence and outcome of intracerebral hemorrhage. *Neurology*. 2016;87(9):870-878. doi:10.1212/WNL.0000000000003031
20. Passero S, Ciacci G, Ulivelli M. The influence of diabetes and hyperglycemia on clinical course after intracerebral hemorrhage. *Neurology*. 2003;61(10):1351-1356. doi:10.1212/01.WNL.0000094326.30791.2D
21. Song EC, Chu K, Jeong SW, et al. Hyperglycemia exacerbates brain edema and perihematomal cell death after intracerebral hemorrhage. *Stroke*. 2003;34(9):2215-2220. doi:10.1161/01.STR.0000088060.83709.2C
22. Sweetnam D, Holmes A, Tennant KA, et al. Diabetes impairs cortical plasticity and functional recovery following Ischemic stroke. *J Neurosci*. 2012;32(15):5132-5143. doi:10.1523/JNEUROSCI.5075-11.2012
23. Tennant KA, Brown CE. Diabetes augments in vivo microvascular blood flow dynamics after stroke. *J Neurosci*. 2013;33(49):19194-19204. doi:10.1523/JNEUROSCI.3513-13.2013
24. McManus ML, Churchwell KB, Strange K. Regulation of Cell Volume in Health and Disease. *N Engl J Med*. 1995;333(19):1260-1267. doi:10.1056/nejm199511093331906
25. Burg MB, Kador PF. Sorbitol, osmoregulation, and the complications of diabetes. *J Clin Invest*. 1988;81(3):635-640. doi:10.1172/JCI113366
26. Lang F. Mechanisms and Significance of Cell Volume Regulation. *J Am Coll Nutr*. 2007;26:613S-623S. doi:10.1080/07315724.2007.10719667
27. Auriat A, Plahta WC, McGie SC, Yan R, Colbourne F. 17β-Estradiol pretreatment reduces bleeding and brain injury after intracerebral hemorrhagic stroke in male rats. *J Cereb Blood Flow Metab*. 2005;25(2):247-256. doi:10.1038/sj.jcbfm.9600026
28. Zheng Y, Hu Q, Manaenko A, et al. 17B-Estradiol Attenuates Hematoma Expansion

Through Estrogen Receptor A/Silent Information Regulator 1/Nuclear Factor-Kappa B Pathway in Hyperglycemic Intracerebral Hemorrhage Mice. *Stroke*. 2015;46(2):485-491. doi:10.1161/STROKEAHA.114.006372

29. Brann DW, Dhandapani K, Wakade C, Mahesh VB, Khan MM. Neurotrophic and neuroprotective actions of estrogen: Basic mechanisms and clinical implications. *Steroids*. 2007;72(5):381-405. doi:10.1016/j.steroids.2007.02.003
30. Murphy SJ, McCullough LD, Smith JM. Stroke in the female: role of biological sex and estrogen. *ILAR J*. 2004;45(2):147-159. doi:10.1093/ilar.45.2.147
31. Goldman JM, Murr AS, Cooper RL. The rodent estrous cycle: Characterization of vaginal cytology and its utility in toxicological studies. *Birth Defects Res Part B - Dev Reprod Toxicol*. 2007;80(2):84-97. doi:10.1002/bdrb.20106
32. Ayus JC, Achinger SG, Arieff A. Brain cell volume regulation in hyponatremia: Role of sex, age, vasopressin, and hypoxia. *Am J Physiol - Ren Physiol*. 2008;295(3). doi:10.1152/ajprenal.00502.2007
33. Shahrokhi N, Khaksari M, Soltani Z, Mahmoodi M, Nakhaee N. Effect of sex steroid hormones on brain edema, intracranial pressure, and neurologic outcomes after traumatic brain injury. *Can J Physiol Pharmacol*. 2010;88(4):414-421. doi:10.1139/Y09-126
34. Takubo Y, Hirai T, Muro S, Kogishi K, Hosokawa M, Mishima M. Age-associated changes in elastin and collagen content and the proportion of types I and III collagen in the lungs of mice. *Exp Gerontol*. 1999;34(3):353-364. doi:10.1016/S0531-5565(99)00017-0
35. Liebeskind DS. Collateral circulation. *Stroke*. 2003;34(9):2279-2284. doi:10.1161/01.STR.0000086465.41263.06
36. Agarwal S, Scoffings DJ, Simon Jones P, et al. Interaction of age with the ischaemic penumbra, leptomeningeal collateral circulation and haemodynamic variables in acute stroke: A pilot study. *J Neurol Neurosurg Psychiatry*. 2013;84(3):271-276.

doi:10.1136/jnnp-2012-303258

37. Kalisvaart ACJ, Wilkinson CM, Gu S, et al. An update to the Monro–Kellie doctrine to reflect tissue compliance after severe ischemic and hemorrhagic stroke. *Sci Rep*. 2020;10(1). doi:10.1038/s41598-020-78880-4

38. von Bartheld CS, Bahney J, Herculano-Houzel S. The search for true numbers of neurons and glial cells in the human brain: A review of 150 years of cell counting. *J Comp Neurol*. 2016;524(18):3865-3895. doi:10.1002/cne.24040

39. Inoue H, Mori SI, Morishima S, Okada Y. Volume-sensitive chloride channels in mouse cortical neurons: Characterization and role in volume regulation. *Eur J Neurosci*. 2005;21(6):1648-1658. doi:10.1111/j.1460-9568.2005.04006.x

40. Vereninov IA, Yurinskaya VE, Vereninov AA. Commentary: How Cells Can Control Their Size by Pumping Ions. *Front Cell Dev Biol*. 2017;5(May):1-14. doi:10.3389/fcell.2017.00072

41. Aiyagari V, Deibert E, Diringer MN. Hypernatremia in the neurologic intensive care unit: how high is too high? *J Crit Care*. 2006;21(2):163-172. doi:10.1016/j.jcrc.2005.10.002

42. Qiu Z, Dubin AE, Mathur J, et al. SWELL1, a plasma membrane protein, is an essential component of volume-regulated anion channel. *Cell*. 2014;157(2):447-458. doi:10.1016/j.cell.2014.03.024

43. Jentsch TJ. VRACs and other ion channels and transporters in the regulation of cell volume and beyond. *Nat Rev Mol Cell Biol*. 2016;17(5):293-307. doi:10.1038/nrm.2016.29

44. Nadeau CA, Dietrich K, Wilkinson CM, et al. Prolonged Blood-Brain Barrier Injury Occurs After Experimental Intracerebral Hemorrhage and Is Not Acutely Associated with Additional Bleeding. *Transl Stroke Res*. 2019;10(3):287-297. doi:10.1007/s12975-018-0636-9

45. Pasantes-Morales H, Cruz-Rangel S. Brain volume regulation: Osmolytes and aquaporin

perspectives. *Neuroscience*. 2010;168(4):871-884. doi:10.1016/j.neuroscience.2009.11.074

46. Williamson MR, Dietrich K, Hackett MJ, et al. Rehabilitation Augments Hematoma Clearance and Attenuates Oxidative Injury and Ion Dyshomeostasis After Brain Hemorrhage. *Stroke*. 2016;48(1):195-203. doi:10.1161/STROKEAHA.116.015404
47. Qureshi AI, Tuhrim S, Broderick JP, Batjer HH, Hondo H, Hanley DF. Spontaneous Intracerebral Hemorrhage. *N Engl J Med*. 2001;344(19):1450-1460. doi:10.1056/NEJM200105103441907
48. Hemphill JC, Bonovich DC, Besmertis L, Manley GT, Johnston SC. The ICH score: A simple, reliable grading scale for intracerebral hemorrhage. *Stroke*. 2001;32(4):891-896. doi:10.1161/01.str.32.4.891
49. Vespa PM, O'Phelan K, Shah M, et al. Acute seizures after intracerebral hemorrhage: A factor in progressive midline shift and outcome. *Neurology*. 2003;60(9):1441-1446. doi:10.1212/01.WNL.0000063316.47591.B4
50. Seitz RJ, Azari NP, Knorr U, Binkofski F, Herzog H, Freund HJ. The role of diaschisis in stroke recovery. *Stroke*. 1999;30(9):1844-1850. doi:10.1161/01.STR.30.9.1844
51. Lord AS, Gilmore E, Choi HA, et al. Time course and predictors of neurological deterioration after intracerebral hemorrhage. *Stroke*. 2015;46(3):647-652. doi:10.1161/STROKEAHA.114.007704
52. Mayer SA, Sacco RL, Shi T, Mohr JP. Neurologic deterioration in noncomatose patients with supratentorial intracerebral hemorrhage. *Neurology*. 1994;44(8):1379-1384. doi:10.1212/wnl.44.8.1379
53. Shoamanesh A, Patrice Lindsay M, Castellucci LA, et al. Canadian stroke best practice recommendations: Management of Spontaneous Intracerebral Hemorrhage, 7th Edition Update 2020. *Int J Stroke*. 2021;16(3):321-341. doi:10.1177/1747493020968424
54. Marinkovic I, Strbian D, Pedrono E, et al. Decompressive craniectomy for intracerebral

hemorrhage. *Neurosurgery*. 2009;65(4):780-786. doi:10.1227/01.NEU.0000351775.30702.A9

55. Maira G, Anile C, Colosimo C, Rossi GF. Surgical treatment of primary supratentorial intracerebral hemorrhage in stuporous and comatose patients. *Neurol Res*. 2002;24(1):54-60. doi:10.1179/016164102101199549

56. Freeman WD. Management of intracranial pressure. *Contin Lifelong Learn Neurol*. 2015;21(5):1299-1323. doi:10.1212/CON.0000000000000235

57. Wilkinson CM, Kung TFC, Jickling GC, Colbourne F. A translational perspective on intracranial pressure responses following intracerebral hemorrhage in animal models. *Brain Hemorrhages*. 2021;2(1):34-48. doi:10.1016/j.hest.2020.10.002

58. Dastur CK, Yu W. Current management of spontaneous intracerebral haemorrhage. *Stroke Vasc Neurol*. 2017;2(1):21-29. doi:10.1136/svn-2016-000047

59. Song M, Yu SP. Ionic Regulation of Cell Volume Changes and Cell Death after Ischemic Stroke. *Transl Stroke Res*. 2014;5(1):17-27. doi:10.1007/s12975-013-0314-x

Bibliography

Chapter 1

1. Barber PA, Zhang J, Demchuk AM, Hill MD, Buchan AM. Why are stroke patients excluded from TPA therapy? An analysis of patient eligibility. *Neurology*. 2001;56(8):1015-1020. doi:10.1212/WNL.56.8.1015
2. Avan A, Hachinski V. Stroke and dementia, leading causes of neurological disability and death, potential for prevention. *Alzheimer's Dement*. 2021;17(6):1072-1076. doi:10.1002/alz.12340
3. Kapral MK, Fang J, Hill MD, et al. Sex differences in stroke care and outcomes: Results from the Registry of the Canadian Stroke Network. *Stroke*. 2005;36(4):809-814. doi:10.1161/01.STR.0000157662.09551.e5
4. Wu H, Wu T, Xu X, Wang J, Wang J. Iron toxicity in mice with collagenase-induced intracerebral hemorrhage. *J Cereb Blood Flow Metab*. 2011;31(5):1243-1250. doi:10.1038/jcbfm.2010.209
5. Duan X, Wen Z, Shen H, Shen M, Chen G. Intracerebral Hemorrhage, Oxidative Stress, and Antioxidant Therapy. *Oxid Med Cell Longev*. 2016;2016:1203285. doi:10.1155/2016/1203285
6. Nadeau CA, Dietrich K, Wilkinson CM, et al. Prolonged Blood-Brain Barrier Injury Occurs After Experimental Intracerebral Hemorrhage and Is Not Acutely Associated with Additional Bleeding. *Transl Stroke Res*. 2019;10(3):287-297. doi:10.1007/s12975-018-0636-9
7. Hemphill JC, Greenberg SM, Anderson CS, et al. Guidelines for the Management of Spontaneous Intracerebral Hemorrhage: A Guideline for Healthcare Professionals from the American Heart Association/American Stroke Association. *Stroke*. 2015;46(7):2032-2060. doi:10.1161/STR.0000000000000069
8. Boulanger JM, Lindsay MP, Gubitz G, et al. Canadian Stroke Best Practice

Recommendations for Acute Stroke Management: Prehospital, Emergency Department, and Acute Inpatient Stroke Care, 6th Edition, Update 2018. *Int J Stroke*. 2018;13(9):949-984. doi:10.1177/1747493018786616

9. Shoamanesh A, Patrice Lindsay M, Castellucci LA, et al. Canadian stroke best practice recommendations: Management of Spontaneous Intracerebral Hemorrhage, 7th Edition Update 2020. *Int J Stroke*. 2021;16(3):321-341. doi:10.1177/1747493020968424
10. Matsukawa H, Shinoda M, Fujii M, et al. Factors associated with lobar vs. non-lobar intracerebral hemorrhage. *Acta Neurol Scand*. 2012;126(2):116-121. doi:10.1111/j.1600-0404.2011.01615.x
11. Sutherland GR, Auer RN. Primary intracerebral hemorrhage. *J Clin Neurosci*. 2006;13(5):511-517. doi:10.1016/j.jocn.2004.12.012
12. Mehndiratta P, Manjila S, Ostergard T, et al. Cerebral amyloid angiopathy-associated intracerebral hemorrhage: Pathology and management. *Neurosurg Focus*. 2012;32(4). doi:10.3171/2012.1.FOCUS11370
13. Yamada M. Cerebral amyloid angiopathy: Emerging concepts. *J Stroke*. 2015;17(1):17-30. doi:10.5853/jos.2015.17.1.17
14. Boehme AK, Esenwa C, Elkind MSV. Stroke Risk Factors, Genetics, and Prevention. *Circ Res*. 2017;120(3):472-495. doi:10.1161/CIRCRESAHA.116.308398
15. Ariesen MJ, Claus SP, Rinkel GJE, Algra A. Risk factors for intracerebral hemorrhage in the general population: A systematic review. *Stroke*. 2003;34(8):2060-2065. doi:10.1161/01.STR.0000080678.09344.8D
16. Roy-O'Reilly M, McCullough LD. Age and sex are critical factors in ischemic stroke pathology. *Endocrinology*. 2018;159(8):3120-3131. doi:10.1210/en.2018-00465
17. Li C, Baek J, Sanchez BN, Morgenstern LB, Lisabeth LD. Temporal trends in age at ischemic stroke onset by ethnicity. *Ann Epidemiol*. 2018;28(10):686-690.e2. doi:10.1016/j.annepidem.2018.07.010

18. An SJ, Kim TJ, Yoon BW. Epidemiology, risk factors, and clinical features of intracerebral hemorrhage: An update. *J Stroke*. 2017;19(1):3-10. doi:10.5853/jos.2016.00864

19. Ikram MA, Wieberdink RG, Koudstaal PJ. International epidemiology of intracerebral hemorrhage. *Curr Atheroscler Rep*. 2012;14(4):300-306. doi:10.1007/s11883-012-0252-1

20. Arboix A, Vall-Llosera A, García-Eroles L, Massons J, Oliveres M, Targa C. Clinical features and functional outcome of intracerebral hemorrhage in patients aged 85 and older. *J Am Geriatr Soc*. 2002;50(3):449-454. doi:10.1046/j.1532-5415.2002.50109.x

21. Mogensen UB, Olsen TS, Andersen KK, Gerds TA. Cause-specific mortality after stroke: Relation to age, sex, stroke severity, and risk factors in a 10-year follow-up study. *J Stroke Cerebrovasc Dis*. 2013;22(7). doi:10.1016/j.jstrokecerebrovasdis.2012.04.006

22. Oyama N, Gona P, Salton CJ, et al. Differential impact of age, sex, and hypertension on aortic atherosclerosis: The Framingham heart study. *Arterioscler Thromb Vasc Biol*. 2008;28(1):155-159. doi:10.1161/ATVBAHA.107.153544

23. Dandapani BK, Suzuki S, Kelley RE, Reyes-Iglesias Y, Duncan RC. Relation between blood pressure and outcome in intracerebral hemorrhage. *Stroke*. 1995;26(1):21-24. doi:10.1161/01.STR.26.1.21

24. Sacco RL, Boden-Albala B, Abel G, et al. Race-ethnic disparities in the impact of stroke risk factors the Northern Manhattan stroke study. *Stroke*. 2001;32(8):1725-1731. doi:10.1161/01.STR.32.8.1725

25. Carson AP, Howard G, Burke GL, Shea S, Levitan EB, Muntner P. Ethnic differences in hypertension incidence among middle-aged and older adults: The multi-ethnic study of atherosclerosis. *Hypertension*. 2011;57(6):1101-1107. doi:10.1161/HYPERTENSIONAHA.110.168005

26. Tsai MH, Kuo CW, Lin TK, et al. Ischemic Stroke Risk Associated with Mitochondrial Haplogroup F in the Asian Population. *Cells*. 2020;9(8). doi:10.3390/cells9081885

27. Wu J cang, Zhang X, Wang J hao, et al. Gene polymorphisms and circulating levels of

the TNF-alpha are associated with ischemic stroke: A meta-analysis based on 19,873 individuals. *Int Immunopharmacol*. 2019;75. doi:10.1016/j.intimp.2019.105827

28. Krishnan K, Beishon L, Berge E, et al. Relationship between race and outcome in Asian, Black, and Caucasian patients with spontaneous intracerebral hemorrhage: Data from the Virtual International Stroke Trials Archive and Efficacy of Nitric Oxide in Stroke trial. *Int J Stroke*. 2018;13(4):362-373. doi:10.1177/1747493017744463
29. Hurn PD, Brass LM. Estrogen and stroke: A balanced analysis. *Stroke*. 2003;34(2):338-341. doi:10.1161/01.STR.0000054051.88378.25
30. Howard VJ, Madsen TE, Kleindorfer DO, et al. Sex and Race Differences in the Association of Incident Ischemic Stroke with Risk Factors. *JAMA Neurol*. 2019;76(2):179-186. doi:10.1001/jamaneurol.2018.3862
31. Caso V, Paciaroni M, Agnelli G, et al. Gender differences in patients with acute ischemic stroke. *Womens Health (Lond Engl)*. 2010;6(1):51-57. doi:10.2217/whe.09.82
32. Zia E, Pessah-Rasmussen H, Khan FA, et al. Risk factors for primary intracerebral hemorrhage: A population-based nested case-control study. *Cerebrovasc Dis*. 2006;21(1-2):18-25. doi:10.1159/000089589
33. Chen CJ, DIng D, Ironside N, et al. Cigarette smoking history and functional outcomes after spontaneous intracerebral hemorrhage. *Stroke*. 2019;50(3):588-594. doi:10.1161/STROKEAHA.118.023580
34. Juvela S, Hillbom M, Palomaki H. Risk factors for spontaneous intracerebral hemorrhage. *Stroke*. 1995;26(9):1558-1564. doi:10.1161/01.STR.26.9.1558
35. Chen CJ, Brown WM, Moomaw CJ, et al. Alcohol use and risk of intracerebral hemorrhage. *Neurology*. 2017;88(21):2043-2051. doi:10.1212/WNL.0000000000003952
36. Choudhury MJH, Chowdhury MTI, Nayeem A, Jahan WA. Modifiable and Non-Modifiable Risk Factors of Stroke: A Review Update. *J Natl Inst Neurosci Bangladesh*. 2015;1(1):22-26. doi:10.3329/jninb.v1i1.22944

37. Larsson SC, Wallin A, Wolk A, Markus HS. Differing association of alcohol consumption with different stroke types: A systematic review and meta-analysis. *BMC Med*. 2016;14(1). doi:10.1186/s12916-016-0721-4

38. Daviet R, Aydogan G, Jagannathan K, et al. Associations between alcohol consumption and gray and white matter volumes in the UK Biobank. *Nat Commun*. 2022;13(1):1-11. doi:10.1038/s41467-022-28735-5

39. Hu G, Sarti C, Jousilahti P, Silventoinen K, Barengo NC, Tuomilehto J. Leisure time, occupational, and commuting physical activity and the risk of stroke. *Stroke*. 2005;36(9):1994-1999. doi:10.1161/01.STR.0000177868.89946.0c

40. Brott T, Thalinger K, Hertzberg V. Hypertension as a risk factor for spontaneous intracerebral hemorrhage. *Stroke*. 1986;17(6):1078-1083. doi:10.1161/01.STR.17.6.1078

41. de Oliveira Manoel AL, Goffi A, Zampieri FG, et al. The critical care management of spontaneous intracranial hemorrhage: A contemporary review. *Crit Care*. 2016;20(1). doi:10.1186/s13054-016-1432-0

42. Egan BM. Adaptive changes in cerebral blood vessels during chronic hypertension. *J Hypertens*. 1992;10(4):399. doi:10.1097/00004872-199204000-00013

43. Hart MN, Heistad DD, Brody MJ. Effect of chronic hypertension and sympathetic denervation on wall/lumen ratio of cerebral vessels. *Hypertension*. 1980;2(4):419-423. doi:10.1161/01.HYP.2.4.419

44. Cipolla MJ, Liebeskind DS, Chan SL. The importance of comorbidities in ischemic stroke: Impact of hypertension on the cerebral circulation. *J Cereb Blood Flow Metab*. 2018;38(12):2129-2149. doi:10.1177/0271678X18800589

45. Webb AJ, Fischer U, Mehta Z, Rothwell PM. Effects of antihypertensive-drug class on interindividual variation in blood pressure and risk of stroke: a systematic review and meta-analysis. *Lancet*. 2010;375(9718):906-915. doi:10.1016/S0140-6736(10)60235-8

46. Amarenco P, Lavallée PC, Labreuche J, Touboul PJ. Stroke prevention, blood cholesterol

and statins. *Acta Neurol Taiwan*. 2018;27(4):90-106. doi:10.1201/b14239-8

47. Cui Q, Naikoo NA. Modifiable and non-modifiable risk factors in ischemic stroke: A meta-analy. *Afr Health Sci*. 2019;19(2):2121-2129. doi:10.4314/ahs.v19i2.36

48. Lind M, Fahlén M, Kosiborod M, Eliasson B, Odén A. Variability of INR and its relationship with mortality, stroke, bleeding and hospitalisations in patients with atrial fibrillation. *Thromb Res*. 2012;129(1):32-35. doi:10.1016/j.thromres.2011.07.004

49. Waddy SP. Disorders of coagulation in stroke. *Semin Neurol*. 2006;26(1):57-64. doi:10.1055/s-2006-933309

50. Odén A, Fahlén M, Hart RG. Optimal INR for prevention of stroke and death in atrial fibrillation: A critical appraisal. *Thromb Res*. 2006;117(5):493-499. doi:10.1016/j.thromres.2004.11.025

51. Xu Y, Gomes T, Mamdani M, et al. Direct Oral Anticoagulant- or Warfarin-Related Major Bleeding: Characteristics, Reversal Strategies, and Outcomes From a Multicenter Observational Study. *Chest*. 2017;152(1):81-91. doi:10.1016/j.chest.2017.02.009

52. Connolly SJ, Ezekowitz MD, Yusuf S, et al. Dabigatran versus Warfarin in Patients with Atrial Fibrillation. *N Engl J Med*. 2009;361(12):1139-1151. doi:10.1056/nejmoa0905561

53. Schols AMR, Schreuder FHBM, Van Raak EPM, et al. Incidence of oral anticoagulant-associated intracerebral hemorrhage in the Netherlands. *Stroke*. 2014;45(1):268-270. doi:10.1161/Strokeaha.113.003003

54. Flaherty ML, Kissela B, Woo D, et al. The increasing incidence of anticoagulant-associated intracerebral hemorrhage. *Neurology*. 2007;68(2):116-121. doi:10.1212/01.wnl.0000250340.05202.8b

55. Kurogi R, Nishimura K, Nakai M, et al. Comparing intracerebral hemorrhages associated with direct oral anticoagulants or warfarin. *Neurology*. 2018;90(13):e1143-e1149. doi:10.1212/WNL.0000000000005207

56. Hemphill JC, Greenberg SM, Anderson CS, et al. Guidelines for the Management of

Spontaneous Intracerebral Hemorrhage: A Guideline for Healthcare Professionals from the American Heart Association/American Stroke Association. *Stroke*. 2015;46(7):2032-2060. doi:10.1161/STR.0000000000000069

57. Fernando SM, Qureshi D, Talarico R, et al. Intracerebral Hemorrhage Incidence, Mortality, and Association with Oral Anticoagulation Use: A Population Study. *Stroke*. Published online 2021:1673-1681. doi:10.1161/STROKEAHA.120.032550

58. Cheung RTF, Zou LY. Use of the original, modified, or new intracerebral hemorrhage score to predict mortality and morbidity after intracerebral hemorrhage. *Stroke*. 2003;34(7):1717-1722. doi:10.1161/01.STR.0000078657.22835.B9

59. Broderick JP, Brott TG, Duldner JE, Tomsick T, Huster G. Volume of intracerebral hemorrhage: A powerful and easy-to-use predictor of 30-day mortality. *Stroke*. 1993;24(7):987-993. doi:10.1161/01.STR.24.7.987

60. Lopresti MA, Bruce SS, Camacho E, et al. Hematoma volume as the major determinant of outcomes after intracerebral hemorrhage. *J Neurol Sci*. 2014;345(1):3-7. doi:10.1016/j.jns.2014.06.057

61. Fujii Y, Takeuchi S, Sasaki O, Minakawa T, Tanaka R. Multivariate analysis of predictors of hematoma enlargement in spontaneous intracerebral hemorrhage. *Stroke*. 1998;29(6):1160-1166. doi:10.1161/01.STR.29.6.1160

62. Specogna A V., Patten SB, Turin TC, Hill MD. Cost of spontaneous intracerebral hemorrhage in Canada during 1 decade. *Stroke*. 2014;45(1):284-286. doi:10.1161/Strokeaha.113.003276

