Efficacy of Rehabilitation in Translational Models of Intracerebral Hemorrhage

by

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Abstract

Intracerebral hemorrhage (ICH), a devastating stroke caused by the rupture of vasculature in the brain, is responsible for <20% of all strokes, yet accounts for a disproportionately high burden of stroke related death and disability. The formation and degradation of the hematoma (blood clot) injures the brain through mechanisms of both immediate mechanical damage (primary injury) and delayed cellular damage (secondary injury). Clinical trials have yet to identify treatments that can reliably lessen injury and impairment after ICH; as such, treatment for hemorrhagic stroke remains limited to medical management and rehabilitation. Although rehabilitation is an essential component of post-stroke care, understanding of the optimal type, timing, and dosage of rehabilitation after stroke is limited.

Preclinical (animal) models are frequently used to explore underlying mechanisms of injury and recovery after stroke, often with the goal of translating research findings to clinical practice. However, no single experimental model fully mimics the pathophysiology of human stroke, or the heterogeneity of location, stroke subtype, impairment, and comorbidities observed in the clinical population. Despite differing mechanisms of injury and patterns of recovery between ischemic and hemorrhagic stroke, most translational stroke research is conducted in experimental models of ischemia. As a result, much of our understanding of recovery after ICH is grounded in the assumption that the spatial and temporal dynamics of injury and recovery after ischemic and hemorrhagic stroke are similar, despite mounting evidence suggesting this is not entirely true. In this thesis I studied how modifiable treatment parameters such as intensity and timing of rehabilitation onset influence recovery in

preclinical models of striatal ICH and focus on how to improve future preclinical rehabilitation studies to advance translational success.

I first investigated whether early, intense enriched rehabilitation (ER) accelerated hematoma clearance and improved neurological and behavioural outcomes in the sub-acute (experiment 1) and chronic (experiment 2) phases of recovery after experimental striatal ICH in rats (Chapter 2). I hypothesized that ER initiated 5 days after ICH would provide enhanced behavioural benefit and accelerate hematoma clearance at 14 days after ICH; furthermore, increasing treatment duration (from 10 to 20 days) would confer greater behavioural and neurological benefit when measured 30 and 60 days after ICH, respectively. Contrary to both the hypothesis and previous findings, I did not detect a significant difference in hematoma clearance, recovery in skilled reaching, or volume of tissue loss compared to untreated controls.

Owing to the difficulties in extending previous findings of rehabilitation accelerated hematoma clearance, I next sought to characterize the overall efficacy of rehabilitation therapies on motor recovery in translational models of ICH through the use of meta-analysis (Chapter 3). Rehabilitation provided modest benefits to motor recovery after ICH, however efficacy varied by treatment type and functional endpoint. In alignment with clinical findings, rehabilitation was most effective in animals with mild-moderate severity ICH. Interestingly, I found a complex relationship between intervention type and timing of treatment onset <7 days after ICH, with interventions initiated between 48 hours-5 days after ICH providing no significant benefit. These results differ from those reported in a similar meta-analysis of rehabilitation in translational models of ischemic stroke, suggesting that response to rehabilitation may vary by stroke subtype. Finally, to address the varied quality in reporting and

experimental design found in the reviewed literature, I developed a roadmap for researchers to follow to improve the quality of future translational rehabilitation research.

Noting that the generalizability of translational research is likely reliant on whether experimental models reflect the pathophysiology and variability in recovery observed in clinical populations, I conducted a retrospective, exploratory post-hoc analysis to assess if proportional recovery occurs in preclinical models of subcortical ICH and whether any biomarkers predict recovery (Chapter 4). I found that proportional recovery does exist after experimental striatal ICH, but to a much lesser extent than reported elsewhere (30% vs. ~70%). Interestingly, differences in recovery after striatal ICH could not be linked to proposed biomarkers of lesion severity, internal capsule damage, or initial impairment.

In this thesis I provide a comprehensive analysis of the efficacy of rehabilitation in translational models of ICH and discuss the complexity of evaluating how modifiable treatment parameters such as timing, intensity, and dose influence treatment efficacy. This work provides evidence for a complex relationship between intervention onset and treatment efficacy after ICH that is likely influenced by treatment type and/or intensity. Furthermore, it suggests that recovery and response to rehabilitation seemingly differ by stroke subtype. Together, these findings provide justification for future research that systematically manipulates intervention parameters and directly compares how recovery and response to treatment differ between ICH and ischemia.

Preface

This thesis is an original work by Britt A. Fedor. Experiments were granted research ethics approval by the University of Alberta Biosciences Animal Care and Use Committee under Animal Use Protocol #960 "Rodent Stroke Studies."

Chapter 2 of this thesis has been published as B. Fedor, A. Kalisvaart, S. Ralhan, T. Kung, M. MacLaren, & F. Colbourne, "Early, Intense Rehabilitation Fails to Improve Outcome After Intra-Striatal Hemorrhage in Rats" in *Neurorehabilitation and Neural Repair* (2022), volume 36, issue 12. B. Fedor was responsible for experimental design, data collection, data analysis, and manuscript writing. A. Kalisvaart, S. Ralhan, T. Kung, and M. MacLaren were involved in data collection and manuscript editing. F. Colbourne was the supervisory author and assisted with experimental design, data analysis, and manuscript writing.

Chapter 3 and Appendix A of this thesis have been published as B. Fedor, N. Sander, M., MacLaren, L. Liddle, C. MacLellan, & F. Colbourne, "Motor rehabilitation provides modest functional benefits after intracerebral hemorrhage: a systematic review and meta-analysis of translational rehabilitation studies" in *Translational Stroke Research* (2023). B. Fedor was responsible for study conceptualization, search strategy development, literature search and screening, data extraction, quality and risk of bias assessments, data analysis, manuscript writing, and figure creation. N. Sander was involved in data extraction, quality and risk of bias assessments, and manuscript editing. M. MacLaren was involved in literature screening, data extraction, and manuscript editing. L. Liddle contributed statistical expertise, expert review, and manuscript editing. C. MacLellan contributed to study conceptualization, expert review,

and manuscript editing. F. Colbourne was the supervisory author and assisted with study conceptualization, literature screening, and manuscript writing.

Dedication

This thesis is dedicated to Hope Fedor, Mavis Stanton, and Dr. Robert Mulcahy who were all endlessly proud I was doing this work … and never failed to ask, “but how are the rats?”

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Thank-you to the people and things (ski pals, cycling, Taylor Swift's ever-expanding discography…) that kept me going when I was a hermit in a dark room full of rats or tucked away for weeks on end at my computer. Mike, Nate, Saylish, and Andy – thank-you for giving me a place to escape from the stresses of grad school. To my friends (Andrea, Shay, Maron, Gráinne, and Syna), family (Dennis, Mary-Jane, Chris, and Andrei), and partner (Dave) – I am eternally grateful for your unwavering support and encouragement throughout this wild ride.

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List of Abbreviations and Acronyms

ADLs	activities of daily living
AE	aerobic exercise
AMPA	α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptor
ANOVA	analysis of variance
AT	acrobatic training
ATP	adenosine triphosphate
AQP	aquaporin
AWB	autologous whole blood model of ICH
BBB	blood-brain barrier
BDNF	brain-derived neurotrophic factor
BI	Barthel Index
BP	blood pressure
CAA	cerebral amyloid angiopathy
CI	confidence interval
CIMT	constraint-induced movement therapy
CNS	central nervous system
COL	collagenase model of ICH
CSPGs	chondroitin sulfate proteoglycans
CST	corticospinal tract
CT	computerized tomography
DALYs	disability adjusted life years
DAMPs	damage associated molecular patterns
DFX	deferoxamine
EE	environmental enrichment
ER	enriched rehabilitation
ET-1	endothelin-1 model of ischemia
EX	exercise
FA	fractional anisotropy
FGF	fibroblast growth factor
FLU	forced limb use

FM	Fugl-Meyer assessment
GAP-43	growth associated protein 43
GCS	Glasgow coma scale
GFAP	glial fibrillary acidic protein
Hb	hemoglobin
HO-1	heme-oxygenase 1
Hp	haptoglobin
HR	hazard ratio
Hpx	hemopexin
ICH	intracerebral hemorrhage
ICP	intracranial pressure
IL	interleukin
iNOS	inducible nitrous oxide synthase
IVH	intraventricular hemorrhage
IQR	interquartile range
LTD	long-term depression
LTP	long-term potentiation
MCAo	middle cerebral artery occlusion model of ischemia
MCID	minimum clinically important difference
MD	mean difference
MDS	motor deficit score
MEP	motor evoked potential
MMP	matrix metalloproteinase
mNSS	modified neurological severity score
MRI	magnetic resonance imaging
mRS	modified Rankin Scale
NDS	neurological deficit score
NGF	nerve growth factor
NIHSS	National Institute of Health Stroke Score
NMDA	N-methyl-D-aspartate receptor
Nrf-2	nuclear factor erythroid 2-related factor 2
NF-κB	nuclear factor-κB

PHZ	perihematoma zone
PPAR-γ	peroxisome proliferator-activated receptor γ
RBC	red blood cell
REACH	skilled reach training
ROS	reactive oxygen species
SAH	subarachnoid hemorrhage
SD	standard deviation
SHR	spontaneously hypertensive rat
SMD	standardized mean difference
SVZ	subventricular zone
TGF-β	transforming growth factor β
TLR	toll-like receptor
TNF-α	tumor necrosis factor-α
tPA	tissue plasminogen activator
Trk	tropomyosin-related kinase
VEGF	vascular endothelial growth factor

Chapter 1 | Introduction

1.1 An Introduction to Stroke

Stroke is a neurological disease characterized by a disruption of blood flow to a region of the central nervous system.[1] Despite many advancements in prevention, diagnosis, treatment, and medical management of stroke in recent decades, it remains the second largest cause of mortality worldwide and a leading cause of disability.[2] Each year more than 62 000 Canadians are treated for stroke.[3] Currently, more than 400 000 Canadians are living with the effects of stroke, and this number is expected to increase to between 654 000 and 726 000 by 2038.[4] While mortality has decreased in recent decades, increases in life expectancy and an aging population have resulted in a greater number of people experiencing and living with the consequences of stroke.[4,5] One of the leading contributors to **disability adjusted life years (DALYs)**, stroke is estimated to contribute to more than 110 million DALYs globally.[6] As upwards of 40% of stroke survivors require some level of assistance to complete **activities of daily living (ADLs)**,[4] stroke is associated with significant economic burden to both patients and the healthcare system.[7]

Stroke is a heterogenous disease that can be broadly broken down into two categories. Ischemic stroke accounts for 80-85% of all strokes and occurs as a result of occlusion of the blood supply.[8,9] Hemorrhagic stroke occurs due to the rupture of vasculature, resulting in infiltration of blood into the surrounding regions (i.e., parenchyma, ventricular system, subarachnoid space). Despite accounting for only 15-20% of all strokes, hemorrhagic stroke is responsible for 49% of the global burden of death from stroke.[3]

1.1.1 Hemorrhagic Stroke

Hemorrhagic stroke is characterized by the sudden rupture of vasculature resulting in rapid extrusion of blood into surrounding tissues and spaces. There are two primary and distinct subtypes: **subarachnoid hemorrhage (SAH)**, where bleeding occurs between the pial and arachnoid membranes in the subarachnoid space; and **intracerebral hemorrhage (ICH)**, also termed intraparenchymal hemorrhage, where bleeding occurs within the brain parenchyma and/or ventricular system **(intraventricular hemorrhage, IVH).** ICH is particularly devastating, with a 30-day mortality rate of nearly 40%, and increasing to 54% at one year.[10] Survivors are frequently left with lasting impairments; it is estimated between 50-60% of survivors live with chronic disability.[9,10] ICH alone makes up 10-15%[11,12] of all strokes and is the focus of this thesis.

Primary ICH occurs due to the rupture of small arterioles as a result of the longstanding effects of hypertension and/or **cerebral amyloid angiopathy (CAA)** on the cerebral vasculature.[13] Subsequently, the vast majority of ICHs occur in deep brain regions (hypertension) or lobar regions (CAA). Secondary ICH accounts for a smaller proportion of cases and occurs as a result of vascular abnormalities and malformations, tumors, or coagulopathies.[13] Damage from ICH is both immediate and protracted and can be categorized into two distinct phases: primary injury and secondary injury. Primary injury describes the immediate mechanical damage that occurs as a result of the rupturing of cerebral vasculature. Extruded blood, often following the path of least resistance (e.g., white matter tracts), causes significant trauma to the neurovascular unit (i.e., neurons, glia, endothelial cells, smooth muscle cells, and pericytes) as the formation of the hematoma (mass of clotted blood) leads to

compression and displacement of surrounding brain tissue.[11,14] Secondary injury occurs in the hours, days, weeks, and months following ictus, as a number of pathological processes disrupt the tightly regulated ecosystem of the brain. Early after injury, many of these processes contribute to the formation of a highly cytotoxic, pro-oxidative, and pro-inflammatory environment that is unfavourable to cell survival, ultimately leading to cell death through mechanisms such as necrosis, apoptosis, ferroptosis, and necroptosis.[15,16] Later, many of these processes contribute to clearance of debris, and the formation of an environment favourable to repair and remodeling. A thorough discussion of primary and secondary injury and how these complex processes contribute to repair and recovery can be found in sections 1.6 Mechanisms of Injury and 1.7 Neural Repair After Stroke.

Acute medical management of ICH prioritizes reversal of coagulopathies, controlling **blood pressure (BP)** and **intracranial pressure (ICP)**, and prevention of hematoma expansion.[17] Despite many promising early results, clinical trials have yet to identify a treatment that reliably lessens injury and impairment after ICH.[18,19] To date, the best treatment for hemorrhagic stroke is prevention, as treatment remains limited to medical management and rehabilitation.[13]

1.1.2 Ischemic Stroke

Ischemic stroke accounts for approximately 80-85% of all strokes[8,9,20] and is characterized by the occlusion of the blood supply, predominantly by thromboembolism (clot) or narrowing of the vasculature.[20] The extent of ischemic infarct relies not only on the location of vascular occlusion, but the duration of hypoperfusion as well as the presence of collateral blood supply and degree of reperfusion.[20] Neurons in the ischemic

core, the region of the infarct irreversibly damaged, inevitably die as blood flow drops to below 10-25% of normal[20] (see Figure 1.1). This disruption in blood flow forms a gradient of hypoperfusion, where perfusion improves moving outward from the core. Neurons in these regions, known as the ischemic penumbra, are often dysfunctional but not entirely unsalvageable – making preservation, restoration, and repair of this tissue the target of many therapies.[21] On a molecular level, impaired blood flow disrupts delivery of oxygen, glucose, and other key nutrients to the surrounding tissue, a series of events known as the ischemic cascade.[9] This disruption leads to a failure in the cell's ability to undergo energy intensive processes key to cellular survival, such as formation of **adenosine triphosphate (ATP)**.[20,22] Mitochondrial stores of ATP are rapidly depleted;[22] without adequate energy the cell is unable to power ion pumps, leading to a disruption in the ion gradient and deterioration of membrane potential.[20] These failures contribute to injurious processes such as glutamatergic excitotoxicity, oxidative stress, production of **reactive oxygen species (ROS)**, and formation of cytotoxic edema. While ischemia and ICH share a number of similar mechanisms of injury and repair, as ICH is the focus of this work, many of the aforementioned mechanisms are discussed in detail in the context of ICH in section 1.6 Mechanisms of Injury.

"Time is brain" is a phrase commonly used to describe the approach to medical management of acute ischemic stroke. Recanalization and reperfusion of occluded tissue is the primary goal, with thrombolysis (e.g., tissue plasminogen activator) and endovascular thrombectomy considered the gold standards for acute ischemic stroke treatment.[23,24] Concerningly, a common complication following ischemic stroke is hemorrhagic transformation, where bleeding occurs following an initial ischemic incident. It is estimated that more than 20% of patients who experience ischemic stroke

will experience hemorrhagic transformation within the first 48 hours;[20] this risk is increased following the use of thrombolytic agents such as tPA.[25] Hemorrhagic transformation is associated with worsened outcomes and increased mortality.[26]

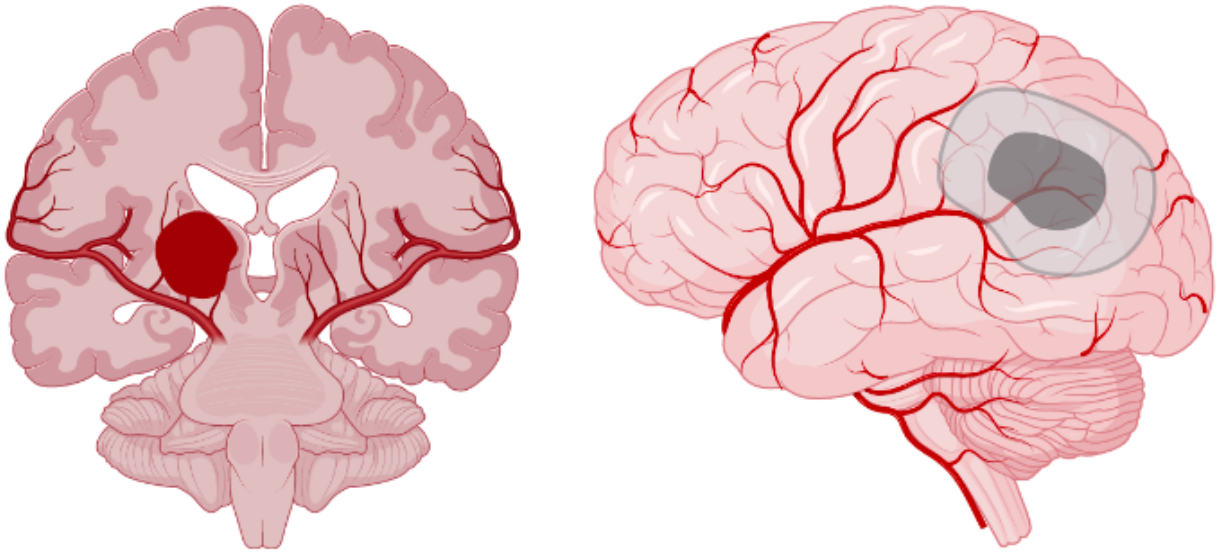

Figure 1-1 Representation of hemorrhagic stroke (left) in deep brain region and ischemic stroke (right) due to occlusion in middle cerebral artery territory. The hematoma, the mass of clotted blood, is depicted in red; the ischemic core, region of irreversible damage, is pictured in dark grey; ischemic penumbra, region of dysfunctional yet potentially salvageable tissue, pictured in light grey [figure created with BioRender]

1.2 Risk Factors

Risk factors for stroke can be broken down into two broad categories: non-modifiable, meaning they cannot be directly altered; or modifiable, meaning factors that can be altered or medically managed. A recent large, international case control study, INTERSTROKE,[27,28] has shed light on many risk factors implicated in both ischemic and hemorrhagic stroke.

1.2.1 Non-modifiable Risk Factors

Multiple non-modifiable risk factors have been identified to elevate risk of ICH including age, sex, presence of CAA, race, ApoE genotype (ε2, ε4), kidney disease, history of cerebral microbleeds, and previous stroke.[10,13,29] Age is the most significant non-modifiable risk factor, with risk of stroke doubling every decade after 55.[9] Aging induces numerous structural and physiological changes to the cerebrovascular system and reduces the central nervous system's capacity for repair.[12] Combined, these factors make the aging brain more susceptible to injury,[12] resulting in worsened outcomes for older individuals. Additionally, older individuals are more likely to live with one or more chronic conditions or comorbidities (hypertension, atrial fibrillation, diabetes, etc.) associated with elevated risk.[12]

Sex differentially effects stroke risk across the lifespan. Overall, the risk of stroke is lower for women, however the distribution of risk is not uniform across the lifespan.[30] A recent province wide study in Ontario found that women <30 years old have a higher risk of stroke relative to their male peers (**hazard ratio, HR**=1.26), likely in part due to the use of oral contraceptives and pregnancy related complications and comorbidities.[30] Interestingly, this study reported that women had a lower risk of ICH across the lifespan, but a greater risk of SAH ≥30 years of age. Stroke risk becomes greater for men in midlife, then approaches equal between the sexes ~80 years of age. These differences in risk across the lifespan are believed to be due to the cytoprotective effects of estrogen, as stroke incidence increases following menopause.[30,31]

Cerebral amyloid angiopathy, a common form of small vessel disease, is another leading risk factor for ICH.[27,32] Accumulation of β-amyloid causes structural changes in the walls of small-medium arteries and arterioles in the brain, increasing the propensity

for “leakiness” or microhemorrhages as vasculature walls become fragmented.[33] This phenomenon is most common in the cortical regions, and is most frequently associated with the occurrence of lobar hemorrhages.[32,33]

Race is also associated with altered stroke risk; Black and Hispanic individuals have an increased risk of all strokes and a greater stroke incidence compared to White individuals.[34] This is believed to be linked to the higher rate of vascular risk factors (e.g., hypertension, atherosclerosis, hypercholesterolemia, diabetes) present in these populations due in part to systemic barriers leading to disparity in access to resources, healthcare, and education.[34] Asian ethnicity is also associated with increased risk of ICH;[35] though the cause for this finding is unclear, some have suggested it may be a result of high rates of uncontrolled hypertension[36] or ApoE polymorphisms (ε4) among Asian populations.[29]

1.2.2 Modifiable Risk Factors

Hypertension increases the risk of ICH by 3-4x,[9] and is the strongest modifiable risk factor associated with elevated risk of ICH.[37,38] Individuals are considered to have hypertension when BP is consistently elevated above 140/90mmHg,[38] though some studies use a more conservative cut-off of 160/90mmHg.[27] Hypertension promotes several changes within brain vasculature such as the development of plaques in arteries and arterioles (i.e., deposits of fat, cholesterol, cellular waste, or clotting products), and lipohyalinosis (thickening of vascular walls resulting in reduced luminal diameter) in penetrating arteries and arterioles supplying white matter. In addition to vascular stiffening, hypertrophy of smooth muscle cells and eutrophic remodeling lead to reductions in luminal space.[38] Combined, these factors make the penetrating arteries of

the brain particularly susceptible to rupture. Luckily, several treatments are available to treat hypertension and lower BP including angiotensin converting enzyme inhibitors, angiotensin receptor blockers, and diuretics. Despite treatment with antihypertensives, approximately one in four people with hypertension will continue to live with treated but uncontrolled hypertension, resulting in greater risk of both all-cause mortality and cerebrovascular disease associated mortality (HR=3.01).[39]

Diabetes is associated with a number of physiological changes to the vasculature that contribute to increased stroke risk, including vascular endothelial dysfunction, increased arterial stiffness, and chronic inflammation.[40] Hyperglycemia, a common symptom of diabetes, is associated with worsened clinical outcomes after stroke in both patients with and without diabetes.[41] Furthermore, diabetes is often present with other comorbidities that elevate stroke risk such as hypertension, atherosclerosis, and sedentary lifestyle.

Atrial fibrillation is another factor that increases stroke risk, as many patients are prescribed anticoagulants to decrease risk of clotting. While anticoagulants reduce the general risk of stroke, the risk of ICH varies among treatments. For example, warfarin, a vitamin K antagonist, is associated with a roughly two-fold greater risk of ICH vs. direct oral anti-coagulants such as dabigatran (thrombin inhibitor) or rivaroxaban (factor Xa inhibitor).[42]

Lifestyle factors both directly and indirectly influence risk of stroke. Regular, moderate exercise is associated with a reduced risk of stroke as it helps to regulate BP, blood glucose, and cholesterol.[43] Additionally, regular exercise reduces the risk of several comorbidities known to increase stroke risk, including hypertension, diabetes, and atrial fibrilation.[43] A number of modifiable behavioural risk factors have also been

identified with increased risk of ICH stroke including current smoking habit, current alcohol consumption and heavy episodic alcohol intake,[44] and use of sympathomimetic drugs (e.g., heroin, cocaine, amphetamines, etc.).[27,28] Cessation or reduction of these activities typically reduces risk attributed to these factors.

1.2.3 Other Risk Factors

Risk of stroke is also influenced by population based risk factors such as socioeconomic status and living in low-middle income countries.[2,10] Use of oral contraceptives, history of migraine, sickle cell disease, and infection (e.g. *Chlamydia pneumoniae*) have also been implicated in risk of stroke, but are not well studied or understood.[9]

1.3 Clinical Presentation & Management

The brain lesion profile, the combination of both lesion location and size, influences the type and severity of the clinical presentation of stroke symptoms.[10,14,45–47] The Canadian Stroke Best Practice Recommendations: Management of Spontaneous Intracerebral Hemorrhage[3] thoroughly outline the protocol for medical diagnosis and management of ICH. I will discuss the key points of these guidelines in brief in this section.