63. Fallenius M, Skrifvars MB, Reinikainen M, et al. Spontaneous Intracerebral Hemorrhage: Factors Predicting Long-Term Mortality after Intensive Care. *Stroke*. 2019;50(9):2336-2343. doi:10.1161/STROKEAHA.118.024560

64. Tuhrim S, Horowitz DR, Sacher M, Godbold JH. Volume of ventricular blood is an important determinant of outcome in supratentorial intracerebral hemorrhage. *Crit Care*

Med. 1999;27(3):617-621. doi:10.1097/00003246-199903000-00045

65. Diringer MN. Intracerebral hemorrhage: Pathophysiology and management. *Crit Care Med*. 1993;21(10):1591-1603. doi:10.1097/00003246-199310000-00032

66. Thiex R, Tsirka SE. Brain edema after intracerebral hemorrhage: mechanisms, treatment options, management strategies, and operative indications. *Neurosurg Focus*. 2007;22(5):E6. doi:10.3171/foc.2007.22.5.7

67. Caliaperumal J, Ma Y, Colbourne F. Intra-parenchymal ferrous iron infusion causes neuronal atrophy, cell death and progressive tissue loss: Implications for intracerebral hemorrhage. *Exp Neurol*. 2012;237(2):363-369. doi:10.1016/j.expneurol.2012.07.001

68. Nguyen AP, Huynh HD, Sjovold SB, Colbourne F. Progressive brain damage and alterations in dendritic arborization after collagenase-induced intracerebral hemorrhage in rats. *Curr Neurovasc Res*. 2008;5(3):171-177. doi:10.2174/156720208785425710

69. Fulda S, Gorman AM, Hori O, Samali A. Cellular stress responses: Cell survival and cell death. *Int J Cell Biol*. Published online 2010. doi:10.1155/2010/214074

70. Felberg RA, Grotta JC, Shirzadi AL, et al. Cell death in experimental intracerebral hemorrhage: The “black hole” model of hemorrhagic damage. *Ann Neurol*. 2002;51(4):517-524. doi:10.1002/ana.10160

71. Caliaperumal J, Brodie S, Ma Y, Colbourne F. Thrombin Causes Neuronal Atrophy and Acute but not Chronic Cell Death. *Can J Neurol Sci*. 2014;41(6):714-720. doi:10.1017/cjn.2014.105

72. Sinar EJ, Mendelow AD, Graham DI, Teasdale GM. Experimental intracerebral hemorrhage: Effects of a temporary mass lesion. *J Neurosurg*. 1987;66(4):568-576. doi:10.3171/jns.1987.66.4.0568

73. Tsivgoulis G, Katsanos AH, Butcher KS, et al. Intensive blood pressure reduction in acute intracerebral hemorrhage: A meta-analysis. *Neurology*. 2014;83(17):1523-1529. doi:10.1212/WNL.0000000000000917

74. Qureshi AI, Palesch YY, Martin R, et al. Effect of systolic blood pressure reduction on hematoma expansion, perihematomal edema, and 3-month outcome among patients with intracerebral hemorrhage: Results from the antihypertensive treatment of acute cerebral hemorrhage study. *Arch Neurol*. 2010;67(5):570-576. doi:10.1001/archneurol.2010.61

75. Goldstein JN, Thomas SH, Frontiero V, et al. Timing of fresh frozen plasma administration and rapid correction of coagulopathy in warfarin-related intracerebral hemorrhage. *Stroke*. 2006;37(1):151-155. doi:10.1161/01.STR.0000195047.21562.23

76. Steiner T, Rosand J, Diringer M. Intracerebral hemorrhage associated with oral anticoagulant therapy: Current practices and unresolved questions. *Stroke*. 2006;37(1):256-262. doi:10.1161/01.STR.0000196989.09900.f8

77. Kirshner H, Schrag M. Management of Intracerebral Hemorrhage: Update and Future Therapies. *Curr Neurol Neurosci Rep*. 2021;21(10). doi:10.1007/s11910-021-01144-9

78. Diringer MN, Skolnick BE, Mayer SA, et al. Thromboembolic events with recombinant activated factor vii in spontaneous intracerebral hemorrhage: Results from the factor seven for acute hemorrhagic stroke (FAST) trial. *Stroke*. 2010;41(1):48-53. doi:10.1161/STROKEAHA.109.561712

79. Mayer SA. Recombinant activated factor VII for acute intracerebral hemorrhage. In: *Stroke*. ; 2007. doi:10.1161/01.STR.0000254499.46122.22

80. Gladstone DJ, Aviv RI, Demchuk AM, et al. Effect of Recombinant Activated Coagulation Factor VII on Hemorrhage Expansion among Patients with Spot Sign-Positive Acute Intracerebral Hemorrhage: The SPOTLIGHT and STOP-IT Randomized Clinical Trials. *JAMA Neurol*. 2019;76(12):1493-1501. doi:10.1001/jamaneurol.2019.2636

81. Ovesen C, Jakobsen JC, Gluud C, et al. Tranexamic Acid for Prevention of Hematoma Expansion in Intracerebral Hemorrhage Patients with or without Spot Sign. *Stroke*. Published online 2021:2629-2636. doi:10.1161/STROKEAHA.120.032426

82. Broderick JP. Haemostatic treatment for intracerebral haemorrhage. *Lancet*.

2018;391(10135):2081-2082. doi:10.1016/S0140-6736(18)31040-7

83. Shuaib A, Amlani S, Kalashyan H, et al. Mobile stroke unit triage of patients with a suspected stroke: A novel solution to reducing suspected stroke admissions in busy emergency departments. *BMJ Innov*. 2018;4(2):54-59. doi:10.1136/bmjinnov-2017-000240

84. Wilkinson CM, Burrell JI, Kuziek JWP, Thirunavukkarasu S, Buck BH, Mathewson KE. Predicting stroke severity with a 3-min recording from the Muse portable EEG system for rapid diagnosis of stroke. *Sci Rep*. 2020;10(1). doi:10.1038/s41598-020-75379-w

85. Belur PK, Chang JJ, He S, Emanuel BA, Mack WJ. Emerging experimental therapies for intracerebral hemorrhage: Targeting mechanisms of secondary brain injury. *Neurosurg Focus*. 2013;34(5). doi:10.3171/2013.2.FOCUS1317

86. Crilly S, Withers SE, Allan SM, Parry-Jones AR, Kasher PR. Revisiting promising preclinical intracerebral hemorrhage studies to highlight repurposable drugs for translation. *Int J Stroke*. 2021;16(2):123-136. doi:10.1177/1747493020972240

87. Baker TS, Durbin J, Troiani Z, et al. Therapeutic hypothermia for intracerebral hemorrhage: Systematic review and meta-analysis of the experimental and clinical literature. *Int J Stroke*. Published online 2021. doi:10.1177/17474930211044870

88. Liddle LJ, Kalisvaart ACJ, Abrahart AH, Almekhlafi M, Demchuk A, Colbourne F. Targeting focal ischemic and hemorrhagic stroke neuroprotection: Current prospects for local hypothermia. *J Neurochem*. 2022;160(1):128-144. doi:10.1111/jnc.15508

89. Sindelar BD, Patel V, Chowdhry S, Bailes JE. A Case Report in Hemorrhagic Stroke: A Complex Disease Process and Requirement for a Multimodal Treatment Approach. *Cureus*. Published online 2018. doi:10.7759/cureus.2976

90. Adeoye O, Broderick JP. Advances in the management of intracerebral hemorrhage. *Nat Rev Neurol*. 2010;6(11):593-601. doi:10.1038/nrneurol.2010.146

91. MacLellan CL, Silasi G, Poon CC, et al. Intracerebral hemorrhage models in rat:

comparing collagenase to blood infusion. *J Cereb Blood Flow Metab*. 2008;28:516-525. doi:10.1038/sj.jcbfm.9600548

92. Rosenberg G a., Mun-Bryce S, Wesley M, Kornfeld M. Collagenase-induced intracerebral hemorrhage in rats. *Stroke*. 1990;21(5):801-807. doi:10.1161/01.STR.21.5.801

93. Bullock R, Mendelow AD, Teasdale GM, Graham DI. Intracranial haemorrhage induced at arterial pressure in the rat. Part 1: Description of technique, ICP changes and neuropathological findings. *Neurol Res*. 1984;6(4):184-188. doi:10.1080/01616412.1984.11739687

94. Krenzlin H, Gresser E, Jussen D, et al. The Cerebral Thrombin System Is Activated after Intracerebral Hemorrhage and Contributes to Secondary Lesion Growth and Poor Neurological Outcome in C57Bl/6 Mice. *J Neurotrauma*. 2020;37(12):1481-1490. doi:10.1089/neu.2019.6582

95. Krafft PR, Rolland WB, Duris K, et al. Modeling intracerebral hemorrhage in mice: Injection of autologous blood or bacterial collagenase. *J Vis Exp*. 2012;(67). doi:10.3791/4289

96. Brouwers HB, Greenberg SM. Hematoma expansion following acute intracerebral hemorrhage. *Cerebrovasc Dis*. 2013;35(3):195-201. doi:10.1159/000346599

97. Dowlatshahi D, Demchuk AM, Flaherty ML, Ali M, Lyden PL, Smith EE. Defining hematoma expansion in intracerebral hemorrhage: Relationship with patient outcomes. *Neurology*. 2011;76(14):1238-1244. doi:10.1212/WNL.0b013e3182143317

98. Xue M, Del Bigio MR. Comparison of brain cell death and inflammatory reaction in three models of intracerebral hemorrhage in adult rats. *J Stroke Cerebrovasc Dis*. 2003;12(3):152-159. doi:10.1016/S1052-3057(03)00036-3

99. Matsushita K, Meng W, Wang X, et al. Evidence for apoptosis after intracerebral hemorrhage in rat striatum. *J Cereb Blood Flow Metab*. 2000;20(2):396-404. doi:10.1097/00004647-200002000-00022

100. Chu K, Jeong SW, Jung KH, et al. Celecoxib induces functional recovery after intracerebral hemorrhage with reduction of brain edema and perihematomal cell death. *J Cereb Blood Flow Metab*. 2004;24(8):926-933. doi:10.1097/01.WCB.0000130866.25040.7D

101. Sims JR, Gharai LR, Schaefer PW, et al. ABC/2 for rapid clinical estimate of infarct, perfusion, and mismatch volumes. *Neurology*. 2009;72(24):2104-2110. doi:10.1212/WNL.0b013e3181aa5329

102. Williamson MR, Colbourne F. Evidence for Decreased Brain Parenchymal Volume After Large Intracerebral Hemorrhages: a Potential Mechanism Limiting Intracranial Pressure Rises. *Transl Stroke Res*. 2017;8(4):386-396. doi:10.1007/s12975-017-0530-x

103. MacLellan CL, Auriat AM, McGie SC, et al. Gauging recovery after hemorrhagic stroke in rats: Implications for cytoprotection studies. *J Cereb Blood Flow Metab*. 2006;26(8):1031-1042. doi:10.1038/sj.jcbfm.9600255

104. Ziai WC, Carhuapoma JR. Intracerebral Hemorrhage. *Contin Lifelong Learn Neurol*. 2018;24(6):1603-1622. doi:10.1212/CON.0000000000000672

105. Adeoye O, Clark JF, Khatri P, Wagner KR, Zuccarello M, Pyne-Geithman GJ. Do Current Animal Models of Intracerebral Hemorrhage Mirror the Human Pathology? *Transl Stroke Res*. 2011;2(1):17-25. doi:10.1007/s12975-010-0037-1

106. Pan H, Guo R, Zhu J, et al. A gene catalogue of the Sprague-Dawley rat gut metagenome. *Gigascience*. 2018;7(5). doi:10.1093/gigascience/giy055

107. Yamori Y, Horie R, Handa H, Sato M, Fukase M. Pathogenetic similarity of strokes in stroke-prone spontaneously hypertensive rats and humans. *Stroke*. 1976;7(1):46-53. doi:10.1161/01.STR.7.1.46

108. Okamoto K, Aoki K. Development of a strain of spontaneously hypertensive rats. *Jpn Circ J*. 1963;27(3):282-293. doi:10.1253/jcj.27.282

109. Wu G, Bao X, Xi G, Keep RF, Thompson BG, Hua Y. Brain injury after intracerebral

hemorrhage in spontaneously hypertensive rats: Laboratory investigation. *J Neurosurg*. 2011;114(6):1805-1811. doi:10.3171/2011.1.JNS101530

110. Sun L, Gao YH, Tian DK, et al. Inflammation of different tissues in spontaneously hypertensive rats. *Sheng Li Xue Bao*. 2006;58(4):318-323.

111. Koletsky S. Obese spontaneously hypertensive rats-A model for study of atherosclerosis. *Exp Mol Pathol*. 1973;19(1):53-60. doi:10.1016/0014-4800(73)90040-3

112. Kurtz TW, Curtis Morris R. Biological variability in wistar-kyoto rats implications for research with the spontaneously hypertensive rat. *Hypertension*. 1987;10(1):127-131. doi:10.1161/01.HYP.10.1.127

113. Smeda JS, VanVliet BN, King SR. Stroke-prone spontaneously hypertensive rats lose their ability to auto-regulate cerebral blood flow prior to stroke. *J Hypertens*. 1999;17(12):1697-1705. doi:10.1097/00004872-199917120-00006

114. Badyal DK, Lata H, Dadhich AP. Animal models of hypertension and effect of drugs. *Indian J Pharmacol*. 2003;35(6):349-362.

115. Gomes PM, Sá RWM, Aguiar GL, et al. Chronic high-sodium diet intake after weaning lead to neurogenic hypertension in adult Wistar rats. *Sci Rep*. 2017;7(1). doi:10.1038/s41598-017-05984-9

116. Lerman LO, Chade AR, Sica V, Napoli C. Animal models of hypertension: An overview. *J Lab Clin Med*. 2005;146(3):160-173. doi:10.1016/j.lab.2005.05.005

117. Smith AJ, Clutton RE, Lilley E, Hansen KEA, Brattelid T. PREPARE: guidelines for planning animal research and testing. *Lab Anim*. 2018;52(2):135-141. doi:10.1177/0023677217724823

118. Kilkenny C, Browne WJ, Cuthill IC, Emerson M, Altman DG. Improving bioscience research reporting: The arrive guidelines for reporting animal research. *Animals*. 2013;4(1):35-44. doi:10.3390/ani4010035

119. Ioannidis JPA. Why most published research findings are false. In: *Getting to Good:*

Research Integrity in the Biomedical Sciences. ; 2018:2-8. doi:10.1371/journal.pmed.0020124

120. Fisher M, Feuerstein G, Howells DW, et al. Update of the stroke therapy academic industry roundtable preclinical recommendations. *Stroke*. 2009;40(6):2244-2250. doi:10.1161/STROKEAHA.108.541128
121. Andrade C. Internal, external, and ecological validity in research design, conduct, and evaluation. *Indian J Psychol Med*. 2018;40(5):498-499. doi:10.4103/IJPSYM.IJPSYM_334_18
122. Dirnagl U. Bench to bedside: The quest for quality in experimental stroke research. *J Cereb Blood Flow Metab*. 2006;26(12):1465-1478. doi:10.1038/sj.jcbfm.9600298
123. Macleod MR, Fisher M, O'Collins V, et al. Good laboratory practice: preventing introduction of bias at the bench. *Stroke*. 2009;40(3). doi:10.1161/STROKEAHA.108.525386
124. Frantzias J, Sena ES, MacLeod MR, Salman RAS. Treatment of intracerebral hemorrhage in animal models: Meta-analysis. *Ann Neurol*. 2011;69(2):389-399. doi:10.1002/ana.22243
125. Crossley NA, Sena E, Goehler J, et al. Empirical evidence of bias in the design of experimental stroke studies: A metaepidemiologic approach. *Stroke*. 2008;39(3):929-934. doi:10.1161/STROKEAHA.107.498725
126. Bosetti F, Koenig JI, Ayata C, et al. Translational Stroke Research: Vision and Opportunities. In: *Stroke*. Vol 48. ; 2017:2632-2637. doi:10.1161/STROKEAHA.117.017112
127. Dirnagl U, Fisher M. International, multicenter randomized preclinical trials in translational stroke research: It's time to act. *J Cereb Blood Flow Metab*. 2012;32(6):933-935. doi:10.1038/jcbfm.2012.51
128. Dirnagl U, Endres M. Found in translation: Preclinical stroke research predicts human

pathophysiology, clinical phenotypes, and therapeutic outcomes. *Stroke*. 2014;45(5):1510-1518. doi:10.1161/STROKEAHA.113.004075

129. Gladstone DJ, Black SE, Hakim AM. Toward wisdom from failure: Lessons from neuroprotective stroke trials and new therapeutic directions. *Stroke*. 2002;33(8):2123-2136. doi:10.1161/01.STR.0000025518.34157.51

130. Chamorro Á, Lo EH, Renú A, Van Leyden K, Lyden PD. The future of neuroprotection in stroke. *J Neurol Neurosurg Psychiatry*. 2021;92(2):129-135. doi:10.1136/jnnp-2020-324283

131. Liddle LJ, Ralhan S, Ward DL, Colbourne F. Translational Intracerebral Hemorrhage Research: Has Current Neuroprotection Research ARRIVEd at a Standard for Experimental Design and Reporting? *Transl Stroke Res*. 2020;11(6):1203-1213. doi:10.1007/s12975-020-00824-x

132. MacLellan CL, Paquette R, Colbourne F. A critical appraisal of experimental intracerebral hemorrhage research. *J Cereb Blood Flow Metab*. 2012;32(4):612-627. doi:10.1038/jcbfm.2012.8

133. Sena ES, Briscoe CL, Howells DW, Donnan GA, Sandercock PAG, MacLeod MR. Factors affecting the apparent efficacy and safety of tissue plasminogen activator in thrombotic occlusion models of stroke: Systematic review and meta-analysis. *J Cereb Blood Flow Metab*. 2010;30(12):1905-1913. doi:10.1038/jcbfm.2010.116

134. O'Collins VE, Donnan GA, MacLeod MR, Howells DW. Hypertension and experimental stroke therapies. *J Cereb Blood Flow Metab*. 2013;33(8):1141-1147. doi:10.1038/jcbfm.2013.88

135. Constantinides C, Murphy K. Molecular and Integrative Physiological Effects of Isoflurane Anesthesia: The Paradigm of Cardiovascular Studies in Rodents using Magnetic Resonance Imaging. *Front Cardiovasc Med*. 2016;3. doi:10.3389/fcvm.2016.00023

136. Sicard K, Shen Q, Brevard ME, et al. Regional cerebral blood flow and BOLD responses

in conscious and anesthetized rats under basal and hypercapnic conditions: Implications for functional MRI studies. *J Cereb Blood Flow Metab*. 2003;23(4):472-481. doi:10.1097/01.WCB.0000054755.93668.20

137. Yang C-F, Yu-Chih Chen M, Chen T-I, Cheng C-F. Dose-dependent effects of isoflurane on cardiovascular function in rats. *Tzu Chi Med J*. 2014;26(3):119-122. doi:10.1016/J.TCMJ.2014.07.005

138. Statler KD, Alexander H, Vagni V, et al. Isoflurane exerts neuroprotective actions at or near the time of severe traumatic brain injury. *Brain Res*. 2006;1076(1):216-224. doi:10.1016/j.brainres.2005.12.106

139. Gigante PR, Appelboom G, Hwang BY, et al. Isoflurane preconditioning affords functional neuroprotection in a murine model of intracerebral hemorrhage. In: *Acta Neurochirurgica, Supplementum*. Springer-Verlag Wien; 2011:141-144. doi:10.1007/978-3-7091-0693-8_23

140. Fluri F, Schuhmann MK, Kleinschnitz C. Animal models of ischemic stroke and their application in clinical research. *Drug Des Devel Ther*. 2015;9:3445-3454. doi:10.2147/DDDT.S56071

141. MacRae I. Preclinical stroke research - Advantages and disadvantages of the most common rodent models of focal ischaemia. *Br J Pharmacol*. 2011;164(4):1062-1078. doi:10.1111/j.1476-5381.2011.01398.x

142. Ruíz-Sandoval JL, Cantú C, Barinagarrementeria F. Intracerebral hemorrhage in young people: Analysis of risk factors, location, causes, and prognosis. *Stroke*. 1999;30(3):537-541. doi:10.1161/01.STR.30.3.537

143. Cho DY, Chen CC, Lee WY, Lee HC, Ho LH. A new Modified Intracerebral Hemorrhage Score for treatment decisions in basal ganglia hemorrhage-a randomized trial. *Crit Care Med*. 2008;36(7):2151-2156. doi:10.1097/CCM.0b013e318173fc99

144. Karthikeyan S, Jeffers MS, Carter A, Corbett D. Characterizing Spontaneous Motor

Recovery Following Cortical and Subcortical Stroke in the Rat. *Neurorehabil Neural Repair*. 2019;33(1):27-37. doi:10.1177/1545968318817823

145. Chen R, Wang X, Anderson CS, et al. Infratentorial Intracerebral Hemorrhage: Relation of Location to Outcome. *Stroke*. 2019;50(5):1257-1259. doi:10.1161/STROKEAHA.118.023766
146. Delcourt C, Sato S, Zhang S, et al. Intracerebral hemorrhage location and outcome among INTERACT2 participants. *Neurology*. 2017;88(15):1408-1414. doi:10.1212/WNL.0000000000003771
147. Samarasekera N, Fonville A, Lerpiniere C, et al. Influence of intracerebral hemorrhage location on incidence, characteristics, and outcome: Population-based study. *Stroke*. 2015;46(2):361-368. doi:10.1161/STROKEAHA.114.007953
148. Sreekrishnan A, Dearborn JL, Greer DM, et al. Intracerebral Hemorrhage Location and Functional Outcomes of Patients: A Systematic Literature Review and Meta-Analysis. *Neurocrit Care*. 2016;25(3):384-391. doi:10.1007/s12028-016-0276-4
149. Henderson VC, Kimmelman J, Fergusson D, Grimshaw JM, Hackam DG. Threats to Validity in the Design and Conduct of Preclinical Efficacy Studies: A Systematic Review of Guidelines for In Vivo Animal Experiments. *PLoS Med*. 2013;10(7). doi:10.1371/journal.pmed.1001489
150. Munafò MR, Nosek BA, Bishop DVM, et al. A manifesto for reproducible science. *Nat Hum Behav*. 2017;1(1). doi:10.1038/s41562-016-0021
151. Hojat M, Xu G. A visitor's guide to effect sizes - Statistical significance versus practical (clinical) importance of research findings. *Adv Heal Sci Educ*. 2004;9(3):241-249. doi:10.1023/B:AHSE.0000038173.00909.f6
152. Vilaró M, Cortés J, Selva-O'Callaghan A, et al. Adherence to reporting guidelines increases the number of citations: The argument for including a methodologist in the editorial process and peer-review. *BMC Med Res Methodol*. 2019;19(1).

doi:10.1186/s12874-019-0746-4

153. Sena ES, Bart van der Worp H, Bath PMW, Howells DW, Macleod MR. Publication bias in reports of animal stroke studies leads to major overstatement of efficacy. *PLoS Biol*. 2010;8(3). doi:10.1371/journal.pbio.1000344

154. Head ML, Holman L, Lanfear R, Kahn AT, Jennions MD. The Extent and Consequences of P-Hacking in Science. *PLoS Biol*. 2015;13(3). doi:10.1371/journal.pbio.1002106

155. Xi G, Fewel ME, Hua Y, Thompson BG, Hoff JT, Keep RF. Intracerebral hemorrhage: Pathophysiology and therapy. *Neurocrit Care*. 2004;1(1):5-18. doi:10.1385/NCC:1:1:5

156. Keep RF, Xiang J, Ennis SR, et al. Blood-brain barrier function in intracerebral hemorrhage. *Acta Neurochir Suppl*. 2008;105(105):73-77. doi:10.1007/978-3-211-09469-3_15

157. Schoknecht K, David Y, Heinemann U. The blood-brain barrier-Gatekeeper to neuronal homeostasis: Clinical implications in the setting of stroke. *Semin Cell Dev Biol*. 2015;38:35-42. doi:10.1016/j.semcdb.2014.10.004

158. Nadeau CA, Dietrich K, Wilkinson CM, et al. Prolonged Blood-Brain Barrier Injury Occurs After Experimental Intracerebral Hemorrhage and Is Not Acutely Associated with Additional Bleeding. *Transl Stroke Res*. 2019;10(3):287-297. doi:10.1007/s12975-018-0636-9

159. Merali Z, Huang K, Mikulis D, Silver F, Kassner A. Evolution of blood-brain-barrier permeability after acute ischemic stroke. *PLoS One*. 2017;12(2). doi:10.1371/journal.pone.0171558

160. Ironside N, Chen CJ, Ding D, Mayer SA, Connolly ES. Perihematomal Edema after Spontaneous Intracerebral Hemorrhage. *Stroke*. 2019;50(6):1626-1633. doi:10.1161/STROKEAHA.119.024965

161. Staykov D, Wagner I, Volbers B, et al. Natural course of perihemorrhagic edema after intracerebral hemorrhage. *Stroke*. 2011;42(9):2625-2629.

doi:10.1161/STROKEAHA.111.618611

162. Rungta RL, Choi HB, Tyson JR, et al. The cellular mechanisms of neuronal swelling underlying cytotoxic edema. *Cell*. 2015;161(3):610-621. doi:10.1016/j.cell.2015.03.029

163. Prabhakaran S, Naidech AM. Ischemic brain injury after intracerebral hemorrhage: A critical review. *Stroke*. 2012;43(8):2258-2263. doi:10.1161/STROKEAHA.112.655910

164. Qureshi AI, Wilson DA, Hanley DF, Traystman RJ. No evidence for an ischemic penumbra in massive experimental intracerebral hemorrhage. *Neurology*. 1999;52(2):266-272. doi:10.1212/wnl.52.2.266

165. Thiex R, Tsirka SE. Brain edema after intracerebral hemorrhage: mechanisms, treatment options, management strategies, and operative indications. *Neurosurg Focus*. 2007;22(5). doi:10.3171/foc.2007.22.5.7

166. Kalisvaart ACJ, Wilkinson CM, Gu S, et al. An update to the Monro–Kellie doctrine to reflect tissue compliance after severe ischemic and hemorrhagic stroke. *Sci Rep*. 2020;10(1). doi:10.1038/s41598-020-78880-4

167. Butcher KS, Baird T, MacGregor L, Desmond P, Tress B, Davis S. Perihematomal edema in primary intracerebral hemorrhage is plasma derived. *Stroke*. 2004;35(8):1879-1885. doi:10.1161/01.STR.0000131807.54742.1a

168. Hoff JT, Xi G. Brain edema from intracerebral hemorrhage. *Acta Neurochir Suppl*. 2003;(86):11-15. doi:10.1007/978-3-7091-0651-8_3

169. Wu S, Yuan R, Wang Y, et al. Early prediction of malignant brain edema after ischemic stroke: A systematic review and meta-analysis. *Stroke*. 2018;49(12):2918-2927. doi:10.1161/STROKEAHA.118.022001

170. Dostovic Z, Dostovic E, Smajlovic D, Ibrahimagic OC, Avdic L. Brain Edema After Ischaemic Stroke. *Med Arch (Sarajevo, Bosnia Herzegovina)*. 2016;70(5):339-341. doi:10.5455/medarh.2016.70.339-341

171. Walcott BP, Kahle KT, Simard JM. Novel Treatment Targets for Cerebral Edema.

Neurotherapeutics. 2012;9(1):65-72. doi:10.1007/s13311-011-0087-4

172. Wang G, Huang H, He Y, Ruan L, Huang J. Bumetanide protects focal cerebral ischemia-reperfusion injury in rat. *Int J Clin Exp Pathol*. 2014;7(4):1487-1494.