1.3.1 Clinical Presentation

ICH has a heterogenous clinical presentation, as no two injuries are identical. Patients typically experience sudden focal neurological deterioration (e.g., difficulty

speaking, muscle weakness, muscle paralysis) frequently accompanied by headache and vomiting.[3,13,48] Many patients experience disorientation or decreased level of consciousness, often related to elevated ICP and compression or distortion of thalamic and or brainstem regions.[48] Lesion location and size influence the clinical presentation and severity of symptoms.[10,14,45,46] Sensorimotor impairments, such as poor fine motor skills, may be present contralateral to injury and indicative of involvement of subcortical structures (e.g., basal ganglia) and/or key white matter tracts (e.g., internal capsule).[48] Higher level processing dysfunction (e.g., neglect, aphasia, hemianopia) may be indicative of cortical involvement in the injury or disruption of connectivity between cortical structures, subcortical structures, and/or related processing regions.[48]

1.3.2 Acute Clinical Management

Clinicians suspecting a patient of stroke perform a rapid neurological exam, either the **National Institute of Health Stroke Score (NIHSS)** if awake or **Glasgow Coma Scale (GCS)** if in a comatose state.[3] Once confirmed stable, neuroimaging by **computed tomography (CT)** or **magnetic resonance imaging (MRI)** is performed to confirm diagnosis. Patients are closely monitored for signs of clinical deterioration or changes in level of consciousness as that is often indicative of complications arising due to hematoma expansion, which occurs in ~30% of ICH patients, or sudden increases in ICP.[3] A primary focus of early post-ICH care is limitation of hematoma expansion and rebleeding, with typical treatment being BP management (i.e., <140-160 mmHg, target tailored to patient) and reversal of coagulopathies.[3] Analysis of pooled data from two large BP management trials, ATACH-II and INTERACT2, found a 10% increase in the odds of favourable outcome for every

10 mmHg decrease in systolic BP in patients with mild-moderate ICH.[49] Surgical intervention, such as external ventricular drainage or hematoma evacuation, may be considered for patients that present with worsening level of consciousness, hydrocephalus, or potential for herniation. Stable patients are admitted to a dedicated stroke unit or neuro-intensive care unit as treatment in these units is well established to improve functional outcomes and reduce mortality.[50,51]

1.3.3 Long-Term Clinical Management

Long-term clinical management of stroke is a complex, multi-faceted risk-assessment process informed by the patient's medical history, level of independence, and presence of comorbidities and risk factors that may increase secondary stroke risk. All patients with stroke should begin rehabilitation once medically stable and able to actively participate.[52] The rehabilitation processes and timeline will look different for every patient, however the overarching goal of post-stroke rehabilitation is to enable individuals with impairment to achieve pre-stroke physical and social functioning.[52] Despite this goal, most patients will never fully recover and will require some level of assistance in their day to day living.[9] Rehabilitation is an ongoing, dynamic process, tailored to the specific needs and goals of each individual patient and may be delivered acutely in hospital settings (i.e., in designated stroke units or in-patient rehabilitation centres), in out-patient settings (i.e., clinic), at home, or in community-based settings (e.g., recreation centres). Further discussion of stroke rehabilitation, delivery, and role of the interdisciplinary care team can be found in section 1.8 Rehabilitation. Lastly, prevention of recurrent stroke is an important component of long-term clinical management that is typically accomplished through a combination of management of

lifestyle factors (e.g., drinking, smoking, level of activity, diet) and/or medical management of physiological risk factors (e.g., resumption or cessation of anticoagulants, continued BP control).[3]

1.4 Recovery After Stroke

In 2017 the Stroke Recovery and Rehabilitation Roundtable established a framework to define five critical time periods after stroke in humans: hyper-acute (0-24 hours), acute (1-7 days), early sub-acute (7 days-3 months), late sub-acute (3-6 months), and chronic (>6 months).[53] These time periods will serve as the framework for discussions of recovery and timing of therapeutic interventions throughout this thesis.

1.4.1 Prognosis & Factors Influencing Recovery

ICH is a particularly devastating stroke, with a 30-day mortality rate of nearly 40%, increasing to 54% at one year.[10] Comparatively, ischemic stroke has a much lower mortality rate, with a 30-day mortality of ~12%, increasing to ~25-30% at one year.[54,55] Despite ischemic stroke accounting for a greater proportion of strokes worldwide, hemorrhagic stroke accounts for a disproportionate amount of the global burden of stroke; in 2013 ischemic stroke was estimated to account for ~47 million DALYs globally whereas hemorrhagic stroke was ~65 million DALYs.[6]

Multiple factors predict mortality after ICH, including hematoma volume, initial severity (e.g., NIHSS or GCS), hematoma location, age, and presence of comorbidities.[8,11,12,56–60] Hematoma volume has been identified as one of the most important predictors of outcome;[56] while a precise value has yet to be determined,

volumes below the range of 20-30 mL are correlated with more favourable outcomes than those >30 mL.[11] Clinically significant hematoma expansion has been observed in ~33% of patients;[61] hematoma volumes >25 mL are more frequently associated with this phenomenon.[11] INTERACT1, a major clinical trial that explored intensive BP management and hematoma expansion, found that each mL increase in volume was associated with a 5% increase in death or disability at 90 days.[61] Hematoma location influences both mortality and morbidity, the consequences of which may be exacerbated by volume.[56] For example, a patient with a small hemorrhage (e.g., ~5 mL) would typically have a good prognosis for recovery, likely with minimal long-term impairment. However this prognosis is not universal – a 5 mL lesion encompassing the internal capsule would likely result in significant long-term motor impairment[47] and a comparably sized hemorrhage in the pons would likely be fatal.[56] Additionally, increasing age further alters risk of mortality after ICH, with odds of in-hospital mortality increasing 6-9% for every decade.[60]

1.4.2 Measuring Recovery

"Recovery" is often used as a catch-all term to describe the multitude of changes in health and performance after injury. Describing all changes (for better or worse) with the single word "recovery" simply does not capture the nuance needed to truly understand and explain how and why an intervention may work. While clinical recovery is not the focus of this thesis, use of a common language to describe the neurorehabilitation process across clinical and preclinical work is essential to furthering translation and collaboration across disciplines. The World Health Organization International Classification of Function provides the necessary framework and

terminology to discuss the changes occurring after stroke at the level of the health condition (neural), level of impairment (structural and functional), and level of function or disability (activity).[62] Furthermore, it allows researchers, clinicians, and therapists to classify change in the context of recovery and compensation, two related but fundamentally distinct components of neural and behavioural change.[63,64] At a neural level, recovery refers to the restoration of function within brain tissue that was initially lost due to stroke. This differs from compensation at the neural level, which is often seen when residual neural tissue takes on a new function that was lost due to stroke. A comparable dichotomy is applied to impairment and disability. At the level of impairment, recovery refers to the capacity to perform a movement in the same manner as before injury, for example using the same motor pattern, timing of movement, and range of motion. In comparison, compensation at the level of impairment refers to performing a movement in a new way, such as activation of different muscles, using a different range of motion, or change in coordination or timing of movement. Similarly, at the level of activity, recovery refers to completing a task in the same way as before (i.e., same movement patterns), whereas compensation refers to achieving a task, albeit through different means (e.g., using two hands to complete a task that previously took one).

Many assessment tools are used to evaluate therapeutic benefit and impact on function after stroke. The most frequently used outcome measure to assess therapeutic benefit after stroke is the **modified Rankin Scale (mRS)**,[65,66] a 7-point scale (0-6, rated from no disability to death) that broadly categorizes an individual's global extent of disability. Many clinical studies use the mRS as their primary endpoint, often with the goal of improving the percentage of patients scoring between 0-2, a range which

encompasses survivors with no symptoms to those living with slight disability. The **Fugl-Meyer assessment (FM)**[67] is a comprehensive examination that measures multiple domains of impairment: motor function, sensory function, balance, range of motion, and pain. Low total scores or low scores within a specific domain indicate greater levels of impairment. The FM is a very common form of assessment in clinical rehabilitation studies as it has sub scales for both the upper extremity (FM-UE) and lower extremity (FM-LE) which can be used to assess rehabilitation strategies targeted directly at the upper limb (e.g., task specific training) or lower limb (e.g., gait training). The **Barthel Index (BI)**[68] is another common assessment that is used to measure functional performance in ADLs for the purpose of evaluating independence. The BI scores various activity domains such as toileting, hygiene, walking, dressing, etc., with low scores indicating greater dependence on external help. Many other assessments exist that focus on a specific domain of impairment or function. Some examples include grip dynamometry (unilateral hand strength), the Action Research Arm Test (unilateral arm and hand coordination), Wolf Motor Function Test (arm and hand coordination, combination movements), Stroke Rehabilitation Assessment of Movement (lower extremity movement and mobility), and the Rivermead Mobility Index (general mobility).[69] Importantly, many of these assessments, such as the FM,[70] have a defined **minimum clinically important difference (MCID)** which represents the smallest change in an outcome that is of value to the patient.[71] As the goal of rehabilitation is to reduce impairment and disability, conceptualizing therapeutic efficacy from the perspective of the patient and not simply analyzing efficacy statistically, is critical to understanding and measuring treatment outcomes.

1.4.3 Typical Course of Recovery

At admission to a rehabilitation program, those with hemorrhagic stroke often present with more severe functional and cognitive impairments than those with ischemic stroke.[57,72] Despite the initial difference, those with ICH have been documented to make greater gains in recovery by the time of discharge.[57,58] Interestingly, this trend may be changing; more recent data has demonstrated equivalent gains in function between hemorrhagic and ischemic patients, which some have attributed to the introduction of acute reperfusion therapies for ischemia.[72,73]

Much of our understanding of the typical course of recovery after stroke comes from The Copenhagen Stroke Study, a seminal characterization of stroke outcome stratified by initial severity in a heterogenous stroke population (92% ischemia, 8% hemorrhage).[74–76] Initial stroke severity is a critical predictor of recovery; patients with mild-moderate stroke have a better prognosis and are more likely to be discharged to home, and have less functional disability and neurological deficits at discharge compared to those with very severe stroke.[74] Overall, ~80% of patients will reach their maximal functional improvement on ADLs ~6 weeks after stroke, in the early-subacute phase, with 95% of patients achieving maximal function within 12.5 weeks.[75] Time course of recovery is further influenced by stroke severity, as those with mild stroke achieve maximal functional and neurological improvement quicker than those with more severe strokes.[75] In patients with mild stroke, best neurological recovery and maximal functional outcome is achieved by ~80% of mild stroke patients within 2.5 weeks and 3 weeks of onset, respectively; comparatively the same milestones will be reached by those with the most severe strokes at 10 and 11.5 weeks from onset.[75] While

the majority of stroke recovery occurs in the early sub-acute phase, there is evidence to support cognitive and functional recovery extending well into the chronic phase.[77]

Although stroke has a rapid impact on the cells and structures at the core of the injury and proximal surrounding regions, such as the penumbra in ischemia or **perihematoma zone (PHZ)** in ICH, the effects on neural function extend far beyond the immediate insult.[78] Abrupt changes in synaptic signalling, such as loss of input due to neuronal death, dysfunction, or degeneration, contribute to disruptions in excitatory-inhibitory balance in both individual neurons and larger neural networks.[79] Distal connected regions, for example cortex, frequently exhibit depressed metabolic activity as a result of alterations in excitatory-inhibitory balance, GABAergic signalling, and functional connectivity, in a phenomenon known as diaschisis.[78,80] Furthermore, increased interhemispheric inhibition and changes in GABAergic signalling, in conjunction with the aforementioned changes, may hamper activity in surviving ipsilesional circuits.[79] Spontaneous recovery, improvement in function driven by underlying biological processes, is greatest in the acute and early-sub acute phases after stroke.[81,82] Amelioration of consequences of stroke that hinder brain function, such as edema or elevated ICP, together with engagement of innate repair processes (e.g., synaptogenesis, unmasking of silent synapses, dendritic branching, and other mechanisms of plasticity discussed in Section 1.7 Neural Repair After Stroke), greatly contribute to the resolution of depressed activity, restoration of lost connectivity, and establishment of new circuitry after stroke.[83]

1.4.4 Proportional Recovery & Predictors of Recovery

Regardless of stroke type or rehabilitation, in the days, weeks, and months following stroke, many survivors (humans and animals alike) will experience a substantial degree of improvement in neurological and behavioural function, an occurrence known as spontaneous recovery.[82] The extent of spontaneous recovery varies from individual to individual, and is often incomplete, frequently leaving survivors with some degree of impairment. Interestingly, the majority of all but the most severely impaired stroke survivors are reported to recover ~70% of their early impairment, a phenomena termed proportional recovery.[84] While this phenomena was initially reported to describe recovery of motor function in the upper limb of a small sample of ischemic stroke patients, it has been observed in the lower extremity[85] as well as non-motor domains, such as aphasia.[86] The principle of proportional recovery suggests that resolution of impairment may be based on an underlying biological mechanism, rather than the presence (or absence) of rehabilitation. Over the last decade, a substantial amount of stroke recovery research has focused on the identification of biomarkers to predict long-term outcomes and potential for recovery.[87] As stroke is a heterogenous disease, understanding who may recover best from a particular neurorehabilitation intervention and why is of critical importance, not only to improving treatment efficacy and patient outcomes but also for proper allocation of healthcare resources.

The **corticospinal tract (CST)**, a white matter pathway essential in voluntary movement, has been identified in many neuroimaging studies as a biomarker predictive of recovery.[87] Neuroimaging studies in both the acute[88] and late sub-acute[89] phases of recovery have shown CST integrity to be predictive of motor recovery. Further, one study also suggested the presence of a predictive injury threshold as patients with >63%

CST injury failed to reach a MCID on functional measures.[89] Presence (or absence) of **motor evoked potentials (MEPs)**, a marker of CST function, early after stroke is also predictive of functional recovery. MEPs elicited by transcranial magnetic stimulation in the paretic arm within 5 days of infarct are associated with spontaneous recovery of impairment regardless of degree of initial impairment.[90] It should be acknowledged that these findings have been reported in ischemic stroke patients; however similar follow up work in a heterogenous population (ischemia, ICH, previous stroke) has found similar results.[91]

Despite frequent discussion and exploration of proportional recovery in clinical populations, only one research group has explored whether proportional recovery is observed in preclinical models of stroke.[92,93] In their large retrospective analysis of rodent recovery data, Jeffers and colleagues reported that proportional recovery exists in rats following **endothelin-1 (ET-1)** induced ischemic stroke, albeit to a somewhat lesser extent (~65%) and only in ~30% of the population.[92] In congruence with clinical findings, smaller lesion size, milder initial impairment, and limited striatal injury were predictive of "fitting" the proportional recovery rule.[92] These findings extend some face validity to the commonly used preclinical ET-1 model of ischemic stroke, however additional exploration in other model populations and stroke subtypes is required to determine the generalizability of these results.

1.5 Preclinical Models of Stroke

Several *in vivo* animal models have been developed to advance our understanding of stroke. Due to the heterogeneity observed in human stroke, a variety of models have been developed specific to stroke subtype, including ischemia, ICH,

SAH, and IVH. While no one model perfectly mimics all the pathological processes that occur due to each type of injury, they provide researchers the opportunity to explore mechanisms of injury and repair, identify potential therapeutic targets, and test new therapies.[94] Infusions of **collagenase (COL)** or blood **(autologous whole blood, AWB)** into the brain parenchyma are the most commonly used models of ICH, although injection of various blood components (e.g., thrombin, $FeCl_2$), the inert microballoon model, and stroke prone spontaneously hypertensive rats are also used.[94–96] Here, I discuss the two most frequently used models of ICH in depth.

1.5.1 The Collagenase Model

The COL model, used in just over 50% of all studies between 2015 and 2019,[97] is the most commonly used model in preclinical ICH research. Developed in 1990 by Rosenberg and colleagues, this model involves infusing bacterial collagenase directly into brain tissue to induce spontaneous bleeding within the brain parenchyma.[98] Collagen type IV is found in abundance in the basal lamina of brain vasculature.[98,99] Injection of collagenase, a **matrix metalloproteinase (MMP)** that degrades collagen, results in localized vascular disruption and bleeding in the capillaries surrounding the injection site.[98] Induction of ICH via COL model is a straightforward, simple surgical procedure that can be completed in under 30 minutes. Under general anesthetic (e.g., isoflurane), the subject is positioned in a stereotaxic frame, the skull is exposed, and a burr hole is drilled into the skull. A fine needle is inserted into the brain and COL is slowly infused, usually over a period of 5-10 minutes. This procedure gives researchers significant flexibility as coordinates, concentration of COL, and volume of infusion can all be modified to produce lesions of varying size, severity, and location.

While lesions may not be identical in all animals due to individual variances in vasculature, the hematomas created by this method are relatively reproducible and typically spherical in shape.

1.5.2 The Autologous Whole Blood Model

The AWB model, the second most commonly used model of ICH, involves infusing autologous blood directly into the brain parenchyma to form a hematoma.[97] First reported in 1982 by Ropper and Zervas[100] and refined multiple times over the following decades,[96] the blood injection model mimics a single, rapid bleeding event. Induction of ICH via the AWB model is somewhat similar to the COL model. Under general anesthetic the subject is positioned in a stereotaxic frame, the skull is exposed, and a burr hole is drilled into the skull. Blood, usually taken from a catheterized femoral or tail vein artery, is then injected into the brain over a period of 5-10 minutes. Similar to the COL model, this procedure gives researchers significant flexibility as coordinates and volume of infusion can all be modified to produce lesions of varying size, severity, and location.

1.5.3 Considerations for Model Selection

While useful for easily, and reliably inducing spontaneous ICH, the COL model does not perfectly mimic clinical ICH. Roughly 30% of ICH patients experience clinically significant hematoma expansion,[61] the majority of which occurs within 6 hours of stroke symptom onset.[101] Bleeding occurs over a period of hours following COL injection,[102] making COL a good model choice for exploring therapies impacting bleeding and mechanisms of hemostasis. However, unlike most clinical hemorrhages, bleeding

following COL induced stroke is more diffuse, coming from many capillaries near the injection location rather than a single site.[96] Further, infusion of an exogenous substance such as collagenase can result in an altered immune response,[103] complicating interpretation of studies of immune response to ICH and immunomodulatory therapies.

Similarly, the AWB model is excellent for modelling a single source of bleeding leading to rapid development of a hematoma;[96] however hematoma expansion, an important clinical symptom, is rarely reported following AWB injection.[104] As such, the AWB model is frequently used for studying consequences of mass effect or neurotoxic effects of blood breakdown. Furthermore, the AWB model often results in hematomas of inconsistent size, shape, and location; infusing blood can be technically challenging (i.e., due to clotting), and it is not uncommon for blood to move up the injection tract following needle retraction or extend bidirectionally along white matter tracts such as the corpus callosum.[102,104]

While both models share many pathological hallmarks of clinical ICH, including **blood-brain barrier (BBB)** dysfunction, edema, and inflammation, the extent of injury and resolution of impairments differs. MacLellan and colleagues[102] published a thorough characterization of the AWB and COL models, using matched hematoma volumes to assess time course of bleeding, progression of injury, and resolution of neurological deficits. They found that the hematoma remains largely stable from about 1 hour onwards following AWB injection. In comparison, the COL model shows a notable increase in hematoma volume between 1-4 hours. COL animals displayed greater BBB dysfunction early after stroke and had fewer surviving neurons in PHZ of ipsilateral striatum and substantia nigra and greater atrophy of ipsilateral cortex and corpus callosum 6 weeks after ICH. Interestingly, by 6 weeks after stroke COL animals show

much greater tissue loss (~50%) than AWB animals, despite having similar early hematoma volumes. Extent and resolution of neurological deficits also differed between models. Although both groups displayed significant deficits, animals in the AWB group largely made a full recovery, however animals in the COL group remained impaired at 28 days. Together, these findings suggest the presence of an extended period of cell death in the COL model that does not occur (or not to the same extent) following AWB injury. No one model perfectly mimics all of the clinical features of ICH, and it is unclear which model better predicts what happens in humans. There are advantages and drawbacks to both the COL and AWB models that must be carefully considered by researchers when designing experiments; as such, the appropriate model may differ based on the specific pathological feature or pattern of recovery being studied.

1.6 Mechanisms of Injury

ICH disrupts the tightly regulated ecosystem of the brain, leading to development of a highly cytotoxic, proinflammatory, and pro-oxidative environment that significantly decreases cell viability.[105] Several pathological processes overlap both spatially and temporally over the hours, days, and weeks after injury and damage the neurovascular unit (i.e., neurons, glia, endothelial cells, smooth muscle cells, and pericytes) and contribute to the prolonged course of cell death observed after ICH. The injurious processes described in the following sections contribute to cell death through a variety of routes, including apoptosis (programmed cell death via caspase activation), necrosis (unprogrammed cell death), necroptosis (caspase-independent programmed necrosis), and ferroptosis (programmed cell death in response to iron characterized by accumulation of lipid peroxides).[15,16] [106]

1.6.1 Primary Injury

The first phase of injury is caused by the immediate mechanical trauma related to the rupture of vasculature and extrusion of blood into the surrounding tissue. Extruded blood infiltrates the surrounding parenchyma, stretching and shearing cells and structures, and often transecting or travelling along nearby white matter.[13,107] Hemostatic mechanisms quickly activate to quell the bleeding, as platelets aggregate at the site of injury and lead to the formation of a platelet plug. Extrinsic and intrinsic coagulation pathways are activated, leading to the production of thrombin, a key enzyme involved in catalyzing fibrinogen into cross-linked fibrin, and crucial in clot stabilization and hematoma formation. While coagulation is essential to stop bleeding, some components (e.g., thrombin, discussed in *1.6.2 Secondary Injury*) are cytotoxic, and further contribute to injury.

Due to the spontaneous nature of ICH, primary injury is particularly difficult to treat. While ICH was once considered to be a rapid and monophasic event, that is not entirely true; serial neuroimaging has identified that >20% of patients will experience hematoma expansion, likely due to protracted bleeding or rebleeding.[108] As such, medical management predominantly focuses on preventing hematoma expansion and rebleeding through reversal of coagulopathy (e.g., those on anticoagulants such as warfarin)[3,13,109] and BP monitoring and management, to varying degrees of success. Although there have been some concerns that aggressive BP management may lead to hypoperfusion or ischemic injury and further worsen outcomes,[13,110] clinical trials such as ICH-ADAPT[111] and INTERACT2[112] have provided evidence that BP management to <150 systolic appears to be safe and may be related to improved functional outcomes.

1.6.2 Secondary Injury

In the aftermath of ICH, damaged and dying neurons release glutamate, triggering the influx of Ca^{2+} via **N-methyl-D-aspartate receptors (NMDA)** and leading to a deterioration in the capacity to regulate membrane potential.[20,113] Without sufficient energy, the cell is unable to power ion pumps, leading to a failure in the cell's ability to undergo energy intensive processes key to cellular survival, such as formation of ATP.[20,22] These failures contribute to injurious processes such as glutamatergic excitotoxicity, oxidative stress, production of ROS, and formation of edema.[114]

Several modes of cell death have been identified to occur after ICH; while not the focus of this thesis, a brief overview is necessary. Mechanical pressure, exerted on tissue by the hematoma, in combination with activation of NMDA by excess glutamate, triggers influx of Ca^{2+}, leading to mitochondrial dysfunction which can trigger death by necrosis.[15] Extrusion of cellular components into the extracellular space as a result of necrosis contributes to the highly inflammatory environment that develops after ICH. Changes in the intra- and extracellular microenvironment, for example in response to oxidative stress, can induce death by apoptosis via activation of caspase-dependent pathways. Binding of **tumor necrosis factor (TNF)**, an inflammatory cytokine released by microglia, leads to the development of the necrosome, influx of Ca^{2+} and Na^{+}, and ultimately death by necroptosis.[15,16] The breakdown of red blood cells and accompanying release of iron, increases the production of ROS; as cellular antioxidant mechanisms become overwhelmed, lipid peroxides accumulate, leading to cell death via ferroptosis.[16]

The formation and presence of the hematoma in the parenchyma and/or ventricular system contributes to mass effect, further compressing and displacing brain

tissue. Depending on stroke severity, ICP within the skull may become dangerously elevated (e.g., >20 mmHg);[115–117] this elevated ICP can have dire consequences, such as severely reduced cerebral blood flow or brain herniation.[118] As more than 20% of patients will experience hematoma expansion within the first ~24 hours after ICH,[108] further elevation of ICP can have deadly consequences. Recently, our lab showed that following severe stroke, neurons in regions distal to injury display marked reductions in cell volume, a space-saving effect we believe limits the effects of high ICP.[116,119,120] This mechanism of widespread "tissue compliance" may be important in reducing ICP related mortality, however it may cause short and long-term neural dysfunction throughout the brain, as markers of subcellular injury (e.g., mitochondrial swelling)[116] have been observed to accompany these changes.