173. Wilkinson CM, Fedor BA, Aziz JR, et al. Failure of bumetanide to improve outcome after intracerebral hemorrhage in rat. *PLoS One*. 2019;14(1):1-20. doi:10.1371/journal.pone.0210660

174. Simard JM, Woo SK, Schwartzbauer GT, Gerzanich V. Sulfonylurea Receptor 1 in Central Nervous System Injury: A Focused Review. *J Cereb Blood Flow Metab*. 2012;32(9):1699-1717. doi:10.1038/jcbfm.2012.91

175. Caffes N, Kurland DB, Gerzanich V, Marc Simard J. Glibenclamide for the treatment of ischemic and hemorrhagic stroke. *Int J Mol Sci*. 2015;16(3):4973-4984. doi:10.3390/ijms16034973

176. Simard JM, Tsymbalyuk N, Tsymbalyuk O, Ivanova S, Yurovsky V, Gerzanich V. Glibenclamide Is Superior to Decompressive Craniectomy in a Rat Model of Malignant Stroke. *Stroke*. 2010;41(3):531-537. doi:10.1161/STROKEAHA.109.572644

177. Sheth K, Elm J, Cheung K, et al. Abstracts for the 10th World Stroke Congress, 2016. *Int J Stroke*. 2016;11(3_suppl):4-288. doi:10.1177/1747493016670567

178. Halnes G, Østby I, Pettersen KH, Omholt SW, Einevoll GT. Electrodiffusive Model for Astrocytic and Neuronal Ion Concentration Dynamics. *PLoS Comput Biol*. 2013;9(12). doi:10.1371/journal.pcbi.1003386

179. Williamson MR, Dietrich K, Hackett MJ, et al. Rehabilitation Augments Hematoma Clearance and Attenuates Oxidative Injury and Ion Dyshomeostasis After Brain Hemorrhage. *Stroke*. 2016;48(1):195-203. doi:10.1161/STROKEAHA.116.015404

180. Xi G, Keep RF, Hoff JT. Erythrocytes and delayed brain edema formation following intracerebral hemorrhage in rats. *J Neurosurg*. 1998;89(6):991-996. doi:10.3171/jns.1998.89.6.0991

181. Wilkinson CM, Brar PS, Balay CJ, Colbourne F. Glibenclamide, a Sur1-Trpm4 antagonist, does not improve outcome after collagenase-induced intracerebral hemorrhage. *PLoS One*. 2019;14(5). doi:10.1371/journal.pone.0215952

182. Dang G, Yang Y, Wu G, Hua Y, Keep RF, Xi G. Early Erythrolysis in the Hematoma After Experimental Intracerebral Hemorrhage. *Transl Stroke Res*. 2017;8(2):174-182. doi:10.1007/s12975-016-0505-3

183. Pleșeru A-M, Mihailă RG. the Role of Thrombin in Central Nervous System Activity and Stroke. *Med Pharm Reports*. 2018;91(4):368-371. doi:10.15386/cjmed-973

184. Nguyen A, Huynh H, Sjovold S, Colbourne F. Progressive Brain Damage and Alterations in Dendritic Arborization after Collagenase-Induced Intracerebral Hemorrhage in Rats. *Curr Neurovasc Res*. 2008;5(3):171-177. doi:10.2174/156720208785425710

185. Cao S, Zheng M, Hua Y, Chen G, Keep RF, Xi G. Hematoma Changes during Clot Resolution after Experimental Intracerebral Hemorrhage. *Stroke*. 2016;47(6):1626-1631. doi:10.1161/STROKEAHA.116.013146

186. Takasugi S, Ueda S, Matsumoto K. Chronological changes in spontaneous intracerebral hematoma — an experimental and clinical study. *Stroke*. 1985;16(4):651-658. doi:10.1161/01.STR.16.4.651

187. Wang J, Wang G, Yi J, et al. The effect of monascin on hematoma clearance and edema after intracerebral hemorrhage in rats. *Brain Res Bull*. 2017;134:24-29. doi:10.1016/j.brainresbull.2017.06.018

188. Huang FP, Xi G, Keep RF, Hua Y, Nemoianu A, Hoff JT. Brain edema after experimental intracerebral hemorrhage: Role of hemoglobin degradation products. *J Neurosurg*. 2002;96(2):287-293. doi:10.3171/jns.2002.96.2.0287

189. Clark JF, Loftspring M, Wurster WL, et al. Bilirubin oxidation products, oxidative stress, and intracerebral hemorrhage. *Acta Neurochir Suppl*. 2008;(105):7-12. doi:10.1007/978-3-211-09469-3_2

190. Zille M, Karuppagounder SS, Chen Y, et al. Neuronal Death after Hemorrhagic Stroke in Vitro and in Vivo Shares Features of Ferroptosis and Necroptosis. *Stroke*. 2017;48(4):1033-1043. doi:10.1161/STROKEAHA.116.015609

191. Lattanzi S, Cagnetti C, Provinciali L, Silvestrini M. Neutrophil-to-Lymphocyte Ratio Predicts the Outcome of Acute Intracerebral Hemorrhage. *Stroke*. 2016;47(6):1654-1657. doi:10.1161/STROKEAHA.116.013627

192. Zhang Z, Zhang Z, Lu H, Yang Q, Wu H, Wang J. Microglial Polarization and Inflammatory Mediators After Intracerebral Hemorrhage. *Mol Neurobiol*. 2017;54(3):1874-1886. doi:10.1007/s12035-016-9785-6

193. Zhou Y, Wang Y, Wang J, Anne Stetler R, Yang QW. Inflammation in intracerebral hemorrhage: From mechanisms to clinical translation. *Prog Neurobiol*. 2014;115(C):25-44. doi:10.1016/j.pneurobio.2013.11.003

194. Khanna A, Kahle KT, Walcott BP, Gerzanich V, Simard JM. Disruption of Ion Homeostasis in the Neurogliovascular Unit Underlies the Pathogenesis of Ischemic Cerebral Edema. *Transl Stroke Res*. 2014;5(1). doi:10.1007/s12975-013-0307-9

195. Wasserman JK, Zhu X, Schlichter LC. Evolution of the inflammatory response in the brain following intracerebral hemorrhage and effects of delayed minocycline treatment. *Brain Res*. 2007;1180(1):140-154. doi:10.1016/j.brainres.2007.08.058

196. Malhotra K, Chang JJ, Khunger A, et al. Minocycline for acute stroke treatment: a systematic review and meta-analysis of randomized clinical trials. *J Neurol*. 2018;265(8):1871-1879. doi:10.1007/s00415-018-8935-3

197. Moskowitz MA, Lo EH, Iadecola C. The science of stroke: Mechanisms in search of treatments. *Neuron*. 2010;67(2):181-198. doi:10.1016/j.neuron.2010.07.002

198. Balami JS, Buchan AM. Complications of intracerebral haemorrhage. *Lancet Neurol*. 2012;11(1):101-118. doi:10.1016/S1474-4422(11)70264-2

199. Klahr AC, Dickson CT, Colbourne F. Seizure Activity Occurs in the Collagenase but not

the Blood Infusion Model of Striatal Hemorrhagic Stroke in Rats. *Transl Stroke Res*. 2014;6(1):29-38. doi:10.1007/s12975-014-0361-y

200. Germonpré C, Proesmans S, Bouckaert C, et al. Acute symptomatic seizures following intracerebral hemorrhage in the rat collagenase model. *Epilepsy Res*. 2020;164. doi:10.1016/j.eplepsyres.2020.106364
201. Gabor AJ, Brooks AG, Scobey RP, Parsons GH. Intracranial pressure during epileptic seizures. *Electroencephalogr Clin Neurophysiol*. 1984;57(6):497-506. doi:10.1016/0013-4694(84)90085-3
202. Vespa PM, Miller C, McArthur D, et al. Nonconvulsive electrographic seizures after traumatic brain injury result in a delayed, prolonged increase in intracranial pressure and metabolic crisis. *Crit Care Med*. 2007;35(12):2830-2836. doi:10.1097/01.CCM.0000295667.66853.BC
203. Wilkinson CM, Kung TFC, Jickling GC, Colbourne F. A translational perspective on intracranial pressure responses following intracerebral hemorrhage in animal models. *Brain Hemorrhages*. 2021;2(1):34-48. doi:10.1016/j.hest.2020.10.002
204. Freeman WD. Management of intracranial pressure. *Contin Lifelong Learn Neurol*. 2015;21(5):1299-1323. doi:10.1212/CON.0000000000000235
205. Badri S, Chen J, Barber J, et al. Mortality and long-term functional outcome associated with intracranial pressure after traumatic brain injury. *Intensive Care Med*. 2012;38(11):1800-1809. doi:10.1007/s00134-012-2655-4
206. Sykora M, Steinmacher S, Steiner T, Poli S, Diedler J. Association of intracranial pressure with outcome in comatose patients with intracerebral hemorrhage. *J Neurol Sci*. 2014;342(1-2):141-145. doi:10.1016/j.jns.2014.05.012
207. Godoy DA, Núñez-Patiño RA, Zorrilla-Vaca A, Ziai WC, Hemphill JC. Intracranial Hypertension After Spontaneous Intracerebral Hemorrhage: A Systematic Review and Meta-analysis of Prevalence and Mortality Rate. *Neurocrit Care*. 2019;31(1):176-187.

doi:10.1007/s12028-018-0658-x

208. Guo T, Ren P, Li X, et al. Neural Injuries Induced by Hydrostatic Pressure Associated with Mass Effect after Intracerebral Hemorrhage. *Sci Rep*. 2018;8(1). doi:10.1038/s41598-018-27275-7

209. Liu Q, Ju WK, Crowston JG, et al. Oxidative stress is an early event in hydrostatic pressure-induced retinal ganglion cell damage. *Investig Ophthalmol Vis Sci*. 2007;48(10):4580-4589. doi:10.1167/iovs.07-0170

210. Pranevicius M, Pranevicius H, Pranevicius O. Cerebral venous steal equation for intracranial segmental perfusion pressure predicts and quantifies reversible intracranial to extracranial flow diversion. *Sci Rep*. 2021;11(1). doi:10.1038/s41598-021-85931-x

211. Bullock R, Brock-Utne J, van Dellen J, Blake G. Intracerebral hemorrhage in a primate model: Effect on regional cerebral blood flow. *Surg Neurol*. 1988;29(2):101-107. doi:10.1016/0090-3019(88)90065-1

212. Tadevosyan A, Kornbluth J. Brain Herniation and Intracranial Hypertension. *Neurol Clin*. 2021;39(2):293-318. doi:10.1016/j.ncl.2021.02.005

213. Rincon F, Mayer SA. Clinical review: Critical care management of spontaneous intracerebral hemorrhage. *Crit Care*. 2008;12(6):237. doi:10.1186/cc7092

214. Hanley DF, Thompson RE, Rosenblum M, et al. Efficacy and safety of minimally invasive surgery with thrombolysis in intracerebral haemorrhage evacuation (MISTIE III): a randomised, controlled, open-label, blinded endpoint phase 3 trial. *Lancet*. 2019;393(10175):1021-1032. doi:10.1016/S0140-6736(19)30195-3

215. Dastur CK, Yu W. Current management of spontaneous intracerebral haemorrhage. *Stroke Vasc Neurol*. 2017;2(1):21-29. doi:10.1136/svn-2016-000047

216. Kamel H, Navi BB, Nakagawa K, Hemphill JC, Ko NU. Hypertonic saline versus mannitol for the treatment of elevated intracranial pressure: A meta-analysis of randomized clinical trials. *Crit Care Med*. 2011;39(3):554-559. doi:10.1097/CCM.0b013e318206b9be

217. Bühler D, Azghandi S, Schüller K, Plesnila N. Effect of decompressive craniectomy on outcome following subarachnoid hemorrhage in mice. *Stroke*. 2015;46(3):819-826. doi:10.1161/STROKEAHA.114.007703

218. Bothwell SW, Janigro D, Patabendige A. Cerebrospinal fluid dynamics and intracranial pressure elevation in neurological diseases. *Fluids Barriers CNS*. 2019;16(1). doi:10.1186/s12987-019-0129-6

219. Uldall M, Botfield H, Jansen-Olesen I, Sinclair A, Jensen R. Acetazolamide lowers intracranial pressure and modulates the cerebrospinal fluid secretion pathway in healthy rats. *Neurosci Lett*. 2017;645:33-39. doi:10.1016/j.neulet.2017.02.032

220. Williamson MR, Wilkinson CM, Dietrich K, Colbourne F. Acetazolamide Mitigates Intracranial Pressure Spikes Without Affecting Functional Outcome After Experimental Hemorrhagic Stroke. *Transl Stroke Res*. 2019;10(4):428-439. doi:10.1007/s12975-018-0663-6

221. Diringer MN. The evolution of the clinical use of osmotic therapy in the treatment of cerebral Edema. In: *Acta Neurochirurgica, Supplementum*. Vol 121. ; 2016:3-6. doi:10.1007/978-3-319-18497-5_1

222. Maira G, Anile C, Colosimo C, Rossi GF. Surgical treatment of primary supratentorial intracerebral hemorrhage in stuporous and comatose patients. *Neurol Res*. 2002;24(1):54-60. doi:10.1179/016164102101199549

223. Hayashi M, Kobayashi H, Kawano H, Handa Y, Hirose S. Treatment of systemic hypertension and intracranial hypertension in cases of brain hemorrhage. *Stroke*. 1988;19(3):314-321. doi:10.1161/01.STR.19.3.314

224. Roberts DJ, Hall RI, Kramer AH, Robertson HL, Gallagher CN, Zygun DA. Sedation for critically ill adults with severe traumatic brain injury: A systematic review of randomized controlled trials. *Crit Care Med*. 2011;39(12):2743-2751. doi:10.1097/CCM.0b013e318228236f

225. John RF, Colbourne F. Delayed localized hypothermia reduces intracranial pressure following collagenase-induced intracerebral hemorrhage in rat. *Brain Res*. 2016;1633:27-36. doi:10.1016/j.brainres.2015.12.033

226. Omileke D, Azarpeykan S, Bothwell SW, et al. Short-duration hypothermia completed prior to reperfusion prevents intracranial pressure elevation following ischaemic stroke in rats. *Sci Rep*. 2021;11(1):1-9. doi:10.1038/s41598-021-01838-7

227. Kawai N, Nakamura T, Okauchi M, Nagao S. Effects of hypothermia on intracranial pressure and brain edema formation: Studies in a rat acute subdural hematoma model. *J Neurotrauma*. 2000;17(3):193-202. doi:10.1089/neu.2000.17.193

228. Kollmar R, Staykov D, Dörfler A, Schellinger PD, Schwab S, Bardutzky J. Hypothermia reduces perihemorrhagic edema after intracerebral hemorrhage. *Stroke*. 2010;41(8):1684-1689. doi:10.1161/STROKEAHA.110.587758

229. Omileke D, Pepperall D, Bothwell SW, et al. Ultra-Short Duration Hypothermia Prevents Intracranial Pressure Elevation Following Ischaemic Stroke in Rats. *Front Neurol*. 2021;12. doi:10.3389/fneur.2021.684353

230. Omileke D, Bothwell SW, Pepperall D, et al. Decreased Intracranial Pressure Elevation and Cerebrospinal Fluid Outflow Resistance: A Potential Mechanism of Hypothermia Cerebroprotection Following Experimental Stroke. *Brain Sci*. 2021;11(12):1589. doi:10.3390/brainsci11121589

231. Eide PK, Sorteberg W. Association among intracranial compliance, intracranial pulse pressure amplitude and intracranial pressure in patients with intracranial bleeds. *Neurol Res*. 2007;29(8):798-802. doi:10.1179/016164107X224132

232. Wilson MH. Monro-Kellie 2.0: The dynamic vascular and venous pathophysiological components of intracranial pressure. *J Cereb Blood Flow Metab*. 2016;36(8):1338-1350. doi:10.1177/0271678X16648711

233. Hua J, Liu P, Kim T, et al. MRI techniques to measure arterial and venous cerebral blood

volume. *Neuroimage*. 2019;187:17-31. doi:10.1016/j.neuroimage.2018.02.027

234. Hopton P, Walsh TS, Lee A. Measurement of cerebral blood volume using near-infrared spectroscopy and indocyanine green elimination. *J Appl Physiol*. 1999;87(5):1981-1987. doi:10.1152/jappl.1999.87.5.1981

235. Castro P, Azevedo E, Sorond F. Cerebral Autoregulation in Stroke. *Curr Atheroscler Rep*. 2018;20(8). doi:10.1007/s11883-018-0739-5

236. LASSEN NA. Cerebral blood flow and oxygen consumption in man. *Physiol Rev*. 1959;39(2):183-238. doi:10.1152/physrev.1959.39.2.183

237. Brassard P, Labrecque L, Smirl JD, et al. Losing the dogmatic view of cerebral autoregulation. *Physiol Rep*. 2021;9(15). doi:10.14814/phy2.14982

238. Mascarenhas S, Vilela GHF, Carlotti C, et al. The new ICP minimally invasive method shows that the monro-kellie doctrine is not valid. In: *Acta Neurochirurgica, Supplementum*. ; 2012. doi:10.1007/978-3-7091-0956-4_21

239. Hiploylee C, Colbourne F. Intracranial pressure measured in freely moving rats for days after intracerebral hemorrhage. *Exp Neurol*. 2014;255:49-55. doi:10.1016/j.expneurol.2014.02.017

240. Wells AJ, Vink R, Helps SC, Knox SJ, Blumbergs PC, Turner RJ. Elevated intracranial pressure and cerebral edema following permanent MCA occlusion in an ovine model. *PLoS One*. 2015;10(6). doi:10.1371/journal.pone.0130512

241. Cohen BA, Knopp EA, Rusinek H, Liu S, Gonen O. Brain compression without global neuronal loss in meningiomas: Whole-brain proton MR spectroscopy report of 2 cases. *Am J Neuroradiol*. 2005;26(9):2178-2182.

242. Hackett MJ, Hollings A, Caine S, et al. Elemental characterisation of the pyramidal neuron layer within the rat and mouse hippocampus. *Metallomics*. 2019;11(1):151-165. doi:10.1039/c8mt00230d

243. Kahle KT, Staley KJ, Nahed B V., et al. Roles of the cation - Chloride cotransporters in

neurological disease. *Nat Clin Pract Neurol*. 2008;4(9):490-503. doi:10.1038/ncpneuro0883

244. Pasantes-Morales H, Cruz-Rangel S. Brain volume regulation: Osmolytes and aquaporin perspectives. *Neuroscience*. 2010;168(4):871-884. doi:10.1016/j.neuroscience.2009.11.074

245. Song M, Yu SP. Ionic Regulation of Cell Volume Changes and Cell Death after Ischemic Stroke. *Transl Stroke Res*. 2014;5(1):17-27. doi:10.1007/s12975-013-0314-x

246. Basavappa S, Ellory JC. The role of swelling-induced anion channels during neuronal volume regulation. *Mol Neurobiol*. 1996;13(2):137-153. doi:10.1007/BF02740638

247. Liedtke W. Molecular mechanisms of TRPV4-mediated neural signaling. In: *Annals of the New York Academy of Sciences*. ; 2008. doi:10.1196/annals.1418.012

248. Jiang H, Sun SX. Cellular pressure and volume regulation and implications for cell mechanics. *Biophys J*. 2013;105(3):609-619. doi:10.1016/j.bpj.2013.06.021

249. Fjell AM, Walhovd KB, Fennema-Notestine C, et al. One-year brain atrophy evident in healthy aging. *J Neurosci*. 2009;29(48):15223-15231. doi:10.1523/JNEUROSCI.3252-09.2009

250. Ayus JC, Achinger SG, Arieff A. Brain cell volume regulation in hyponatremia: Role of sex, age, vasopressin, and hypoxia. *Am J Physiol - Ren Physiol*. 2008;295(3). doi:10.1152/ajprenal.00502.2007

251. Trachtman H. Cell volume regulation: a review of cerebral adaptive mechanisms and implications for clinical treatment of osmolal disturbances: II. *Pediatr Nephrol*. 1992;6(1):104-112. doi:10.1007/BF00856852

Chapter 2

1. Feigin VL, Lawes CM, Bennett DA, Barker-Collo SL, Parag V. Worldwide stroke incidence and early case fatality reported in 56 population-based studies: a systematic

review. *Lancet Neurol*. 2009;8(4):355-369. doi:10.1016/S1474-4422(09)70025-0

2. Tian Y, Wang Z, Jia Y, et al. Intracranial pressure variability predicts short-term outcome after intracerebral hemorrhage: A retrospective study. *J Neurol Sci*. 2013;330(1-2):38-44. doi:10.1016/j.jns.2013.04.001
3. Sykora M, Steinmacher S, Steiner T, Poli S, Diedler J. Association of intracranial pressure with outcome in comatose patients with intracerebral hemorrhage. *J Neurol Sci*. 2014;342(1-2):141-145. doi:10.1016/j.jns.2014.05.012
4. Bullock R, Brock-Utne J, van Dellen J, Blake G. Intracerebral hemorrhage in a primate model: Effect on regional cerebral blood flow. *Surg Neurol*. 1988;29(2):101-107. doi:10.1016/0090-3019(88)90065-1
5. Zeng J, Zheng P, Tong W, Fang W. Decreased risk of secondary brain herniation with intracranial pressure monitoring in patients with haemorrhagic stroke. *BMC Anesthesiol*. 2014;14:2-5. doi:10.1186/1471-2253-14-19
6. Cooper DJ, Rosenfeld J V, Murray L, et al. Patient outcomes at twelve months after early decompressive craniectomy for diffuse traumatic brain injury in the Randomized DECRA Clinical Trial. *J Neurotrauma*. 2020;816:810-816. doi:10.1089/neu.2019.6869
7. Misra UK, Kalita J, Ranjan P, Mandal SK. Mannitol in intracerebral hemorrhage: A randomized controlled study. *J Neurol Sci*. 2005;234(1-2):41-45. doi:10.1016/j.jns.2005.03.038
8. Hanley DF, Thompson RE, Rosenblum M, et al. Efficacy and safety of minimally invasive surgery with thrombolysis in intracerebral haemorrhage evacuation (MISTIE III): a randomised, controlled, open-label, blinded endpoint phase 3 trial. *Lancet*. 2019;393(10175):1021-1032. doi:10.1016/S0140-6736(19)30195-3
9. Rincon F, Mayer SA. Clinical review: Critical care management of spontaneous intracerebral hemorrhage. *Crit Care*. 2008;12(6):237. doi:10.1186/cc7092
10. Diringer MN. Intracerebral hemorrhage: Pathophysiology and management. *Crit Care*