Cerebral edema is a significant contributor to post-ICH morbidity and mortality and develops rapidly over the first 24 hours, peaking ~3 days after ICH in animal models[118] and ~5-7 days in humans.[14] Within minutes of injury, dysfunctional and irreparably damaged cells in the PHZ begin to experience an influx of ions (e.g., Na^+, Cl^-),[118,121] leading to cell swelling (cytotoxic edema), as water from the extracellular space follows the ions into the cell. As hemostasis is reached, clot retraction results in the extrusion of serum into the surrounding tissue, contributing to early perihematomal edema and ionic dyshomeostasis.[110,122] The presence of this serum, in combination with ionic imbalances that extend well past the hematoma/PHZ border and BBB dysfunction, create a driving force for vasogenic edema to develop.[123] Later, erythrocyte lysis and **hemoglobin (Hb)** related toxicity contribute to delayed edema formation.[124] Depending on stroke severity, mass effect and edema can contribute to dangerously elevated ICP, often with deleterious consequences such as brain herniation and

ultimately death.[46,110] Additional complications of mass effect include reduced cerebral blood flow leading to concerns of localized regions of ischemia that may further exacerbate cell death.[104,110] While there is evidence for reduced metabolic rate and oxygen demand in the PHZ, evidence suggests this region is likely experiencing hypoperfusion rather than true ischemia.[110]

While necessary to coagulation and clot stabilization, thrombin has been widely implicated early in secondary injury as it leads to infiltration of inflammatory cells, BBB dysfunction, neuronal atrophy, and cell death.[46,125] Extravasated blood components, such as complement components, coagulation factors, and other bioactive molecules contribute to the development of a neurotoxic environment. Approximately 24 hours after ictus, red blood cell lysis begins, and over the following weeks, Hb is degraded into its breakdown products iron (Fe^{2+}) and heme via the **heme oxygenase-1 (HO-1)** pathway, resulting in the production of ROS. The presence of this free iron further contributes to oxidative stress, inflammation, and BBB breakdown as Fenton reactions lead to the production of free radicals.[104,105,114] The excess levels of ROS produced as a result of injury overwhelm the anti-oxidant capacity of cells, leading to dysfunction (i.e., oxidative damage to mitochondria, lipid peroxidation, DNA fragmentation) and ultimately cell death.[114]

There is a marked inflammatory effect after ICH, as blood components are released into the extracellular space alongside the release of products of **damage associated molecular patterns (DAMPs)** (e.g., nucleic acids, ATP, various neurotransmitters) by necrotic neurons.[104] Microglia, the macrophages of the brain, are activated from their ramified state as early as 1 hour after ICH,[126] as DAMPs and various blood breakdown components (e.g., heme, thrombin, fibrin) act on the family of **toll-**

like receptors (TLRs) found on microglia. TLRs interact with the **nuclear factor-κB (NF-κB)** signaling pathway and have been implicated in a variety of proinflammatory responses after brain injury.[104,127] TLR4 is noticeably upregulated beginning as early as 6 hours after ICH[128] and is associated with worsened outcomes; inhibiting TLR4 has been shown to reduce inflammation and neurological deficits following ICH in mice,[129] generating interest in TLR4 as a potential therapeutic target. In addition to activation through TLR/NF-κB pathways, microglia can also be activated through various protein kinases resulting in increased production of TNF-α and through the endocytosing of erythrocytes via scavenger receptors such as CD36.[104] Activation by the aforementioned pathways is implicated in expression of the M1 microglial phenotype, considered to be proinflammatory and pro-damage due to the production of chemokines, inflammatory cytokines (e.g., TNF-α; **interleukin (IL)**-1β, IL-6), ROS, and **inducible nitrous oxide synthase (iNOS)**, all of which contribute to early BBB dysfunction.[127] Infiltration of peripheral leukocytes, in particular neutrophils, rapidly follows the activation of microglia [24] and further contributes to the production of inflammatory mediators and degradation of the cellular environment through the subsequent release of various MMPs, most notably MMP-9.[130] MMPs contribute to the worsening of BBB function by degrading structural components of the extracellular matrix and surrounding vasculature (i.e., collagen, laminin, fibronectin) and weaking proteins critical to the integrity of tight junctions.[130] Blood brain barrier breakdown is both a consequence and contributor to secondary injury; as the compromised BBB becomes permissive to larger molecules (e.g., albumin) and infiltrating cells, osmotic gradients are disrupted and further contribute to the development of vasogenic edema and the formation of a neurotoxic environment.[124]

Astrocytes play a fundamental role in the regulation of the brain environment and maintenance of homeostasis under normal physiological conditions.[131] In addition to providing neurotrophic support, astrocytes are essential to the regulation of cerebral blood flow,[132] maintenance of BBB integrity,[133] and clearance of metabolites from the brain via the glymphatic system.[134] Under physiological conditions, astrocytes play a major role in glutamate reuptake.[135] Following ICH, thrombin and plasmin activate protease-activated receptor 1 (PAR-1) on astrocytes,[136] leading to rapid remodeling of the neuropil around glutamatergic synapses.[137] While this has the effect of helping in the rapid removal of extracellular glutamate released by dead and dying neurons, it has the capacity to impair **long term potentiation (LTP),** a key mechanism of plasticity, by reducing glutamate receptor activation.[137]

Though likely an oversimplification, astrocytes appear to adopt proinflammatory (A1) and anti-inflammatory (A2) phenotypes, similar to their microglia counterparts.[127,131] Presence of thrombin and release of signaling molecules, such as TNF-α and IL-1β by M1 activated microglia, trigger reactive astrogliosis and a transition to the A1 proinflammatory phenotype. Astrocytes also express several TLRs; much like what occurs in microglia, exposure to DAMPs activates similar downstream pathways (e.g., NF-κB).[138] Within 1-3 days after ICH, activated astrocytes aggregate in the dysfunctional and inflammatory PHZ[127] and further amplify inflammation through the expression of inflammatory factors, exacerbating BBB breakdown.[131] IL-1β influences the expression of **vascular endothelial growth factor A (VEGF-A)** by A1 astrocytes, which ultimately results in the destruction of tight junctions,[138] while heme and thrombin act through surface receptors to induce the expression of MMPs.[138] Additionally, changes in the localization of **aquaporins (AQPs)** on astrocytic endfeet

occur as early as 1 hour after stroke, and appear to play a role in the formation of edema.[138] Key hallmarks of this process known as reactive astrogliosis include cellular hypertrophy, increased astrocyte proliferation,[139] elevated **glial fibrillary acidic protein (GFAP)**, and the upregulation of **chondroitin sulfate proteoglycans (CSPGs)**.[140] CSPGs play an important role in neurodevelopment, however following injury they are associated with the collapse of neuronal growth cones and inhibition of axonal regeneration.[141,142] Severe reactive astrogliosis is associated with the formation of a 'glial scar,'[142] a pathological feature that occurs across **central nervous system (CNS)** injury in both the brain and spinal cord. The glial scar has two important but conflicting roles in recovery: early, it serves to seal off the lesion site to prevent the infiltration of toxic and inflammatory substances released by glial cells and necrotic neurons into nearby undamaged tissue; later this seal impedes the regeneration of injured neurons through the lesion area due to both physical and biochemical barriers.[141,142] While astrocytes contribute to the worsening of the inflammatory environment early after stroke, they also play a critical role in repair and regeneration, which will be discussed in section *1.7 Neural Repair After Stroke*.

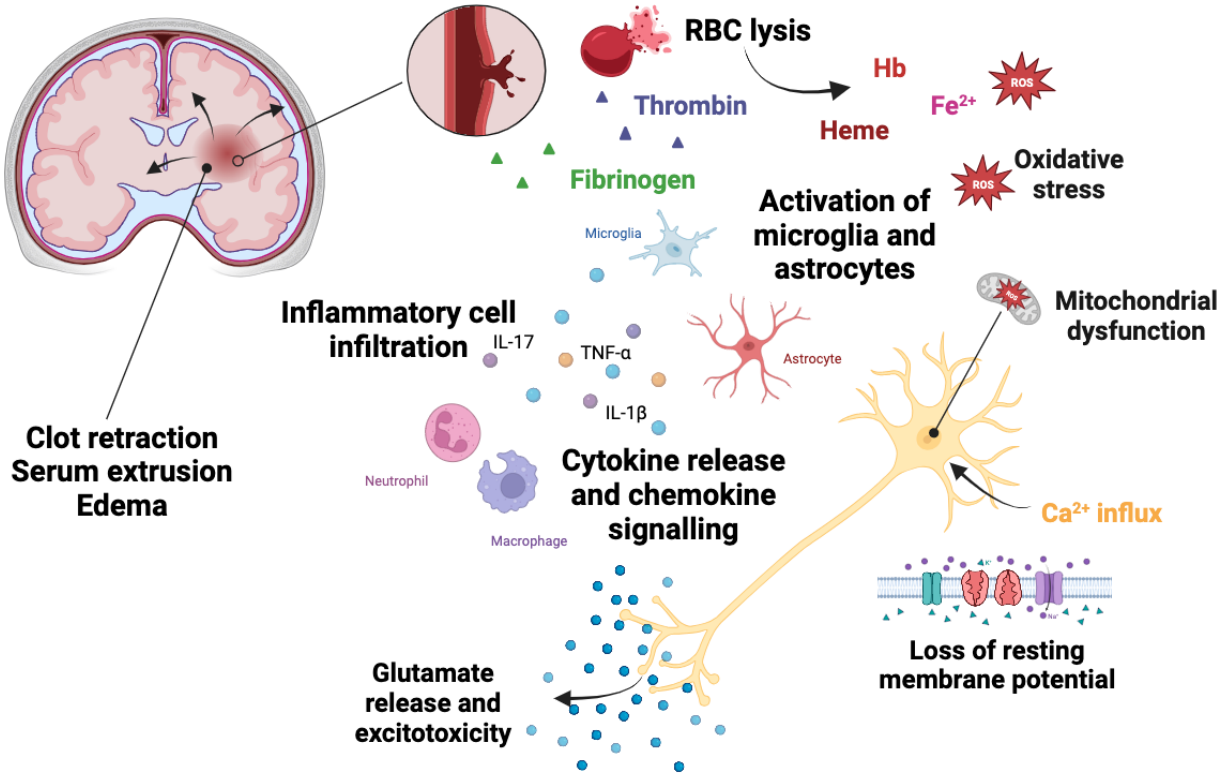

Figure 1-2 Sudden unexpected bleeding and the resulting hematoma trigger several parallel physiological cascades that contribute to secondary injury after ICH. Together, these mechanisms contribute to the formation of a highly cytotoxic, proinflammatory environment resulting in damage to components of the neurovascular unit (neurons, glia, endothelial cells, smooth muscle cells, and pericytes) and cell death through modes such as apoptosis, necrosis, necroptosis, and ferroptosis [figure created with BioRender]

1.7 Neural Repair After Stroke

The role of inflammation and the immune system is two-fold in the days and weeks following ICH. In the first hours and days after injury, the brain's tightly regulated environment becomes highly neurotoxic as a series of inflammatory cascades are triggered. Early after injury, microglia, neutrophils, and astrocytes produce a variety of bioactive molecules that contribute to the formation of a highly proinflammatory, cytotoxic environment. In the early phase, the proportion of microglia and astrocytes skews to pro-inflammatory M1/A1 phenotypes; as time passes, this proportion changes

to favour the more amenable to repair, M2/A2 phenotypes. In this second phase, we see increases in expression of phagocytic receptors, and release of various anti-inflammatory cytokines and growth factors. In this later stage, the now dominant M2/A2 microglia and astrocytes play an important role in endogenous recovery and repair.[131] Many cells and substrates appear to have a biphasic effect following stroke: early inflammatory, later pro-repair.

1.7.1 Hematoma Clearance

As discussed in section *1.6.2 Secondary Injury*, many of the breakdown products of the hematoma contribute to cytotoxicity and cell death after ICH. Here, I will discuss the major forms in which blood cells and their components are cleared from the brain tissue following ICH: erythrolysis and phagocytosis.

Erythrolysis, the breakdown of **red blood cells (RBCs)**, begins within a few hours of ICH onset.[143] Early erythrolysis is initiated by the complement system, a component of the innate immune system that responds in the presence of pathogens and indicators of injury.[144] Activation of the complement cascade leads to the creation of the membrane attack complex, which latches onto the surface of RBCs and forms a pore in the cell membrane, ultimately leading to lysis and the expulsion of cell contents (e.g., Hb, peroxiredoxin-2, carbonic anhydrase 1), and in turn creating a neurotoxic environment and triggering neuroinflammation.[145] Interestingly, it has been documented that **spontaneously hypertensive rats (SHRs)** have increased early erythrolysis after ICH (~24 hours) compared to normotensive controls,[146] which suggest that hypertension may aggravate early injury through cellular mechanisms. As such, it

has been postulated that BP management may improve outcomes by reducing potential for secondary injury via decreased early erythrolysis.[144]

Erythrophagocytosis, the phagocytosis of red blood cells via microglia and infiltrating macrophages, is essential to hematoma clearance after ICH. Unlike erythrolysis, where toxic cell contents are spilled into the extracellular space, erythrophagocytosis results in the containment of the otherwise harmful components of RBC breakdown. While this may prevent some secondary injury, activation of microglia and macrophages triggers the release of numerous cytokines that may be either pro-inflammatory (e.g., IL-1β, IL-6, IL-12, TNF-α) or anti-inflammatory (e.g., IL-4, IL-10, TGF-β) in nature.[145] Furthermore, sustained phagocytosis of RBCs is associated with decreased macrophage viability, likely as a consequence of oxidative stress due to the production of ROS exceeding the antioxidant capacity of the cells.[147] Many pathways and receptors have been implicated in erythrocytosis; while not the focus of this thesis, I will discuss a few in detail.

The glycoprotein CD47, a receptor found on the surface of erythrocytes, plays an important role in RBC phagocytosis as it functions as a "don't eat me" signal. As such, there has been much interest in CD47 as a potential therapeutic target, and it has been explored in multiple models of ICH.[148–152] In a mouse model, wild type or CD47 knockout blood was infused to induce ICH; CD47 knockout animals had quicker clot resolution than their wild type counterparts.[148] Assessment of CD47 in a porcine model of ICH documented decreases in CD47 expression within the hematoma over time, suggesting that changes in CD47 expression may be linked to endogenous regulation of hematoma clearance.[149] Several additional studies in both young and aged rats have demonstrated that blocking CD47 is linked to improved hematoma clearance.[150–152]

Our early understanding of microglia led to a dichotomous categorization of M1/M2, or a broad concept of proinflammatory vs. anti-inflammatory. Our knowledge of microglial polarization has expanded significantly in recent years, to recognize that they exist on a spectrum where they can be found in a number of morphologies (e.g., rod-like, spherical, amoeboid, or an intermediate) and phenotypes (i.e., M1, M0 (ramified), M2a/b/c).[153] While present to some extent throughout the course of injury, the M2 microglia phenotype begins to dominate the PHZ environment approximately one week after ICH as IL-4, IL-10, and **transforming growth factor-β (TGF-β)** are released by other immune cells. It is unclear what precisely triggers the phenotypic switch, as the mechanisms have proven hard to elucidate. ROS produced by M1 microglia during the early inflammatory period appear to activate the **nuclear factor erythroid 2-related factor 2 (Nrf-2)** pathway, a transcription factor crucially important in the regulation of a variety of anti-oxidant processes and detoxification enzymes.[105] For example, activation of the Nrf-2 pathway by sulforaphane has been shown to reduce protein and lipid oxidative damage and improve neurological deficits following ICH; Nrf-2 knockout significantly worsens ICH injury.[154] Nrf-2 works in a complimentary fashion with **peroxisome proliferator-activated receptor-γ (PPAR-γ)** to regulate numerous antioxidant pathways, most notable to ICH, the HO-1 pathway. Together, Nrf-2 and PPAR-γ upregulate the expression of IL-4 and CD36, which play key roles in facilitating microglial phagocytosis (i.e., hematoma clearance) and tissue repair. In addition, Nrf-2 activation results in elevated **haptoglobin (Hp)** and **hemopexin (Hpx)**, both of which play a role in hematoma clearance.[105] Binding of Hb to Hp creates stable iron sequestering Hp-Hb complexes which can then be engulfed

by CD163 expressing microglia; binding of heme to Hpx forms stable Hpx-heme complexes which are then taken up through CD91 mediated endocytosis.[155]

Mechanical disruption of blood flow (e.g., damage to vasculature) and changes in tissue perfusion and blood flow, due to mass effect or elevated ICP, facilitate the need for the development of collateral vasculature in and around the hematoma. Astrocytes play an essential role in post-stroke angiogenesis and reestablishment of blood flow in impaired regions. Astrocytic upregulation of VEGF beings ~2 days after ICH, peaks at 21 days, then begins to return to normal, with new vessels beginning to appear around and penetrating into the hematoma starting ~7 days after ICH in rats.[156] Aerobic exercise (i.e., running) has been documented to increase angiogenesis in naïve rodents[157,158] as well as those recovering from ischemic stroke,[159] making it an interesting target for therapeutic intervention. Recently, meningeal lymphangiogenesis has been implicated in improved hematoma clearance after ICH for its role in metabolite and potentially erythrocyte clearance.[160] Changes in meningeal lymphatic vessel morphology and function have been observed ~7-10 days and persisting to 60 days following both AWB and COL ICH in mice.[160] These changes were associated with reduced hematoma volume and neurological deficits; loss of function via meningeal lymphatic vessel ligation impeded hematoma resolution.[160]

Astrocytes are critical to neural repair after brain injury.[138] Crosstalk between astrocytes and microglia is a constant, ongoing process, both under normal physiological conditions and after injury. In both the acute and sub-acute phases of recovery, activity in one population triggers a reciprocal, often complementary action in the other. For example, as the brain response to injury shifts to a less proinflammatory state, release of TGF-β by reactive astrocytes serves to blunt the activation of the NF-κB

pathway by microglia, in turn reducing the production of proinflammatory molecules and creating an environment more favourable to repair.[138] Following ICH or thrombin induced injury, astrocytic upregulation of HO-1 occurs, a pathway important in hematoma clearance. In addition to their role in hematoma clearance, reactive astrocytes release several growth promoting molecules such as **brain derived neurotrophic factor (BDNF)**, **nerve growth factor (NGF)**, and **fibroblast growth factor (FGF)** which activate **tropomyosin-related kinase (Trk)** pathways TrkB, TrkA, and FGF pathways (respectively) to improve tolerance to injury and promote survival.[138] These and other growth factors important in plasticity will be discussed in section *1.7.2 Neuroplasticity*.

As blood components are neurotoxic, much of recent research has selected the hematoma or its components as a target for therapeutic intervention. Many recent preclinical and some clinical studies have broadly attempted to achieve one or more of the following goals: prevent hematoma expansion, evacuate the hematoma, accelerate hematoma resolution, prevent release of neurotoxic components from the hematoma, or prevent uptake of neurotoxins.[107] While often showing promising preclinical results, these agents regularly fail to translate to clinical success.[161] Iron chelators, agents designed to sequester iron and reduce damage related to oxidative stress and production of free radicals, have been explored both preclinically and clinically. Most notably, there has been mixed efficacy for the use of the iron chelator **deferoxamine (DFX)**;[143] some have shown neuroprotective benefit preclinically,[162,163] while others have had less success.[164,165] Phase II clinical trial i-DEF, which administered DFX to patients following ICH, found no significant difference in favourable outcomes (mRS 0-2) at 90 days between those treated with DFX compared to placebo.[166] Recently, some have targeted

the effects of iron toxicity through the administration of lactoferrin (an iron binding protein) and modulation of neutrophils; preclinical results show promise in augmenting hematoma clearance and improving behaviour.[167–169] Other studies aimed at reducing secondary injury have attempted to alter the proinflammatory and anti-inflammatory balance after ICH.[170] The beneficial anti-inflammatory effects of M2 polarized microglia have made them the subject of interest for therapeutic targets, and there has been some study to date with agents that alter reactive cell immunity, such as minocycline (shown to decrease microglial activation after ICH)[171,172] and fingolimod (shown to reduce lymphocyte infiltration after ICH).[173] While it may seem logical to tamper down the polarization of M1 microglia in favour of M2 expression or prevent the infiltration of immune cells, it is important to recognize that they work in a complementary fashion. Although M1 microglia contribute to widespread inflammation and dysfunction, they also allow for the destruction of obstructive matrix components and clearance of dead neurons that may impede the growth and remodeling of surviving circuitry.[153] It is possible that without these effects, mechanisms of endogenous and activity dependent repair and plasticity may not be possible.

Despite promising preclinical results, clinical trials aimed at reducing hematoma expansion through the administration of Factor VIIa, SPOTLIGHT and STOP-IT, have failed to find benefit on their primary endpoints.[174] Surgical hematoma evacuation has been another area of interest, however clinical trials such as STICH II (open surgery)[175] and MISTIE II (minimally invasive surgery plus alteplase)[176] have failed to find benefit on their primary endpoints. It is unclear why translation has largely failed; while one could argue that the risks of surgery may outweigh the benefits, it is equally possible that the ICH models used in the preclinical studies do not mimic human pathology (e.g.,

limited delayed cell death in the AWB model could be more reflective of human injury than the protracted cell death observed in the COL model). Still, other large clinical trials of surgical hematoma evacuation are currently underway to assess functional improvement and mortality outcomes, including ENRICH (early hematoma evacuation using minimally invasive surgery), MIND and DIST (testing of minimally invasive Artemis Neuro evacuation device), and EVACUATE (ultra-early minimally invasive hematoma evacuation).[177]

1.7.2 Neuroplasticity

Neuroplasticity refers to the multitude of changes that occur in the brain from a micro level (e.g., receptor expression, cellular excitability) to a macro level (e.g., changes in cortical connectivity, alterations in motor maps) in response to intrinsic or extrinsic stimuli. It is the adaptive, and sometimes maladaptive, process by which both structure and function can be changed in the brain. There is a preponderance of evidence that has suggested a 'critical window' for recovery occurs after brain injury[81,178–181] During this time, numerous spontaneous processes of recovery are upregulated to create an environment that is more favourable to repair the injured brain.[81] Some have likened this to the critical period observed in early neurodevelopment; while many similar processes (e.g., synaptogenesis, synaptic pruning, dendritic branching, angiogenesis) are harnessed for recovery after injury, repair in the mature brain is not simply a recapitulation of early neurodevelopment.[81,182,183] Training (i.e., rehabilitation) is an essential component of post-stroke care as promoting recovery and/or teaching compensation are critical to re-establishing independence and success in activities of daily living.[184] Importantly, this training is vital to restoring and repairing the injured

brain, as a variety of experience dependent processes reinforce both motor learning and memory.[185] While a considerable amount has been learned about the CNS response to injury in recent decades, much of our understanding comes from studies of ischemic stroke. Although there is significant overlap in the mechanisms of injury and repair between ischemic stroke, hemorrhagic stroke, traumatic brain injury, and spinal cord injury, our understanding of the specific time course of mechanisms and molecules that contribute to or inhibit recovery following ICH remains somewhat unclear. Owing to distinct differences in pathophysiology and time course of injury, there is reason to expect some differing responses between ICH and ischemia.

To understand the scale in which change is happening in the brain as a response to injury, we must first recognize that changes occur at the level of both individual neurons (micro level) and populations of neurons (macro level). In an excellent review on neural plasticity and neurorehabilitation, Warraich and Kleim categorize neural plasticity into four related but distinct categories: individual structural changes, individual functional changes, structural population changes, and functional population changes.[63] Collectively, these structural and functional changes are harnessed to promote recovery through restoration and re-engagement of intact and dysfunctional regions, recruitment of uninjured regions (both proximal and distal), and retraining regions to perform new functions.[63]

While motor and sensory cortices follow a somatotopic organization, diffuse connections to proximal and distal regions exist throughout the brain. This redundancy in connectivity and neural function is integral to the brain's ability to repair itself, as it serves as a scaffold for recovery of function by allowing mapping onto regions responsible for comparable functions, both proximally (e.g., ipsilesional; within nearby

populations of neurons) and more distally (e.g., contralesional; in multiple representations across distinct brain regions).[63] Under normal physiological conditions, movement is largely controlled via unilateral activation of relevant sensory and motor cortices in the contralateral hemisphere; while ipsilateral pathways exist, bilateral activation is limited. After injury these ipsilateral pathways can become "unmasked," either by reductions in inhibitory input from connected neurons[186,187] or by becoming the only source of input to a damaged pathway.[188] Recent advances suggest that the capacity to recruit and form new connections may lie in the unique distribution of **α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptors (AMPA)** and NMDA receptors on dendritic processes and filopodia; silent synapses, previously thought to be limited in the mature brain, appear to be abundant on the AMPA receptor-less filopodia.[189] Interestingly, these silent synapses can be recruited into functional synapses through activity, following principles of Hebbian plasticity,[190] the idea that "neurons that fire together, wire together."

In both healthy and injured brains, activity-dependent excitation and co-activation of neurons leads to alterations in neural pathways through mechanisms such as **long-term potentiation (LTP)**[191] and **long-term depression (LTD)**,[192] whereby high synaptic activity produces stronger connections (LTP) and lower activity produces weaker connections (LTD).[193] These connections are consolidated and stabilized through structural changes, such as alterations in number of synapses, addition/removal of dendritic spines, and axonal sprouting, all mechanisms which contribute to the remodeling of peri-infarct circuitry after stroke and refinement of neural networks.[81,193] It should be noted that while diffuse connections exist throughout the brain, proximity of recruited tissue to the site of injury and injury severity both

influence outcome and extent of rewiring. Recruitment of uninjured ipsilesional neurons (e.g., neurons that maximally respond to one stimulus, but generate sub-threshold potentials in response to a different stimulus) is associated with better, more complete recovery than recruitment of connected contralesional regions.[21,81] As smaller strokes may spare a greater percentage of neighboring circuitry, activation of the contralesional hemisphere is believed to play a greater role in recovery with increasing stroke severity.[81,194]

Changes in neural excitability and activation occur on both a micro and macro level following stroke and include alterations in the intrinsic excitability of neurons, excitatory post-synaptic potentials, receptive field specificity, and neural network activity.[63,81] Hyperexcitability and a loss of inhibition, either due to intrinsic changes and/or loss of input, is commonly observed after stroke. Homeostatic plasticity, a collection of mechanisms that work together to stabilize neuronal networks through the regulation of neuronal excitability, is important in restoring synaptic activity in abnormally functioning circuits.[81] Homeostatic plasticity is believed to play a role in ameliorating some of these disturbances, through mechanisms such as axonal sprouting, and changes in dendritic arborization and spine density.[193] Unlike Hebbian plasticity, homeostatic plasticity utilizes a negative feedback system to regulate synaptic efficacy; high activity within a circuit results in weakened connections, low activity results in strengthened connections.[193]

Under physiological conditions, the adult CNS is not permissive to axonal growth due to the presence of inhibitory molecules such as Nogo and myelin associated glycoprotein (myelin associated proteins), various CSPGs (extracellular matrix proteins), and ephrins and semaphorins (growth cone inhibitors).[182] After stroke, the

glial scar serves as a further physical and biochemical barrier to repair; however, the surrounding peri-infarct region experiences remarkable changes in the extracellular environment that make it more amenable to repair,[182] both through a reduction in inhibitory molecules (e.g., CSPGs) and increase in growth promoting factors.[182,195] For example, **growth-associated protein-43 (GAP43)**, a growth cone protein important in axonal sprouting, is upregulated as early as 3 days after ischemic stroke, with elevated levels lasting for ~1 month.[182] It is postulated that these environmental changes that allow for axonal sprouting contribute to the altered and expanded cortical maps that have been observed in experiments of cortical remapping after injury. As such, there has been growing interest in exploration of pharmacological agents that block or digest inhibitory molecules (e.g., anti-Nogo-A,[196,197] chondroitinase ABC[198,199]) or increase neurotrophic factors such as BDNF and NGF (e.g., memantine,[200] intranasal NGF[201]) as a means of improving recovery. While many have reported promising preclinical results, the clinical utility of these agents is uncertain.[202,203]

Other regenerative processes are also believed to contribute to recovery after brain injury. Both angiogenesis, the development of new blood vessels, and vascular remodelling appear to aid in repair after stroke.[204] Recently, serial *in vivo* imaging has been used to document spatiotemporal changes in vascular structure, blood flow, and behavioural recovery after focal cortical ischemia.[205] Vascular plasticity in the peri-infarct region (i.e., formation and/or modulation of collateral capillaries) was largely restricted to the first two weeks after stroke and correlated with the re-establishment of blood flow; furthermore, the extent of blood flow restoration was coupled with the extent of motor recovery.[205] It is unclear to what extent angiogenesis directly contributes to recovery after ICH; however, in addition to re-establishing blood flow, it

has been hypothesized that angiogenesis, in part due to VEGF related increases in vascular permeability, helps to facilitate immune cell infiltration, contributing to removal of cellular and hematoma related debris.[204,206,207]

Angiogenesis is also involved in facilitating neurogenesis after stroke via the neurovascular niche,[208] guiding migrating neuroblasts from the **subventricular zone (SVZ)** away from the rostral migratory stream and towards nearby regions of injury, such as the striatum[209] or cortex.[208] Interestingly, despite their early role in BBB degradation, evidence suggests that MMPs have a pleiotropic effect in recovery. In later stages, MMPs appear to aid in neuroblast migration from the SVZ[204] to injured regions; inhibition of MMPs during this time has been reported to prevent migration.[210] While in theory neurogenesis should lead to significant improvement in function of injured tissue, many new cells fail to integrate into the existing circuitry, with ~80% of new cells not surviving longer than 6 weeks.[211] Conflicting results have been reported in studies modulating neurogenesis after stroke, some report benefit with enhanced neurogenesis while others report deleterious effects, leading to questions as to what extent neurogenesis contributes to both behavioural and cognitive recovery after stroke (for a comprehensive review see Ceanga et al. 2021[212]).

Principles of learning and memory can be harnessed to facilitate motor recovery after a variety of brain injuries. In an excellent discussion of experience dependent plasticity, Kleim and Jones outline 10 principles that influence neurorehabilitation.[185] As stroke causes significant disruption to the circuitry of the brain, the principles “use it or lose it” and “use it or improve it” speak to the importance of activating these disrupted neural networks; without activation, limited recovery of function is possible, as the brain may interpret the lack of engagement in these circuits as a signal to degrade

existing cellular and synaptic infrastructure in favour of retraining for other purposes. Time is also of utmost importance. Evidence suggests the presence of a "critical window" for recovery exists in both animals[178] and humans,[181] although the specific, most suitable time course of intervention remains somewhat unclear (see section 1.8.2 Critical Factors for discussion). Specificity, transference, and interference are principles that are heavily intertwined with one another. The manner of training dictates the type and location in which plastic changes occur, the degree to which these experiences translate to other abilities, and enhances the acquisition of a similar skill or behaviour. Conversely, the manner of training can interfere with the acquisition of new skills or restoration of previous patterns of activity, for example maladaptive plasticity as a response to repetitive use of compensatory behaviours. Training repetition and intensity are essential factors that drive lasting change within neural circuits; these principles also heavily influence both transference and interference of motor learning. Age plays a critical factor in plastic responses to experience, as several mechanisms of plasticity (e.g., synaptogenesis, cortical remapping, neurogenesis), are altered with increasing age.[213] As risk of stroke increases with age, the reduced endogenous response to injury in older individuals may contribute to reduced rehabilitative efficacy. For example, microglial activity and morphology is impacted by age, which may result in impaired lesion resolution. Following ICH, activation and infiltration of microglia into the PHZ is delayed in aged animals; at 1 day post ICH, aged animals have less activated microglia in the PHZ than younger animals, however by 3 days aged animals have greater, more pronounced microglial response, spread more diffusely throughout the brain (i.e., farther from injury).[214] Finally, much like in learning and memory processes, stimulus salience appears to play an important role in modulating plasticity and consolidation of

motor learning. Research findings in animals and humans all seems to support these principles, and of course, these are also obvious in clinical practice (e.g., therapies seek engaging tasks, they provide considerable repetition and so on).

In the next section, 1.8 Rehabilitation, I will discuss rehabilitation and the changes it drives in both brain and behaviour. Neither behaviour or measures of neural plasticity alone can tell us how the brain is adapting to treatment, it is only when paired together can we begin to understand how mechanisms may contribute to action.[63]

1.8 Rehabilitation

Despite improvements in acute clinical care, many survivors are left with lasting impairments after stroke, including muscle weakness, impaired consciousness or cognition, dysphagia, and incontinence.[215] Roughly 70-75% of all stroke patients will have some degree of motor deficits in their upper or lower limbs three months after stroke;[215] in the long term, of those who survive 10 years, 20-30% will continue to have poor outcomes.[216] Rehabilitation remains our greatest strategy for improving independence and quality of life in survivors.

Neurorehabilitation harnesses several principles of experience dependent plasticity to achieve the broad goals of restoring, recruiting, and repairing the circuitry in the injured brain. Many factors influence rehabilitation success, yet clinicians and researchers alike are unsure of the optimal treatment parameters that could maximize treatment efficacy and functional benefit to survivors. Although clinical rehabilitation is not the focus of this thesis, a brief discussion of clinical rehabilitation and delivery methods is required to better understand the obstacles researchers and clinicians face in translating preclinical findings to clinical settings. In this section, I will discuss critical

factors related to rehabilitation, common rehabilitation treatments, methods of clinical assessment, and how preclinical scientists model clinical treatments.

1.8.1 Clinical Rehabilitation Delivery

Rehabilitative care is delivered by an interdisciplinary team of healthcare providers, including doctors, nurses, neuropsychologists, physical therapists, occupational therapists, recreation therapists, speech language pathologists, and social workers.[69] These specialists play a critical, yet difficult to quantify, role in patient success; therapist engagement and feedback shape the delivery of rehabilitative training and may influence the extent to which patients actively participate in their own recovery. In addition, patient's family members and friends may provide caregiving and rehabilitative assistance in later stages of recovery. Rehabilitative goals are tailored to the individual and address the impact of impairments related to loss of function (e.g., hemiparesis, limb impairment, dysphagia, spasticity, bladder or bowel issues) and limitations of activity or participation (e.g., completing self-care activities, ADLs, use of mobility devices).[69] Impairments that may impact communication (e.g., aphasia, vision loss, hearing loss) or influence motivation (e.g., post-stroke depression, apathy, anxiety, fatigue) must be considered in the design and delivery of rehabilitation.[217]

Owing to the overwhelming heterogeneity in impairment observed in stroke survivors, a number of treatment modalities are used to promote motor recovery that consider both the degree of impairment (minor vs. severe) and impact on function (e.g., gait, posture, muscle strength). A variety of treatments have been explored to improve motor skills after stroke, including strength training, balance training, gait training (assisted or independent treadmill walking), repetitive task specific training,

constraint-induced movement therapy (CIMT), and others. A comprehensive discussion and critical review of the evidence for each treatment modality and its effects on a variety of clinical outcome measures can be found in the most recent Evidence Based Review of Stroke Research.[218]

1.8.2 Critical factors

Expanding our earlier discussion on principles of experience dependent plasticity, therapy type, timing, intensity, and frequency are all integral components of a rehabilitative treatment. Timing, the initiation of treatment onset, has been the topic of much focus in recent years. On a broad level, earlier treatment is often regarded as better, as both preclinical[178] and clinical[181] evidence show greater treatment efficacy with early vs. delayed treatment. However, caution must be taken when interpreting this concept, as very early intervention may negatively impact outcomes. For example, CIMT immediately following cortical lesion in rats has been documented to cause severe chronic behavioural deficits[219] and aggravate injury,[219,220] likely due to use-dependent localized hyperthermia.[220] Relatedly, the VECTORS clinical trial found that higher intensity CIMT initiated early after stroke (i.e., <14 days) was associated with less functional improvement at 90 days than more moderate doses of CIMT or usual care.[221] The AVERT clinical trial also found evidence that frequent, high dose, early out of bed mobilization after stroke was associated with decreased odds of favourable outcome.[222] Optimal timing may also vary by stroke subtype and severity; subgroup analysis from the AVERT trial suggests that effects of early, intense mobilization may be worse in those with ICH or severe stroke, although this analysis was underpowered to make any

definitive claims. Determination of the best timing and protocol for early mobility interventions is ongoing, with results of the AVERT-DOSE trial expected in late 2023.

Treatment intensity is an important component in rehabilitative training, yet it is often both poorly defined and reported in the literature. Some report intensity as the amount of time spent in therapy per day, others the number of consecutive hours of therapy, and others still as the number of repetitions completed and/or rate at which a therapy is participated in (e.g., in animals, repetitions in a reaching task or speed and distance of treadmill running; in humans, perceived exertion, heart rate). In an attempt to reduce variability in the definition and interpretation of therapeutic intensity, in 2012 the American Congress of Rehabilitation Medicine Stroke Movement Interventions Subcommittee defined intensity as "the amount of physical or mental work put forth by the client during a particular movement or series of movements, exercise, or activity during a defined period of time."[223] In the case of clinical interventions, subjective measures (e.g., Borg rating of perceived exertion scale), or objective measures (e.g., heart rate, rate of maximal oxygen consumption) may be used to describe intensity. Although both measures may be impacted by the effects of stroke (e.g., cognitive deficits may impact subjective measures, autonomic functions may impact physiological measures), this definition allows for a broad discussion on treatment intensity.

Within the animal literature, intensity is often poorly defined and woefully under reported (see Chapter 3 for a more nuanced discussion). This is likely due in part to methodological limitations, for example a measure of perceived intensity such as the Borg rating is not feasible in animal models, and measuring heart rate or oxygen consumption in small, moving animals is technically challenging. As there appears to be no standardized approach to reporting treatment intensity, it may be more appropriate

for basic and clinical scientists alike to report and discuss both treatment intensity and dose (i.e., the total amount of treatment received over the intervention period)[224] in a manner similar to how parameters of pharmacological interventions are reported.[225] As measures of intensity as defined by clinicians lack equivalent translational counterparts, intensity has been defined and reported in varying ways in the preclinical literature, including but not limited to: time spent in restraint for CIMT,[220,226–228] speed of running in forced running,[229–235] and number of successful reaches in **enriched rehabilitation (ER)**.[236,237]

Findings from preclinical studies suggest that a minimum threshold of intensity must be met for the benefits of rehabilitation to be produced. Using the ET-1 model of ischemia, Maclellan et al. found that modest levels of ER training did not induce improvements in skilled reaching in cortical, striatal, cortico-striatal, or **middle cerebral artery occlusion (MCAo)** stroke; it was only when the number of repetitions (i.e., intensity) was increased did animals show significant behavioural improvement after MCAo stroke.[236] While there is some support that greater intensity is associated with improved outcomes clinically, overall there is limited evidence that higher intensity therapy is more beneficial than standard care (level of evidence 1a and 1b).[218] While this is in direct contrast to what has been reported in preclinical literature, one possible reason for this discrepancy is the much lower level of rehabilitation delivered in clinical settings.[238] Animals completing ER often complete a skilled reaching task >200 times per day; this level of repetition is not documented in the clinical literature.

Treatment dosing parameters are fundamental to understanding the total dose of rehabilitation a subject has received. Treatment duration and frequency are important

components of rehabilitation that influence the efficacy of a given treatment intervention. Treatment duration encompasses two factors: the length of a single session (e.g., minutes, hours) as well as the period over which treatment occurs (e.g., days, weeks, months).[223] Treatment frequency describes how often the treatment is administered within the given period of intervention.[223] For example, an ER protocol where animals completed four 15-minute skilled reaching sessions per day for two weeks would have a single treatment duration of 15 minutes, a treatment period of two weeks, and a frequency of 4 sessions per day (alternatively described as 56 sessions in a two-week period).

1.8.3 Translational Models of Rehabilitation After ICH

Animal studies of rehabilitation provide researchers the opportunity to explore the impacts of treatment at both a behavioural and biological level, offering insight into the mechanisms by which rehabilitation may facilitate recovery. Here, six commonly used translational rehabilitation therapies and the neurobiological changes that have been associated with their use are discussed. A summary of common rehabilitation paradigms used in preclinical research and the parameters often modified between studies can be found in **Table 1-1**.

Table 1-1 Common Preclinical Rehabilitation Therapies Used in ICH Research

Type	Description	Modifiable Parameters	Adjunct Therapies
Constraint-induced movement therapy (CIMT)	Unimpaired limb is restrained (commonly via casting or bracing) for a pre-specified period each day	Duration of daily limb restraint	EE, skilled reach training
Environmental enrichment (EE)	A form of social housing that introduces novel items for rodents to interact with (e.g., running wheels, toys, ramps, tubes) to create a more wild like environment	Novel items, duration of sessions or total period in EE housing	CIMT, skilled reach training
Skilled reach training (REACH)	Massed practice training of fine motor skills (e.g., Montoya staircase task, single pellet reaching task, tray task, modified reaching task)	Task, duration of sessions or total period, frequency of sessions, number of repetitions	EE, CIMT
Aerobic exercise (AE)	Forced or voluntary running that involves the use of treadmills or running wheels	Forced vs. voluntary, speed of running, duration of sessions or total period, distance	EE, skilled reach training
Enriched rehabilitation (ER)	A combination therapy comprised of skilled reach training and EE housing	Task, duration of sessions or total period, frequency of sessions, time in EE housing	
Acrobatic training (AT)	A motor skills training protocol consisting of several tasks requiring gross motor coordination (e.g., walking across a grid, rope ladder, rope, parallel bars, or barriers)	Tasks, frequency of sessions, number of repetitions	

Environmental enrichment (EE) is a form of social housing that introduces novel items into the environment for an animal to interact with, such as running wheels, toys, ramps, tubes, etc. As standard laboratory housing has little in the way of enrichment (i.e., only bedding and a tube/box for hiding), the goal of EE housing is to create a more stimulating, wild like environment for its inhabitants. In 1996 Johansson and Ohlsson were the first to show that EE housing after ischemic stroke provided superior behavioural benefit compared to social housing alone or individual housing with access to a running wheel,[239] a finding supported by work in other models of brain injury.[240] Use of EE after ICH has been explored as an adjunct to other treatments such as amphetamine[241] and estradiol.[242] In both studies EE was reported to be associated

with improved walking outcomes, but failed to provide neuroprotective benefit or superior improvement to skilled reaching.

Skilled reach training (REACH) is a widely used form of massed practice training of upper limb fine motor skills. Several variations of this training exist, but all share three common features: the subject must extend their impaired limb to reach for a target (usually a small pellet or seed, arguably a delicious treat for a rat), grasp the target, then retract the limb while holding the target to complete a successful retrieval (and eat the target as a treat). Importantly, these tasks can be used to assess both impairment and disability depending on method of data collection and endpoint selection. Disability can be measured by assessing task success (e.g., pellet retrieval success rate) and impairment can be measured using kinematic analysis (i.e., videotaping and reviewing movement quality and patterns). Two frequently used training methods are the Montoya Staircase test and the Single pellet reaching task. The Montoya Staircase test[243] involves the use of a 7-step, small plexiglass stair baited with pellets and placed in the testing apparatus (3 pellets/stair, 21 total on each side). Rats are placed in the apparatus and given 15 minutes to retrieve as many pellets as possible. Similarly, the single pellet reaching task[244] involves training the subject to reach through a small (~1 cm) slot to retrieve a target (pellet or seed). Both of these therapies may also be used for determining paw preference before surgical intervention and/or functional assessment. For example, in the case of the Montoya staircase, rats are given 15 minutes to retrieve as many pellets as possible; the number of remaining pellets on each side is then recorded at the end of the session for each limb.

Constraint-induced movement therapy is a technique used to reduce learned non-use in the impaired limb, as the unimpaired limb is restrained for up to 90% of

waking hours over the course of the intervention period.[245] To harness principles of experience dependent plasticity and encourage cortical reengagement and/or reorganization, constraint is paired with concentrated, repetitive training using the impaired limb and maximized through techniques like shaping. In a rodent model of ICH, use of CIMT (constraint paired with exercise) beginning 7 days after stroke resulted in better behavioural outcomes (ladder walking, skilled reaching) and reduced lesion volume compared to either constraint or exercise alone.[226] In a study of severe ICH, early therapeutic hypothermia was paired with CIMT initiated 14 days after ICH to assess whether the combined use of early TH and CIMT was superior to either therapy alone.[227] CIMT was found to provide small functional benefits, with slightly greater improvement in skilled reaching when paired with TH; interestingly, no neuroprotective benefit was found in any group.[227] Other studies of **forced limb use (FLU)**, constraint of unimpaired limb without paired training, have reported a variety of results. One study using FLU beginning 1 day after ICH found greater improvement in early reaching movements and ladder walking, but no effect on gross motor skills, limb use asymmetry, or lesion volume.[228] A follow up study comparing early FLU (initiated 1 day after ICH) with late FLU (initiated 17 days after ICH) found that early FLU improved both reaching and stepping success in the impaired limb, but late FLU did not.[246] Neither early nor late FLU was shown to be neuroprotective, however early FLU was associated with cellular and molecular level changes in the ipsilesional motor cortex such as increased dendritic arborization and elevated expression of ΔFosB (a transcription factor associated with neural activity), BDNF, and GAP-43.[246] Others have demonstrated alterations and expansions of cortical maps accompanied by increased axonal

projections to ipsilesional cortex, and implicated both the cortico-rubral[247] and cortico-reticular tracts[248] as playing a role in mediating FLU induced recovery.

Aerobic exercise (AE) is a behavioural intervention that is frequently used after ICH to drive neuroplastic changes in the brain.[229–235,249] Aerobic exercise is typically broken down into two categories: voluntary exercise and forced exercise. Under Voluntary exercise, animals have access to a running wheel, often in their home cage, that they may use at their leisure over a designated period of time (e.g., 30 minutes, 24 hours) or number of sessions. In comparison, forced exercise involves the placement of the animal on a running apparatus (i.e., treadmill, rotarod, rotating wheel) set at a prespecified speed (e.g., 10 m/min) for a given length of time (e.g., 30 minutes) and frequency (e.g., 4 sessions/day). Dozens of studies have explored the effects of AE following ischemia with mixed results. Some have reported little to no benefit, others have reported modest reductions in lesion volume, inflammation, and oxidative damage, improvements in behavioural deficits, and increases in angiogenesis, neurogenesis, and expression of important pro-plasticity neurotrophic factors such as BDNF.[250] Results following ICH have also been mixed. Some have reported positive behavioural benefits including improvement to the motor deficit score,[119,121,136] beam walking,[231,233] and ladder walking.[234] Others have reported neurobiological changes thought to underlie behavioural improvement such as increases in dendritic length and arborization in the contralateral striatum;[249] lessened ipsilesional dendritic atrophy;[231] and increased cortical thickness, neuronal density, and dendritic length and complexity in contralateral motor cortex.[234] Others have found less benefit. One study explored the use of forced exercise as both pre- and post- ICH treatment; neither paradigm was beneficial, and increased post-ICH treatment duration was associated with worsened

outcomes.[229] Another assessed forced exercise after ICH initiated early (24 hours) and with delay (1 week); early forced exercise improved performance on the rotarod test, but did not improve performance in other behavioural measures or alter hematoma resolution.[230] Despite assessing both timing and duration of treatment, another study found limited benefit of forced exercise, although there was a trend that suggested earlier intervention was superior to later intervention regardless of treatment duration.[232] Compiled, the data on AE suggests that it may be capable of providing some behavioural benefit and upregulating mechanisms of plasticity, however it is likely insufficient as a standalone treatment.

Enriched rehabilitation is a combination therapy that combines REACH with EE housing as a means of combining the benefits derived from elements of EE (i.e., upregulation of plasticity) with the benefits of mass practice skill training (i.e., restoration, recruitment, and rewiring of functional movement circuits). First described in Biernaskie & Corbett for use after ischemic stroke,[251] ER was found to substantially improve both skilled reaching and walking, as well as enhance dendritic complexity in pyramidal cells of the motor cortex. Follow up of this study found that initiating ER early after injury (i.e., 5 vs. 30 days) was associated with greater behavioural improvement and enhanced plasticity in the contralateral hemisphere, suggesting the presence of a critical window for intervention after brain injury.[178] The use of ER has since been explored after ICH in both the COL[252–254] and AWB models.[255] A study of ER beginning 7 days after COL ICH demonstrated that ER provides behavioural benefit in skilled reaching tasks and walking, and substantially reduces the volume of tissue lost.[252] A follow up study showed similar behavioural improvements to skilled reaching and walking, as well as enhanced dendritic complexity with ER, yet did not observe a

neuroprotective benefit.[253] A similar study of AWB induced ICH found comparable behavioural benefits, but did not observe neuroprotective benefit or increased dendritic length in the contralateral striatum.[255] Others have tried to explore the mechanisms that may drive the benefits derived of ER, suggesting that it may come from reducing cell death,[254] possibly by accelerating hematoma clearance and reducing oxidative stress.[256]

Acrobatic training (AT), occasionally referred to in the literature as motor skills training, is a rehabilitative therapy comprised of a subset of 5 tasks that require gross motor coordination, and to a lesser extent fine motor control. Tasks included in this protocol include walking across an elevated rope ladder, an elevated grid platform, a single thick rope, two parallel bars, and a series of irregular platforms.[257] Following COL ICH, AT has been reported to accelerate improvements in gross motor function and fine motor function, as well as increase neural activity and plasticity.[257–259] Mechanistic studies into AT have suggested these behavioural improvements may be driven by increased synaptogenesis,[257] expression of structural proteins such as MAP2 (a cytoskeletal protein important in dendritic structure),[258] and alterations in AMPA expression.[259]

Despite the development of preclinical therapies aimed at emulating critical factors of clinical rehabilitation delivery (e.g., therapy type, timing, frequency, intensity), preclinical researchers have struggled to consistently obtain behavioural and neurological benefit from rehabilitation. Treatment parameters, individual characteristics (e.g., age, stroke type, insult severity, comorbidities), and experimental design (e.g., stroke model, endpoint selection, population, etc.) all contribute to the challenges researchers face in attempting to translate preclinical findings into clinical success.

1.9 Considerations for Translational Research

Preclinical animal research plays a fundamental role in our understanding of the basic mechanisms of disease, injury, and repair. Despite many encouraging preclinical results in recent decades, translation of preclinical findings into clinical success remains extremely low. In order to understand the obstacles to therapeutic translation in the current research environment, we must first understand the translational pipeline and the "valley of death"[260] that occurs in the transition between basic science (i.e., at the bench) and early clinical studies (i.e., phase I/II proof of concept, safety, and dosage studies). Both experimental factors (e.g., study design, population, methodology, analysis, reporting) and systemic factors (e.g., funding, lack of interdisciplinary research teams, publication bias) contribute to the lack of efficacy and high failure rate when moving research from preclinical to clinical settings.[260] In this section I will discuss the challenges researchers and clinicians face in transitioning ideas through the research pipeline and discuss opportunities that may improve the rate of successful translation.