Med. 1993;21(10):1591-1603. doi:10.1097/00003246-199310000-00032

11. Guo T, Ren P, Li X, et al. Neural Injuries Induced by Hydrostatic Pressure Associated with Mass Effect after Intracerebral Hemorrhage. *Sci Rep*. 2018;8(1). doi:10.1038/s41598-018-27275-7
12. Gebel JM, Jauch EC, Brott TG, et al. Relative edema volume is a predictor of outcome in patients with hyperacute spontaneous intracerebral hemorrhage. *Stroke*. 2002;33(11):2636-2641. doi:10.1161/01.STR.0000035283.34109.EA
13. Lee KR, Colon GP, Betz AL, Keep RF, Kim S, Hoff JT. Edema from intracerebral hemorrhage: The role of thrombin. *J Neurosurg*. 1996;84(1):91-96. doi:10.3171/jns.1996.84.1.0091
14. Zheng H, Chen C, Zhang J, Hu Z. Mechanism and therapy of brain edema after intracerebral hemorrhage. *Cerebrovasc Dis*. 2016;42(3-4):155-169. doi:10.1159/000445170
15. Urday S, Beslow LA, Dai F, et al. Rate of Perihematomal Edema Expansion Predicts Outcome after Intracerebral Hemorrhage. *Crit Care Med*. 2016;44(4):790-797. doi:10.1097/CCM.0000000000001553
16. Stokum JA, Gerzanich V, Simard JM. Molecular pathophysiology of cerebral edema. *J Cereb Blood Flow Metab*. 2016;36(3):513-538. doi:10.1177/0271678X15617172
17. Ironside N, Chen CJ, Ding D, Mayer SA, Connolly ES. Perihematomal Edema after Spontaneous Intracerebral Hemorrhage. *Stroke*. 2019;50(6):1626-1633. doi:10.1161/STROKEAHA.119.024965
18. Williamson MR, Colbourne F. Evidence for Decreased Brain Parenchymal Volume After Large Intracerebral Hemorrhages: a Potential Mechanism Limiting Intracranial Pressure Rises. *Transl Stroke Res*. 2017;8(4):386-396. doi:10.1007/s12975-017-0530-x
19. Volbers B, Giede-Jeppe A, Gerner ST, et al. Peak perihemorrhagic edema correlates with functional outcome in intracerebral hemorrhage. *Neurology*. 2018;90(12):e1005-e1012.

doi:10.1212/WNL.0000000000005167

20. Hiploylee C, Colbourne F. Intracranial pressure measured in freely moving rats for days after intracerebral hemorrhage. *Exp Neurol*. 2014;255:49-55. doi:10.1016/j.expneurol.2014.02.017

21. Simard JM, Kent TA, Chen M, Tarasov K V., Gerzanich V. Brain oedema in focal ischaemia: molecular pathophysiology and theoretical implications. *Lancet Neurol*. 2007;6(3):258-268. doi:10.1016/S1474-4422(07)70055-8

22. Keep RF, Andjelkovic A V., Xi G. Cytotoxic and Vasogenic Brain Edema. In: *Primer on Cerebrovascular Diseases: Second Edition*. ; 2017. doi:10.1016/B978-0-12-803058-5.00029-1

23. Thiex R, Tsirka SE. Brain edema after intracerebral hemorrhage: mechanisms, treatment options, management strategies, and operative indications. *Neurosurg Focus*. 2007;22(5):E6. doi:10.3171/foc.2007.22.5.7

24. Walcott BP, Kahle KT, Simard JM. Novel Treatment Targets for Cerebral Edema. *Neurotherapeutics*. 2012;9(1):65-72. doi:10.1007/s13311-011-0087-4

25. Lodhia KR, Shakui P, Keep RF. Hydrocephalus in a rat model of intraventricular hemorrhage. *Acta Neurochir Suppl*. 2006;(96):207-211. doi:10.1007/3-211-30714-1_45

26. Helbok R, Olson DW, Le Roux P, et al. Intracranial Pressure and Cerebral Perfusion Pressure Monitoring in Non-TBI Patients: Special Considerations. *Neurocrit Care*. 2014;21(2):85-94. doi:10.1007/s12028-014-0040-6

27. Vespa PM, Miller C, McArthur D, et al. Nonconvulsive electrographic seizures after traumatic brain injury result in a delayed, prolonged increase in intracranial pressure and metabolic crisis. *Crit Care Med*. 2007;35(12):2830-2836. doi:10.1097/01.CCM.0000295667.66853.BC

28. Gabor AJ, Brooks AG, Scobey RP, Parsons GH. Intracranial pressure during epileptic seizures. *Electroencephalogr Clin Neurophysiol*. 1984;57(6):497-506. doi:10.1016/0013-

4694(84)90085-3

29. Malkinson TJ, Veale WL, Cooper KE. Fever and intracranial pressures. *Brain Res Bull*. 1985;15(3):315-319. doi:10.1016/0361-9230(85)90157-1

30. Puccio AM, Fischer MR, Jankowitz BT, Yonas H, Darby JM, Okonkwo DO. Induced normothermia attenuates intracranial hypertension and reduces fever burden after severe traumatic brain injury. *Neurocrit Care*. 2009;11(1):82-87. doi:10.1007/s12028-009-9213-0

31. Scaravilli V, Tinchero G, Citerio G. Fever management in SAH. *Neurocrit Care*. 2011;15(2):287-294. doi:10.1007/s12028-011-9588-6

32. Papo I, Janny P, Caruselli G, Colnet G, Luongo A. Intracranial pressure time course in primary intracerebral hemorrhage. *Neurosurgery*. 1979;4(6):504-511. doi:10.1227/00006123-197906000-00002

33. Juvela S, Kase CS. Advances in intracerebral hemorrhage management. *Stroke*. 2006;37(2):301-304. doi:10.1161/01.STR.0000200445.68303.25

34. Sinar EJ, Mendelow AD, Graham DI, Teasdale GM. Experimental intracerebral hemorrhage: Effects of a temporary mass lesion. *J Neurosurg*. 1987;66(4):568-576. doi:10.3171/jns.1987.66.4.0568

35. Monro A. Observations on structure and functions of the ner- vous system. *Edinburgh: Creech and Johnson*. Published online 1783.

36. Mokri B. The Monro-Kellie hypothesis: Applications in CSF volume depletion. *Neurology*. 2001;56(12):1746-1748. doi:10.1212/WNL.56.12.1746

37. Reinstrup P, Ryding E, Ohlsson T, Dahm PL, Uski T. Cerebral blood volume (CBV) in humans during normo- and hypocapnia: Influence of nitrous oxide (N2O). *Anesthesiology*. 2001;95(5):1079-1082. doi:10.1097/00000542-200111000-00009

38. Bolon B, Butt MT. Appendix 6: Comparative and Correlative Neurobiological Parameters for the Brain. In: *Fundamental Neuropathology for Pathologists and Toxicologists*. ; 2011:551-554. doi:10.1002/9780470939956.app6

39. Pardridge WM. Drug transport in brain via the cerebrospinal fluid. *Fluids Barriers CNS*. 2011;8(1). doi:10.1186/2045-8118-8-7

40. Sandor P, Cox-van Put J, de Jong W, de Wied D. Continuous measurement of cerebral blood volume in rats with the photoelectric technique: Effect of morphine and naloxone. *Life Sci*. 1986;39(18):1657-1665. doi:10.1016/0024-3205(86)90163-3

41. Sakka L, Coll G, Chazal J. Anatomy and physiology of cerebrospinal fluid. *Eur Ann Otorhinolaryngol Head Neck Dis*. 2011;128(6):309-316. doi:10.1016/j.anorl.2011.03.002

42. Beard DJ, Murtha LA, McLeod DD, Spratt NJ. Intracranial Pressure and Collateral Blood Flow. *Stroke*. 2016;47(6):1695-1700. doi:10.1161/STROKEAHA.115.011147

43. Dunn LT. Raised intracranial pressure. *Neurol Pract*. 2002;73(1). doi:10.1136/jnnp.73.suppl_1.i23

44. Eide PK, Sorteberg W. Association among intracranial compliance, intracranial pulse pressure amplitude and intracranial pressure in patients with intracranial bleeds. *Neurol Res*. 2007;29(8):798-802. doi:10.1179/016164107X224132

45. Hariri RJ, Firlick AD, Shepard SR, et al. Traumatic brain injury, hemorrhagic shock, and fluid resuscitation: Effects on intracranial pressure and brain compliance. *J Neurosurg*. 1993;79(3):421-427. doi:10.3171/jns.1993.79.3.0421

46. Fan JY, Kirkness C, Vicini P, Burr R, Mitchell P. Intracranial pressure waveform morphology and intracranial adaptive capacity. *Am J Crit Care*. 2008;17(6):545-554. doi:10.4037/ajcc2008.17.6.545

47. Zazulia AR, Diringer MN, Videen TO, et al. Hypoperfusion without ischemia surrounding acute intracerebral hemorrhage. *J Cereb Blood Flow Metab*. 2001;21(7):804-810. doi:10.1097/00004647-200107000-00005

48. Nath FP, Jenkins A, Mendelow AD, Graham DI, Teasdale GM. Early hemodynamic changes in experimental intracerebral hemorrhage. *J Neurosurg*. 1986;65(5):697-703. doi:10.3171/jns.1986.65.5.0697

49. Qureshi AI, Wilson DA, Hanley DF, Traystman RJ. No evidence for an ischemic penumbra in massive experimental intracerebral hemorrhage. *Neurology*. 1999;52(2):266-272. doi:10.1212/wnl.52.2.266

50. Bothwell SW, Janigro D, Patabendige A. Cerebrospinal fluid dynamics and intracranial pressure elevation in neurological diseases. *Fluids Barriers CNS*. 2019;16(1). doi:10.1186/s12987-019-0129-6

51. Wan S, Hua Y, Keep RF, Hoff JT, Xi G. Deferoxamine reduces CSF free iron levels following intracerebral hemorrhage. *Acta Neurochir Suppl*. 2006;(96):199-202. doi:10.1007/3-211-30714-1_43

52. Bolte AC, Dutta AB, Hurt ME, et al. Meningeal lymphatic dysfunction exacerbates traumatic brain injury pathogenesis. *Nat Commun*. 2020;11(1). doi:10.1038/s41467-020-18113-4

53. Sun BL, Xia ZL, Wang JR, et al. Effects of blockade of cerebral lymphatic drainage on regional cerebral blood flow and brain edema after subarachnoid hemorrhage. In: *Clinical Hemorheology and Microcirculation*. ; 2006.

54. He G, Lü T, Lu B, et al. Perivascular and perineural extension of formed and soluble blood elements in an intracerebral hemorrhage rat model. *Brain Res*. 2012;1451:10-18. doi:10.1016/j.brainres.2012.02.069

55. Gaberel T, Gakuba C, Goulay R, et al. Impaired glymphatic perfusion after strokes revealed by contrast-enhanced MRI: A new target for fibrinolysis? *Stroke*. 2014;45(10):3092-3096. doi:10.1161/STROKEAHA.114.006617

56. Schlunk F, Greenberg SM. The Pathophysiology of Intracerebral Hemorrhage Formation and Expansion. *Transl Stroke Res*. 2015;6(4):257-263. doi:10.1007/s12975-015-0410-1

57. Davis SM, Broderick J, Hennerici M, et al. Hematoma growth is a determinant of mortality and poor outcome after intracerebral hemorrhage. *Neurology*. 2006;66(8):1175-1181. doi:10.1212/01.wnl.0000208408.98482.99

58. Zazulia AR, Diringer MN, Derdeyn CP, Powers WJ. Progression of mass effect after intracerebral hemorrhage. *Stroke*. 1999;30(6):1167-1173. doi:10.1161/01.STR.30.6.1167

59. Heuer GG, Smith MJ, Elliott JP, Winn HR, Leroux PD. Relationship between intracranial pressure and other clinical variables in patients with aneurysmal subarachnoid hemorrhage. *J Neurosurg*. 2004;101(3):408-416. doi:10.3171/jns.2004.101.3.0408

60. Liddle LJ, Ralhan S, Ward DL, Colbourne F. Translational Intracerebral Hemorrhage Research: Has Current Neuroprotection Research ARRIVEd at a Standard for Experimental Design and Reporting? *Transl Stroke Res*. 2020;11(6):1203-1213. doi:10.1007/s12975-020-00824-x

61. Lampl Y, Gilad R, Eshel Y, Sarova-Pinhas I. Neurological and functional outcome in patients with supratentorial hemorrhages; a prospective study. *Stroke*. 1995;26(12):2249-2253. doi:10.1161/01.STR.26.12.2249

62. Butcher K, Laidlaw J. Current intracerebral haemorrhage management. *J Clin Neurosci*. 2003;10(2):158-167. doi:10.1016/S0967-5868(02)00324-7

63. Tian Y, Wang Z, Jia Y, et al. Intracranial pressure variability predicts short-term outcome after intracerebral hemorrhage: A retrospective study. *J Neurol Sci*. 2013;330(1-2):38-44. doi:10.1016/j.jns.2013.04.001

64. Sykora M, Steinmacher S, Steiner T, Poli S, Diedler J. Association of intracranial pressure with outcome in comatose patients with intracerebral hemorrhage. *J Neurol Sci*. 2014;342(1-2):141-145. doi:10.1016/j.jns.2014.05.012

65. Rosenberg G a., Mun-Bryce S, Wesley M, Kornfeld M. Collagenase-induced intracerebral hemorrhage in rats. *Stroke*. 1990;21(5):801-807. doi:10.1161/01.STR.21.5.801

66. Ropper AH, Zervas NT. Cerebral blood flow after experimental basal ganglia hemorrhage. *Ann Neurol*. 1982;11(3):266-271. doi:10.1002/ana.410110306

67. MacLellan CL, Silasi G, Poon CC, et al. Intracerebral hemorrhage models in rat: comparing collagenase to blood infusion. *J Cereb Blood Flow Metab*. 2008;28:516-525.

doi:10.1038/sj.jcbfm.9600548

68. Hanci M. Blood degradation products play a role in cerebral ischemia caused by acute subdural hematoma. *J Neurosurg Sci*. 1997;41(4):379-385.

69. Lee KR, Kawai N, Kim S, Sagher O, Hoff JT. Mechanisms of edema formation after intracerebral hemorrhage: Effects of thrombin on cerebral blood flow, blood-brain barrier permeability, and cell survival in a rat model. *J Neurosurg*. 1997;86(2):272-278. doi:10.3171/jns.1997.86.2.0272

70. Lee KR, Drury I, Vitarbo E, Hoff JT. Seizures induced by intracerebral injection of thrombin: a model of intracerebral hemorrhage. *J Neurosurg*. 1997;87(1):73-78. doi:10.3171/jns.1997.87.1.0073

71. Caliaperumal J, Ma Y, Colbourne F. Intra-parenchymal ferrous iron infusion causes neuronal atrophy, cell death and progressive tissue loss: Implications for intracerebral hemorrhage. *Exp Neurol*. 2012;237(2):363-369. doi:10.1016/j.expneurol.2012.07.001

72. Caliaperumal J, Brodie S, Ma Y, Colbourne F. Thrombin Causes Neuronal Atrophy and Acute but not Chronic Cell Death. *Can J Neurol Sci*. 2014;41(6):714-720. doi:10.1017/cjn.2014.105

73. Lee JM, Zhai G, Liu Q, et al. Vascular permeability precedes spontaneous intracerebral hemorrhage in stroke-prone spontaneously hypertensive rats. *Stroke*. 2007;38(12):3289-3291. doi:10.1161/STROKEAHA.107.491621

74. Davis LA, Smeda JS. Captopril treatment temporarily restores cerebral blood flow autoregulation in spontaneously hypertensive rats after hemorrhagic stroke. *J Cardiovasc Pharmacol*. 2010;56(3):255-262. doi:10.1097/FJC.0b013e3181e8af62

75. Kirkman MA, Allan SM, Parry-Jones AR. Experimental intracerebral hemorrhage: Avoiding pitfalls in translational research. *J Cereb Blood Flow Metab*. 2011;31(11):2135-2151. doi:10.1038/jcbfm.2011.124

76. John RF, Colbourne F. Delayed localized hypothermia reduces intracranial pressure

following collagenase-induced intracerebral hemorrhage in rat. *Brain Res*. 2016;1633:27-36. doi:10.1016/j.brainres.2015.12.033

77. Kingman TA, Mendelow AD, Graham DI, Teasdale GM. Experimental intracerebral mass: Description of model, intracranial pressure changes and neuropathology. *J Neuropathol Exp Neurol*. 1988;47(2):128-137. doi:10.1097/00005072-198803000-00005

78. Karuppagounder SS, Alin L, Chen Y, et al. N-acetylcysteine targets 5 lipoxygenase-derived, toxic lipids and can synergize with prostaglandin E2 to inhibit ferroptosis and improve outcomes following hemorrhagic stroke in mice. *Ann Neurol*. 2018;84(6):854-872. doi:10.1002/ana.25356

79. Zhu W, Gao Y, Chang CF, Wan JR, Zhu SS, Wang J. Mouse models of intracerebral hemorrhage in ventricle, cortex, and hippocampus by injections of autologous blood or collagenase. *PLoS One*. 2014;9(5). doi:10.1371/journal.pone.0097423

80. Lekic T, Tang J, Zhang JH. Rat model of intracerebellar hemorrhage. *Acta Neurochir Suppl*. 2008;(105):131-134. doi:10.1007/978-3-211-09469-3_27

81. Shrestha BK, Guo X, Ma L, et al. Rat Brainstem Hemorrhage Model: Key Points to Success in Modeling. *World Neurosurg*. 2018;117:e106-e116. doi:10.1016/j.wneu.2018.05.195

82. Sloan MA, Sila CA, Mahaffey KW, et al. Prediction of 30-day mortality among patients with thrombolysis-related intracranial hemorrhage. *Circulation*. 1998;98(14):1376-1382. doi:10.1161/01.CIR.98.14.1376

83. Dastur CK, Yu W. Current management of spontaneous intracerebral haemorrhage. *Stroke Vasc Neurol*. 2017;2(1):21-29. doi:10.1136/svn-2016-000047

84. Ziai WC, Thompson CB, Mayo S, et al. Intracranial Hypertension and Cerebral Perfusion Pressure Insults in Adult Hypertensive Intraventricular Hemorrhage: Occurrence and Associations With Outcome. *Crit Care Med*. 2019;47(8):1125-1134. doi:10.1097/CCM.0000000000003848

85. Wilkinson CM, Kalisvaart ACJ, Kung TFC, et al. The collagenase model of intracerebral hemorrhage in awake, freely moving animals: The effects of isoflurane. *Brain Res*. 2020;1728:146593. doi:10.1016/j.brainres.2019.146593

86. Klahr AC, Dickson CT, Colbourne F. Seizure Activity Occurs in the Collagenase but not the Blood Infusion Model of Striatal Hemorrhagic Stroke in Rats. *Transl Stroke Res*. 2014;6(1):29-38. doi:10.1007/s12975-014-0361-y

87. Rudy TA, Westergaard JL, Yaksh TL. Hyperthermia produced by simulated intraventricular hemorrhage in the cat. *Exp Neurol*. 1978;58(2):296-310. doi:10.1016/0014-4886(78)90142-5

88. Wilkinson CM, Fedor BA, Aziz JR, et al. Failure of bumetanide to improve outcome after intracerebral hemorrhage in rat. *PLoS One*. 2019;14(1):1-20. doi:10.1371/journal.pone.0210660

89. MacLellan CL, Paquette R, Colbourne F. A critical appraisal of experimental intracerebral hemorrhage research. *J Cereb Blood Flow Metab*. 2012;32(4):612-627. doi:10.1038/jcbfm.2012.8

90. Qureshi AI, Wilson DA, Traystman RJ. Treatment of elevated intracranial pressure in experimental intracerebral hemorrhage: Comparison between mannitol and hypertonic saline. *Neurosurgery*. 1999;44(5):1055-1064. doi:10.1097/00006123-199905000-00064

91. Qureshi AI, Wilson DA, Hanley DF, Traystman RJ. Pharmacologic reduction of mean arterial pressure does not adversely affect regional cerebral blood flow and intracranial pressure in experimental intracerebral hemorrhage. *Crit Care Med*. 1999;27(5):965-971. doi:10.1097/00003246-199905000-00036

92. Kobari M, Gotoh F, Tomita M, et al. Bilateral hemispheric reduction of cerebral blood volume and blood flow immediately after experimental cerebral hemorrhage in cats. *Stroke*. 1988;19(8):991-996. doi:10.1161/01.STR.19.8.991

93. Tomita H, Ito U, Ohno K, Hirakawa K. Chronological changes in brain edema induced by

experimental intracerebral hematoma in cats. *Acta Neurochir Suppl (Wien)*. 1994;60:558-560. doi:10.1007/978-3-7091-9334-1_154

94. Scheller MS, Tateishi A, Drummond JC, Zornow MH. The effects of sevoflurane on cerebral blood flow, cerebral metabolic rate for oxygen, intracranial pressure, and the electroencephalogram are similar to those of isoflurane in the rabbit. *Anesthesiology*. 1988;68(4):548-551. doi:10.1097/00000542-198804000-00012

95. Karwacki Z, Kowiański P, Moryś J, Dziewiątkowski J, Kaczmarek E, Suchorzewska J. Effect of sevoflurane on intracranial pressure and cardiovascular function in rabbits with experimental intracerebral haematoma. *Med Sci Monit*. 2001;7(2):212-217.

96. Jasien J V., Samuels BC, Johnston JM, Downs JC. Diurnal cycle of translaminar pressure in nonhuman primates quantified with continuous wireless telemetry. *Investig Ophthalmol Vis Sci*. 2020;61(2). doi:10.1167/iovs.61.2.37

97. Silasi G, MacLellan CL, Colbourne F. Use of telemetry blood pressure transmitters to measure intracranial pressure (ICP) in freely moving rats. *Curr Neurovasc Res*. 2009;6(1):62-69. doi:10.2174/156720209787466046

98. Kim KD, Chang CH, Choi BY, Jung YJ. Mortality and real cause of death from the nonlesional intracerebral hemorrhage. *J Korean Neurosurg Soc*. 2014;55(1):1-4. doi:10.3340/jkns.2014.55.1.1

99. Magistris F, Bazak S, Martin J. Intracerebral Hemorrhage: Pathophysiology, Diagnosis and Management. *McMaster Univ Med J*. Published online 2013.

100. Broderick JP, Brott TG, Duldner JE, Tomsick T, Huster G. Volume of intracerebral hemorrhage: A powerful and easy-to-use predictor of 30-day mortality. *Stroke*. 1993;24(7):987-993. doi:10.1161/01.STR.24.7.987

101. James ML, Warner DS, Laskowitz DT. Preclinical models of intracerebral hemorrhage: A translational perspective. *Neurocrit Care*. 2008;9(1):139-152. doi:10.1007/s12028-007-9030-2

102. Hayashi M, Kobayashi H, Kawano H, Handa Y, Hirose S. Treatment of systemic hypertension and intracranial hypertension in cases of brain hemorrhage. *Stroke*. 1988;19(3):314-321. doi:10.1161/01.STR.19.3.314

103. Shintani S, Umezato M, Toba Y, et al. Pharmacological properties of buprenorphine, a new analgesic agent. Part II. *Folia Pharmacol Jpn*. 1982;79(3):173-191. doi:10.1254/fpj.79.173

104. Kamel H, Hemphill JC. Characteristics and sequelae of intracranial hypertension after intracerebral hemorrhage. *Neurocrit Care*. 2012;17(2):172-176. doi:10.1007/s12028-012-9744-7

105. Shahrokhi N, Khaksari M, Soltani Z, Mahmoodi M, Nakhaee N. Effect of sex steroid hormones on brain edema, intracranial pressure, and neurologic outcomes after traumatic brain injury. *Can J Physiol Pharmacol*. 2010;88(4):414-421. doi:10.1139/Y09-126

106. Gong Y, Hua Y, Keep RF, Hoff JT, Xi G. Intracerebral hemorrhage: Effects of aging on brain edema and neurological deficits. In: *Stroke*. ; 2004. doi:10.1161/01.STR.0000145485.67827.d0

107. Hamezah HS, Durani LW, Ibrahim NF, et al. Volumetric changes in the aging rat brain and its impact on cognitive and locomotor functions. *Exp Gerontol*. 2017;99:69-79. doi:10.1016/j.exger.2017.09.008

108. Sheth K, Elm J, Cheung K, et al. Abstracts for the 10th World Stroke Congress, 2016. *Int J Stroke*. 2016;11(3_suppl):4-288. doi:10.1177/1747493016670567

109. Uldall M, Juhler M, Skjolding AD, Kruuse C, Jansen-Olesen I, Jensen R. A novel method for long-term monitoring of intracranial pressure in rats. *J Neurosci Methods*. 2014;227:1-9. doi:10.1016/j.jneumeth.2014.01.036

110. Wagner I, Volbers B, Kloska S, Doerfler A, Schwab S, Staykov D. Sex differences in perihemorrhagic edema evolution after spontaneous intracerebral hemorrhage. *Eur J*

Neurol. 2012;19(11):1477-1481. doi:10.1111/j.1468-1331.2011.03628.x

111. Jiang C, Zuo F, Wang Y, et al. Progesterone exerts neuroprotective effects and improves long-term neurologic outcome after intracerebral hemorrhage in middle-aged mice. *Neurobiol Aging*. 2016;42:13-24. doi:10.1016/j.neurobiolaging.2016.02.029

112. Chen Z, Xi G, Mao Y, Keep RF, Hua Y. Effects of progesterone and testosterone on ICH-induced brain injury in rats. In: *Acta Neurochirurgica, Supplementum*. ; 2011. doi:10.1007/978-3-7091-0693-8_48

113. Ariesen MJ, Claus SP, Rinkel GJE, Algra A. Risk factors for intracerebral hemorrhage in the general population: A systematic review. *Stroke*. 2003;34(8):2060-2065. doi:10.1161/01.STR.0000080678.09344.8D

114. Immink R V., Van Den Born BJH, Van Montfrans GA, Koopmans RP, Karemaker JM, Van Lieshout JJ. Impaired cerebral autoregulation in patients with malignant hypertension. *Circulation*. 2004;110(15):2241-2245. doi:10.1161/01.CIR.0000144472.08647.40

115. Smeda JS, VanVliet BN, King SR. Stroke-prone spontaneously hypertensive rats lose their ability to auto-regulate cerebral blood flow prior to stroke. *J Hypertens*. 1999;17(12):1697-1705. doi:10.1097/00004872-199917120-00006

116. Lapchak PA, Zhang JH, Noble-Haeusslein LJ. RIGOR Guidelines: Escalating STAIR and STEPS for Effective Translational Research. *Transl Stroke Res*. 2013;4(3):279-285. doi:10.1007/s12975-012-0209-2

117. Fisher M, Feuerstein G, Howells DW, et al. Update of the stroke therapy academic industry roundtable preclinical recommendations. *Stroke*. 2009;40(6):2244-2250. doi:10.1161/STROKEAHA.108.541128

118. Kawoos U, Gu M, Lankasky J, McCarron RM, Chavko M. Effects of exposure to blast overpressure on intracranial pressure and blood-brain barrier permeability in a rat model. *PLoS One*. 2016;11(12). doi:10.1371/journal.pone.0167510

119. Kawoos U, Meng X, Huang SM, Rosen A, McCarron RM, Chavko M. Telemetric

intracranial pressure monitoring in blast-induced traumatic brain injury. *IEEE Trans Biomed Eng*. 2014;61(3):841-847. doi:10.1109/TBME.2013.2291239

120. Kawoos U, McCarron RM, Chavko M. Protective effect of N-acetylcysteine amide on blast-induced increase in intracranial pressure in rats. *Front Neurol*. 2017;8(JUN). doi:10.3389/fneur.2017.00219

121. Lin JS, Liu JHK. Circadian variations in intracranial pressure and translaminar pressure difference in sprague-dawley rats. *Investig Ophthalmol Vis Sci*. 2010;51(11):5739-5743. doi:10.1167/iovs.10-5542

122. Jiang J, Tyssebotn I. Measurement of cerebrospinal fluid pressure in conscious rats. *Undersea Hyperb Med*. 1997;24(1):39-43.