1.9.1 Experimental Design & Quality Considerations

Recent estimates suggest between 75-90% of preclinical research findings cannot be reproduced.[261] Incomplete reporting of methodology of experimental models, populations, laboratory settings, assessments, and analysis all contribute to a lack of methods and results reproducibility; inappropriate statistical practices (e.g., ignoring multiplicity, violating test assumptions), incomplete/selective reporting of results, and a failure to contextualize findings within the broader literature (e.g., through systematic review and meta-analysis) lessen inferential reproducibility.[260–262] Experimental design

and methodology therefore must take into account factors that influence both internal and external validity, carefully selecting appropriate endpoints to answer the experimental question, while being mindful of confounds that may influence experimental results or generate spurious findings.

Selecting a stroke model to create an injury that not only mimics the population of interest but also generates an appropriate injury profile is a fundamental component of rigorous study design.[263] For example, while both the COL and AWB methods induce ICH, if studying the impact of a treatment on tissue loss (i.e., rehabilitation), the COL model may be more appropriate as it displays considerably more tissue loss over a longer duration of time.[102] While difficult to definitively prove similar cellular and molecular changes occur in clinical populations, owing in part to limited resolution of neuroimaging techniques and invasiveness required to assess equivalent endpoints, a recent serial longitudinal neuroimaging study has reported evolving and long-term microstructural (i.e., decreased **fractional anisotropy, FA**) and physiological (i.e., cerebral blood flow) changes following ICH.[264] Other important considerations for stroke model selection include location and severity, as both factors impact recovery and the type of deficits observed.[11] Stroke severity is of particular importance in studies assessing behavioural outcomes; too mild an injury may risk ceiling effects, too severe may risk floor effects.[265] Despite potential confounds, it is essential to assess therapies in severe stroke, as survivors of these strokes are most likely to live with ongoing impairment and disability.

Clinical populations display significant heterogeneity both in individual patient characteristics (e.g., age, sex, comorbidities) as well as injury profile and degree of impairment. Despite these facts, preclinical stroke research is dominated by the use of

young, male rats as the model population.[97] While use of homogenous model populations in early stages may be beneficial for proof of concept (e.g., less variability and higher statistical power), failure to evaluate treatment efficacy in other model populations (e.g., females, aged animals, with comorbidities) may limit the generalizability of findings and ultimately contribute to reduced translational success.[263]

Careful and appropriate endpoint selection is therefore an essential component of rigorous study design. Endpoint selection is particularly critical for translational rehabilitation research – not only should studies be designed to assess clinically relevant endpoints (e.g., assessing fine motor skills by measuring success in a skilled reaching task), but also be appropriately timed, and sensitive to both the behaviour of interest, and the amelioration of expected deficits over time.[263] For example, use of the composite **neurological deficit score (NDS)** would be appropriate for assessing early differences in gross function between groups (<2 weeks after ICH), but not for long-term assessment (>2 weeks) as test sensitivity decreases over time due to amelioration of deficits attributed to spontaneous recovery.[266] Similarly, use of the ladder walking task would be suitable for assessing gross locomotor function, yet inappropriate for assessing muscle strength or endurance. Researchers must also consider how the lesion location and severity may impact the ability to participate in an assessment. For example, animals displaying signs of spatial neglect may perform poorly on a test due to lack of attention or awareness, not exclusively due to motor impairment; attributing these gains to therapeutic efficacy may lead to overestimation of treatment effects. Furthermore, sensorimotor impairments or motivational impairments (e.g., as a result of post-stroke depression or anxiety, or disinterest in a task) may reduce participation in an assessment or training task, complicating

interpretation of reported effects. Finally, as many assessments are very good at detecting the presence of injury but cannot reliably distinguish between more subtle gradations in injury (e.g., mild vs. moderate stroke), using a battery of assessments is recommended.[267]

Addressing sources of observer confirmation bias, through procedures such as randomization and blinding (to treatment and/or during assessment and/or analysis), is important in preventing overestimation of effect sizes.[268] Clear disclosure of mortality and data exclusions is important to ensuring transparency in research reporting and statistical interpretation.[269] For example, not reporting mortality or excluding many animals due to severity of impairment may inadvertently disguise the effects of a treatment on survival. A common problem across preclinical neuroscience research is the use of small group sizes, and as a result, low statistical power;[269] indeed, a recent review of preclinical ICH neuroprotection studies found that the median group size used in behavioural endpoints is 8.[97] While the use of small group sizes may be justified as making research quicker, more feasible, and less expensive, their use has the insidious consequence of not only reducing the chance of detecting a true effect, but also that a statistically significant result is true.[269] The practice of *a priori* identification of primary endpoints, planned statistical analysis, and power calculations to determine group sizes, are all strategies recommended to improve experimental rigor and transparency.[269]

Despite the publication of guidelines designed to improve reporting of preclinical studies and the quality of stroke research (ARRIVE,[270] STAIR,[271] RIGOR[272]), research quality remains a major concern across the stroke field. As translational research often creates the foundation for later clinical studies, lack of reproducibility is of grave concern; basing clinical studies on data that fails to replicate within the original model

organism (e.g., mouse, rat, pig) may result in costly failures in our attempt to translate findings to human populations.

1.9.2 Systemic Considerations

Several systemic scientific issues also contribute to the failures in translational stroke research. The current culture of "publish or perish" rewards those who publish frequently, often regardless of quality.[261] Limited funding, combined with granting agencies and academic journals that often favour novelty over replication or publication of negative findings, has led to an overwhelming positive publication bias in the field of neuroscience.[261,273] In an analysis of 525 animal stroke studies, only 2% reported no significant effect on infarct volume, a common primary endpoint in preclinical work.[273] Selective reporting and "p-hacking" (repeating or modifying statistical analysis until a favourable result is achieved) are two statistical problems that further contribute to this overt positive publication bias.[274] Furthermore, common practice of reporting statistical significance (i.e., p-values) provides limited information on the magnitude of a treatment effect.[275] Despite passing through the peer review process, many papers continue to be published that use inappropriate statistical methods (e.g., using a t-test on ordinal data) or inappropriate statistical comparisons (e.g., lack of comparison to appropriate control groups), suggesting that gaps in statistical expertise exist at the level of both the research group and across the field as a whole. To combat these concerns, several experts have called for the use of pre-planned statistical analysis, reporting of effect sizes and confidence intervals, and the incorporation of designated statisticians into research groups and animal ethics boards to aid in experimental design and planning.[261,269,276–278] Despite widespread use in clinical research, frequent use of meta-

analysis has not been adopted to the same extent in the preclinical literature, although it appears to be growing considerably.[269] While time consuming, completion of a meta-analysis of the available preclinical data for a given therapeutic intervention should be a requirement prior to advancing to clinical trial as it may prevent the costly exploration of treatments based on questionable results or effect sizes. However, meta-analysis does not replace the need for high quality research; heterogeneity and quality of primary studies influence the quality of meta-analysis and may limit their accuracy and utility.[275] Finally, the lack of interdisciplinary research teams (e.g., groups consisting of basic scientists, clinicians, and PTs/OTs etc.), contributes to a unidirectional approach to the translational research pipeline.[63] Collaborative research units allow for much larger studies[279] and create a two-way flow of information,[263] allowing for better understanding of preclinical research and clinical implementation, which should ultimately lead to the design of experiments that better reflect clinically relevant protocols and assessments, and hopefully, result in greater translational success.

1.9.3 Considerations for ICH & Neurorehabilitation Research

Much of our understanding of stroke injury, recovery, and treatment comes from ischemia. While ICH and ischemia are related and share many similar mechanisms of damage and repair, it is likely that they differ to some degree and therefore may be more (or less) amenable to treatment than other subtypes. For example, a recent study of reactive gliosis after stroke found that following ischemia, astrocytes display greater phagocytic activity than after ICH, and play a larger role in synapse elimination following ischemia.[280]

No one model perfectly mimics clinical ICH, and while each model has its advantages, translational neurorehabilitation researchers must be aware of the differences between models and select appropriate endpoints to assess therapeutic efficacy. The COL model is documented to have a protracted period of cell death, where lesion volume substantially increases from 1-6 weeks after ICH; comparatively, the AWB model shows a relatively stable lesion profile with little change from 1-6 weeks post-ICH.[94] These differences in injury profile and time course may influence therapeutic efficacy. Despite behavioural benefit, rehabilitation interventions beginning 1 week after ICH have not shown an effect on neuroprotection in the AWB model.[255] Conversely, the COL model gives a longer window for therapeutic intervention, and rehabilitation starting 1 week after ICH has shown both behavioural[252,253] and neuroprotective benefit[252] (albeit inconsistently).[253]

Clinical practice should be taken into consideration in experimental design and analysis, particularly with respect to inclusion of control groups. While feasible to withhold treatment (i.e., no treatment control) in experimental studies, clinical interventions are compared with usual care (i.e., standard accepted practice). Recent meta-analysis of laboratory housing conditions found conventional housing (e.g., shoebox type plexiglass cages with limited enrichment or socialization) to increase distress and mortality rates compared to animals housed in enriched environments.[281] While further research is required, it is possible that this disparity in housing conditions (e.g., enriched housing vs. conventional solo housing) contributes to inflated effect sizes in comparisons between treatment and control groups.

Drug trials should serve as a potent reminder that treatment cannot take a “one size fits all” approach; not every patient will benefit from every therapy, making the

identification of biomarkers essential to our understanding of who may benefit from a therapy and why.[282,283] As we move into the age of precision medicine, there continues to be a lack of stratification of patients in stroke recovery clinical trials,[282] despite increased understanding of predictive biomarkers of functional recovery[284] (e.g., MEPs as a measure of CST integrity).[90] The same appears to be true in preclinical research. Study populations are typically homogenous and explore treatment following mild-moderate stroke, with little manipulation of lesion location or volume; regardless of treatment success, few treatments are followed up with variations to population, lesion size, lesion location, or presence of comorbidities.

Timing of intervention onset is a critical component of any treatment and understanding the spatial and temporal dynamics of the multitude of endogenous mechanisms of injury and repair in experimental populations is extremely complex. Although there is evidence for a similar critical window of recovery in humans,[181] the timing of spontaneous recovery is not identical, which presents a particularly difficult hurdle in translating time sensitive therapies. For example, the timeline of hematoma resolution in animals is much quicker (~21 days) than in humans, where resolution may occur over an extended period of 1-3 months.[264] Similarly, in animal models most spontaneous recovery is observed within the first month[81] (although sometimes in the first week),[102] whereas in humans this same period appears to last ~3-6 months.[285]

Although many models of rehabilitation have been created for preclinical use, an often-ignored factor in preclinical rehabilitation is the role the rehabilitation specialist (or caregiver) plays in therapeutic delivery. Therapists often serve as a coach, encouraging active participation and mental engagement in tasks, providing feedback by use of verbal or physical cues, and easily modifying a task to meet the patient's

abilities.[286] A comparable level of feedback, correction, and motivation is a difficult challenge to address in preclinical settings. It is possible that despite the best effort of researchers, preclinical research paradigms may be promoting compensation rather than true recovery.[287] However, promoting compensation may not be inherently bad; while some activity may have maladaptive consequences (e.g., training of the non-impaired limb early after stroke may result in worsening of function in the impaired limb),[288] these changes may be beneficial in those with poor prognosis (i.e., severe stroke).[287]

Finally, while many clinical assessments, such as the FM,[70] have a defined MCID which represents the smallest change in an outcome that is of value to the patient,[71] the same has not been established in preclinical settings. Preclinical researchers must therefore be cautious in interpreting results; a statistically significant result does not necessarily equate to a biologically meaningful change in behaviour. As such, we must rely upon intuition (e.g., a 25% improvement should be meaningful) and statistical estimates (e.g., Cohen's d values) in our interpretations of efficacy.

1.10 Thesis Objectives

Broadly, the goal of this thesis was to assess the efficacy of rehabilitation after experimental ICH. What began as an investigation into a potential mechanism of rehabilitation-induced neuroprotection slowly morphed into an exploration of efficacy of preclinical motor rehabilitation therapies after ICH and a commentary on factors impeding translational success.

Chapter 2 is comprised of two experiments assessing efficacy of ER after ICH, in the sub-acute and chronic phases of stroke recovery. Experiment 1 aimed to assess

whether initiating ER 5 days after ICH accelerated hematoma clearance early after stroke, as previously reported by Williamson and colleagues.[256] I hypothesized that ER beginning 5 days after ICH would provide enhanced behavioural benefit and accelerate hematoma clearance at 14 days after ICH. Further, I hypothesized that animals completing ER in the dark would participate at a higher intensity, conferring greater benefit than those who completed training in the light. Experiment 2 aimed to assess whether manipulating treatment duration (10 vs. 20 days) conferred greater behavioural and neurological benefit when measured in the chronic phase of recovery.

Chapter 3 is a systematic review and meta-analysis of motor rehabilitation interventions delivered in preclinical ICH studies. Although comprehensive analyses of clinical rehabilitation strategies and their efficacy are commonplace (i.e., Evidence Based Review of Stroke Rehabilitation), the same cannot be said for translational stroke rehabilitation research. While some have explored the effects of post-stroke rehabilitation on functional recovery,[289] neuroprotection,[250,289] or neuroplasticity,[290] a comprehensive review of the effects of post-stroke rehabilitation on motor recovery has not been conducted in translational models of ICH. Owing to differing mechanisms and patterns of injury between ischemia and ICH, and that the majority of stroke rehabilitation research is conducted following ischemia, I first assessed the overall efficacy of motor rehabilitation after experimental ICH. Next, I explored how efficacy was altered by factors such as intervention type, timing of onset, dose, and stroke severity. Lastly, in response to the varied quality of reporting and experimental design found in the reviewed literature, I developed a roadmap for researchers to follow aimed at improving the quality of preclinical rehabilitation research at each step of the scientific process.

Chapter 4 is a retrospective, exploratory post-hoc analysis that assessed whether proportional recovery occurs in preclinical models of subcortical ICH. Clinical literature supports that the majority of stroke survivors will recover ~70% of their initial impairment after stroke.[84–86,90,91,291–293] A retrospective analysis of recovery after ET-1 induced ischemic stroke in rats has also found evidence to support this phenomenon, albeit to a somewhat lesser extent (~66%) and in a much lower proportion of the study population (~30%). However, it is unclear to what extent proportional recovery occurs after ICH as all previous studies used only ischemic populations, or mixed populations with <15% hemorrhagic stroke patients. As there is mixed evidence on whether patterns of recovery differ by stroke subtype, [57,58,72,294–296] I explored whether recovery in skilled reaching is proportional to initial impairment after experimentally induced striatal ICH and sought to evaluate whether clinically relevant factors such as severity, lesion size, and internal capsule damage predicted recovery.

Finally, Chapter 5 provides a summary and general discussion of findings related to rehabilitation and motor recovery in translational models of ICH. Limitations of the presented work and hurdles that must be overcome within the field are reviewed, and considerations for future research are discussed.

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Chapter 2 | Early, intense rehabilitation fails to improve outcome after intra-striatal hemorrhage in rats

2.1 Introduction

Intracerebral hemorrhage (ICH), caused by the rupture of vasculature in the brain, accounts for 10-20% of all strokes.[1] This sudden extrusion of blood into surrounding tissue causes immediate mechanical trauma and delayed secondary injury. Although there are overlapping mechanisms of injury with ischemia, unique processes contribute to the protracted cell death observed in models of ICH.[2,3] Pre-clinical studies illustrate progressive tissue loss occurring for weeks after collagenase-induced ICH,[2,4] accompanied by decreases in cortical thickness and white matter atrophy. Degradation of the hematoma into cytotoxic components, such as heme and iron, causes inflammation, formation of reactive oxygen species, and a disrupted blood brain barrier.[5] Similarly, intra-cerebral iron infusion alone causes progressive brain injury.[6]

To date, rehabilitation (rehab) remains our best treatment to promote recovery after stroke, yet much of our understanding of rehab comes from ischemic stroke, with more limited work exploring rehab exclusively after ICH.[7] Animal[8] and clinical[9] evidence suggests that individuals recovering from ICH show greater early improvements than those recovering from ischemic stroke;[10] however, as acute treatment for ischemic stroke has improved, this finding has been challenged.[11] There is a moderate body of animal research exploring rehab and recovery after ICH,[12–16] and some of these studies have varied treatment parameters.[12,15,17] From that limited work, it is difficult to optimize treatment protocols (e.g., timing and intensity of intervention) or to identify key underlying mechanisms. Thus, we must rely upon the known principles of learning and memory that can be harnessed to drive plasticity in the injured brain. Rehab utilizing optimal timing, specificity, repetition, intensity, and salience, all

principles of experience-dependent plasticity, seem to best drive recovery after brain injury[18] and it seems reasonable that the same should apply to ICH.

As with treatment parameters, the means by which rehab improves outcome have yet to be fully elucidated after ICH. Not surprisingly, rehab increases neurotrophic factors[19] leading to the growth of spines and dendrites,[20] synaptogenesis,[21] and sometimes neurogenesis,[22] (but see[20]). DeBow and colleagues also discovered a neuroprotective effect with using constraint induced movement therapy (CIMT) following ICH.[12] Despite the simplicity, running alone appears to be neuroprotective,[14] but not all studies agree.[23] Like CIMT, enriched rehabilitation (ER),[24] a combination of skill training and environmental enrichment (EE), mitigated cell death after ICH while augmenting recovery.[25] Both CIMT and ER align with several principles of experience-dependent plasticity, such as specificity, repetition, and intensity. As for timing, most ischemia and ICH studies begin rehab within the first 7 days after stroke,[13,20,25–27] a time of heightened plasticity.[28]

Recently, accelerated hematoma clearance (mechanism unknown) with attenuated iron levels has been identified as a potential way by which rehab lessens injury (less oxidative stress) and neural dysfunction (less ionic dyshomeostasis).[29] Drug-augmented clearance[30,31] as well as surgical removal[32,33] of the hematoma have been topics of interest for years,[34] as reducing hematoma breakdown products should minimize secondary injury. For instance, pre-clinical studies administering lactoferrin show promise in augmenting hematoma clearance and improving behaviour.[35–37] It makes sense then that rehab's known benefits (promoting synaptogenesis, etc.) might synergize with the neuroprotective and restorative effects of augmented hematoma clearance.

We must acknowledge that our treatment and mechanistic understanding of rehab, especially in the small ICH rehab sub-field, must be viewed in light of the fact that many pre-clinical stroke studies have shown interesting results, only to fail when re-tested or applied in a clinical setting.[38] Numerous questionable practices, such as poor reporting, cherry picking data, and the use of small group sizes, all lead to overestimated effect sizes. Those issues, coupled with the more common failure to publish negative results, all contribute to the "replication crisis" in biomedical research.[39] Thus additional high-quality studies are needed.

Here, we used a translationally rigorous design to explore whether treatment intensity and duration impact the efficacy of ER following ICH in rats. We were principally concerned about the neuroprotective effects of rehab, as we believe that this neuroprotective effect is biologically meaningful and at least partially underlies the better behavioural benefit reported in previous studies. As the basal ganglia is a common site of ICH in humans,[40] we infused collagenase into the striatum to cause an ICH. This is a well-characterized[41] and common model in ICH rehab studies, which have shown that rehab lessens secondary injury.[12,25] Our first study assessed whether ER performed in the light or dark phase of the housing cycle altered rehab intensity and efficacy. Experimental work in ischemia suggests that rehab delivered in the dark leads to greater engagement in training and better outcomes than ER in the light phase.[27] We used a hemoglobin assay to determine residual hematoma volume 14 days after ICH, as a comparable treatment was previously shown to accelerate hematoma clearance at 14 and 21 days after ICH in rat.[29] Our second experiment explored whether increasing treatment duration (10 vs. 20 days of ER) provided superior behavioural benefit and neuroprotection when measured 60 days after ICH. We hypothesized that longer ER

treatment would enhance behavioural and neuroprotective benefits owing to the ongoing secondary injury in this model.[2,3,6]

2.2 Methods

2.2.1 Animals

One hundred twenty-eight male Sprague-Dawley rats (250-300 g, ~2-4 months old) were obtained from Charles River (Saint Constant, Quebec). All procedures were approved by the Biosciences Animal Care and Use Committee at the University of Alberta (Protocol 960) and complied with Canadian Council of Animal Care Guidelines. Researchers adhered to the ARRIVE guidelines,[42] except when blinding was not possible (e.g., during ER delivery and behaviour assessment).

Rats were group housed 4 per cage in standard plexiglass cages (37 cm by 47 cm by 20 cm) with 2 standardized rat retreats (tubes) per cage in temperature- and humidity-controlled rooms, with lights on from 7 am-7 pm (standard light cycle) or 7 pm-7 am (dark cycle). In the week preceding behaviour training, groups assigned to the dark cycle condition (all interventions completed in the dark), were transitioned to this schedule over a period of 4 days. Each day, the start of the light cycle was delayed by 3 hours (10 am-10 pm, 1 pm-1 am, 4 pm-4 am, 7 pm-7 am). To reduce animal stress, behavioural training was not performed until 2 days after transition. Rats assigned to ER groups were housed under the same conditions as control groups (CON), except during EE. Rodents were fed standard rat chow (Purina) with water ad libitum. Food and water were available ad libitum during acclimation phase, light cycle transition, pre- and post-surgery, and outside periods of behavioural testing. To reduce stress and

familiarize animals to researchers, all animals received two 10-minute handling sessions with each researcher prior to the start of behaviour training.

Table 2-1 Group sizes, exclusions, and endpoints analyzed

Experiment	Group	Size	Exclusions	Endpoint Analysis (n/group)
Experiment 1: 14 Day Survival	Dark Rehab (ER-D10)	16	Complete exclusions: 3 Partial exclusions: 3 (ladder)	Staircase: n=13 Beam: n=13 Ladder: n=10 Hematoma volume: n=13
	Light Rehab (ER-L10)	16	Complete exclusions: 3 Partial exclusions: 0	Staircase: n=13 Beam: n=13 Ladder: n=13 Hematoma volume: n=13
	Dark Control (CON-D)	12	Complete exclusions: 1 Partial exclusions: 1 (beam)	Staircase: n=11 Beam: n=10 Ladder: n=11 Hematoma volume: n=11
	Light Control (CON-L)	12	Complete exclusions: 3 Partial exclusions: 1 (beam)	Staircase: n=9 Beam: n=8 Ladder: n=9 Hematoma volume: n=9
	N=56 Complete exclusions: n=10 (7 failed to reach baseline behaviour criteria; 3 technical error) Partial exclusions: n=2 (beam – camera malfunction); 3 (ladder – camera malfunction)			
Experiment 2: 60 Day Survival	Rehab-10 (ER-D10)	24	Complete exclusions: 0 Partial exclusions: 1 (staircase), 1 (corpus callosum)	Staircase: n=23 Lesion volume: n=24 Corpus callosum: n=23
	Rehab-20 (ER-D20)	24	Complete exclusions: 1 Partial exclusions: 2 (staircase), 1 (lesion volume), 2 (corpus callosum)	Staircase: n=21 Lesion volume: n=22 Corpus callosum: n=22
	Control (CON-D)	24	Complete exclusions: 1 Partial exclusions: 0	Staircase: n=23 Lesion volume: n=23 Corpus callosum: n=23
	N=72 Complete exclusions: n=2 (premature death) Partial exclusions: n=3 (staircase – incomplete assessment), 1 (lesion volume – poor tissue quality), 3 (corpus callosum – poor tissue quality)			

In experiment 1, rats (n=56) were indiscriminately assigned to cages by animal care staff and were later randomized by cage (random number generator) to dark (D) or light (L) housing condition and transitioned to dark cycle housing appropriately (Figure 2-1A). Following behaviour training and ICH induction, cages were further randomized to ER (ER-D10, ER-L10) or no treatment control (CON-D, CON-L) as noted in Table 2-1.

In experiment 2, all animals (n=72) were transitioned to dark cycle housing (Figure 2-1B) as there was a small but non-significant trend that ER-D10 animals performed better at skilled reaching on day 14. Following behaviour training and ICH, cages were randomized to 10 days of ER (ER-D10), 20 days of ER (ER-D20), or control (CON-D) condition (Table 2-1).

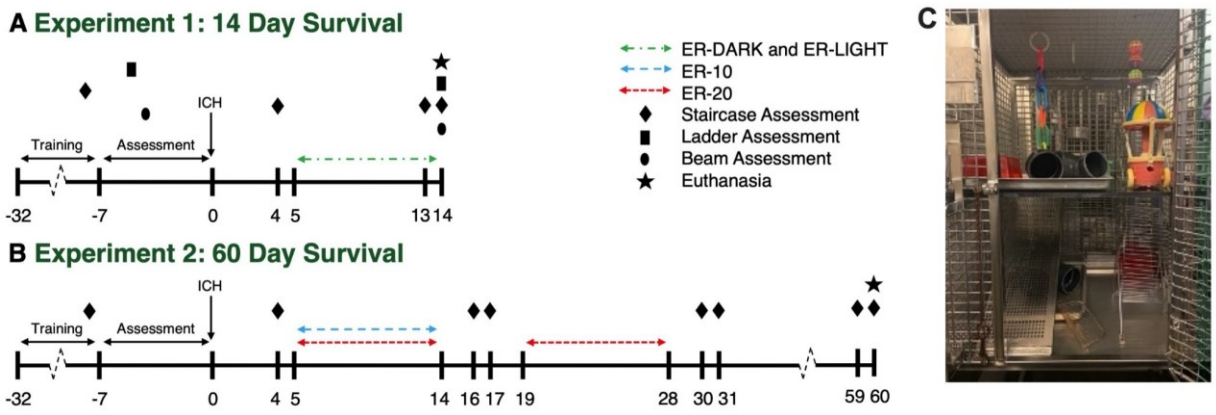

Figure 2-1 Experimental Timeline. **A** Experiment 1: investigation of effects of ER on residual hematoma volume and behaviour 14 days after collagenase-induced ICH. **B** Experiment 2: investigation of effect of altering ER duration (10 or 20 days) on lesion volume and behaviour in the chronic phase of recovery after collagenase ICH. **C** Dual-level environmental enrichment housing. Each level contained a running wheel for rodents to use at their leisure, as well as tubes for hiding, and novelty toys. To encourage exploratory behaviour, food location was changed daily, and novelty items were changed twice weekly (e.g., balls, chains, wooden blocks, cars). Cages were made of wire rungs to allow climbing between levels of the cage and included a ramp to allow more impaired animals to walk to upper level

2.2.2 Skilled Reach Training & Montoya Staircase Test

To encourage participation in behavioural training and testing, animals were food restricted to 90% of their free feeding weight with water available ad libitum in their home cages. Animals were trained on the staircase test[43] for 4 weeks prior to stroke (5 days per week, 2 trials daily, 15-minutes each). Baseline assessment of skilled reaching and paw preference was determined on the last 3 days of training (average of the last 6 trials). Based on a priori exclusion criteria, animals that failed to successfully retrieve a minimum average of 9 pellets on at least one side were excluded from analysis but remained in the study and continued to receive treatment under their assigned protocol to prevent disruption to cage hierarchy and social dynamics.

All animals completed skilled reach testing prior to ICH (baseline testing), and after ICH on day 4, prior to the start of treatment. In experiment 1, skilled reaching was assessed over 4 trials on day 13 and 14 (2 trials/day, replacing the 7 am and 11 am rehab sessions on those days). In experiment 2, skilled reaching was assessed over 4 trials on days 16 and 17 (mid-point assessment) and days 30 and 31 (final assessment). Scores represent the average number of pellets retrieved across 4 trials.

2.2.3 Beam Walking Task

Following completion of skilled reach training, animals were trained to cross a horizontal beam, as previously described.[3] The final trial was video recorded and scored to determine baseline ability. Briefly, animals were scored as: 0 (rat fell off beam <10 s), 1 (could not place impaired limb on beam, stayed on >10 s), 2 (unable to cross beam but able to place impaired limb and maintain balance), 3 (able to cross beam but dragged impaired limb), 4 (able to cross beam, placed impaired limb on beam 1+ time), 5

(crossed beam with >50% slip rate on impaired limb), 6 (crossed beam with <50% slip rate on impaired limb), or 7 (crossed beam with 2 or less slips of impaired limb).[3] Following all reach training sessions and reach assessment trials on day 14, rats completed one post-treatment beam walking assessment. Recordings were scored by a blinded researcher.

2.2.4 Ladder Walking Task

Following completion of skilled reach training, rats were trained to cross a horizontal ladder apparatus with rungs spaced 1-5 cm apart, and scored as previously described.[44] The final 4 trials were recorded to determine baseline walking ability. To avoid the ladder walking serving as rehab, post-ICH trials were only completed on D14, after all skilled reaching and beam walking assessments were complete. Per a priori exclusion criteria, animals that failed to successfully cross the ladder a minimum of 2 times in either the baseline or day 14 assessments were excluded from analysis. The percentage of successful steps was calculated for the contralateral forelimb and averaged across all included trials:

$$\%\ successful\ steps\ = \frac{\#\ of\ successful\ steps}{\#\ of\ total\ steps} x\ 100$$

2.2.5 Surgery

For ICH, animals were anesthetized with isoflurane (graded induction to 4%, 2-3% maintenance) and placed in a stereotaxic frame.[45] A midline incision was made over the scalp to expose the surface of the skull and a burr hole was made 0.5 mm anterior and 3.5 mm lateral to Bregma,[46,47] contralateral to the dominant paw (established

during baseline reach testing). An infusion of 1.2 μL of 0.6 U bacterial collagenase (Type IV-S, Sigma) was injected 6.5 mm deep into the striatum over 5 minutes (26-gauge Hamilton syringe). The syringe remained in place for an additional 5 minutes to prevent backflow. The burr hole was then sealed with a small screw, and the scalp was closed. Temperature was monitored throughout the procedure via rectal probe and maintained at 37±0.5 ºC via heating pad. All animals received Marcaine (0.5 mL S.C., Pfizer Canada) for pain management at the incision site. Rats were provided with a wet mashed rat chow following surgery and recovery was monitored through daily weighing and health checks.

2.2.6 Enriched Rehabilitation & Training Intensity

Animals in ER training completed four 15-minute sessions of task specific training daily. Using a modified reaching apparatus,[13] rats performed skilled reach training in individual plexiglass boxes. During these sessions, animals had access to ~200 reward pellets (Dustless Precision Pellets, Primate Purified Diet, Banana Flavor, 45 mg, Bio-Serv) in the well corresponding to their impaired limb. The start of each session was separated by 1.5 hours (7 am, 8:30 am, 10:00 am, 11:30 am). The weight of pellets consumed in each session was converted into the equivalent number of pellets retrieved during the session. Pellets retrieved was used to approximate rehab intensity.

Following completion of task specific training and daily feeding, rats assigned to ER were removed from their standard housing cages and placed in EE cages for 6 hours per day (1-7 pm). Animals completed EE within their light cycle, meaning that animals assigned to standard light cycle completed their EE during the light phase (experiment 1) and animals in the dark cycle completed EE in the dark (experiment 1, 2). Cages used

for EE were dual level modified wire primate cages (71 cm by 71 cm by 89 cm) that included different types of wood shavings, small toys (changed twice per week for novelty), two running wheels, and a ramp between levels (Figure 2-1C).

2.2.7 Hematoma Volume Assay

In Experiment 1, animals were deeply anaesthetized under isoflurane and euthanized by decapitation 14 days post-ICH. Blood volume of ipsilateral and contralateral hemispheres was measured using a spectrophotometric hemoglobin assay,[48] as previously modified.[4,45] To control for blood in the vasculature, hematoma volume (μL) was calculated as:

$$hematoma\ volume = ipsilateral\ hemisphere\ blood\ volume - contralateral\ hemisphere\ blood\ volume$$

2.2.8 Total Tissue Loss & White Matter Quantification

In Experiment 2, animals were euthanized at 60 days post-ICH and lesion volume was calculated.[4,49] Briefly, animals were overdosed with sodium pentobarbital (Euthanyl, 100 mg/kg I.P., Bimedia-MTC) and transcardially perfused with saline followed by 10% neutral buffered formalin. Brains were extracted and fixed in formalin for at least a week, then transferred to a 30% sucrose solution for cryoprotection ~72 hours before cryosectioning. Coronal sections 20 μm-thick were taken every 200 μm, and stained with cresyl violet for lesion volume analysis and white matter atrophy.[49] Fiji (ImageJ) software[50] was used to quantify both total tissue loss (mm^3) and white matter

atrophy in the corpus callosum (mm^3) by a blinded researcher. Total volume of tissue loss was calculated as:

$$volume\ of\ tissue\ loss = volume\ of\ normal\ hemisphere - volume\ of\ injured\ hemisphere,$$

$$volume\ of\ normal\ hemisphere = average\ (area\ of\ hemisphere - area\ of\ ventricle - area\ of\ lesion)\ x\ interval\ between\ sections\ x\ \#\ of\ sections$$

$$volume\ of\ injured\ hemisphere = average\ (area\ of\ hemisphere - area\ of\ ventricle - area\ of\ lesion)\ x\ interval\ between\ sections\ x\ \#\ of\ sections$$

To assess white matter atrophy, the corpus callosum in each hemisphere was traced from AP: +1.5 to -0.5, with landmarks identified using the WHS rat brain atlas (v1.01, RRID: SCR_017124).[51] This location encompassed 1 mm of tissue anterior and posterior to the site of collagenase injection and was reliably present in all brains. Volume of corpus callosum in each hemisphere was calculated as:

$$volume\ of\ corpus\ callosum = average\ (area\ of\ corpus\ callosum)\ x\ interval\ between\ sections\ x\ \#\ of\ sections$$

2.2.9 Statistical Analysis

In experiment 1, group sizes were selected based on previous work.[29] In experiment 2, group sizes were determined by a priori power calculation with reference

to the most common marker of neuroprotection, and our primary endpoint, lesion volume. Using effect size and variance reported in similar work,[25] group sizes of n=24 were calculated to give at least 80% power to detect a 30% reduction in lesion volume with alpha set at 0.05.

All data was analyzed using GraphPad Prism (version 9.3.1 for Mac, GraphPad Software, San Diego, California). Baseline data was analyzed using ANOVA to assess group differences; when no baseline differences were found, all raw data was analyzed and reported. In experiment 1, two-way ANOVA was used to assess baseline reaching data, ladder walking success, rehab intensity, and hematoma volume. Three-way ANOVA was used to compare repeated measures reaching data. Beam walking data was analyzed using the Kruskal-Wallis test at baseline and day 14. In experiment 2, one-way ANOVA was used to assess baseline reaching data, hemispheric differences in corpus callosum, and lesion volume. Two-way ANOVA was used to compare repeated measures reaching data. When multiple groups were compared, ANOVA with Tukey's or Sidak's post hoc test was used. Data are reported as mean ± 95% confidence interval (CI), except for the beam walking data which are reported as median ± interquartile range (IQR).

2.3 Results

2.3.1 Mortality & Exclusions

See Table 2-1 for analyzed group sizes and exclusions; note that no animals were excluded based on their post-injury level of impairment.

There was no unexpected mortality in experiment 1. Ten animals were fully excluded from analysis, while an additional 5 animals were partially excluded and removed from analysis (beam test, n=2; ladder walking task, n=3).

In experiment 2, 2 animals died unexpectedly during surgery, presumably due to complications with anaesthetic dosage. Seven animals were partially excluded and removed from analysis (skilled reaching, n=3; lesion volume, n=1; corpus callosum volume, n=3).

2.3.2 Experiment 1

2.3.2.1 Reaching success

There was no significant difference in baseline reaching abilities among groups (Figure 2-2A; light cycle, $p=0.8910$; treatment, $p=0.8491$; interaction, $p=0.2626$). As anticipated, all groups were impaired on day 4 after ICH (time main effect, $p<0.0001$) and impairment persisted to day 14 ($p<0.0001$, two-way ANOVA with Tukey's multiple comparisons test). Three-way ANOVA detected a main effect of time ($p<0.0001$) but no main effect of light cycle ($p=0.3286$), treatment ($p=0.6246$), or any interaction ($p\geq0.1371$). While the ER-D10 group retrieved the greatest number of pellets on average at day 14, this result was not significantly different from the other groups ($p\geq0.2171$, two-way ANOVA with Tukey's multiple comparisons test).

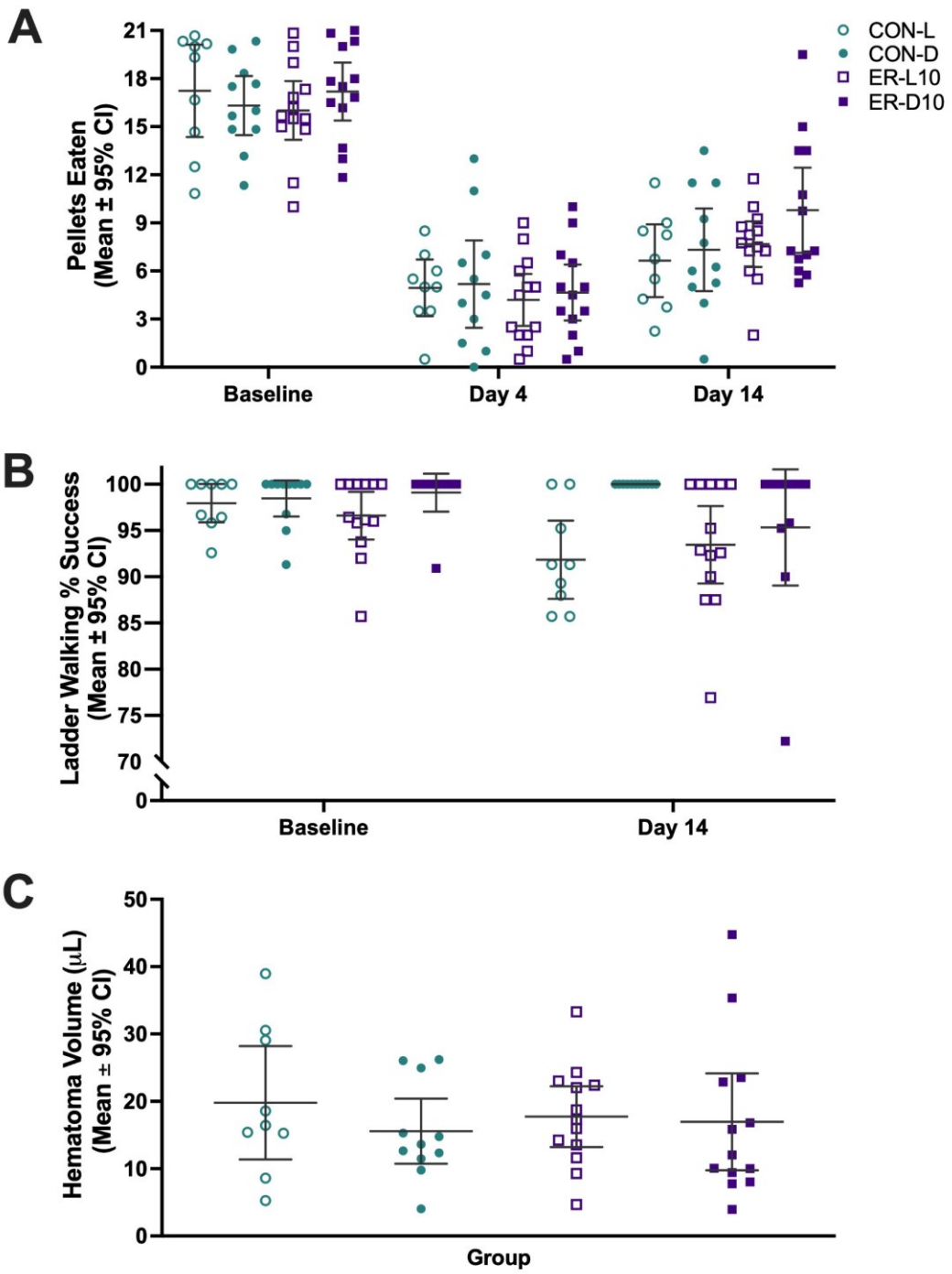

Figure 2-2 Results of Experiment 1. **A** Reaching success in staircase task. All groups displayed notable impairment after ICH (vs. baseline, persistent out to 14 days) but no effect of light cycle or treatment was detected. **B** Percent success of correct paw placement of contralateral forelimb on the ladder walking task. Three-way ANOVA detected a main effect of time and light cycle but not treatment. **C** Residual hematoma volume (µL) measured 14 days after ICH. Two-way ANOVA did not detect an effect of light cycle or treatment. Individual data points are shown along with the mean ± 95% CI

2.3.2.2 Rehabilitation Intensity

Rats gradually increased pellets retrieved over time (time main effect, $p<0.0001$), (Figure 2-3A). Light cycle did not alter reaching intensity (light cycle main effect, $p=0.2918$), however an interaction was present ($p=0.0015$). Sidak's multiple comparisons test determined the groups were significantly different only at day 14 ($p=0.0167$). Rats tended to reach somewhat more on the initial daily rehab session, and this declined over the four sessions within each day of rehab, presumably as they got satiated. This pattern of results varied modestly over days and groups, but without any meaningful pattern or obvious explanation for the failure to find behavioural improvements. The average number of pellets retrieved per trial from day 5-14 was 118.3 (95% CI: 97.71, 138.8) in the ER-L10 group and 107.1 (95% CI: 94.95, 119.3) in the ER-D10 group.

2.3.2.3Beam Walking Task

All groups performed similarly at baseline ($p=0.5855$) and at day 14 ($p=0.6729$). Median beam walking score was 7 (7-7 IQR) in all groups at baseline. Nearly all animals received a perfect test score at day 14, suggesting that the beam walking assessment was not particularly sensitive to our injury. Median beam walking score was 7 (7-7 IQR) in ER-D10, ER-L10, and CON-D and 7 (6.5-7 IQR) in CON-L (data not shown). Rehab did not impact beam walking success.

2.3.2.4 Ladder Walking Task

Two-way ANOVA showed all groups performed similarly at baseline (light cycle, $p=0.1530$; treatment, $p=0.7316$; interaction, $p=0.3441$). With no baseline differences,

raw data were analyzed (Figure 2-2B). Three-way ANOVA detected a time main effect ($p=0.0095$) and light cycle ($p=0.0060$) but failed to detect a treatment effect ($p=0.4062$) or interactions ($p\geq0.0570$). Animals in the dark performed better than animals in the light, and this effect was likely driven by the uniform scores in the CON-D group at day 14. Owing to heterogeneity of variance in some of these data, we further analyzed it with several statistical tests (e.g., t-tests and non-parametric statistics). Post-hoc analysis revealed that the ladder task was not particularly sensitive to the stroke, and as such, we could not clearly assess whether rehab impacted walking success. For instance, there was no significant time effect (baseline vs. day 14) for just the control groups, nor was there any evidence of benefit with either rehab treatment on the test day (statistics not shown).

2.3.2.5 Hematoma volume

Residual hematoma volume at day 14 was ~17 μL on average (Figure 2-2C). Two-way ANOVA did not detect an effect of light cycle ($p=0.3874$), treatment ($p=0.9073$), or interaction ($p=0.5478$). Thus, rehab did not impact hematoma resolution.

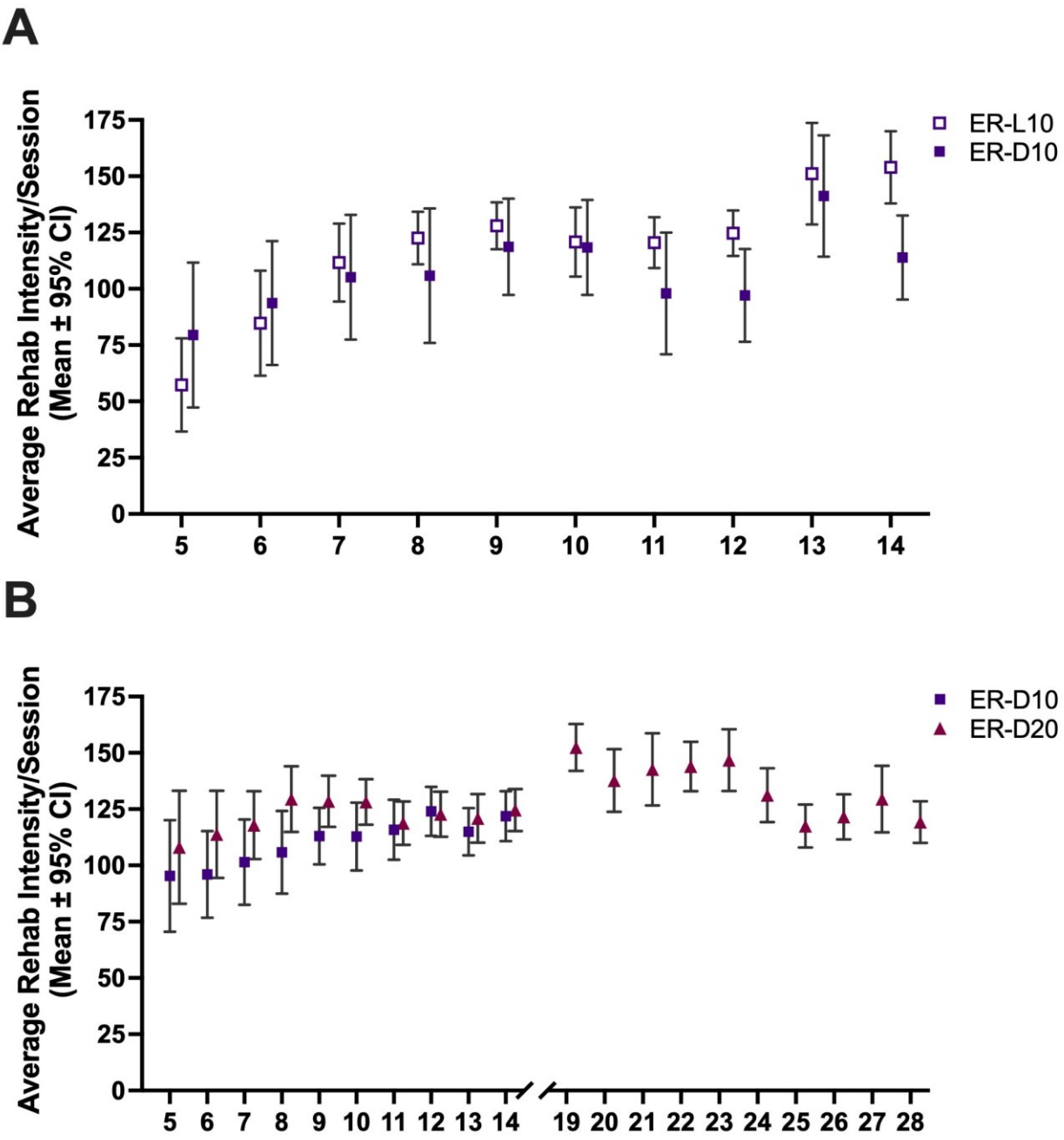

Figure 2-3 Rehabilitation Intensity. Figures represent the average number of pellets successfully retrieved per training session on each day. **A** Experiment 1: rats in ER-L10 and ER-D10 groups completed 4 daily rehab training sessions days 4-12 and 2 sessions on days 13-14. **B** Experiment 2: rats in ER-D10 and ER-D20 groups completed 4 daily rehab training sessions days 4-14. Rats in ER-20 completed an additional 10 days of ER days 19-28. All data presented as mean ± 95% CI

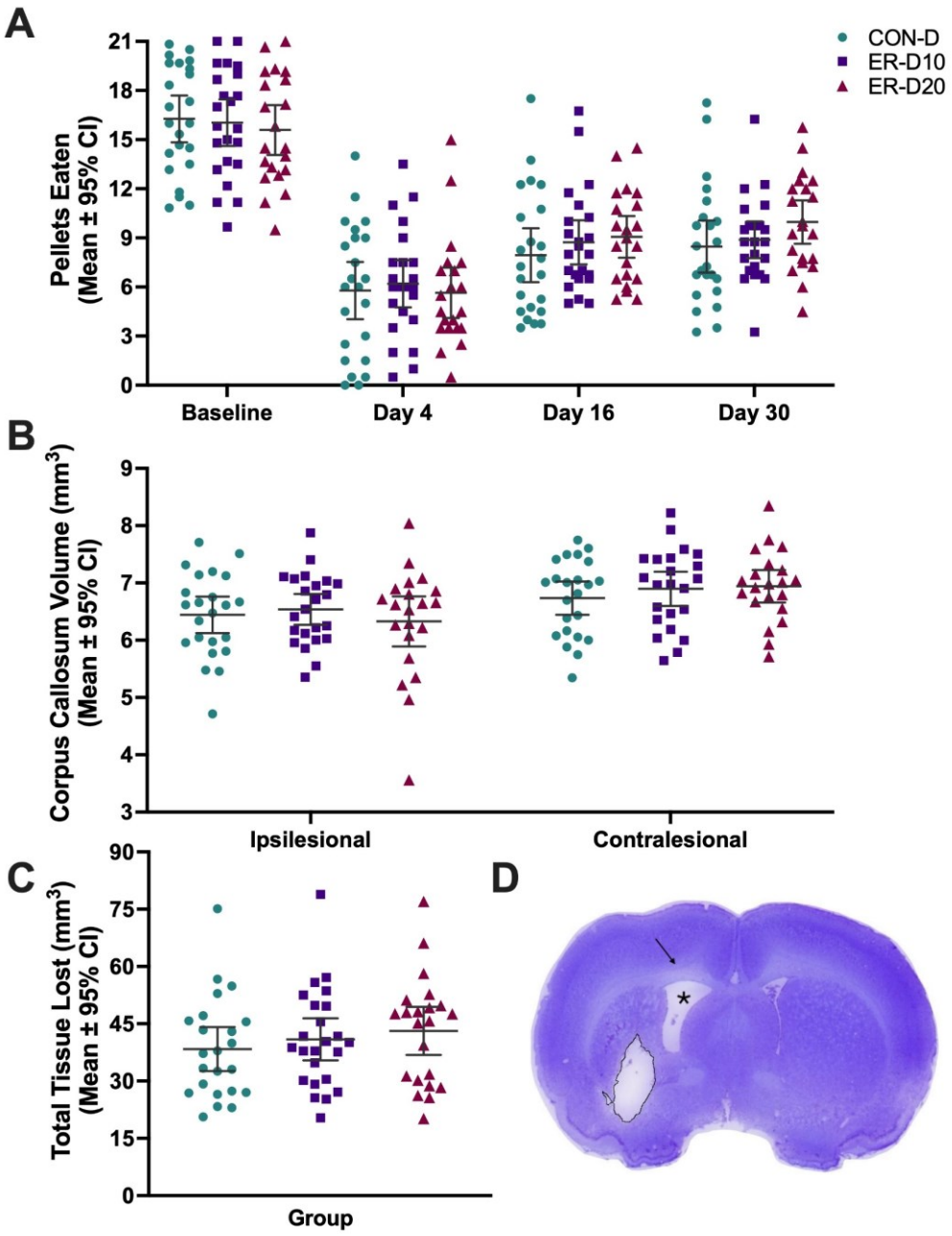

Figure 2-4 Results of Experiment 2. **A** Reaching success in the staircase task was not improved by rehab. All groups displayed notable impairment after ICH (day 4). Multiple comparisons showed a main effect of time at all levels of comparison, except between day 16 and 30. We failed to detect an effect of treatment. **B** The volume of ipsilesional corpus callosum was smaller than contralateral; no effect of treatment was detected. **C** Lesion Volume (mm^3) measured 60 days after ICH; no effect of treatment was detected. **D** Coronal section of rat brain at 60 days displaying lesion cavity (outlined in black), ipsilesional ventriculomegaly (*), and atrophy of ipsilesional corpus callosum (black arrow). Subject in image had a total lesion volume of 38.8 mm^3, approximately the average observed across groups. Individual data points are shown along with the mean ± 95% CI

2.3.3 Experiment 2

2.3.3.1 Reaching Success

All groups performed similarly at baseline ($p=0.7906$, Figure 2-4A). As anticipated, all groups displayed impairment after ICH (time main effect, $p<0.0001$) but we failed to detect a treatment effect ($p=0.8275$) or interaction ($p=0.3673$). Tukey's multiple comparisons test showed a main effect of time at all levels of comparison ($p<0.0001$), except between day 16 and 30 ($p=0.1293$). Thus, rehab did not improve reaching success, which mirrors the results from our 14-day survival experiment.