123. Malkinson TJ, Cooper KE, Veale WL. Cerebrospinal fluid pressure in conscious rats during prostaglandin E1 fever. *Am J Physiol - Regul Integr Comp Physiol*. 1990;258(3 27-3). doi:10.1152/ajpregu.1990.258.3.r783

124. Alshuhri MS, Gallagher L, McCabe C, Holmes WM. Change in CSF Dynamics Responsible for ICP Elevation After Ischemic Stroke in Rats: a New Mechanism for Unexplained END? *Transl Stroke Res*. 2020;11(2):310-318. doi:10.1007/s12975-019-00719-6

125. Eide PK. Comparison of simultaneous continuous intracranial pressure (ICP) signals from ICP sensors placed within the brain parenchyma and the epidural space. *Med Eng Phys*. 2008;30(1):34-40. doi:10.1016/j.medengphy.2007.01.005

126. Zwienenberg M, Gong QZ, Lee LL, Berman RF, Lyeth BG. ICP monitoring in the rat: Comparison of monitoring in the ventricle, brain parenchyma, and cistema magna. *J Neurotrauma*. 1999;16(11):1095-1102. doi:10.1089/neu.1999.16.1095

127. Williamson MR, Wilkinson CM, Dietrich K, Colbourne F. Acetazolamide Mitigates Intracranial Pressure Spikes Without Affecting Functional Outcome After Experimental Hemorrhagic Stroke. *Transl Stroke Res*. 2019;10(4):428-439. doi:10.1007/s12975-018-

0663-6

128. Guild SJ, McBryde FD, Malpas SC. Recording of intracranial pressure in conscious rats via telemetry. *J Appl Physiol*. 2015;119(5):576-581. doi:10.1152/japplphysiol.00165.2015

129. Hemphill JC, Greenberg SM, Anderson CS, et al. Guidelines for the Management of Spontaneous Intracerebral Hemorrhage: A Guideline for Healthcare Professionals from the American Heart Association/American Stroke Association. *Stroke*. 2015;46(7):2032-2060. doi:10.1161/STR.0000000000000069

130. Goren S, Kahveci N, Alkan T, Goren B, Korfali E. The effects of sevoflurane and isoflurane on intracranial pressure and cerebral perfusion pressure after diffuse brain injury in rats. *J Neurosurg Anesthesiol*. 2001;13(2):113-119. doi:10.1097/00008506-200104000-00008

131. Bar-Joseph G, Guilburd Y, Tamir A, Guilburd JN. Effectiveness of ketamine in decreasing intracranial pressure in children with intracranial hypertension: Clinical article. *J Neurosurg Pediatr*. 2009;4(1):40-46. doi:10.3171/2009.1.PEDS08319

132. Albanèse J, Arnaud S, Rey M, Thomachot L, Alliez B, Martin C. Ketamine decreases intracranial pressure and electroencephalographic activity in traumatic brain injury patients during propofol sedation. *Anesthesiology*. 1997;87(6):1328-1334. doi:10.1097/00000542-199712000-00011

133. Pinaud M, Lelausque JN, Chetanneau A, Fauchoux N, Menegalli D, Souron R. Effects of propofol on cerebral hemodynamics and metabolism in patients with brain trauma. *Anesthesiology*. 1990;73(3):404-409. doi:10.1097/00000542-199009000-00007

134. Jennett WB, Barker J, Fitch W, McDowall DG. Effect of anaesthesia on intracranial pressure in patients with space-occupying lesions. *Lancet*. 1969;1(7585):61-64. doi:10.1097/00132586-197002000-00037

135. Bazin JE. Effects of anaesthetic agents on intracranial pressure. In: *Annales Francaises d'Anesthesie et de Reanimation*. ; 1997. doi:10.1016/S0750-7658(97)81477-3

136. Starcevic VP, Morrow BA, Farner LA, Keil LC, Severs WB. Long-term recording of cerebrospinal fluid pressure in freely behaving rats. *Brain Res*. 1988;462(1):112-117. doi:10.1016/0006-8993(88)90592-6

137. Irvine RJ, White J, Chan R. The influence of restrains on blood pressure in the rat. *J Pharmacol Toxicol Methods*. 1997;38(3):157-162. doi:10.1016/S1056-8719(97)00081-6

138. Wilkinson CM, Kalisvaart ACJ, Kung TFC, et al. The collagenase model of intracerebral hemorrhage in awake, freely moving animals: The effects of isoflurane. *Brain Res*. 2020;1728:146593. doi:10.1016/j.brainres.2019.146593

139. Mandell EC, Zimmermann E. Continuous measurement of cerebrospinal fluid pressure in unrestrained rats. *Physiol Behav*. 1980;24(2):399-402. doi:10.1016/0031-9384(80)90105-5

140. Chen CJ, Ding D, Ironside N, et al. Intracranial pressure monitoring in patients with spontaneous intracerebral hemorrhage. *J Neurosurg*. 2020;132(6):1854-1864. doi:10.3171/2019.3.JNS19545

141. Subramaniam S, Hill MD. Controversies in medical management of intracerebral hemorrhage. *Can J Neurol Sci*. 2005;32 Suppl 2:S13-21. http://www.ncbi.nlm.nih.gov/pubmed/16450804

142. Maira G, Anile C, Colosimo C, Rossi GF. Surgical treatment of primary supratentorial intracerebral hemorrhage in stuporous and comatose patients. *Neurol Res*. 2002;24(1):54-60. doi:10.1179/016164102101199549

143. Aucoin PJ, Kotilainen HR, Gantz NM, Davidson R, Kellogg P, Stone B. Intracranial pressure monitors. Epidemiologic study of risk factors and infections. *Am J Med*. 1986;80(3):369-376. doi:10.1016/0002-9343(86)90708-4

144. North B, Reilly P. Comparison among three methods of intracranial pressure recording. *Neurosurgery*. 1986;18(6):730-732. doi:10.1227/00006123-198606000-00009

145. Rajajee V, Vanaman M, Fletcher JJ, Jacobs TL. Optic nerve ultrasound for the detection

of raised intracranial pressure. *Neurocrit Care*. 2011;15(3):506-515. doi:10.1007/s12028-011-9606-8

146. de Oliveira Manoel AL, Goffi A, Zampieri FG, et al. The critical care management of spontaneous intracranial hemorrhage: A contemporary review. *Crit Care*. 2016;20(1). doi:10.1186/s13054-016-1432-0

147. Marinkovic I, Strbian D, Pedrono E, et al. Decompressive craniectomy for intracerebral hemorrhage. *Neurosurgery*. 2009;65(4):780-786. doi:10.1227/01.NEU.0000351775.30702.A9

148. Bühler D, Azghandi S, Schüller K, Plesnila N. Effect of decompressive craniectomy on outcome following subarachnoid hemorrhage in mice. *Stroke*. 2015;46(3):819-826. doi:10.1161/STROKEAHA.114.007703

149. Kuczynski AM, Marzoughi S, Al Sultan AS, et al. Therapeutic Hypothermia in Acute Ischemic Stroke-a Systematic Review and Meta-Analysis. *Curr Neurol Neurosci Rep*. 2020;20(5):13. doi:10.1007/s11910-020-01029-3

150. Sacco S, Marini C, Carolei A. Medical treatment of intracerebral hemorrhage. *Neurol Sci*. 2004;25(SUPPL. 1). doi:10.1007/s10072-004-0206-7

151. Mendelow AD, Gregson BA, Rowan EN, et al. Early surgery versus initial conservative treatment in patients with spontaneous supratentorial lobar intracerebral haematomas (STICH II): A randomised trial. *Lancet*. 2013;382(9890):397-408. doi:10.1016/S0140-6736(13)60986-1

152. Alberio N, Cicero S, Iacopino DG, et al. Minimally Invasive Management of Spontaneous Supratentorial Intracerebral Lobar Hemorrhages by a “Homemade” Endoscopic Strategy: The Evangelical Doctrine of “Venite ad Me” Allied to the Legacy of King Leonida. *World Neurosurg*. 2019;122:638-647. doi:10.1016/j.wneu.2018.11.136

153. Li Y, Yang R, Li Z, et al. Surgical Evacuation of Spontaneous Supratentorial Lobar Intracerebral Hemorrhage: Comparison of Safety and Efficacy of Stereotactic Aspiration,

Endoscopic Surgery, and Craniotomy. *World Neurosurg*. 2017;105:332-340. doi:10.1016/j.wneu.2017.05.134

154. Ziai WC, Toung TJK, Bhardwaj A. Hypertonic saline: First-line therapy for cerebral edema? *J Neurol Sci*. 2007;261(1-2):157-166. doi:10.1016/j.jns.2007.04.048

155. Mirski MA, Denchev DI, Schnitzer MS, Hanley DF. Comparison between hypertonic saline and mannitol in the reduction of elevated intracranial pressure in a rodent model of acute cerebral injury. *J Neurosurg Anesthesiol*. 2000;12(4):334-344. doi:10.1097/00008506-200010000-00006

156. Kamel H, Navi BB, Nakagawa K, Hemphill JC, Ko NU. Hypertonic saline versus mannitol for the treatment of elevated intracranial pressure: A meta-analysis of randomized clinical trials. *Crit Care Med*. 2011;39(3):554-559. doi:10.1097/CCM.0b013e318206b9be

157. Qureshi AI, Suri MFK, Ringer AJ, Guterman LR, Hopkins LN. Regional intraparenchymal pressure differences in experimental intracerebral hemorrhage: Effect of hypertonic saline. *Crit Care Med*. 2002;30(2):435-441. doi:10.1097/00003246-200202000-00028

158. Da Silva JC, De Melo Tavares De Lima F, Valença MM, Cirne De Azevedo Filho HR. Hypertonic saline more efficacious than mannitol in lethal intracranial hypertension model. *Neurol Res*. 2010;32(2):139-143. doi:10.1179/174313209X405119

159. Schreibman DL, Hong CM, Keledjian K, et al. Mannitol and Hypertonic Saline Reduce Swelling and Modulate Inflammatory Markers in a Rat Model of Intracerebral Hemorrhage. *Neurocrit Care*. 2018;29(2):253-263. doi:10.1007/s12028-018-0535-7

160. Glimåker M, Johansson B, Halldorsdottir H, et al. Neuro-intensive treatment targeting intracranial hypertension improves outcome in severe bacterial meningitis: An intervention-control study. *PLoS One*. 2014;9(3). doi:10.1371/journal.pone.0091976

161. Mitchell P, Gregson BA, Vindlacheruvu RR, Mendelow AD. Surgical options in ICH including decompressive craniectomy. *J Neurol Sci*. 2007;261(1-2):89-98. doi:10.1016/j.jns.2007.04.040

162. Guo G, Guo W, Feng Y, et al. Therapeutic time window of minimally invasive surgery for intracerebral hemorrhage. *Brain Hemorrhages*. 2020;(xxxx):4-6. doi:10.1016/j.hest.2020.07.002

163. Fukuhara T, Luciano MG, Brant CL, Klauscie J. Effects of ventriculoperitoneal shunt removal on cerebral oxygenation and brain compliance in chronic obstructive hydrocephalus. *J Neurosurg*. 2001;94(4):573-581. doi:10.3171/jns.2001.94.4.0573

164. Miller JM, McAllister JP. Reduction of astrogliosis and microgliosis by cerebrospinal fluid shunting in experimental hydrocephalus. *Cerebrospinal Fluid Res*. 2007;4. doi:10.1186/1743-8454-4-5

165. Lopez Valdes E, Hernandez Lain A, Calandre L, Grau M, Cabello A, Gomez-Escalonilla C. Time window for clinical effectiveness of mass evacuation in a rat balloon model mimicking an intraparenchymatous hematoma. *J Neurol Sci*. 2000;174(1):40-46. doi:10.1016/S0022-510X(99)00288-9

166. Nehls DG, Mendelow AD, Graham DI, Teasdale GM. Experimental intracerebral hemorrhage: Early removal of a spontaneous mass lesion improves late outcome. *Neurosurgery*. 1990;27(5):674-682. doi:10.1227/00006123-199011000-00002

167. Deinsberger W, Vogel J, Fuchs C, Auer LM, Kuschinsky W, Böker DK. Fibrinolysis and aspiration of experimental intracerebral hematoma reduces the volume of ischemic brain in rats. *Neurol Res*. 1999;21(5):517-523. doi:10.1080/01616412.1999.11740968

168. Thiex R, Küker W, Jungbluth P, et al. Minor inflammation after surgical evacuation compared with fibrinolytic therapy of experimental intracerebral hemorrhages. *Neurol Res*. 2005;27(5):493-498. doi:10.1179/016164105X17369

169. Wu G, Sheng F, Wang L, Wang F. The pathophysiological time window study of performing minimally invasive procedures for the intracerebral hematoma evacuation in rabbit. *Brain Res*. 2012;1465:57-65. doi:10.1016/j.brainres.2012.04.005

170. Rohde V, Rohde I, Thiex R, et al. Fibrinolysis therapy achieved with tissue plasminogen

activator and aspiration of the liquefied clot after experimental intracerebral hemorrhage: Rapid reduction in hematoma volume but intensification of delayed edema formation. *J Neurosurg*. 2002;97(4):954-962. doi:10.3171/jns.2002.97.4.0954

171. Kolecka M, Ondreka N, Moritz A, Kramer M, Schmidt MJ. Effect of acetazolamide and subsequent ventriculo-peritoneal shunting on clinical signs and ventricular volumes in dogs with internal hydrocephalus. *Acta Vet Scand*. 2015;57(1). doi:10.1186/s13028-015-0137-8
172. Uldall M, Botfield H, Jansen-Olesen I, Sinclair A, Jensen R. Acetazolamide lowers intracranial pressure and modulates the cerebrospinal fluid secretion pathway in healthy rats. *Neurosci Lett*. 2017;645:33-39. doi:10.1016/j.neulet.2017.02.032
173. Wilkinson HA, Rosenfeld S. Furosemide and mannitol in the treatment of acute experimental intracranial hypertension. *Neurosurgery*. 1983;12(4):405-410. doi:10.1227/00006123-198304000-00006
174. Miller TB, Wilkinson HA, Rosenfeld SA, Furuta T. Intracranial hypertension and cerebrospinal fluid production in dogs: Effects of furosemide. *Exp Neurol*. 1986;94(1):66-80. doi:10.1016/0014-4886(86)90272-4
175. Scotton WJ, Botfield HF, Westgate CSJ, et al. Topiramate is more effective than acetazolamide at lowering intracranial pressure. *Cephalalgia*. 2019;39(2):209-218. doi:10.1177/0333102418776455
176. Kahveci FS, Kahveci N, Alkan T, Goren B, Korfali E, Ozluk K. Propofol versus isoflurane anesthesia under hypothermic conditions: Effects on intracranial pressure and local cerebral blood flow after diffuse traumatic brain injury in the rat. *Surg Neurol*. 2001;56(3):206-214. doi:10.1016/S0090-3019(01)00555-9
177. Clubb RJ, Maxwell RE, Chou SN. Experimental brain injury in the dog. The pharmacological effects of pentobarbital and sodium nitroprusside. *J Neurosurg*. 1980;52(2):189-196. doi:10.3171/jns.1980.52.2.0189

178. Hayashi M, Kobayashi H, Kawano H, Handa Y, Kabuto M. The effects of local intraparenchymal pentobarbital on intracranial hypertension following experimental subarachnoid hemorrhage. *Anesthesiology*. 1987;66(6):758-765. doi:10.1097/00000542-198706000-00009

179. Villa F, Iacca C, Molinari AF, et al. Inhalation versus endovenous sedation in subarachnoid hemorrhage patients: Effects on regional cerebral blood flow. *Crit Care Med*. 2012;40(10):2797-2804. doi:10.1097/CCM.0b013e31825b8bc6

180. Roberts DJ, Hall RI, Kramer AH, Robertson HL, Gallagher CN, Zygun DA. Sedation for critically ill adults with severe traumatic brain injury: A systematic review of randomized controlled trials. *Crit Care Med*. 2011;39(12):2743-2751. doi:10.1097/CCM.0b013e318228236f

181. Choi HA, Badjatia N, Mayer SA. Hypothermia for acute brain injury - Mechanisms and practical aspects. *Nat Rev Neurol*. 2012;8(4):214-222. doi:10.1038/nrneurol.2012.21

182. Hemmen TM, Lyden PD. Induced hypothermia for acute stroke. In: *Stroke*. ; 2007. doi:10.1161/01.STR.0000247920.15708.fa

183. Kawai N, Nakamura T, Okauchi M, Nagao S. Effects of hypothermia on intracranial pressure and brain edema formation: Studies in a rat acute subdural hematoma model. *J Neurotrauma*. 2000;17(3):193-202. doi:10.1089/neu.2000.17.193

184. John RF, Williamson MR, Dietrich K, Colbourne F. Localized Hypothermia Aggravates Bleeding in the Collagenase Model of Intracerebral Hemorrhage. *Ther Hypothermia Temp Manag*. 2015;5(1):19-25. doi:10.1089/ther.2014.0020

185. Wowk S, Fagan KJ, Ma Y, Nichol H, Colbourne F. Examining potential side effects of therapeutic hypothermia in experimental intracerebral hemorrhage. *J Cereb Blood Flow Metab*. 2017;37(8):2975-2986. doi:10.1177/0271678X16681312

186. Murtha LA, McLeod DD, Pepperall D, et al. Intracranial pressure elevation after ischemic stroke in rats: Cerebral edema is not the only cause, and short-duration mild hypothermia

is a highly effective preventive therapy. *J Cereb Blood Flow Metab*. 2015;35(4):592-600. doi:10.1038/jcbfm.2014.230

187. Murtha LA, Mcleod DD, Mccann SK, et al. Short-duration hypothermia after ischemic stroke prevents delayed intracranial pressure rise. *Int J Stroke*. 2014;9(5):553-559. doi:10.1111/ijs.12181

188. Moore EM, Nichol AD, Bernard SA, Bellomo R. Therapeutic hypothermia: Benefits, mechanisms and potential clinical applications in neurological, cardiac and kidney injury. *Injury*. 2011;42(9):843-854. doi:10.1016/j.injury.2011.03.027

189. Kollmar R, Staykov D, Dörfler A, Schellinger PD, Schwab S, Bardutzky J. Hypothermia reduces perihemorrhagic edema after intracerebral hemorrhage. *Stroke*. 2010;41(8):1684-1689. doi:10.1161/STROKEAHA.110.587758

190. Staykov D, Wagner I, Volbers B, Doerfler A, Schwab S, Kollmar R. Mild prolonged hypothermia for large intracerebral hemorrhage. *Neurocrit Care*. 2013;18(2):178-183. doi:10.1007/s12028-012-9762-5

191. Citerio G, Prisco L, Oddo M, et al. International prospective observational study on intracranial pressure in intensive care (ICU): The SYNAPSE-ICU study protocol. *BMJ Open*. 2019;9(4). doi:10.1136/bmjopen-2018-026552

192. Giraudet F, Longeras F, Mulliez A, et al. Noninvasive detection of alarming intracranial pressure changes by auditory monitoring in early management of brain injury: A prospective invasive versus noninvasive study. *Crit Care*. 2017;21(1). doi:10.1186/s13054-017-1616-2

193. Seule M, Sikorski C, Sakowitz O, et al. Evaluation of a New Brain Tissue Probe for Intracranial Pressure, Temperature, and Cerebral Blood Flow Monitoring in Patients with Aneurysmal Subarachnoid Hemorrhage. *Neurocrit Care*. 2016;25(2):193-200. doi:10.1007/s12028-016-0284-4

194. Preethi J, Bidkar PU, Cherian A, et al. Comparison of total intravenous anesthesia vs.

inhalational anesthesia on brain relaxation, intracranial pressure, and hemodynamics in patients with acute subdural hematoma undergoing emergency craniotomy: a randomized control trial. *Eur J Trauma Emerg Surg*. 2021;47(3):831-837. doi:10.1007/s00068-019-01249-4

195. Wilkinson CM, Brar PS, Balay CJ, Colbourne F. Glibenclamide, a Sur1-Trpm4 antagonist, does not improve outcome after collagenase-induced intracerebral hemorrhage. *PLoS One*. 2019;14(5). doi:10.1371/journal.pone.0215952
196. Jiang B, Li L, Chen Q, et al. Role of Glibenclamide in Brain Injury After Intracerebral Hemorrhage. *Transl Stroke Res*. 2017;8(2):183-193. doi:10.1007/s12975-016-0506-2
197. Uddin MS, Mamun A Al, Jakaria M, et al. Emerging promise of sulforaphane-mediated Nrf2 signaling cascade against neurological disorders. *Sci Total Environ*. 2020;707. doi:10.1016/j.scitotenv.2019.135624
198. Zhao X, Sun G, Zhang J, et al. Transcription factor Nrf2 protects the brain from damage produced by intracerebral hemorrhage. *Stroke*. 2007;38(12):3280-3286. doi:10.1161/STROKEAHA.107.486506
199. Schmidt-Pogoda A, Bonberg N, Koecke MHM, et al. Why Most Acute Stroke Studies Are Positive in Animals but Not in Patients: A Systematic Comparison of Preclinical, Early Phase, and Phase 3 Clinical Trials of Neuroprotective Agents. *Ann Neurol*. 2020;87(1):40-51. doi:10.1002/ana.25643
200. Tuhrim S, Horowitz DR, Sacher M, Godbold JH. Volume of ventricular blood is an important determinant of outcome in supratentorial intracerebral hemorrhage. *Crit Care Med*. 1999;27(3):617-621. doi:10.1097/00003246-199903000-00045
201. Roman MJ, Pini R, Pickering TG, Devereux RB. Non-invasive measurements of arterial compliance in hypertensive compared with normotensive adults. *J Hypertens*. 1992;10:S115-S118. doi:10.1097/00004872-199208001-00029

Chapter 3

1. Feigin VL, Lawes CM, Bennett DA, Barker-Collo SL, Parag V. Worldwide stroke incidence and early case fatality reported in 56 population-based studies: a systematic review. *Lancet Neurol*. 2009;8(4):355-369. doi:10.1016/S1474-4422(09)70025-0
2. van Asch CJ, Luitse MJ, Rinkel GJ, van der Tweel I, Algra A, Klijn CJ. Incidence, case fatality, and functional outcome of intracerebral haemorrhage over time, according to age, sex, and ethnic origin: a systematic review and meta-analysis. *Lancet Neurol*. 2010;9(2):167-176. doi:10.1016/S1474-4422(09)70340-0
3. MacLellan CL, Paquette R, Colbourne F. A critical appraisal of experimental intracerebral hemorrhage research. *J Cereb Blood Flow Metab*. 2012;32(4):612-627. doi:10.1038/jcbfm.2012.8
4. Jiang M, Sun L, Feng DX, et al. Neuroprotection provided by isoflurane pre-conditioning and post-conditioning. *Med Gas Res*. 2017;7(1):48-55. doi:10.4103/2045-9912.202910
5. Statler KD, Alexander H, Vagni V, et al. Isoflurane exerts neuroprotective actions at or near the time of severe traumatic brain injury. *Brain Res*. 2006;1076(1):216-224. doi:10.1016/j.brainres.2005.12.106
6. Tsurugizawa T, Takahashi Y, Kato F. Distinct effects of isoflurane on basal BOLD signals in tissue/vascular microstructures in rats. *Sci Rep*. 2016;6. doi:10.1038/srep38977
7. Archer DP, Walker AM, McCann SK, Moser JJ, Appireddy RM. Anesthetic Neuroprotection in Experimental Stroke in Rodents: A Systematic Review and Meta-analysis. *Anesthesiology*. 2017;126(4):653-665. doi:10.1097/ALN.0000000000001534
8. Seto A, Taylor S, Trudeau D, et al. Induction of ischemic stroke in awake freely moving mice reveals that isoflurane anesthesia can mask the benefits of a neuroprotection therapy. *Front Neuroenergetics*. 2014;6(APR). doi:10.3389/fnene.2014.00001
9. Ramachandra V, Moore C, Kaur N, Carli F. Effect of halothane, enflurane and isoflurane on body temperature during and after surgery. *Br J Anaesth*. 1989;62(4):409-414.