2.3.3.2 Rehabilitation Intensity

The average number of pellets retrieved per trial from day 5-14 was 110.2 (95% CI: 103.1, 117.4) in the ER-D10 group and 121.3 (95% CI: 116.3,126.2) in the ER-D20 group and did not differ significantly on any day ($p \geq 0.3556$; Figure 2-3B). The average number of pellets retrieved per trial from day 19-28 was 134.4 (95% CI: 125.7, 143.1). As in Experiment 1, rats tended to reach somewhat more on the initial daily rehab session, and this declined over the four sessions within each day of rehab, presumably as they got satiated. Again, this pattern of results varied modestly over days and groups, but without any meaningful pattern.

2.3.3.3 White Matter Quantification

The volume of corpus collosum remaining in the ICH hemisphere was significantly smaller than in the contralesional hemisphere ($p=0.001$) in all groups. No effect of treatment was detected (treatment, $p=0.6898$; interaction, $p=0.5495$, Figure 2-4B).

2.3.3.4 Volume of Tissue Lost

All groups had significant tissue loss at 60 days as a result of cell death in striatum, white matter loss, and atrophy (Figure 2-4C). Treatment duration did not impact the volume of tissue lost ($p=0.5021$), with an average of ~40 mm^3 volume across groups.

2.3.4 Post-Hoc Pooled Analysis

Data from both experiments were pooled into ER10 (n=70) and CON (n=43). A two-way ANOVA revealed a main effect of time ($p<0.0001$), but no significant treatment effect ($p=0.5318$) or interaction ($p=0.066$). Compared to baseline, rats retrieved 10.85 fewer pellets (95% CI: 9.82, 11.88) on day 4, and 7.93 fewer pellets (95% CI: 6.96, 8.90) on day 14/16. Groups were not significantly different after ICH (day 4, $p=0.999$; day 14/16, $p=0.146$); treatment effect size on day 14/16 was an improvement of 1.32 pellets retrieved (95% CI: -0.31, 2.95) vs. day 4). Thus, rehab did not notably change reaching success. This analysis had >99% power to detect a 3-pellet difference (one level of the staircase), a minimum effect we believe would signify biological importance.

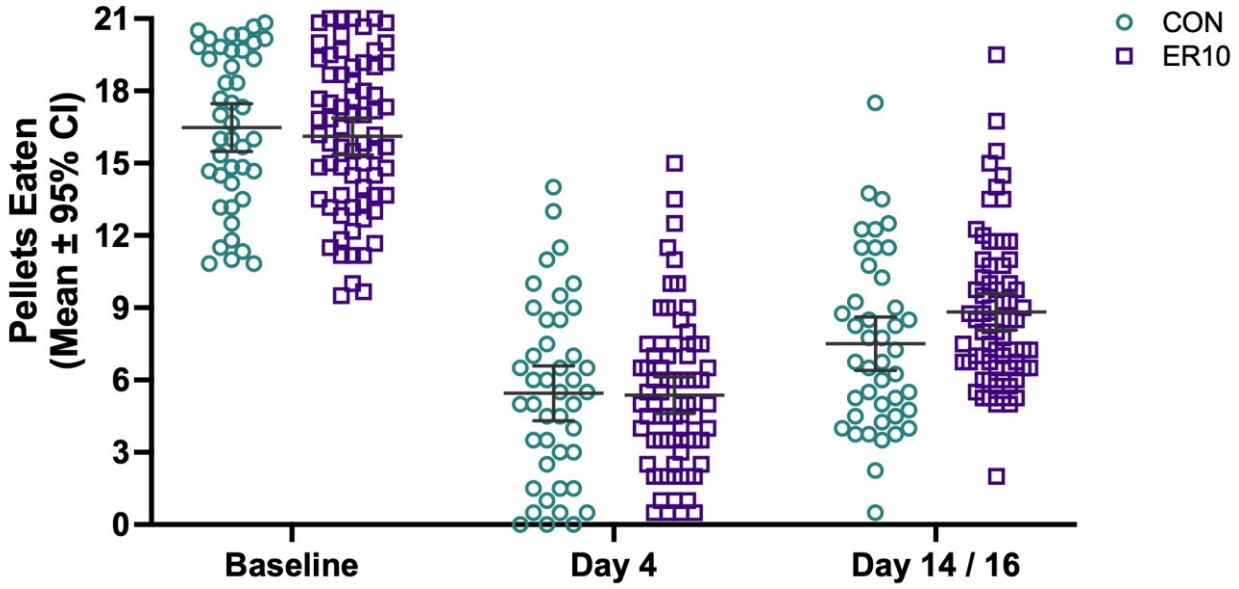

Figure 2-5 Post-hoc analysis of pooled data. Data from both experiments were pooled into ER10 (n=70) and CON (n=43) groups. A main effect of time (stroke-induced impairment) was readily evident on day 4 (10.85 fewer pellets retrieved [95% CI: 9.82, 11.88] vs. baseline), and on day 14/16 (7.93 fewer pellets retrieved [95% CI: 6.96, 8.90] vs. baseline). No significant effect of treatment was present (treatment effect size on day 14/16 was an improvement of 1.32 pellets retrieved [95% CI: -0.31, 2.95] vs. day 4). Despite >99% statistical power to detect a 3-pellet difference, one level of the staircase task and presumably a minimum biologically meaningful effect, groups were not statistically different from each other after ICH. Individual data points are shown along with the mean ± 95% CI

2.4 Discussion

Despite high-intensity training at a level and complexity comparable to previous work,[20,52] three rehab protocols (ER-D10, ER-L10, ER-D20) failed to improve behavioral recovery after striatal ICH on our primary behavioural endpoint: skilled reaching. Contrary to previous work,[25,29] rehab did not affect hematoma resolution or cell death. Our results highlight the difficulty of improving functional recovery of skilled movements after striatal bleeds, and the difficulty with reproducing or extending previous work. The latter undoubtedly contributes to translational failures.[39]

ER has been accepted as an effective intervention after experimental ischemic[24,26,27] and hemorrhagic strokes.[13,20,25] Likewise, other rehab methods, such as EE[53] and running[14,54] have been found beneficial. However, with these protocols, negative findings have been reported, and likely there are similar unpublished data. For instance, EE was of minimal benefit in several ICH studies[55,56] and forced running was ineffective in one study.[23] Studies also vary in the choice of behavioural tests and the timing of assessment; these factors may influence the apparent level of benefit observed (e.g., one test may be more sensitive to smaller treatment effects). Additionally, timing of assessment, differing sensitivity to lesion size, and degree of transference between rehab and skill assessment tasks may account for some of the reported differences in functional outcomes. Negative findings are generally dismissed owing to potential statistical (e.g., lack of power and bad luck), model (e.g., stroke severity), assessment (e.g., type and timing of testing), and treatment protocol issues (e.g., timing and intensity of rehab). Notwithstanding the aforementioned factors, the imprecise estimates of treatment effects, arising from small sample sizes, should not be underestimated for its contribution to study-to-study variability in outcome. In this study, we expected all of our ER treatments to improve skilled reaching given that comparable studies reported substantial effects in this ICH model[20,25,52] and the autologous whole blood model.[13] Since we used similar or identical methods (ICHs, rehab methods, and assessments) to past studies, one might surmise that statistical issues were at play, including bad luck or inadequate power. On the former, we made four independent tests of whether ER improves reaching and assessed both early (~14 days) and late (~31 days) into recovery. Further, we explored whether increasing ER treatment from 10 to 20 days would improve outcome. It did not. The net result yields

no evidence for benefit despite multiple comparisons employing large groups sizes. Additional post-hoc analysis of ER and CON data pooled from both experiments (>99% power to detect a 3-pellet difference) showed only a slight non-significant trend in skilled reaching improvement in favour of ER when measured ~2 weeks after ICH, which is not reasonably expected to be of biological significance. As such, the publication of negative data is crucial[39] to be considered along with other data in meta-analyses in order to accurately gauge treatment efficacy. As well, only with definitive evidence of efficacy can we truly make progress on confidently attributing cause to any mechanism(s) of action.

Our histological analysis showed typical ICH damage including: a lesion cavity, ventriculomegaly and hemispheric atrophy. Contrary to earlier work[25,29] and regardless of duration, ER failed to reduce injury. Use of ER also did not affect hematoma resolution in contrast to Williamson et al.'s study, where ER beginning one week after ICH substantially accelerated hematoma clearance in two experiments.[29] As previous work characterizing recovery after ICH demonstrated comparable behavioural impairment and tissue loss,[3] we are confident that we induced a moderate ICH in these studies that should have been amenable to treatment (e.g., no floor or ceiling effects in the reaching task). Despite using ER during a well-established period of ongoing injury,[2,4,52] the absence of behavioural and histological benefit in this study may ultimately stem from the lack of effect on hematoma clearance for whatever reason. If so, we surmise that when hematoma clearance is accelerated, injury is attenuated and behaviour is improved.

Interestingly, we found no meaningful difference in the average rehab intensity between groups who completed intervention in the light or dark phase of their housing

cycles. This differs from MacLellan and colleagues, who found that following ischemia, rats completing ER during the dark phase were more engaged in rehab than those that completed the same task in the light phase.[27] It should be noted that not all rehab protocols successfully replicate beneficial findings – in fact, the MacLellan study initially investigated rehab in the standard light cycle and failed to obtain benefit. As rats in the dark group completed ~230 successful reaches vs. ~150 in the light, it was postulated that a certain threshold of intensity must be met to drive recovery. Rats in our experiments certainly exceeded this threshold, often reaching successfully more than 400 times/day. Perhaps treatment-induced recovery after ICH differs from ischemia, including that high therapeutic intensity may negatively impact rehab efficacy. Location is also likely a factor, with striatal injury perhaps less amenable to rehab than injury involving the cortex.[27]

Many consider earlier interventions to be more beneficial to recovery than delayed interventions,[26] with early mobilization being a common recommendation in clinical guidelines.[57] However, the best timing, intensity, and frequency of intervention is yet to be elucidated. Evidence from the AVERT clinical trial found that higher dose, early, intense out of bed mobilization in the first days after stroke may reduce odds of favourable outcome.[58] Subgroup analysis showed this effect to be most prominent for those with severe stroke and ICH,[58] with further analysis showing that increased frequency (number of mobilization events) but not increased intensity (time out of bed) was associated with improved outcomes.[59] While other pre-clinical studies have reported benefit of various rehab paradigms beginning as early as 1 day after ICH, many fail to report the key parameters necessary to interpret treatment dose, such as treatment frequency and intensity. This poses a challenge to researchers and clinicians

attempting to translate pre-clinical findings into clinical success. Here, we utilized a well-established therapy, with minor modifications to previously published work. Animals actively engaged with this treatment (both in reaching and EE) and overall achieved a high dose of therapy beginning at a time thought to be safe, yet we were unable to find comparable results to similar studies. Perhaps differences among treatment protocols (e.g., a day 5 vs. day 7 start; 6 hours EE vs. 10 hours EE; training sessions spaced 1.5h vs. 2h apart; treatment in light vs. dark) underlie study outcome differences (e.g., longer EE may provide additional benefits). However, these findings likely speak to the "replication crisis" of biomedical research, where reducing variability in our groups to achieve higher internal validity, often comes at the sacrifice of external validity and therapeutic translation.[60] If so, these results speak to the finicky and challenging nature of translating therapeutic parameters to work in humans.

2.5 Conclusion

Despite group sizes up to three times the average typically reported in pre-clinical ICH neuroprotection research (n=8),[38] our results show that intense ER beginning 5 days after ICH failed to improve outcome when assessed at day 14, during a period of ongoing cell death, and out to day 60, after cell death is believed to be complete. These results underscore the importance of studying rehab after ICH, and the necessity for future work to be conducted with higher power and factors that may impact translation in mind.

2.6 References

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Chapter 3 | Motor rehabilitation provides modest functional benefits after intracerebral hemorrhage: a systematic review and meta-analysis of translational rehabilitation studies

3.1 Introduction

Stroke is a leading cause of death and disability worldwide,[1] with >12 million cases reported annually.[2]Despite representing only 10-20% of all cases, hemorrhagic stroke is responsible for ~60% of the global burden of stroke.[3,4] Intracerebral hemorrhage (ICH), caused by the rupture of cerebral vasculature and bleeding into the brain, is particularly devastating due to high mortality and disability. Analysis of burden of disease by stroke subtype highlights the disproportionate impact of ICH: while ischemia is associated with 4.6-5.9 disability-adjusted-life-years (DALYs),[5] ICH ranges from 8.1-12.6 DALYs.[6] Although advances in the diagnosis, treatment, and management of stroke have led to decreased mortality in recent decades, 50-60% of survivors live with persistent impairment or disability.[4,7] Tasks that require high levels of dexterity or motor coordination can be challenging for survivors, as ~80% will experience some degree of transient or permanent paresis in one or more limbs.[8] As a result, many survivors live with impairments that limit participation in activities of daily living, making functions that enable independence (e.g., walking, reaching, grasping, and using the impaired limb) common targets of rehabilitation.

Neurorestorative interventions, such as physical and occupational therapy, attempt to harness principles of experience dependent plasticity to restore, recruit, and retrain circuitry in the injured brain,[9] thereby improving function and lessening disability. Several motor rehabilitation interventions have been used in preclinical settings to gain insight into functional and neurological recovery after ICH. Environmental enrichment (EE; or enriched housing) is social housing that introduces novel elements (e.g., toys, tubes, running wheels, ramps, multiple levels) to create a more stimulating cage environment. Early studies of EE after ischemic stroke found that

treated rodents showed greater behavioural recovery compared to those in social housing alone or solo housing with running wheel access.[10] Skilled reach training (REACH) uses massed practice of forelimb fine motor skills through repetition of tasks like the Montoya Staircase test,[11] tray task,[12] and single pellet reaching task.[12] Two related therapies, forced limb use (FLU) and constraint-induced movement therapy (CIMT), involve restraint of the unimpaired limb to encourage use of the impaired limb, thereby preventing learned non-use.[13] Unlike FLU, CIMT pairs restraint with task specific training (e.g., REACH) and/or an exercise (EX) battery (e.g., REACH, wheel running, ladder walking, etc.) to maximize treatment efficacy through massed practice. Aerobic exercise (AE) is a running-based intervention; under voluntary exercise conditions, animals have free access to a running apparatus (e.g., running wheel) over a designated period. In contrast, under forced exercise, animals are placed into a running apparatus (e.g., treadmill, rotarod, rotating wheel) where the device is set to a prespecified speed or distance over the intervention period. Enriched rehabilitation (ER), combines REACH with EE to synergize the effects of both therapies.[14] Finally, acrobatic training (AT; or motor skills training) is a complex rehabilitation paradigm comprised of elevated rope ladder walking, elevated grid platform walking, traversing a thick rope, traversing parallel bars, and crossing a series of irregular platforms.[15]

Current guidelines recommend all individuals begin rehabilitation once medically stable and able to actively participate in treatment.[16] However, despite numerous promising clinical trials and preclinical studies exploring rehabilitation after stroke, few certainties exist regarding optimal treatment type, timing, or dose.[17] Clinical studies must often rely on surrogate measures to explore mechanisms of recovery, making preclinical studies often better suited for exploration of mechanisms, in part owing to

the complexity, cost, and ethics of conducting such research in patients. Although an essential component of post-stroke care for all patients, most insight into treatment and recovery after stroke has been gained from animal models of cerebral ischemia.[18] These studies provide evidence for a critical period after stroke where endogenous repair processes are heightened and rehabilitation interventions are most effective,[19] a phenomenon later supported with clinical evidence.[20] Preclinical studies have also demonstrated that early and intense rehabilitation can exacerbate injury and worsen functional outcomes after cortical lesion, likely triggered by use-dependent responses.[21,22] However, when given with a short delay, others have reported that a critical threshold of intensity must be met for rehabilitation to mediate functional recovery.[23] No single experimental model of injury can perfectly reproduce the heterogeneous clinical pathology and presentation of stroke; therefore, these experimental findings may not hold true across all types of brain injury. Although some have explored the effect of post-stroke rehabilitation on functional recovery, neuroprotection, or neuroplasticity,[24–26] a comprehensive review of the effects of post-stroke rehabilitation on recovery of motor function has not been conducted for preclinical ICH. Owing to fundamental differences in mechanisms of injury between ischemia and ICH (e.g., greater role of mechanical injury and neurotoxicity in ICH) and the differential impact of additional mediators (e.g., post-stroke fever),[27] calls for subtype specific exploration of treatment and rehabilitative therapies are well justified.[28,29]

This systematic review and meta-analysis aimed to: (1) identify and characterize common motor rehabilitation interventions used after preclinical *in vivo* models of ICH;

(2) assess the scientific and translational quality of this literature; and (3) analyze the efficacy of post-ICH rehabilitation on recovery of motor function.

3.2 Methods

Our search protocol was developed using PRISMA guidelines and adapted from the PICOS (Patients, Intervention, Comparison, Outcomes, Study designs) framework and registered with PROSPERO (CRD42021227134). The search strategy was developed to identify all articles that used an animal population to model ICH (P), a post-stroke motor rehabilitation intervention (I), compared to no-treatment (C), and evaluated rehabilitation efficacy in at least one motor outcome (O) following experimental induction of ICH (S).

Table 3-1 Search Term Keywords

Search	Term	Keywords
S1	Rehabilitation	rehabilitation OR rehab OR exercise OR motor-therapy OR physical-therap* OR physiotherap* OR aerobic-training OR running OR walking OR treadmill* OR constraint-induced-movement-therapy OR mobilization OR mobilisation OR forced-use-therapy OR enrichment OR environmental-enrichment OR enriched-rehabilitation OR training OR reach* OR grasp*
S2	Stroke Type	cerebral-hemorrhage* OR cerebral-haemorrhage* OR intracerebral-hemorrhage* OR intracerebral- haemorrhage* OR intracranial-hemorrhage* OR intracranial-haemorrhage* OR intracerebral-bleed OR cerebral-hematoma* OR hemorrhagic-stroke* OR haemorrhagic-stroke*
S3	Population	rat OR rats OR mouse OR mice OR rodent* OR primate OR canine OR murine OR non-human OR animal-model

3.2.1 Search Strategy

An electronic records search of the databases Academic Search Complete, Medline, EMBASE, CINAHL, and PubMed Central was completed on March 12, 2021, and again December 14, 2022, to identify all eligible records published up to December 14, 2022. Search terms (Table 3-1) were compiled by subject: rehabilitation, stroke type, and population. To ensure accuracy, search term formatting was tailored to each database (see Appendix A). Results were entered into Covidence software (Veritas Health Innovation, Melbourne, Australia) and duplicates were removed. Two reviewers (BF, MM/FC) screened titles and abstracts against *a priori* criteria (Table 3-2); articles proceeded to full-text review in cases of disagreement. Full-text review was completed by two reviewers (BF, MM/FC); disagreements were discussed, and if agreement was not reached a tie-breaking vote was completed (FC).

Table 3-2 Inclusion and Exclusion Criteria for Abstract and Full-Text Screening

Screening	Include	Exclude
Abstract	1. Stroke type is ICH 2. Study type is animal 3. Therapy is motor rehabilitation intervention	1. Stroke type is not ICH 2. Not an animal study 3. No behavioural intervention *If unclear from abstract, study continued to full text review
Full Text	1. Stroke type is ICH 2. Study type is animal 3. Therapy is motor rehabilitation intervention 4. Motor outcome assessment present post-treatment 5. Control group present 6. Full text available in English	1. Stroke type is not ICH 2. Not an animal study 3. No post-stroke behavioural intervention 4. No motor outcome assessment post-treatment 5. No appropriate comparator group present 6. Full text unavailable in English

3.2.2 Inclusion and Exclusion Criteria

Articles that used an *in vivo* animal model of ICH regardless of species, strain, co-morbidities, or ICH model were eligible; clinical studies, *in vitro* studies, or *in vivo* studies that did not include motor assessment or were completed in a non-ICH animal model of stroke were ineligible. If rehabilitation was delivered pre-stroke, not a motor intervention, failed to assess motor function (e.g., learning or memory task, physiological outcome), or was paired with an adjuvant treatment (e.g., drug, hypothermia) it was ineligible. Articles that did not have an appropriate comparator group (i.e., no treatment group), only compared to another rehabilitation intervention, or were unavailable in English were excluded.

3.2.3 Data Extraction

Descriptive characteristics were extracted by one reviewer (BF) and validated by a second (MM/NS). Extracted characteristics included author names, publication year, animal population (species, strain, sex, age, co-morbidities), ICH model, anaesthetic, survival time(s), use of blinding and randomization, rehabilitation type, behavioural outcomes measured (e.g., reaching success, ladder walking error rate, spontaneous forelimb use, etc.), and histological outcomes of severity (i.e., lesion volume, hematoma volume). Treatment parameters (i.e., type, timing, period, duration, frequency, intensity, and dose) were operationalized (Table 3-3) to create a standardized terminology for interpreting and extracting data.[30] Extracted parameters were used to calculate total treatment dose and reported as the total number of repetitions or running distance achieved over the intervention period and total time in treatment (i.e., hours).

Efficacy of post-ICH rehabilitation on motor function was our primary meta-analytical endpoint. A motor outcome was eligible for meta-analysis if data were available from ≥3 articles that assessed the same domain of recovery (e.g., skilled reaching) using the same or equivalent tasks (e.g., reaching success in the staircase task or single pellet task). Motor outcomes were grouped into forelimb, locomotor, and composite neurobehavioural assessments. Forelimb assessments included skilled reaching success (e.g., staircase test, single pellet task) and spontaneous use of the impaired forelimb (i.e., cylinder task). Locomotor assessments included walking success (e.g., success or error rate in ladder walking, beam walking score), walking speed, and distance travelled. Composite neurobehavioural assessments included global impairment rating scales such as the neurological deficit score (NDS), motor deficit score (MDS), and modified neurological severity score (mNSS). While test batteries and scoring systems differ among these assessments, all rate performance in multiple tests to create a single score representing impairment across several functional domains (e.g., paw asymmetry, grip strength, mobility, balance, response to stimuli, etc.). Mean and standard deviation (SD) were extracted for all treatment and control groups for parametric data (i.e., skilled reaching, ladder walking), with median and interquartile range (IQR) extracted for non-parametric data (i.e., beam walking, composite neurobehavioural tests). When data was not explicitly reported, values were measured and calculated from figures using WebPlotDigitizer (version 4.6, Ankit Rohatgi, 2022).