doi:10.1093/bja/62.4.409

10. Xie GM, Lauber R, Zbinden AM. Nitrous oxide decreases solubility of isoflurane and halothane in blood. *Anesth Analg*. 1993;77(4):761-765. doi:10.1213/00000539-199310000-00018
11. Duke T, Caulkett NA, Tataryn JM. The effect of nitrous oxide on halothane, isoflurane and sevoflurane requirements in ventilated dogs undergoing ovariohysterectomy. *Vet Anaesth Analg*. 2006;33(6):343-350. doi:10.1111/j.1467-2995.2005.00274.x
12. Chen M, Olsen JI, Stolk JA, Schweizer MP, Sha M, Ueda I. An in vivo 19F NMR study of isoflurane elimination as a function of age in rat brain. *NMR Biomed*. 1992;5(3):121-126. doi:10.1002/nbm.1940050304
13. MacLellan CL, Silasi G, Poon CC, et al. Intracerebral hemorrhage models in rat: comparing collagenase to blood infusion. *J Cereb Blood Flow Metab*. 2008;28:516-525. doi:10.1038/sj.jcbfm.9600548
14. Matchett GA, Allard MW, Martin RD, Zhang JH. Neuroprotective effect of volatile anesthetic agents: Molecular mechanisms. *Neurol Res*. 2009;31(2):128-134. doi:10.1179/174313209X393546
15. Wang H, Li P, Xu N, et al. Paradigms and mechanisms of inhalational anesthetics mediated neuroprotection against cerebral ischemic stroke. *Med Gas Res*. 2016;6(4):194-205. doi:10.4103/2045-9912.196901
16. Khatibi NH, Ma Q, Rolland W, et al. Isoflurane posttreatment reduces brain injury after an intracerebral hemorrhagic stroke in mice. *Anesth Analg*. 2011;113(2):343-348. doi:10.1213/ANE.0b013e31821f9524
17. Gigante PR, Appelboom G, Hwang BY, et al. Isoflurane preconditioning affords functional neuroprotection in a murine model of intracerebral hemorrhage. In: *Acta Neurochirurgica, Supplementum*. ; 2011. doi:10.1007/978-3-7091-0693-8_23
18. Constantinides C, Murphy K. Molecular and Integrative Physiological Effects of Isoflurane

Anesthesia: The Paradigm of Cardiovascular Studies in Rodents using Magnetic Resonance Imaging. *Front Cardiovasc Med*. 2016;3. doi:10.3389/fcvm.2016.00023

19. Sicard K, Shen Q, Brevard ME, et al. Regional cerebral blood flow and BOLD responses in conscious and anesthetized rats under basal and hypercapnic conditions: Implications for functional MRI studies. *J Cereb Blood Flow Metab*. 2003;23(4):472-481. doi:10.1097/01.WCB.0000054755.93668.20
20. Yang C-F, Yu-Chih Chen M, Chen T-I, Cheng C-F. Dose-dependent effects of isoflurane on cardiovascular function in rats. *Tzu Chi Med J*. 2014;26(3):119-122. doi:10.1016/J.TCMJ.2014.07.005
21. Wolberg AS, Meng ZH, Monroe DM, Hoffman M. A systematic evaluation of the effect of temperature on coagulation enzyme activity and platelet function. *J Trauma - Inj Infect Crit Care*. 2004;56(6):1221-1228. doi:10.1097/01.TA.0000064328.97941.FC
22. Khurram S, Hayes I, Buggy D. Pharmacology of anaesthetic agents I: intravenous anaesthetic agents. *Contin Educ Anesth Crit Care Pain*. 2014;4(3):100-105. doi:10.1093/bjaceaccp/mkt039
23. Colbourne F, Nurse SM, Corbett D. Temperature changes associated with forebrain ischemia in the gerbil. *Brain Res*. 1993;602(2):264-267. doi:10.1016/0006-8993(93)90691-F
24. Moore EM, Nichol AD, Bernard SA, Bellomo R. Therapeutic hypothermia: Benefits, mechanisms and potential clinical applications in neurological, cardiac and kidney injury. *Injury*. 2011;42(9):843-854. doi:10.1016/j.injury.2011.03.027
25. Klahr AC, Dickson CT, Colbourne F. Seizure Activity Occurs in the Collagenase but not the Blood Infusion Model of Striatal Hemorrhagic Stroke in Rats. *Transl Stroke Res*. 2014;6(1):29-38. doi:10.1007/s12975-014-0361-y
26. Klahr AC, Dietrich K, Dickson CT, Colbourne F. Prolonged Localized Mild Hypothermia Does Not Affect Seizure Activity after Intracerebral Hemorrhage in Rats. *Ther*

Hypothermia Temp Manag. 2016;6(1):40-47. doi:10.1089/ther.2015.0028

27. Amzica F. Basic physiology of burst-suppression. *Epilepsia*. 2009;50:38-39. doi:10.1111/j.1528-1167.2009.02345.x

28. Hentschke H, Raz A, Krause BM, Murphy CA, Banks MI. Disruption of cortical network activity by the general anaesthetic isoflurane. *Br J Anaesth*. 2017;119(4):685-696. doi:10.1093/bja/aex199

29. Vega-Zelaya L, Pastor J, De Sola RG, Ortega GJ. Disrupted ipsilateral network connectivity in temporal lobe epilepsy. *PLoS One*. 2015;10(10). doi:10.1371/journal.pone.0140859

30. Castillo J, Dávalos A, Álvarez-Sabín J, et al. Molecular signatures of brain injury after intracerebral hemorrhage. *Neurology*. 2002;58(4):624-629. doi:10.1212/WNL.58.4.624

31. Hiploylee C, Colbourne F. Intracranial pressure measured in freely moving rats for days after intracerebral hemorrhage. *Exp Neurol*. 2014;255:49-55. doi:10.1016/j.expneurol.2014.02.017

32. Paxinos G, Watson C. Index of Structures. *Rat Brain Stereotaxic Coord*. Published online 1982:7-9. doi:10.1016/b978-0-12-547620-1.50008-4

33. Williams LR, Vahlsing HL, Lindamood T, Varon S, Gage FH, Manthorpe M. A small-gauge cannula device for continuous infusion of exogenous agents into the brain. *Exp Neurol*. 1987;95(3):743-754. doi:10.1016/0014-4886(87)90313-X

34. Shah AA, Sjovold T, Treit D. Inactivation of the medial prefrontal cortex with the GABA A receptor agonist muscimol increases open-arm activity in the elevated plus-maze and attenuates shock-probe burying in rats. *Brain Res*. 2004;1028(1):112-115. doi:10.1016/j.brainres.2004.08.061

35. Sotocinal SG, Sorge RE, Zaloum A, et al. The Rat Grimace Scale: A partially automated method for quantifying pain in the laboratory rat via facial expressions. *Mol Pain*. 2011;7. doi:10.1186/1744-8069-7-55

36. MacLellan CL, Auriat AM, McGie SC, et al. Gauging recovery after hemorrhagic stroke in rats: Implications for cytoprotection studies. *J Cereb Blood Flow Metab*. 2006;26(8):1031-1042. doi:10.1038/sj.jcbfm.9600255

37. Williamson MR, Colbourne F. Evidence for Decreased Brain Parenchymal Volume After Large Intracerebral Hemorrhages: a Potential Mechanism Limiting Intracranial Pressure Rises. *Transl Stroke Res*. 2017;8(4):386-396. doi:10.1007/s12975-017-0530-x

38. Oliver V, De Rantere D, Ritchie R, Chisholm J, Hecker KG, Pang DSJ. Psychometric assessment of the rat grimace scale and development of an analgesic intervention score. *PLoS One*. 2014;9(5). doi:10.1371/journal.pone.0097882

39. Schneider LE, Henley KY, Turner OA, Pat B, Niedzielko TL, Floyd CL. Application of the Rat Grimace Scale as a Marker of Supraspinal Pain Sensation after Cervical Spinal Cord Injury. *J Neurotrauma*. 2017;34(21):2982-2993. doi:10.1089/neu.2016.4665

40. Saine L, Hélie P, Vachon P. Effects of fentanyl on pain and motor behaviors following a collagenase-induced intracerebral hemorrhage in rats. *J Pain Res*. 2016;9:1039-1048. doi:10.2147/JPR.S121415

41. Esposito E, Mandeville ET, Lo EH. Lower doses of isoflurane treatment has no beneficial effects in a rat model of intracerebral hemorrhage. *BMC Neurosci*. 2013;14. doi:10.1186/1471-2202-14-129

42. Ferland C, Veilleux-Lemieux D, Vachon P. Effects of buprenorphine on intracerebral collagenase-induced hematoma in Sprague-Dawley rats. *J Am Assoc Lab Anim Sci*. 2007;46(3):13-16. http://www.embase.com/search/results?subaction=viewrecord&from=export&id=L47068011%5Cnhttp://limo.libis.be/resolver?&sid=EMBASE&issn=15596109&id=doi:&atitle=Effects+of+buprenorphine+on+intracerebral+collagenase-induced+hematoma+in+Sprague-Dawley+rats&stit

43. Scheller MS, Tateishi A, Drummond JC, Zornow MH. The effects of sevoflurane on

cerebral blood flow, cerebral metabolic rate for oxygen, intracranial pressure, and the electroencephalogram are similar to those of isoflurane in the rabbit. *Anesthesiology*. 1988;68(4):548-551. doi:10.1097/00000542-198804000-00012

44. Silasi G, MacLellan CL, Colbourne F. Use of telemetry blood pressure transmitters to measure intracranial pressure (ICP) in freely moving rats. *Curr Neurovasc Res*. 2009;6(1):62-69. doi:10.2174/156720209787466046

45. Su TM, Lee TH, Chen WF, Lee TC, Cheng CH. Contralateral acute epidural hematoma after decompressive surgery of acute subdural hematoma: Clinical features and outcome. *J Trauma - Inj Infect Crit Care*. 2008;65(6):1298-1302. doi:10.1097/TA.0b013e31815885d9

46. DeVries AC, Joh HD, Bernard O, et al. Social stress exacerbates stroke outcome by suppressing Bcl-2 expression. *Proc Natl Acad Sci U S A*. 2001;98(20):11824-11828. doi:10.1073/pnas.201215298

47. Shah QA, Ezzeddine MA, Qureshi AI. Acute hypertension in intracerebral hemorrhage: Pathophysiology and treatment. *J Neurol Sci*. 2007;261(1-2):74-79. doi:10.1016/j.jns.2007.04.036

48. Durand JL, Hosinking W, Jelicks LA. Time course of effects of inhalation anesthesia on blood glucose level in male and female C57BL/6 mice. *Horm Metab Res*. 2009;41(4):339-341. doi:10.1055/s-0028-1112114

49. Fogelholm R, Murros K, Rissanen A, Avikainen S. Admission blood glucose and short term survival in primary intracerebral haemorrhage: A population based study. *J Neurol Neurosurg Psychiatry*. 2005;76(3):349-353. doi:10.1136/jnnp.2003.034819

50. Kagansky N, Levy S, Knobler H. The role of hyperglycemia in acute stroke. *Arch Neurol*. 2001;58(8):1209-1212. doi:10.1001/archneur.58.8.1209

51. Márquez C, Nadal R, Armario A. The hypothalamic-pituitary-adrenal and glucose responses to daily repeated immobilisation stress in rats: Individual differences.

Neuroscience. 2004;123(3):601-612. doi:10.1016/j.neuroscience.2003.10.016

52. De Herdt V, Dumont F, Hénon H, et al. Early seizures in intracerebral hemorrhage: Incidence, associated. *Neurology*. 2011;77(20):1794-1800. doi:10.1212/WNL.0b013e31823648a6

53. Kogure K, Schwartzman RJ. Seizure Propagation and ATP Depletion in the Rat Stroke Model. *Epilepsia*. 1980;21(1):63-72. doi:10.1111/j.1528-1157.1980.tb04045.x

54. Detsch O, Vahle-Hinz C, Kochs E, Siemers M, Bromm B. Isoflurane induces dose-dependent changes of thalamic somatosensory information transfer. *Brain Res*. 1999;829(1-2):77-89. doi:10.1016/S0006-8993(99)01341-4

55. Jia F, Yue M, Chandra D, Homanics GE, Goldstein PA, Harrison NL. Isoflurane is a potent modulator of extrasynaptic GABAA receptors in the thalamus. *J Pharmacol Exp Ther*. 2008;324(3):1127-1135. doi:10.1124/jpet.107.134569

56. Vahle-Hinz C, Detsch O, Siemers M, Kochs E. Contributions of GABAergic and glutamatergic mechanisms to isoflurane-induced suppression of thalamic somatosensory information transfer. *Exp Brain Res*. 2007;176(1):159-172. doi:10.1007/s00221-006-0604-6

57. Bar-Klein G, Klee R, Brandt C, et al. Isoflurane prevents acquired epilepsy in rat models of temporal lobe epilepsy. *Ann Neurol*. 2016;80(6):896-908. doi:10.1002/ana.24804

58. Ito BM, Sato S, Kufta C V., Tran D. Effect of isoflurane and enflurane on the electrocorticogram of epileptic patients. *Neurology*. 1988;38(6):924-928. doi:10.1212/wnl.38.6.924

59. Veronesi MC, Kubek DJ, Kubek MJ. Isoflurane exacerbates electrically evoked seizures in amygdala-kindled rats during recovery. *Epilepsy Res*. 2008;82(1):15-20. doi:10.1016/j.eplepsyres.2008.06.007

60. Glass PSA, Gan TJ, Howell S, Ginsberg B. Drug interactions: Volatile anesthetics and opioids. In: *Journal of Clinical Anesthesia*. ; 1997. doi:10.1016/S0952-8180(97)00122-0

61. Wood M. Pharmacokinetic Drug Interactions in Anaesthetic Practice. *Clin Pharmacokinet*. 1991;21(4):285-307. doi:10.2165/00003088-199121040-00005

62. Bhardwaj A, Castro AF, Alkayed NJ, Hurn PD, Kirsch JR. Anesthetic choice of halothane versus propofol impact on experimental perioperative stroke. *Stroke*. 2001;32(8):1920-1925. doi:10.1161/01.STR.32.8.1920

63. Zausinger S, Baethmann A, Schmid-Elsaesser R. Anesthetic methods in rats determine outcome after experimental focal cerebral ischemia: Mechanical ventilation is required to obtain controlled experimental conditions. *Brain Res Protoc*. 2002;9(2):112-121. doi:10.1016/S1385-299X(02)00138-1

64. Colbourne F, Auer RN, Sutherland GR. Characterization of postischemic behavioral deficits in gerbils with and without hypothermic neuroprotection. *Brain Res*. 1998;803(1-2):69-78. doi:10.1016/S0006-8993(98)00612-X

Chapter 4

1. Ikram MA, Wieberdink RG, Koudstaal PJ. International epidemiology of intracerebral hemorrhage. *Curr Atheroscler Rep*. 2012;14(4):300-306. doi:10.1007/s11883-012-0252-1

2. Patel TR, Schielke GP, Hoff JT, Keep RF, Lorris Betz A. Comparison of cerebral blood flow and injury following intracerebral and subdural hematoma in the rat. *Brain Res*. 1999;829(1-2):125-133. doi:10.1016/S0006-8993(99)01378-5

3. Williamson MR, Dietrich K, Hackett MJ, et al. Rehabilitation Augments Hematoma Clearance and Attenuates Oxidative Injury and Ion Dyshomeostasis After Brain Hemorrhage. *Stroke*. 2016;48(1):195-203. doi:10.1161/STROKEAHA.116.015404

4. Nadeau CA, Dietrich K, Wilkinson CM, et al. Prolonged Blood-Brain Barrier Injury Occurs After Experimental Intracerebral Hemorrhage and Is Not Acutely Associated with Additional Bleeding. *Transl Stroke Res*. 2019;10(3):287-297. doi:10.1007/s12975-018-0636-9

5. Nguyen AP, Huynh HD, Sjovold SB, Colbourne F. Progressive brain damage and alterations in dendritic arborization after collagenase-induced intracerebral hemorrhage in rats. *Curr Neurovasc Res*. 2008;5(3):171-177. doi:10.2174/156720208785425710
6. Del Bigio MR, Yan HJ, Buist R, Peeling J. Experimental intracerebral hemorrhage in rats: Magnetic resonance imaging and histopathological correlates. *Stroke*. 1996;27(12):2312-2320. doi:10.1161/01.str.27.12.2312
7. Klahr AC, Dickson CT, Colbourne F. Seizure Activity Occurs in the Collagenase but not the Blood Infusion Model of Striatal Hemorrhagic Stroke in Rats. *Transl Stroke Res*. 2014;6(1):29-38. doi:10.1007/s12975-014-0361-y
8. Merkler AE, Gialdini G, Lerario MP, et al. Population-Based Assessment of the Long-Term Risk of Seizures in Survivors of Stroke. *Stroke*. 2018;49(6):1319-1324. doi:10.1161/STROKEAHA.117.020178
9. Mehta RI, Tosun C, Ivanova S, et al. Sur1-Trpm4 Cation Channel Expression in Human Cerebral Infarcts. *J Neuropathol Exp Neurol*. 2015;74(8):835-849. doi:10.1097/NEN.0000000000000223
10. Simard JM, Woo SK, Schwartzbauer GT, Gerzanich V. Sulfonylurea Receptor 1 in Central Nervous System Injury: A Focused Review. *J Cereb Blood Flow Metab*. 2012;32(9):1699-1717. doi:10.1038/jcbfm.2012.91
11. Simard JM, Kahle KT, Gerzanich V. Molecular mechanisms of microvascular failure in central nervous system injury—synergistic roles of NKCC1 and SUR1/TRPM4. *J Neurosurg*. 2010;113(3):622-629. doi:10.3171/2009.11.JNS081052
12. Simard JM, Geng Z, Kyoon Woo S, et al. Glibenclamide reduces inflammation, vasogenic edema, and caspase-3 activation after subarachnoid hemorrhage. *J Cereb Blood Flow Metab*. 2009;29(2):317-330. doi:10.1038/jcbfm.2008.120
13. Simard JM, Sheth KN, Kimberly WT, et al. Glibenclamide in cerebral ischemia and stroke. *Neurocrit Care*. 2014;20(2):319-333. doi:10.1007/s12028-013-9923-1

14. Sheth K, Elm J, Cheung K, et al. Abstracts for the 10th World Stroke Congress, 2016. *Int J Stroke*. 2016;11(3_suppl):4-288. doi:10.1177/1747493016670567

15. DeWitt D, Hawkins BE, Dixon CE, et al. Preclinical Testing of Therapies for Traumatic Brain Injury. *J Neurotrauma*. 2018;2754:neu.2018.5778. doi:10.1089/neu.2018.5778

16. Jiang B, Li L, Chen Q, et al. Role of Glibenclamide in Brain Injury After Intracerebral Hemorrhage. *Transl Stroke Res*. 2017;8(2):183-193. doi:10.1007/s12975-016-0506-2

17. Zhou F, Liu Y, Yang B, Hu Z. Neuroprotective potential of glibenclamide is mediated by antioxidant and anti-apoptotic pathways in intracerebral hemorrhage. *Brain Res Bull*. 2018;142(June):18-24. doi:10.1016/j.brainresbull.2018.06.006

18. Rathor MY, Rani MFA, Jamalludin AR, Amran M, Shahrin TCA, Shah A. Prediction of functional outcome in patients with primary intracerebral hemorrhage by clinical-computed tomographic correlations. *J Res Med Sci*. 2012;17(11):1056-1062.

19. MacLellan CL, Auriat AM, McGie SC, et al. Gauging recovery after hemorrhagic stroke in rats: Implications for cytoprotection studies. *J Cereb Blood Flow Metab*. 2006;26(8):1031-1042. doi:10.1038/sj.jcbfm.9600255

20. Ghasami K, Rezvanfar MR, Faraji F, Mohammadbeigi A, Rafiee M. Impact of glibenclamide versus Insulin on neurological and functional outcomes of hemorrhagic stroke in diabetic patients. *Neurol Asia*. 2013;18(2):137-141.

21. MacLellan CL, Silasi G, Poon CC, et al. Intracerebral hemorrhage models in rat: comparing collagenase to blood infusion. *J Cereb Blood Flow Metab*. 2008;28:516-525. doi:10.1038/sj.jcbfm.9600548

22. Rosenberg GA, Estrada E, Kelley RO, Kornfeld M. Bacterial collagenase disrupts extracellular matrix and opens blood-brain barrier in rat. *Neurosci Lett*. 1993;160(1):117-119. doi:10.1016/0304-3940(93)90927-D

23. Keep RF, Zhou N, Xiang J, Andjelkovic A V., Hua Y, Xi G. Vascular disruption and blood-brain barrier dysfunction in intracerebral hemorrhage. *Fluids Barriers CNS*. 2014;11(1).

doi:10.1186/2045-8118-11-18

24. Corbett D, Carmichael ST, Murphy TH, et al. Enhancing the Alignment of the Preclinical and Clinical Stroke Recovery Research Pipeline: Consensus-Based Core Recommendations from the Stroke Recovery and Rehabilitation Roundtable Translational Working Group ∗. *Neurorehabil Neural Repair*. 2017;31(8):699-707. doi:10.1177/1545968317724285

25. MacLellan CL, Paquette R, Colbourne F. A critical appraisal of experimental intracerebral hemorrhage research. *J Cereb Blood Flow Metab*. 2012;32(4):612-627. doi:10.1038/jcbfm.2012.8

26. Smits P, Bijlstra PJ, Russel FGM, Lutterman JA, Thien T. Cardiovascular effects of sulphonylurea derivatives. In: *Diabetes Research and Clinical Practice*. Vol 31. ; 1996. doi:10.1016/0168-8227(96)01230-2

27. DeBow SB, Clark DL, MacLellan CL, Colbourne F. Incomplete Assessment of Experimental Cytoprotectants in Rodent Ischemia Studies. *Can J Neurol Sci*. 2003;30(4):368-374. doi:10.1017/S0317167100003097

28. Wilkinson CM, Fedor BA, Aziz JR, et al. Failure of bumetanide to improve outcome after intracerebral hemorrhage in rat. *PLoS One*. 2019;14(1):1-20. doi:10.1371/journal.pone.0210660

29. Williamson MR, Wilkinson CM, Dietrich K, Colbourne F. Acetazolamide Mitigates Intracranial Pressure Spikes Without Affecting Functional Outcome After Experimental Hemorrhagic Stroke. *Transl Stroke Res*. 2019;10(4):428-439. doi:10.1007/s12975-018-0663-6

30. Klahr AC, Nadeau CA, Colbourne F. Temperature Control in Rodent Neuroprotection Studies: Methods and Challenges. *Ther Hypothermia Temp Manag*. 2017;7(1):42-49. doi:10.1089/ther.2016.0018

31. Rosenberg G a., Mun-Bryce S, Wesley M, Kornfeld M. Collagenase-induced intracerebral

hemorrhage in rats. *Stroke*. 1990;21(5):801-807. doi:10.1161/01.STR.21.5.801

32. Paxinos G, Watson C. Index of Structures. *Rat Brain Stereotaxic Coord*. Published online 1982:7-9. doi:10.1016/b978-0-12-547620-1.50008-4

33. Simard JM, Woo SK, Tsymbalyuk N, et al. Glibenclamide-10-h Treatment Window in a Clinically Relevant Model of Stroke. *Transl Stroke Res*. 2012;3(2):286-295. doi:10.1007/s12975-012-0149-x

34. Elster AD, Moody DM. Early cerebral infarction: Gadopentetate dimeglumine enhancement. *Radiology*. 1990;177(3):627-632. doi:10.1148/radiology.177.3.2243961

35. Williamson MR, Colbourne F. Evidence for Decreased Brain Parenchymal Volume After Large Intracerebral Hemorrhages: a Potential Mechanism Limiting Intracranial Pressure Rises. *Transl Stroke Res*. 2017;8(4):386-396. doi:10.1007/s12975-017-0530-x

36. Caffes N, Kurland DB, Gerzanich V, Marc Simard J. Glibenclamide for the treatment of ischemic and hemorrhagic stroke. *Int J Mol Sci*. 2015;16(3):4973-4984. doi:10.3390/ijms16034973

37. Walcott BP, Kahle KT, Simard JM. Novel Treatment Targets for Cerebral Edema. *Neurotherapeutics*. 2012;9(1):65-72. doi:10.1007/s13311-011-0087-4

38. Chodobski A, Zink BJ, Szmydynger-Chodobska J. Blood-Brain Barrier Pathophysiology in Traumatic Brain Injury. *Transl Stroke Res*. 2011;2(4):492-516. doi:10.1007/s12975-011-0125-x

39. Jha RM, Molyneaux BJ, Jackson TC, et al. Glibenclamide produces region-dependent effects on cerebral edema in a combined injury model of traumatic brain injury and hemorrhagic shock in mice. *J Neurotrauma*. 2018;35(17):2125-2135. doi:10.1089/neu.2016.4696

40. Zhang J, Yang Y, Sun H, Xing Y. Hemorrhagic transformation after cerebral infarction: Current concepts and challenges. *Ann Transl Med*. 2014;2(8). doi:10.3978/j.issn.2305-5839.2014.08.08

41. Katzman R, Clasen R, Klatzo I, Meyer J., Pappius H., Waltz A. Report of Joint Committee for Stroke Resources. IV. Brain edema in stroke. *Stroke*. 1977;8(4):512-540. doi:10.1161/01.STR.8.4.512

42. Yao L, Xue X, Yu P, Ni Y, Chen F. Evans Blue Dye: A Revisit of Its Applications in Biomedicine. *Contrast Media Mol Imaging*. 2018;2018. doi:10.1155/2018/7628037

43. Saunders NR, Dziegielewska KM, Møllgård K, Habgood MD. Markers for blood-brain barrier integrity: How appropriate is Evans blue in the twenty-first century and what are the alternatives? *Front Neurosci*. 2015;9(OCT). doi:10.3389/fnins.2015.00385

44. Wang HL, Lai TW. Optimization of Evans blue quantitation in limited rat tissue samples. *Sci Rep*. 2014;4. doi:10.1038/srep06588

45. Yang GY, Chen SF, Kinouchi H, Chan PH, Weinstein PR. Edema, cation content, and atpase activity after middle cerebral artery occlusion in rats. *Stroke*. 1992;23(9):1331-1336. doi:10.1161/01.STR.23.9.1331

46. Rungta RL, Choi HB, Tyson JR, et al. The cellular mechanisms of neuronal swelling underlying cytotoxic edema. *Cell*. 2015;161(3):610-621. doi:10.1016/j.cell.2015.03.029

47. Hladky SB, Barrand MA. *Fluid and Ion Transfer across the Blood–Brain and Blood–Cerebrospinal Fluid Barriers; a Comparative Account of Mechanisms and Roles*. Vol 13.; 2016. doi:10.1186/s12987-016-0040-3

48. Simard JM, Tsymbalyuk N, Tsymbalyuk O, Ivanova S, Yurovsky V, Gerzanich V. Glibenclamide Is Superior to Decompressive Craniectomy in a Rat Model of Malignant Stroke. *Stroke*. 2010;41(3):531-537. doi:10.1161/STROKEAHA.109.572644

49. Button KS, Ioannidis JPA, Mokrysz C, et al. Power failure: Why small sample size undermines the reliability of neuroscience. *Nat Rev Neurosci*. 2013;14(5):365-376. doi:10.1038/nrn3475

50. Begley CG, Ioannidis JPA. Reproducibility in science: Improving the standard for basic and preclinical research. *Circ Res*. 2015;116(1):116-126.

doi:10.1161/CIRCRESAHA.114.303819

51. Ioannidis JPA. Why most published research findings are false. *Get to Good Res Integr Biomed Sci*. Published online 2018:2-8. doi:10.1371/journal.pmed.0020124

52. Macleod MR, Fisher M, O'collins V, et al. Good laboratory practice: Preventing introduction of bias at the bench. *Int J Stroke*. 2009;4(1):3-5. doi:10.1111/j.1747-4949.2009.00241.x

53. Colbourne F, Auer RN, Sutherland GR. Characterization of postischemic behavioral deficits in gerbils with and without hypothermic neuroprotection. *Brain Res*. 1998;803(1-2):69-78. doi:10.1016/S0006-8993(98)00612-X

Chapter 5

1. Sutherland GR, Auer RN. Primary intracerebral hemorrhage. *J Clin Neurosci*. 2006;13(5):511-517. doi:10.1016/j.jocn.2004.12.012

2. Qureshi AI, Tuhrim S, Broderick JP, Batjer HH, Hondo H, Hanley DF. Spontaneous Intracerebral Hemorrhage. *N Engl J Med*. 2001;344(19):1450-1460. doi:10.1056/NEJM200105103441907

3. Kaplan NM, Victor RG. *Kaplan's Clinical Hypertension: Eleventh Edition*.; 2014.

4. Schlunk F, Greenberg SM. The Pathophysiology of Intracerebral Hemorrhage Formation and Expansion. *Transl Stroke Res*. 2015;6(4):257-263. doi:10.1007/s12975-015-0410-1

5. Mirsen T. Acute treatment of hypertensive intracerebral hemorrhage. *Curr Treat Options Neurol*. 2010;12(6):504-517. doi:10.1007/s11940-010-0096-3

6. An SJ, Kim TJ, Yoon BW. Epidemiology, risk factors, and clinical features of intracerebral hemorrhage: An update. *J Stroke*. 2017;19(1):3-10. doi:10.5853/jos.2016.00864

7. Brouwers HB, Chang Y, Falcone GJ, et al. Predicting hematoma expansion after primary intracerebral hemorrhage. *JAMA Neurol*. 2014;71(2):158-164. doi:10.1001/jamaneurol.2013.5433

8. Kroin JS, Nagalla SKS, Buvanendran A, McCarthy RJ, Tuman KJ, Ivankovich AD. The mechanisms of intracranial pressure modulation by epidural blood and other injectates in a postdural puncture rat model. *Anesth Analg*. 2002;95(2):423-429. doi:10.1213/00000539-200208000-00035

9. Ganten U, Rascher W, Lang RE, et al. Development of a new strain of spontaneously hypertensive rats homozygous for hypothalamic diabetes insipidus. *Hypertension*. 1983;5(2):I-119-I-128. doi:10.1161/01.hyp.5.2_pt_2.i119

10. Pravenec M, Křen V, Landa V, et al. Recent progress in the genetics of spontaneously hypertensive rats. *Physiol Res*. 2014;63(SUPPL.). doi:10.33549/physiolres.932622

11. Okamoto K, Aoki K. Development of a strain of spontaneously hypertensive rats. *Jpn Circ J*. 1963;27(3):282-293. doi:10.1253/jcj.27.282

12. Duale H, Waki H, Howorth P, Kasparov S, Teschemacher AG, Paton JFR. Restraining influence of A2 neurons in chronic control of arterial pressure in spontaneously hypertensive rats. *Cardiovasc Res*. 2007;76(1):184-193. doi:10.1016/j.cardiores.2007.06.018

13. Wu G, Bao X, Xi G, Keep RF, Thompson BG, Hua Y. Brain injury after intracerebral hemorrhage in spontaneously hypertensive rats: Laboratory investigation. *J Neurosurg*. 2011;114(6):1805-1811. doi:10.3171/2011.1.JNS101530

14. Nagaoka A, Sudo K, Orita S, Kikuchi K, Aramaki Y. Hematological studies on the spontaneously hypertensive rats with special reference to the development of thrombosis. *Jpn Circ J*. 1971;35(11):1379-1390. doi:10.1253/jcj.35.11_1379

15. Lerman LO, Chade AR, Sica V, Napoli C. Animal models of hypertension: An overview. *J Lab Clin Med*. 2005;146(3):160-173. doi:10.1016/j.lab.2005.05.005

16. Sarikonda K V., Watson RE, Opara OC, DiPette DJ. Experimental animal models of hypertension. *J Am Soc Hypertens*. 2009;3(3):158-165. doi:10.1016/j.jash.2009.02.003

17. Budd JT. Adult secondary hypertension: What the primary care provider needs to know.

Consultant. 2021;61(3):E1-E8. doi:10.25270/con.2020.09.00006

18. Saxena T, Ali AO, Saxena M. Pathophysiology of essential hypertension: an update. *Expert Rev Cardiovasc Ther*. 2018;16(12):879-887. doi:10.1080/14779072.2018.1540301

19. Mokri B. The Monro-Kellie hypothesis: Applications in CSF volume depletion. *Neurology*. 2001;56(12):1746-1748. doi:10.1212/WNL.56.12.1746

20. Wilson MH. Monro-Kellie 2.0: The dynamic vascular and venous pathophysiological components of intracranial pressure. *J Cereb Blood Flow Metab*. 2016;36(8):1338-1350. doi:10.1177/0271678X16648711

21. Williamson MR, Colbourne F. Evidence for Decreased Brain Parenchymal Volume After Large Intracerebral Hemorrhages: a Potential Mechanism Limiting Intracranial Pressure Rises. *Transl Stroke Res*. 2017;8(4):386-396. doi:10.1007/s12975-017-0530-x

22. Kalisvaart ACJ, Wilkinson CM, Gu S, et al. An update to the Monro–Kellie doctrine to reflect tissue compliance after severe ischemic and hemorrhagic stroke. *Sci Rep*. 2020;10(1). doi:10.1038/s41598-020-78880-4

23. Maiër B, Kubis N. Hypertension and Its Impact on Stroke Recovery: From a Vascular to a Parenchymal Overview. *Neural Plast*. 2019;2019. doi:10.1155/2019/6843895

24. Nelson DO, Boulant JA. Altered CNS neuroanatomical organization of spontaneously hypertensive (SHR) rats. *Brain Res*. 1981;226(1-2):119-130. doi:10.1016/0006-8993(81)91087-8

25. Lehr RP, Browning RA, Myers JH. Gross morphological brain differences between wistar-Kyoto and spontaneously hypertensive rats. *Clin Exp Hypertens*. 1980;2(1):123-127. doi:10.3109/10641968009038555

26. Tajima A, Hans FJ, Livingstone D, et al. Smaller local brain volumes and cerebral atrophy in spontaneously hypertensive rats. *Hypertension*. 1993;21(1):105-111. doi:10.1161/01.HYP.21.1.105

27. Sabbatini M, Strocchi P, Vitaioli L, Amenta F. The hippocampus in spontaneously hypertensive rats: A quantitative microanatomical study. *Neuroscience*. 2000;100(2):251-258. doi:10.1016/S0306-4522(00)00297-9

28. Hwang IK, Yoon YS, Choi JH, et al. Doublecortin-immunoreactive neuronal precursors in the dentate gyrus of spontaneously hypertensive rats at various age stages: Comparison with Sprague-Dawley rats. *J Vet Med Sci*. 2008;70(4):373-377. doi:10.1292/jvms.70.373

29. Kurtz TW, Curtis Morris R. Biological variability in wistar-kyoto rats implications for research with the spontaneously hypertensive rat. *Hypertension*. 1987;10(1):127-131. doi:10.1161/01.HYP.10.1.127

30. Rosenberg G a., Mun-Bryce S, Wesley M, Kornfeld M. Collagenase-induced intracerebral hemorrhage in rats. *Stroke*. 1990;21(5):801-807. doi:10.1161/01.STR.21.5.801

31. MacLellan CL, Silasi G, Poon CC, et al. Intracerebral hemorrhage models in rat: comparing collagenase to blood infusion. *J Cereb Blood Flow Metab*. 2008;28:516-525. doi:10.1038/sj.jcbfm.9600548

32. Wilkinson CM, Kalisvaart ACJ, Kung TFC, et al. The collagenase model of intracerebral hemorrhage in awake, freely moving animals: The effects of isoflurane. *Brain Res*. 2020;1728:146593. doi:10.1016/j.brainres.2019.146593

33. Sotocinal SG, Sorge RE, Zaloum A, et al. The Rat Grimace Scale: A partially automated method for quantifying pain in the laboratory rat via facial expressions. *Mol Pain*. 2011;7. doi:10.1186/1744-8069-7-55

34. Colbourne F, Auer RN, Sutherland GR. Characterization of postischemic behavioral deficits in gerbils with and without hypothermic neuroprotection. *Brain Res*. 1998;803(1-2):69-78. doi:10.1016/S0006-8993(98)00612-X

35. Wilkinson CM, Fedor BA, Aziz JR, et al. Failure of bumetanide to improve outcome after intracerebral hemorrhage in rat. *PLoS One*. 2019;14(1):1-20. doi:10.1371/journal.pone.0210660

36. Paxinos G, Watson C. Index of Structures. *Rat Brain Stereotaxic Coord*. Published online 1982:7-9. doi:10.1016/b978-0-12-547620-1.50008-4

37. MacLellan CL, Auriat AM, McGie SC, et al. Gauging recovery after hemorrhagic stroke in rats: Implications for cytoprotection studies. *J Cereb Blood Flow Metab*. 2006;26(8):1031-1042. doi:10.1038/sj.jcbfm.9600255

38. Napper RMA. Total number is important: Using the disector method in design-based stereology to understand the structure of the rodent brain. *Front Neuroanat*. 2018;12. doi:10.3389/fnana.2018.00016

39. Liddle LJ, Dirks CA, Almekhlafi M, Colbourne F. An Ambiguous Role for Fever in Worsening Outcome After Intracerebral Hemorrhage. *Transl Stroke Res*. 2022;(0123456789). doi:10.1007/s12975-022-01010-x

40. Bedussi B, Naessens DMP, De Vos J, et al. Enhanced interstitial fluid drainage in the hippocampus of spontaneously hypertensive rats. *Sci Rep*. 2017;7(1). doi:10.1038/s41598-017-00861-x

41. Mortensen KN, Sanggaard S, Mestre H, et al. Impaired glymphatic transport in spontaneously hypertensive rats. *J Neurosci*. 2019;39(32):6365-6377. doi:10.1523/JNEUROSCI.1974-18.2019

42. Colbourne F, Corbett D, Zhao Z, Yang J, Buchan AM. Prolonged but delayed postischemic hypothermia: A long-term outcome study in the rat middle cerebral artery occlusion model. *J Cereb Blood Flow Metab*. 2000;20(12):1702-1708. doi:10.1097/00004647-200012000-00009

43. Fasmer OB, Johansen EB. Patterns of motor activity in spontaneously hypertensive rats compared to Wistar Kyoto rats. *Behav Brain Funct*. 2016;12(1). doi:10.1186/s12993-016-0117-9

44. Kim P, Choi I, dela Pena IC, et al. A simple behavioral paradigm to measure impulsive behavior in an animal model of attention deficit hyperactivity disorder (ADHD) of the

spontaneously hypertensive rats. *Biomol Ther*. 2012;20(1):125-131. doi:10.4062/biomolther.2012.20.1.125

Chapter 6

1. Kearney PM, Whelton M, Reynolds K, Whelton PK, He J. Worldwide prevalence of hypertension: A systematic review. *J Hypertens*. 2004;22(1):11-19. doi:10.1097/00004872-200401000-00003
2. Keage HAD, Carare RO, Friedland RP, et al. Population studies of sporadic cerebral amyloid angiopathy and dementia: A systematic review. *BMC Neurol*. 2009;9. doi:10.1186/1471-2377-9-3
3. Camacho E, Lopresti MA, Bruce S, et al. The role of age in intracerebral hemorrhages. *J Clin Neurosci*. 2015;22(12):1867-1870. doi:10.1016/j.jocn.2015.04.020
4. An SJ, Kim TJ, Yoon BW. Epidemiology, risk factors, and clinical features of intracerebral hemorrhage: An update. *J Stroke*. 2017;19(1):3-10. doi:10.5853/jos.2016.00864
5. Liddle LJ, Ralhan S, Ward DL, Colbourne F. Translational Intracerebral Hemorrhage Research: Has Current Neuroprotection Research ARRIVEd at a Standard for Experimental Design and Reporting? *Transl Stroke Res*. 2020;11(6):1203-1213. doi:10.1007/s12975-020-00824-x
6. Wilkinson CM, Kung TFC, Jickling GC, Colbourne F. A translational perspective on intracranial pressure responses following intracerebral hemorrhage in animal models. *Brain Hemorrhages*. 2021;2(1):34-48. doi:10.1016/j.hest.2020.10.002
7. Kamel H, Hemphill JC. Characteristics and sequelae of intracranial hypertension after intracerebral hemorrhage. *Neurocrit Care*. 2012;17(2):172-176. doi:10.1007/s12028-012-9744-7
8. Rincon F, Mayer SA. Clinical review: Critical care management of spontaneous intracerebral hemorrhage. *Crit Care*. 2008;12(6):237. doi:10.1186/cc7092

9. Chen CJ, Ding D, Ironside N, et al. Intracranial pressure monitoring in patients with spontaneous intracerebral hemorrhage. *J Neurosurg*. 2020;132(6):1854-1864. doi:10.3171/2019.3.JNS19545

10. Pedersen SH, Lilja-Cyron A, Andresen M, Juhler M. The Relationship Between Intracranial Pressure and Age—Chasing Age-Related Reference Values. *World Neurosurg*. 2018;110:e119-e123. doi:10.1016/j.wneu.2017.10.086

11. Murtha LA, Yang Q, Parsons MW, et al. Cerebrospinal fluid is drained primarily via the spinal canal and olfactory route in young and aged spontaneously hypertensive rats. *Fluids Barriers CNS*. 2014;11(1). doi:10.1186/2045-8118-11-12

12. Pakkenberg B, Gundersen HJG. Neocortical neuron number in humans: Effect of sex and age. *J Comp Neurol*. 1997;384(2):312-320. doi:10.1002/(SICI)1096-9861(19970728)384:2<312::AID-CNE10>3.0.CO;2-K

13. Finch CE. Neuron atrophy during aging: programmed or sporadic? *Trends Neurosci*. 1993;16(3):104-110. doi:10.1016/0166-2236(93)90134-8

14. Pakkenberg B, Pelvig D, Marner L, et al. Aging and the human neocortex. In: *Experimental Gerontology*. Vol 38. ; 2003:95-99. doi:10.1016/S0531-5565(02)00151-1

15. Schwab S, Aschoff A, Spranger M, Albert F, Hacke W. The value of intracranial pressure monitoring in acute hemispheric stroke. *Neurology*. 1996;47(2):393-398. doi:10.1212/WNL.47.2.393

16. De Vis JB, Zwanenburg JJ, van der Kleij LA, et al. Cerebrospinal fluid volumetric MRI mapping as a simple measurement for evaluating brain atrophy. *Eur Radiol*. 2016;26(5):1254-1262. doi:10.1007/s00330-015-3932-8

17. Erten-Lyons D, Dodge HH, Woltjer R, et al. Neuropathologic basis of age-associated brain atrophy. *JAMA Neurol*. 2013;70(5):616-622. doi:10.1001/jamaneurol.2013.1957

18. Czosnyka M, Balestreri M, Steiner L, et al. Age, intracranial pressure, autoregulation, and outcome after brain trauma. *J Neurosurg*. 2005;102(3):450-454.

doi:10.3171/jns.2005.102.3.0450

19. Czosnyka M, Hutchinson PJ, Balestreri M, Hiler M, Smielewski P, Pickard JD. Monitoring and interpretation of intracranial pressure after head injury. *Acta Neurochir Suppl*. 2006;(96):114-118. doi:10.1007/3-211-30714-1_26
20. Williamson MR, Colbourne F. Evidence for Decreased Brain Parenchymal Volume After Large Intracerebral Hemorrhages: a Potential Mechanism Limiting Intracranial Pressure Rises. *Transl Stroke Res*. 2017;8(4):386-396. doi:10.1007/s12975-017-0530-x
21. Kalisvaart ACJ, Wilkinson CM, Gu S, et al. An update to the Monro–Kellie doctrine to reflect tissue compliance after severe ischemic and hemorrhagic stroke. *Sci Rep*. 2020;10(1). doi:10.1038/s41598-020-78880-4
22. Ankolekar S, Rewell S, Howells DW, Bath PMW. The influence of stroke risk factors and comorbidities on assessment of stroke therapies in humans and animals. *Int J Stroke*. 2012;7(5):386-397. doi:10.1111/j.1747-4949.2012.00802.x
23. Ergul A, Hafez S, Fouda A, Fagan SC. Impact of Comorbidities on Acute Injury and Recovery in Preclinical Stroke Research: Focus on Hypertension and Diabetes. *Transl Stroke Res*. 2016;7(4):248-260. doi:10.1007/s12975-016-0464-8
24. Wu G, Bao X, Xi G, Keep RF, Thompson BG, Hua Y. Brain injury after intracerebral hemorrhage in spontaneously hypertensive rats: Laboratory investigation. *J Neurosurg*. 2011;114(6):1805-1811. doi:10.3171/2011.1.JNS101530
25. MacLellan CL, Silasi G, Poon CC, et al. Intracerebral hemorrhage models in rat: comparing collagenase to blood infusion. *J Cereb Blood Flow Metab*. 2008;28:516-525. doi:10.1038/sj.jcbfm.9600548
26. Wilkinson CM, Brar PS, Balay CJ, Colbourne F. Glibenclamide, a Sur1-Trpm4 antagonist, does not improve outcome after collagenase-induced intracerebral hemorrhage. *PLoS One*. 2019;14(5). doi:10.1371/journal.pone.0215952
27. Rosenberg G a., Mun-Bryce S, Wesley M, Kornfeld M. Collagenase-induced intracerebral

hemorrhage in rats. *Stroke*. 1990;21(5):801-807. doi:10.1161/01.STR.21.5.801

28. Paxinos G, Watson C. Index of Structures. *Rat Brain Stereotaxic Coord*. Published online 1982:7-9. doi:10.1016/b978-0-12-547620-1.50008-4

29. Williamson MR, Wilkinson CM, Dietrich K, Colbourne F. Acetazolamide Mitigates Intracranial Pressure Spikes Without Affecting Functional Outcome After Experimental Hemorrhagic Stroke. *Transl Stroke Res*. 2019;10(4):428-439. doi:10.1007/s12975-018-0663-6

30. Wilkinson CM, Fedor BA, Aziz JR, et al. Failure of bumetanide to improve outcome after intracerebral hemorrhage in rat. *PLoS One*. 2019;14(1):1-20. doi:10.1371/journal.pone.0210660

31. Wilkinson CM, Kalisvaart ACJ, Kung TFC, et al. The collagenase model of intracerebral hemorrhage in awake, freely moving animals: The effects of isoflurane. *Brain Res*. 2020;1728:146593. doi:10.1016/j.brainres.2019.146593

32. Napper RMA. Total number is important: Using the disector method in design-based stereology to understand the structure of the rodent brain. *Front Neuroanat*. 2018;12. doi:10.3389/fnana.2018.00016

33. Emerich DF, Mcdermott P, Krueger P, et al. Locomotion of aged rats: Relationship to neurochemical but not morphological changes in nigrostriatal dopaminergic neurons. *Brain Res Bull*. 1993;32(5):477-486. doi:10.1016/0361-9230(93)90294-L

34. Pinto E. Blood pressure and ageing. *Postgrad Med J*. 2007;83(976):109-114. doi:10.1136/pgmj.2006.048371

35. Harrap SB, Van Der Merwe WM, Griffin SA, Macpherson F, Lever AF. Brief angiotensin converting enzyme inhibitor treatment in young spontaneously hypertensive rats reduces blood pressure long-term. *Hypertension*. 1990;16(6):603-614. doi:10.1161/01.HYP.16.6.603

36. Okamoto K, Aoki K. Development of a strain of spontaneously hypertensive rats. *Jpn Circ*

J. 1963;27(3):282-293. doi:10.1253/jcj.27.282

37. Gottschalk A, Scafidi S, Toung TJK. Brain water as a function of age and weight in normal rats. *PLoS One*. 2021;16(9 September). doi:10.1371/journal.pone.0249384

38. Zelenina M, Zelenin S, Aperia A. Water Channels (Aquaporins) and Their Role for Postnatal Adaptation. *Pediatr Res*. 2005;57(5):47R-53R. doi:10.1203/01.PDR.0000159572.79074.0B

39. Wozniak JR, Lim KO. Advances in white matter imaging: A review of in vivo magnetic resonance methodologies and their applicability to the study of development and aging. *Neurosci Biobehav Rev*. 2006;30(6):762-774. doi:10.1016/j.neubiorev.2006.06.003

40. Gong Y, Hua Y, Keep RF, Hoff JT, Xi G. Intracerebral hemorrhage: Effects of aging on brain edema and neurological deficits. In: *Stroke*. ; 2004. doi:10.1161/01.STR.0000145485.67827.d0

41. Staykov D, Wagner I, Volbers B, et al. Natural course of perihemorrhagic edema after intracerebral hemorrhage. *Stroke*. 2011;42(9):2625-2629. doi:10.1161/STROKEAHA.111.618611

42. Volbers B, Willfarth W, Kuramatsu JB, et al. Impact of Perihemorrhagic Edema on Short-Term Outcome After Intracerebral Hemorrhage. *Neurocrit Care*. 2016;24(3):404-412. doi:10.1007/s12028-015-0185-y

43. Tajima A, Hans FJ, Livingstone D, et al. Smaller local brain volumes and cerebral atrophy in spontaneously hypertensive rats. *Hypertension*. 1993;21(1):105-111. doi:10.1161/01.HYP.21.1.105

44. Maiër B, Kubis N. Hypertension and Its Impact on Stroke Recovery: From a Vascular to a Parenchymal Overview. *Neural Plast*. 2019;2019. doi:10.1155/2019/6843895

45. Becker KJ. Strain-Related Differences in the Immune Response: Relevance to Human Stroke. *Transl Stroke Res*. 2016;7(4):303-312. doi:10.1007/s12975-016-0455-9

46. Lee RMKW, Delaney KH, Lu M. Perindopril treatment prolonged the lifespan of

spontaneously hypertensive rats. *J Hypertens*. 1995;13(4):471-476. doi:10.1097/00004872-199504000-00013

47. Brower M, Grace M, Kotz CM, Koya V. Comparative analysis of growth characteristics of Sprague Dawley rats obtained from different sources. *Lab Anim Res*. 2015;31(4):166. doi:10.5625/lar.2015.31.4.166

48. Boland T, Henderson G V., Gibbons FK, et al. Hypernatremia at Hospital Discharge and Out of Hospital Mortality Following Primary Intracerebral Hemorrhage. *Neurocrit Care*. 2016;25(1):110-116. doi:10.1007/s12028-015-0234-6

49. Liu J, Li Q, Ren J, Liang X, Zhang Q, Han Y. Association of Sex with Serum Potassium, Sodium, and Calcium Disorders after Hypertensive Intracerebral Hemorrhage. *World Neurosurg*. 2020;141:e367-e373. doi:10.1016/j.wneu.2020.05.137

50. Vereninov IA, Yurinskaya VE, Vereninov AA. Commentary: How Cells Can Control Their Size by Pumping Ions. *Front Cell Dev Biol*. 2017;5(May):1-14. doi:10.3389/fcell.2017.00072

51. Pasantes-Morales H, Cruz-Rangel S. Brain volume regulation: Osmolytes and aquaporin perspectives. *Neuroscience*. 2010;168(4):871-884. doi:10.1016/j.neuroscience.2009.11.074

52. Hultström M. Development of structural kidney damage in spontaneously hypertensive rats. *J Hypertens*. 2012;30(6):1087-1091. doi:10.1097/HJH.0b013e328352b89a

53. Kim SW. Hypernatemia: Successful treatment. *Electrolyte Blood Press*. 2006;4(2):66-71. doi:10.5049/EBP.2006.4.2.66

54. Oparil S, Schmieder RE. New Approaches in the Treatment of Hypertension. *Circ Res*. 2015;116(6):1074-1095. doi:10.1161/CIRCRESAHA.116.303603

Chapter 7

1. An SJ, Kim TJ, Yoon BW. Epidemiology, risk factors, and clinical features of intracerebral

hemorrhage: An update. *J Stroke*. 2017;19(1):3-10. doi:10.5853/jos.2016.00864

2. Wilkinson CM, Kung TFC, Jickling GC, Colbourne F. A translational perspective on intracranial pressure responses following intracerebral hemorrhage in animal models. *Brain Hemorrhages*. 2021;2(1):34-48. doi:10.1016/j.hest.2020.10.002
3. Tadevosyan A, Kornbluth J. Brain Herniation and Intracranial Hypertension. *Neurol Clin*. 2021;39(2):293-318. doi:10.1016/j.ncl.2021.02.005
4. Kalisvaart ACJ, Wilkinson CM, Gu S, et al. An update to the Monro–Kellie doctrine to reflect tissue compliance after severe ischemic and hemorrhagic stroke. *Sci Rep*. 2020;10(1). doi:10.1038/s41598-020-78880-4
5. Wilson MH. Monro-Kellie 2.0: The dynamic vascular and venous pathophysiological components of intracranial pressure. *J Cereb Blood Flow Metab*. 2016;36(8):1338-1350. doi:10.1177/0271678X16648711
6. Williamson MR, Colbourne F. Evidence for Decreased Brain Parenchymal Volume After Large Intracerebral Hemorrhages: a Potential Mechanism Limiting Intracranial Pressure Rises. *Transl Stroke Res*. 2017;8(4):386-396. doi:10.1007/s12975-017-0530-x
7. Juvela S, Hillbom M, Palomaki H. Risk factors for spontaneous intracerebral hemorrhage. *Stroke*. 1995;26(9):1558-1564. doi:10.1161/01.STR.26.9.1558
8. Ventura-Antunes L, Mota B, Herculano-Houzel S. Different scaling of white matter volume, cortical connectivity, and gyrification across rodent and primate brains. *Front Neuroanat*. 2013;(MARCH). doi:10.3389/fnana.2013.00003
9. Dóczi T. Volume regulation of the brain tissue-a survey. *Acta Neurochir (Wien)*. 1993;121(1-2):1-8. doi:10.1007/BF01405174
10. Mitsios N, Gaffney J, Krupinski J, et al. Expression of signaling molecules associated with apoptosis in human ischemic stroke tissue. *Cell Biochem Biophys*. 2007;47(1):73-85. doi:10.1385/CBB:47:1:73
11. Frugier T, Taylor JM, McLean C, et al. Evidence for the recruitment of autophagic

vesicles in human brain after stroke. *Neurochem Int*. 2016;96:62-68. doi:10.1016/j.neuint.2016.02.016

12. White K, Yang P, Li L, Farshori A, Medina AE, Zielke HR. Effect of Postmortem Interval and Years in Storage on RNA Quality of Tissue at a Repository of the NIH NeuroBioBank. *Biopreserv Biobank*. 2018;16(2):148-157. doi:10.1089/bio.2017.0099
13. Yerby MS, Sundsten JW, Larson EB, Wu SA, Sumi SM. A new method of measuring brain atrophy: The effect of aging in its application for diagnosing dementia. *Neurology*. 1985;35(9):1316-1320. doi:10.1212/wnl.35.9.1316
14. Hamano K, Iwasaki N, Kawashima K, Takita H. Volumetric quantification of brain volume in children using sequential CT scans. *Neuroradiology*. 1990;32(4):300-303. doi:10.1007/BF00593049
15. Kim JH, Kim YS, Kim SH, et al. Contralateral Hemispheric Brain Atrophy After Primary Intracerebral Hemorrhage. *World Neurosurg*. 2017;102:56-64. doi:10.1016/j.wneu.2017.02.105
16. Butcher KS, Jeerakathil T, Hill M, et al. The intracerebral hemorrhage acutely decreasing arterial pressure trial. *Stroke*. 2013;44(3):620-626. doi:10.1161/STROKEAHA.111.000188
17. Butcher K, Jeerakathil T, Emery D, et al. The Intracerebral Haemorrhage Acutely Decreasing Arterial Pressure Trial: ICH ADAPT. *Int J Stroke*. 2010;5(3):227-233. doi:10.1111/j.1747-4949.2010.00431.x
18. Li X, Morgan PS, Ashburner J, Smith J, Rorden C. The first step for neuroimaging data analysis: DICOM to NIfTI conversion. *J Neurosci Methods*. 2016;264:47-56. doi:10.1016/j.jneumeth.2016.03.001
19. Yushkevich PA, Piven J, Hazlett HC, et al. User-guided 3D active contour segmentation of anatomical structures: Significantly improved efficiency and reliability. *Neuroimage*. 2006;31(3):1116-1128. doi:10.1016/j.neuroimage.2006.01.015

20. Gomes AF, Brasil DM, Silva AIV, Freitas DQ, Haiter-Neto F, Groppo FC. Accuracy of ITK-SNAP software for 3D analysis of a non-regular topography structure. *Oral Radiol*. 2020;36(2):183-189. doi:10.1007/s11282-019-00397-y

21. Yushkevich PA, Gerig G. ITK-SNAP: An Intractive Medical Image Segmentation Tool to Meet the Need for Expert-Guided Segmentation of Complex Medical Images. *IEEE Pulse*. 2017;8(4):54-57. doi:10.1109/MPUL.2017.2701493

22. Yuan Q, Rubic M, Seah J, et al. Do maternal opioids reduce neonatal regional brain volumes: A pilot study. *J Perinatol*. 2014;34(12):909-913. doi:10.1038/jp.2014.111

23. Fjell AM, Walhovd KB, Fennema-Notestine C, et al. One-year brain atrophy evident in healthy aging. *J Neurosci*. 2009;29(48):15223-15231. doi:10.1523/JNEUROSCI.3252-09.2009

24. Sele S, Liem F, Mérillat S, Jäncke L. Age-related decline in the brain: a longitudinal study on inter-individual variability of cortical thickness, area, volume, and cognition. *Neuroimage*. 2021;240. doi:10.1016/j.neuroimage.2021.118370

25. De Vis JB, Zwanenburg JJ, van der Kleij LA, et al. Cerebrospinal fluid volumetric MRI mapping as a simple measurement for evaluating brain atrophy. *Eur Radiol*. 2016;26(5):1254-1262. doi:10.1007/s00330-015-3932-8

26. Czosnyka M, Czosnyka Z, Momjian S, Pickard JD. Cerebrospinal fluid dynamics. *Physiol Meas*. 2004;25(5). doi:10.1088/0967-3334/25/5/R01

27. Mokri B. The Monro-Kellie hypothesis: Applications in CSF volume depletion. *Neurology*. 2001;56(12):1746-1748. doi:10.1212/WNL.56.12.1746

28. Zazulia AR, Diringer MN, Derdeyn CP, Powers WJ. Progression of mass effect after intracerebral hemorrhage. *Stroke*. 1999;30(6):1167-1173. doi:10.1161/01.STR.30.6.1167

29. de Lucas EM, Sánchez E, Gutiérrez A, et al. CT protocol for acute stroke: Tips and tricks for general radiologists. *Radiographics*. 2008;28(6):1673-1687. doi:10.1148/rg.286085502

30. Park KY, Kim JH, Chung CS, et al. Cortical sulcal effacement on brain CT associated with cerebral hyperperfusion after carotid artery stenting. *J Neurol Sci*. 2007;260(1-2):83-86. doi:10.1016/j.jns.2007.04.010

31. Steinmetz H, Staiger JF, Schlaug G, Huang Y, Jäncke L. Corpus callosum and brain volume in women and men. *Neuroreport*. 1995;6(7):1002-1004. doi:10.1097/00001756-199505090-00013

32. Allen JS, Damasio H, Grabowski TJ. Normal neuroanatomical variation in the human brain: An MRI-volumetric study. *Am J Phys Anthropol*. 2002;118(4):341-358. doi:10.1002/ajpa.10092

33. Ward MA, Carlsson CM, Trivedi MA, Sager MA, Johnson SC. The effect of body mass index on global brain volume in middle-aged adults: A cross sectional study. *BMC Neurol*. 2005;5. doi:10.1186/1471-2377-5-23

34. Bayat PD, Ghanbari A, Sohouli P, Amiri S, Sari PA. Correlación del tamaño craneal y volumen del cerebro, con edad, peso, talla e índice de masa corporal de los estudiantes de Ciencias Médicas de Arak. *Int J Morphol*. 2012;30(1):157-161. doi:10.4067/S0717-95022012000100028

35. Adeoye O, Broderick JP. Advances in the management of intracerebral hemorrhage. *Nat Rev Neurol*. 2010;6(11):593-601. doi:10.1038/nrneurol.2010.146

36. Ziai WC, Carhuapoma JR. Intracerebral Hemorrhage. *Contin Lifelong Learn Neurol*. 2018;24(6):1603-1622. doi:10.1212/CON.0000000000000672

37. Tian Y, Wang Z, Jia Y, et al. Intracranial pressure variability predicts short-term outcome after intracerebral hemorrhage: A retrospective study. *J Neurol Sci*. 2013;330(1-2):38-44. doi:10.1016/j.jns.2013.04.001

38. MacLellan CL, Silasi G, Poon CC, et al. Intracerebral hemorrhage models in rat: comparing collagenase to blood infusion. *J Cereb Blood Flow Metab*. 2008;28:516-525. doi:10.1038/sj.jcbfm.9600548

39. Erten-Lyons D, Dodge HH, Woltjer R, et al. Neuropathologic basis of age-associated brain atrophy. *JAMA Neurol*. 2013;70(5):616-622. doi:10.1001/jamaneurol.2013.1957

40. Kroin JS, Nagalla SKS, Buvanendran A, McCarthy RJ, Tuman KJ, Ivankovich AD. The mechanisms of intracranial pressure modulation by epidural blood and other injectates in a postdural puncture rat model. *Anesth Analg*. 2002;95(2):423-429. doi:10.1213/00000539-200208000-00035

41. Yang GY, Betz AL, Chenevert TL, Brunberg JA, Hoff JT. Experimental intracerebral hemorrhage: Relationship between brain edema, blood flow, and blood-brain barrier permeability in rats. *J Neurosurg*. 1994;81(1):93-102. doi:10.3171/jns.1994.81.1.0093

42. Staykov D, Wagner I, Volbers B, et al. Natural course of perihemorrhagic edema after intracerebral hemorrhage. *Stroke*. 2011;42(9):2625-2629. doi:10.1161/STROKEAHA.111.618611

Chapter 8

1. Gigante PR, Appelboom G, Hwang BY, et al. Isoflurane preconditioning affords functional neuroprotection in a murine model of intracerebral hemorrhage. In: *Acta Neurochirurgica, Supplementum*. ; 2011. doi:10.1007/978-3-7091-0693-8_23

2. Bar-Klein G, Klee R, Brandt C, et al. Isoflurane prevents acquired epilepsy in rat models of temporal lobe epilepsy. *Ann Neurol*. 2016;80(6):896-908. doi:10.1002/ana.24804

3. Wilkinson CM, Kalisvaart ACJ, Kung TFC, et al. The collagenase model of intracerebral hemorrhage in awake, freely moving animals: The effects of isoflurane. *Brain Res*. 2020;1728:146593. doi:10.1016/j.brainres.2019.146593

4. Yang CF, Yu-Chih Chen M, Chen TI, Cheng CF. Dose-dependent effects of isoflurane on cardiovascular function in rats. *Tzu Chi Med J*. Published online 2014. doi:10.1016/j.tcmj.2014.07.005

5. Seto A, Taylor S, Trudeau D, et al. Induction of ischemic stroke in awake freely moving

mice reveals that isoflurane anesthesia can mask the benefits of a neuroprotection therapy. *Front Neuroenergetics*. 2014;6(APR). doi:10.3389/fnene.2014.00001

6. Wilkinson CM, Brar PS, Balay CJ, Colbourne F. Glibenclamide, a Sur1-Trpm4 antagonist, does not improve outcome after collagenase-induced intracerebral hemorrhage. *PLoS One*. 2019;14(5). doi:10.1371/journal.pone.0215952
7. Hoff JT, Xi G. Brain edema from intracerebral hemorrhage. *Acta Neurochir Suppl*. 2003;(86):11-15. doi:10.1007/978-3-7091-0651-8_3
8. Simard JM, Sheth KN, Kimberly WT, et al. Glibenclamide in cerebral ischemia and stroke. *Neurocrit Care*. 2014;20(2):319-333. doi:10.1007/s12028-013-9923-1
9. Stokum JA, Kwon MS, Woo SK, et al. SUR1-TRPM4 and AQP4 form a heteromultimeric complex that amplifies ion/water osmotic coupling and drives astrocyte swelling. *Glia*. 2018;66(1):108-125. doi:10.1002/glia.23231
10. Woo SK, Tsymbalyuk N, Tsymbalyuk O, Ivanova S, Gerzanich V, Simard JM. SUR1-TRPM4 channels, not KATP, mediate brain swelling following cerebral ischemia. *Neurosci Lett*. 2020;718. doi:10.1016/j.neulet.2019.134729
11. Kung TFC, Wilkinson CM, Dirks CA, Jickling GC, Colbourne F. Glibenclamide does not improve outcome following severe collagenase-induced intracerebral hemorrhage in rats. *PLoS One*. 2021;16(6 June). doi:10.1371/journal.pone.0252584
12. Jiang B, Li L, Chen Q, et al. Role of Glibenclamide in Brain Injury After Intracerebral Hemorrhage. *Transl Stroke Res*. 2017;8(2):183-193. doi:10.1007/s12975-016-0506-2
13. Jiang B, Zhang Y, Wang Y, et al. Glibenclamide Attenuates Neuroinflammation and Promotes Neurological Recovery After Intracerebral Hemorrhage in Aged Rats. *Front Aging Neurosci*. 2021;13. doi:10.3389/fnagi.2021.729652
14. Xu F, Shen G, Su Z, He Z, Yuan L. Glibenclamide ameliorates the disrupted blood–brain barrier in experimental intracerebral hemorrhage by inhibiting the activation of NLRP3 inflammasome. *Brain Behav*. 2019;9(4). doi:10.1002/brb3.1254

15. Akiyama H, Meyer JS, Mortel KF, Yasuo Terayama, Thornby JI, Konno S. Normal human aging: Factors contributing to cerebral atrophy. *J Neurol Sci*. 1997;152(1):39-49. doi:10.1016/S0022-510X(97)00141-X

16. Gur RC, Gunning-Dixon FM, Turetsky BI, Bilker WB, Gur RE. Brain region and sex differences in age association with brain volume: A quantitative MRI study of healthy young adults. *Am J Geriatr Psychiatry*. 2002;10(1):72-80. doi:10.1097/00019442-200201000-00009

17. Liddle LJ, Ralhan S, Ward DL, Colbourne F. Translational Intracerebral Hemorrhage Research: Has Current Neuroprotection Research ARRIVEd at a Standard for Experimental Design and Reporting? *Transl Stroke Res*. 2020;11(6):1203-1213. doi:10.1007/s12975-020-00824-x

18. Seyhan AA. Lost in translation: the valley of death across preclinical and clinical divide – identification of problems and overcoming obstacles. *Transl Med Commun*. 2019;4(1). doi:10.1186/s41231-019-0050-7

19. Boulanger M, Poon MTC, Wild SH, Al-Shahi Salman R. Association between diabetes mellitus and the occurrence and outcome of intracerebral hemorrhage. *Neurology*. 2016;87(9):870-878. doi:10.1212/WNL.0000000000003031

20. Passero S, Ciacci G, Ulivelli M. The influence of diabetes and hyperglycemia on clinical course after intracerebral hemorrhage. *Neurology*. 2003;61(10):1351-1356. doi:10.1212/01.WNL.0000094326.30791.2D

21. Song EC, Chu K, Jeong SW, et al. Hyperglycemia exacerbates brain edema and perihematomal cell death after intracerebral hemorrhage. *Stroke*. 2003;34(9):2215-2220. doi:10.1161/01.STR.0000088060.83709.2C

22. Sweetnam D, Holmes A, Tennant KA, et al. Diabetes impairs cortical plasticity and functional recovery following Ischemic stroke. *J Neurosci*. 2012;32(15):5132-5143. doi:10.1523/JNEUROSCI.5075-11.2012

23. Tennant KA, Brown CE. Diabetes augments in vivo microvascular blood flow dynamics after stroke. *J Neurosci*. 2013;33(49):19194-19204. doi:10.1523/JNEUROSCI.3513-13.2013

24. McManus ML, Churchwell KB, Strange K. Regulation of Cell Volume in Health and Disease. *N Engl J Med*. 1995;333(19):1260-1267. doi:10.1056/nejm199511093331906

25. Burg MB, Kador PF. Sorbitol, osmoregulation, and the complications of diabetes. *J Clin Invest*. 1988;81(3):635-640. doi:10.1172/JCI113366

26. Lang F. Mechanisms and Significance of Cell Volume Regulation. *J Am Coll Nutr*. 2007;26:613S-623S. doi:10.1080/07315724.2007.10719667

27. Auriat A, Plahta WC, McGie SC, Yan R, Colbourne F. 17β-Estradiol pretreatment reduces bleeding and brain injury after intracerebral hemorrhagic stroke in male rats. *J Cereb Blood Flow Metab*. 2005;25(2):247-256. doi:10.1038/sj.jcbfm.9600026

28. Zheng Y, Hu Q, Manaenko A, et al. 17B-Estradiol Attenuates Hematoma Expansion Through Estrogen Receptor A/Silent Information Regulator 1/Nuclear Factor-Kappa B Pathway in Hyperglycemic Intracerebral Hemorrhage Mice. *Stroke*. 2015;46(2):485-491. doi:10.1161/STROKEAHA.114.006372

29. Brann DW, Dhandapani K, Wakade C, Mahesh VB, Khan MM. Neurotrophic and neuroprotective actions of estrogen: Basic mechanisms and clinical implications. *Steroids*. 2007;72(5):381-405. doi:10.1016/j.steroids.2007.02.003

30. Murphy SJ, McCullough LD, Smith JM. Stroke in the female: role of biological sex and estrogen. *ILAR J*. 2004;45(2):147-159. doi:10.1093/ilar.45.2.147

31. Goldman JM, Murr AS, Cooper RL. The rodent estrous cycle: Characterization of vaginal cytology and its utility in toxicological studies. *Birth Defects Res Part B - Dev Reprod Toxicol*. 2007;80(2):84-97. doi:10.1002/bdrb.20106

32. Ayus JC, Achinger SG, Arieff A. Brain cell volume regulation in hyponatremia: Role of sex, age, vasopressin, and hypoxia. *Am J Physiol - Ren Physiol*. 2008;295(3).

doi:10.1152/ajprenal.00502.2007

33. Shahrokhi N, Khaksari M, Soltani Z, Mahmoodi M, Nakhaee N. Effect of sex steroid hormones on brain edema, intracranial pressure, and neurologic outcomes after traumatic brain injury. *Can J Physiol Pharmacol*. 2010;88(4):414-421. doi:10.1139/Y09-126

34. Takubo Y, Hirai T, Muro S, Kogishi K, Hosokawa M, Mishima M. Age-associated changes in elastin and collagen content and the proportion of types I and III collagen in the lungs of mice. *Exp Gerontol*. 1999;34(3):353-364. doi:10.1016/S0531-5565(99)00017-0

35. Liebeskind DS. Collateral circulation. *Stroke*. 2003;34(9):2279-2284. doi:10.1161/01.STR.0000086465.41263.06

36. Agarwal S, Scoffings DJ, Simon Jones P, et al. Interaction of age with the ischaemic penumbra, leptomeningeal collateral circulation and haemodynamic variables in acute stroke: A pilot study. *J Neurol Neurosurg Psychiatry*. 2013;84(3):271-276. doi:10.1136/jnnp-2012-303258

37. Kalisvaart ACJ, Wilkinson CM, Gu S, et al. An update to the Monro–Kellie doctrine to reflect tissue compliance after severe ischemic and hemorrhagic stroke. *Sci Rep*. 2020;10(1). doi:10.1038/s41598-020-78880-4

38. von Bartheld CS, Bahney J, Herculano-Houzel S. The search for true numbers of neurons and glial cells in the human brain: A review of 150 years of cell counting. *J Comp Neurol*. 2016;524(18):3865-3895. doi:10.1002/cne.24040

39. Inoue H, Mori SI, Morishima S, Okada Y. Volume-sensitive chloride channels in mouse cortical neurons: Characterization and role in volume regulation. *Eur J Neurosci*. 2005;21(6):1648-1658. doi:10.1111/j.1460-9568.2005.04006.x

40. Vereninov IA, Yurinskaya VE, Vereninov AA. Commentary: How Cells Can Control Their Size by Pumping Ions. *Front Cell Dev Biol*. 2017;5(May):1-14. doi:10.3389/fcell.2017.00072

41. Aiyagari V, Deibert E, Diringer MN. Hypernatremia in the neurologic intensive care unit: how high is too high? *J Crit Care*. 2006;21(2):163-172. doi:10.1016/j.jcrc.2005.10.002

42. Qiu Z, Dubin AE, Mathur J, et al. SWELL1, a plasma membrane protein, is an essential component of volume-regulated anion channel. *Cell*. 2014;157(2):447-458. doi:10.1016/j.cell.2014.03.024

43. Jentsch TJ. VRACs and other ion channels and transporters in the regulation of cell volume and beyond. *Nat Rev Mol Cell Biol*. 2016;17(5):293-307. doi:10.1038/nrm.2016.29

44. Nadeau CA, Dietrich K, Wilkinson CM, et al. Prolonged Blood-Brain Barrier Injury Occurs After Experimental Intracerebral Hemorrhage and Is Not Acutely Associated with Additional Bleeding. *Transl Stroke Res*. 2019;10(3):287-297. doi:10.1007/s12975-018-0636-9

45. Pasantes-Morales H, Cruz-Rangel S. Brain volume regulation: Osmolytes and aquaporin perspectives. *Neuroscience*. 2010;168(4):871-884. doi:10.1016/j.neuroscience.2009.11.074

46. Williamson MR, Dietrich K, Hackett MJ, et al. Rehabilitation Augments Hematoma Clearance and Attenuates Oxidative Injury and Ion Dyshomeostasis After Brain Hemorrhage. *Stroke*. 2016;48(1):195-203. doi:10.1161/STROKEAHA.116.015404

47. Qureshi AI, Tuhrim S, Broderick JP, Batjer HH, Hondo H, Hanley DF. Spontaneous Intracerebral Hemorrhage. *N Engl J Med*. 2001;344(19):1450-1460. doi:10.1056/NEJM200105103441907

48. Hemphill JC, Bonovich DC, Besmertis L, Manley GT, Johnston SC. The ICH score: A simple, reliable grading scale for intracerebral hemorrhage. *Stroke*. 2001;32(4):891-896. doi:10.1161/01.str.32.4.891

49. Vespa PM, O'Phelan K, Shah M, et al. Acute seizures after intracerebral hemorrhage: A factor in progressive midline shift and outcome. *Neurology*. 2003;60(9):1441-1446.

doi:10.1212/01.WNL.0000063316.47591.B4

50. Seitz RJ, Azari NP, Knorr U, Binkofski F, Herzog H, Freund HJ. The role of diaschisis in stroke recovery. *Stroke*. 1999;30(9):1844-1850. doi:10.1161/01.STR.30.9.1844

51. Lord AS, Gilmore E, Choi HA, et al. Time course and predictors of neurological deterioration after intracerebral hemorrhage. *Stroke*. 2015;46(3):647-652. doi:10.1161/STROKEAHA.114.007704

52. Mayer SA, Sacco RL, Shi T, Mohr JP. Neurologic deterioration in noncomatose patients with supratentorial intracerebral hemorrhage. *Neurology*. 1994;44(8):1379-1384. doi:10.1212/wnl.44.8.1379

53. Shoamanesh A, Patrice Lindsay M, Castellucci LA, et al. Canadian stroke best practice recommendations: Management of Spontaneous Intracerebral Hemorrhage, 7th Edition Update 2020. *Int J Stroke*. 2021;16(3):321-341. doi:10.1177/1747493020968424

54. Marinkovic I, Strbian D, Pedrono E, et al. Decompressive craniectomy for intracerebral hemorrhage. *Neurosurgery*. 2009;65(4):780-786. doi:10.1227/01.NEU.0000351775.30702.A9

55. Maira G, Anile C, Colosimo C, Rossi GF. Surgical treatment of primary supratentorial intracerebral hemorrhage in stuporous and comatose patients. *Neurol Res*. 2002;24(1):54-60. doi:10.1179/016164102101199549

56. Freeman WD. Management of intracranial pressure. *Contin Lifelong Learn Neurol*. 2015;21(5):1299-1323. doi:10.1212/CON.0000000000000235

57. Wilkinson CM, Kung TFC, Jickling GC, Colbourne F. A translational perspective on intracranial pressure responses following intracerebral hemorrhage in animal models. *Brain Hemorrhages*. 2021;2(1):34-48. doi:10.1016/j.hest.2020.10.002

58. Dastur CK, Yu W. Current management of spontaneous intracerebral haemorrhage. *Stroke Vasc Neurol*. 2017;2(1):21-29. doi:10.1136/svn-2016-000047

59. Song M, Yu SP. Ionic Regulation of Cell Volume Changes and Cell Death after Ischemic

Stroke. *Transl Stroke Res*. 2014;5(1):17-27. doi:10.1007/s12975-013-0314-x