As reporting multiple experiments or intervention groups within the same article is common in preclinical rehabilitation, group sizes, treatment parameters, outcomes, and timing of outcome assessment were extracted for each intervention within an article

that met inclusion criteria. When multiple intervention groups were extracted from an article, groups were identified as Author (year)a, Author (year)b, etc.

Table 3-3 Standardized Terminology and Definitions for Preclinical Rehabilitation Interventions

Parameter	Definition	Report As
Type	Activities/tasks that make up the intervention and how they are delivered	Descriptive characteristics
Timing	Onset of rehabilitation after ICH	Hours, days, or weeks after stroke induction (ICH surgery=day 0)
Period	Time over which the intervention occurs (between onset and end of therapy)	Hours, days, or weeks
Duration	The length of a single treatment session, defined for each activity/task in the intervention	Mins, hours, days, or weeks
Frequency	How often the treatment was administered within the treatment period, defined for each activity/task in the intervention	Sessions/day and days/week
Intensity	A measure that provides an estimate of treatment participation/exertion, defined for each activity/task in the intervention	AE: walking/running speed (m/s), walking/running distance (m) EE: time in enrichment (hours/day) FLU: time in restraint (hours/day) REACH: number of repetitions (average number of pellets retrieved per trial) AT: walking distance (m), number of repetitions ER: see EE + REACH EX: see AE, REACH; may also require walking distance (m) and/or number of repetitions CIMT: see FLU + REACH
Total Treatment Dose	The total amount of treatment received over the intervention period, reported for each activity/task in the intervention; calculated using treatment period, duration, frequency, and intensity	AE: total walking/running distance (m) EE: total time in enrichment (hours) FLU: total time in restraint (hours) REACH: total number of repetitions completed (pellets retrieved) AT: total walking distance (m), total number of repetitions ER: see EE + REACH EX: see AE, REACH; may also require total walking distance (m) and/or total number of repetitions CIMT: see FLU + REACH

3.2.4 Study Quality and Risk of Bias

Study quality was assessed using the CAMARADES checklist,[31] with articles rated as yes, unclear, or no for their compliance. Two small modifications were made to the checklist to adapt it for our use: blinded ICH-induction or post ICH-randomization (checklist item 4) and inclusion of comorbidities relevant to ICH (checklist item 7) such as old age, hypertension, diabetes, etc. The SYRCLE Risk of Bias tool and accompanying signalling questions were used to judge each article across multiple domains of bias.[32] Articles were rated for each domain as low-, unclear-, or high risk. Caregiver blinding (performance bias) was not rated, as it is near impossible for preclinical researchers to be blinded to rehabilitation delivery. A rating of unclear was given when reviewers deemed there was insufficient and/or inconsistent reporting of detail to accurately judge compliance with the checklist item or signalling question. For both CAMARADES and SYRCLE assessments, two independent reviewers (BF/NS) rated each article, with rating disagreements resolved through discussion. Owing to inclusion of several articles from the authors' laboratory, FC was excluded from the assessment process to prevent unpublished details from influencing reviewer judgements.

3.2.5 Statistical Analysis

Statistical analyses were conducted in R (v.4.3.0; R Core Team, 2023) using RStudio (v.2023.3.1.446; Posit Team, 2023) and the tidyverse,[33] meta,[34] and dmetar packages.[35,36] Due to variations in experimental designs and intervention protocols, effect sizes were calculated using random effects meta-analysis with the DerSimonian-Laird estimator and the inverse variance method for weighting. Subgroups were determined by intervention type (AT, AE, CIMT+FLU, ER, REACH) – if an intervention

type was explored in <3 articles, it was relegated to OTHER. Rehabilitation efficacy was assessed overall and by subtype for three domains of motor recovery: skilled reaching, spontaneous impaired forelimb use, and locomotor function. To account for small samples sizes and variations in methodology, skilled reaching and ladder walking effect sizes were calculated as Hedge's *G* standardized mean difference (SMD) with 95% confidence intervals (CI).[37] When necessary (i.e., when ladder data was reported as error rates), a correction factor of -1 was applied to the data to maintain consistency in direction of effect across interventions.[37] As all articles that assessed spontaneous impaired forelimb use in the cylinder task reported results as percent impaired forelimb use, effect sizes were calculated as mean difference (MD) with 95% CI. Egger regression was used to assess asymmetry in the funnel plots and possible publication bias; trim-and-fill analysis was conducted if asymmetry was detected. *A priori* sensitivity analyses were conducted to evaluate the impact of study quality on both treatment efficacy and heterogeneity in our results – interventions from articles that scored <4 on the CAMARADES checklist were removed and the updated datasets were re-analyzed as above. To explore the impact of experimental design and treatment parameters on rehabilitation efficacy, secondary analyses were completed for each endpoint using subgroups differentiated by timing of treatment onset, stroke severity, total treatment dose, and CAMARADES score.

3.3 Results

Our search identified 1124 articles (944 March 2021, 180 December 2022). Following screening and full-text review, 30 articles met the eligibility criteria (Figure 3-1).

3.3.1 Descriptive Characteristics

Experimental design characteristics were generally homogenous (Table A-1 Appendix A). All used rodents (Figure 3-2a) <1 year old; 28/30 used males, whereas 1 used females, and 1 was unspecified. The collagenase model was heavily favored (29/30) over the autologous whole blood model, with injury predominately targeting the striatum (26/30). Thirteen articles reported ≥2 eligible intervention groups (i.e., not confounded by adjuvant treatments), resulting in the identification of 48 rehabilitation interventions that assessed efficacy of post-ICH rehabilitation on motor recovery (Figure 3-2b). Histological assessment of stroke severity (lesion or hematoma volume) was included in the methods of 34/48 interventions; however, we could not identify the full range of stroke severity studied as results were often unreported or unclear (e.g., assessed in one brain slice). Rehabilitation interventions were grouped into six categories: AE,[38–47] ER,[48–53] CIMT+FLU,[54–58] REACH,[59–62] AT,[15,63,64] and OTHER (complex exercise,[54] EE,[65] walking,[59,61] swimming[66]). Treatment onset ranged from 6 hours to 17 days post-ICH. Most interventions assessed efficacy in ≥2 behavioural endpoints (35/48); timing of latest functional efficacy assessment ranged from 25 hours to 60 days. Total time spent in treatment ranged from 2 hours to 49 days. Table 3-4 describes the modifiable treatment parameters, total treatment dose, largest group size analyzed in functional endpoints, and comparator group for each of the 48 interventions.

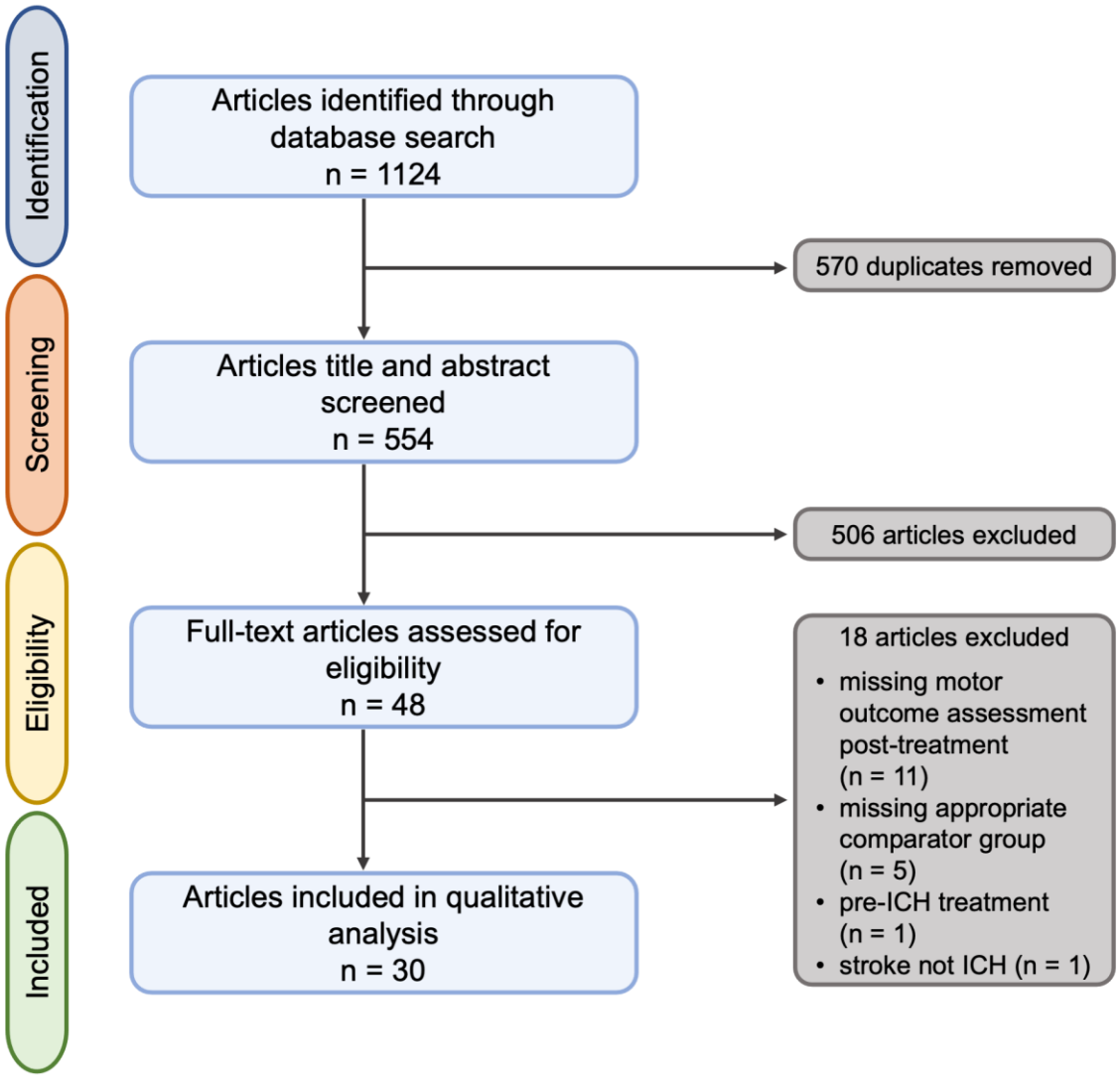

Figure 3-1 PRISMA flowchart of records identified through database searching

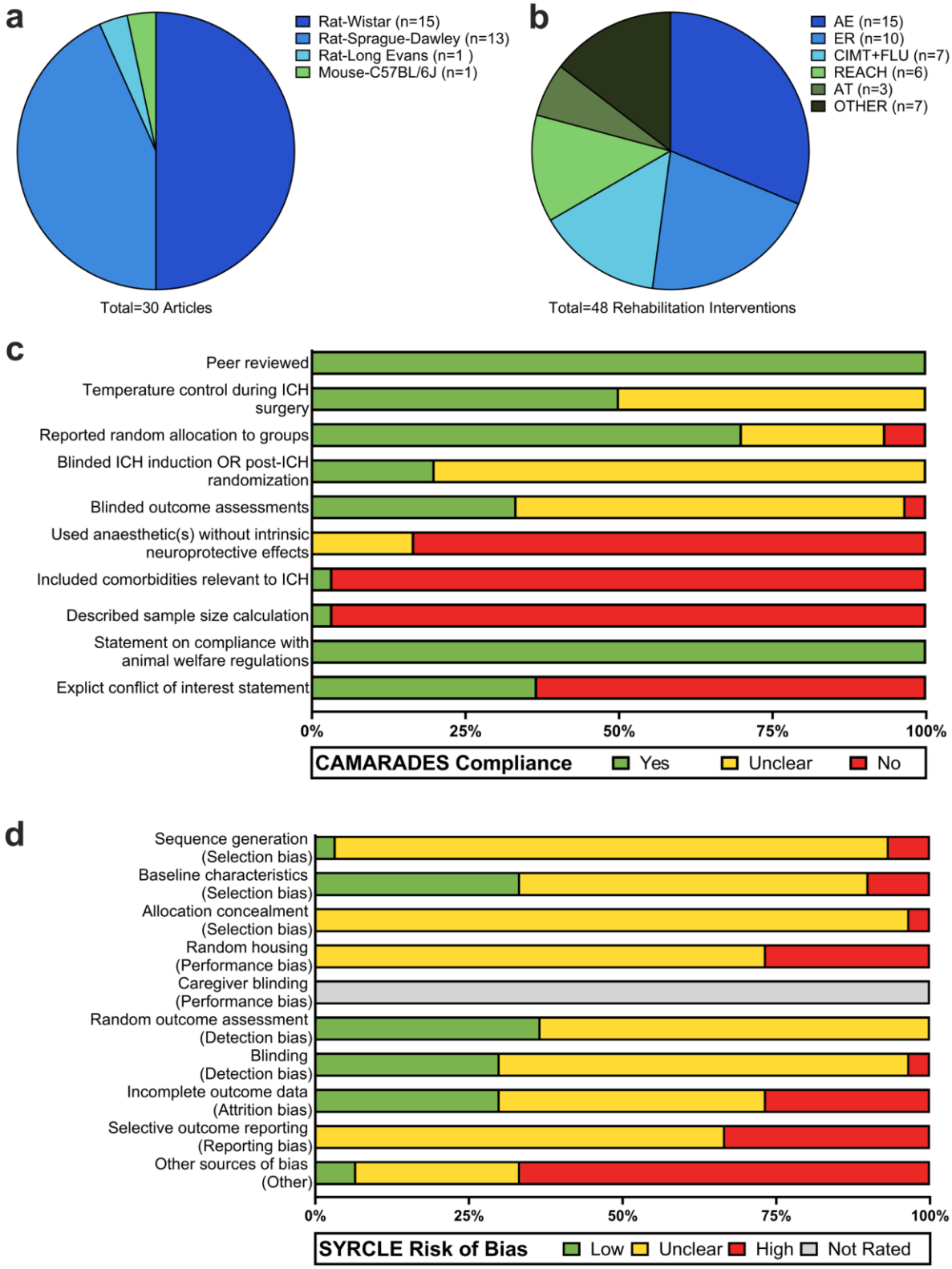
a
Rat-Wistar (n=15)
Rat-Sprague-Dawley (n=13)
Rat-Long Evans (n=1)
Mouse-C57BL/6J (n=1)
Total=30 Articles
b
AE (n=15)
ER (n=10)
CIMT+FLU (n=7)
REACH (n=6)
AT (n=3)
OTHER (n=7)
Total=48 Rehabilitation Interventions
c
Peer reviewed
Temperature control during ICH surgery
Reported random allocation to groups
Blinded ICH induction OR post-ICH randomization
Blinded outcome assessments
Used anaesthetic(s) without intrinsic neuroprotective effects
Included comorbidities relevant to ICH
Described sample size calculation
Statement on compliance with animal welfare regulations
Explict conflict of interest statement
0%
25%
50%
75%
100%
CAMARADES Compliance
Yes
Unclear
No
d
Sequence generation (Selection bias)
Baseline characteristics (Selection bias)
Allocation concealment (Selection bias)
Random housing (Performance bias)
Caregiver blinding (Performance bias)
Random outcome assessment (Detection bias)
Blinding (Detection bias)
Incomplete outcome data (Attrition bias)
Selective outcome reporting (Reporting bias)
Other sources of bias (Other)
0%
25%
50%
75%
100%
SYRCLE Risk of Bias
Low
Unclear
High
Not Rated

Figure 3-2 Summary of experimental characteristics, study quality, and risk of bias in eligible articles (n=30). **A** Model population (species and strain); no article reported use of multiple species or strains. **B** Breakdown of the types of rehabilitation interventions (n=48) used after preclinical ICH where 48 unique intervention groups were identified across 30 articles. **C** Summary of article quality assessed by compliance with 10 item CAMARADES checklist (n=30). Article quality ranged considerably (2-8), with a median score of 4. **D** Summary of SYRCLE Risk of Bias tool (n=30). Risk of bias was predominately unclear, as articles often lacked sufficient detail to determine how/if risk of bias was minimized

Table 3-4 Descriptive Characteristics of Rehabilitation Interventions

Author Year	Type [n]	Intervention Description	Onset [Period]	Frequency	Duration (Session)	Intensity	Treatment Dose	Control, Housing [n]	Lesion Location [Volume]
DeBow 2003a[54]	FLU [n=9]	Unimpaired limb restrained	7 days [7 days]	FLU [1 continuous session daily]	FLU [8 hours]	FLU [8 hours/day]	FLU [56 hours]	ICH, solo housed [n=11*] *shared DeBow 2003 a,b,c	Striatum [44 mm^3 at 60 days]
DeBow 2003b[54]	CIMT [n=11]	Unimpaired limb restrained + Exercise [REACH (tray task), cylinder, ladder walking, wheel running]	7 days [7 days]	FLU [1 continuous session daily] EX [1 session daily]	FLU [8 hours] EX* [1 hour] *REACH [30 mins]; cylinder [10 mins]; ladder walking [10 mins]; AE [10 mins]	FLU [8 hours/day] EX* [varied] *REACH [repetitions NR]; cylinder [NR]; ladder walking [3x 1m ladder crosses/session]; AE [10 m in 10 mins]	FLU [56 hours] EX* [7 hours] *REACH [repetitions NR; 3.5 hours]; cylinder [70 mins]; ladder walking [21 m, 70 mins]; AE [70 m, 70 mins]	ICH, solo housed [n=11*] *shared DeBow 2003 a,b,c	Striatum [44 mm^3 at 60 days]
DeBow 2003c[54]	EX [n=9]	Exercise [REACH (tray task), cylinder, ladder walking, wheel running]	7 days [7 days]	EX [1 session daily]	EX* [1 hour] *REACH [30 mins]; cylinder [10 mins]; ladder walking [10 mins]; AE [10 mins]	EX* [varied] *REACH [repetitions NR]; cylinder [NR]; ladder walking [3x 1m ladder crosses/session]; AE [10 m in 10 mins]	EX* [7 hours] *REACH [repetitions NR; 3.5 hours]; cylinder [70 mins]; ladder walking [21 m, 70 mins]; AE [70 m, 70 mins]	ICH, solo housed [n=11*] *shared DeBow 2003 a,b,c	Striatum [44 mm^3 at 60 days]
MacLellan 2005[55]	CIMT [n=15]	Unimpaired limb restrained + Exercise [REACH (tray task), wheel running]	14 days [7 days]	FLU [1 continuous session daily] EX [1 session daily]	FLU [8 hours] EX* [1 hour] *REACH [30 mins]; AE [30 mins]	FLU [8 hours/day] EX* [varied] *REACH [repetitions NR]; AE [speed NR]	FLU [56 hours] EX* [7 hours] *REACH [repetitions NR, 3.5 hours]; AE [distance NR, 3.5 hours]	ICH, housing unknown [n=15]	Striatum [81 mm^3 at 60 days]

Author Year	Type [n]	Intervention Description	Onset [Period]	Frequency	Duration (Session)	Intensity	Treatment Dose	Control, Housing [n]	Lesion Location [Volume]
Auriat 2006[38]	AE [n=17]	Forced running [Motorized wheel]	14 days [14 days]	AE [1 session daily, 5 days/week]	AE [60 mins]	Week 1 [5.5 m/min for 60 mins], week 2 [5.5 m/min for 5 min, 11 m/min for 55 mins]	AE [4812.5 m, 10 hours]	ICH, solo housed [n=17]	Striatum [91 mm^3 at 49 days]
Auriat 2008[48]	ER [n=14]	EE housing + Exercise [REACH (tray task), beam walking]	5 days [EE], 7 days [EX] [25 days [EE], 5 days [EX]	EE [1 continuous session] EX [1 session daily day 7, 9, 11]	EE [24 hours] EX* [30+ mins] *REACH [30 mins]; beam walking [NR]	EE [24 hours/day (excluding training)] EX* [varied] *REACH [repetitions NR]; beam walking [5x 1.1m beam crosses/session]	EE [~600 hours] EX* [~1.5 hours] *REACH [repetitions NR, 1.5 hours]; beam walking [16.5m, time NR]	ICH, group housed [n=15]	Striatum [40 mm^3 at 30 days]
Nguyen 2008[65]	EE [n=14]	EE housing	7 days [49 days]	EE [1 continuous session]	EE [24 hours]	EE [24 hours/day]	EE [1176 hours]	ICH, group housed [n=16]	Striatum [68 mm^3 at 57 days]
Auriat 2009[49]	ER [n=16]	EE housing + REACH [modified Montoya staircase]	7 days [14 days]	EE [1 continuous session daily] REACH [4 sessions daily, 5 days/week]	EE [15 hours] REACH [15 mins]	EE [15 hours/day] REACH [repetitions NR]	EE [150 hours] REACH [repetitions NR, 10 hours]	ICH, pair housed [n=16]	Striatum [32 mm^3 at 46 days]
Auriat 2010a[50]	ER [n=13]	EE housing + REACH [modified Montoya staircase]	7 days [14 days]	EE [1 continuous session daily] REACH [4 sessions daily, 5 days/week]	EE [15 hours] REACH [15 mins]	EE [15 hours/day] REACH [repetitions NR]	EE [150 hours] REACH [repetitions NR, 10 hours]	ICH, group housed [n=13]	Striatum [not assessed]

Author Year	Type [n]	Intervention Description	Onset [Period]	Frequency	Duration (Session)	Intensity	Treatment Dose	Control, Housing [n]	Lesion Location [Volume]
Auriat 2010b[50]	ER [n=16]	EE housing + REACH [modified Montoya staircase]	7 days [14 days]	EE [1 continuous session daily] REACH [4 sessions daily, 5 days/week]	EE [15 hours] REACH [15 mins]	EE [15 hours/day] REACH [repetitions NR]	EE [150 hours] REACH [repetitions NR, 10 hours]	ICH, group housed [n=16]	Striatum [28 mm^3 at 32 days]
Takamatsu 2010[39]*	AE [n=NR]	Forced running [treadmill]	4 days [11 days]	AE [1 session daily]	AE [30 mins]	AE [9 m/min]	AE [2970 m, 5.5 hours]	ICH, housing unknown [n=NR]	Striatum [~60% of striatal volume lost at 15 days]
Ishida 2011[56]	FLU [n=8]	Unimpaired limb restrained	24 hours [7 days]	FLU [1 continuous session]	FLU [24 hours]	FLU [24 hours/day]	FLU [168 hours]	ICH, group housed [n=9]	Internal capsule [7 mm^3 at 37 days]
MacLellan 2011[51]	ER [n=16]	EE housing + REACH [modified Montoya staircase]	7 days [14 days]	EE [1 continuous session daily] REACH [4 sessions daily with 2-hour interval, 5 days/week]	EE [15 hours] REACH [15 mins]	EE [15 hours/day] REACH [repetitions NR]	EE [150 hours] REACH [repetitions NR, 10 hours]	ICH, group housed [n=14]	Striatum [~8% tissue loss in ipsilesional hemisphere at 49 days]
Mestriner 2011a[59]	REACH [n=12]	REACH [modified Montoya staircase]	7 days [28 days]	REACH [1 session daily, 5 days/week]	REACH [40 mins]	REACH [repetitions NR]	REACH [repetitions NR, 13 hours 20 mins]	ICH, group housed [n=12*] *shared Mestriner 2011a,b	Striatum [56 mm^3 at 28 days]

Author Year	Type [n]	Intervention Description	Onset [Period]	Frequency	Duration (Session)	Intensity	Treatment Dose	Control, Housing [n]	Lesion Location [Volume]
Mestriner 2011b[59]	WALK [n=12]	Walk training [treadmill]	7 days [28 days]	WALK [1 session daily, 5 days/week]	WALK [40 mins]	WALK [1.8 m/min]	WALK [1440 m, 13 hours 20 mins]	ICH, group housed [n=12*] *shared Mestriner 2011a,b	Striatum [56 mm^3 at 28 days]
Kim 2012a[60]*	REACH [n=15]	REACH [single pellet task]	Unclear [unclear]	REACH [1 session daily, 6 days/week]	REACH [15 mins]	REACH [repetitions NR]	Cannot determine	ICH, housing unknown [n=15*] *shared Kim 2012a,b	Striatum [lesion ~12% of total brain volume, timing unclear]
Kim 2012b[60]*	REACH -ipsi [n=15]	REACH [single pellet task - unimpaired paw]	Unclear [unclear]	REACH [1 session daily, 6 days/week]	REACH [15 mins]	REACH [repetitions NR]	Cannot determine	ICH, housing unknown [n=15*] *shared Kim 2012a,b	Striatum [lesion ~12% of total brain volume, timing unclear]
Santos 2013a[61]	REACH [n=8]	REACH [single pellet task]	7 days [28 days]	REACH [1 session daily, 5 days/week]	REACH [40 mins]	REACH [repetitions NR]	REACH [repetitions NR, 13 hours 20 mins]	ICH, group housed [n=8*] *shared Santos 2013a,b	Striatum [unclear – analysis conducted in one tissue slice]
Santos 2013b[61]	WALK [n=8]	Walk training [treadmill]	7 days [28 days]	WALK [1 session daily, 5 days/week]	WALK [40 mins]	WALK [1.8 m/min]	WALK [1440 m, 13 hours 20 mins]	ICH, group housed [n=8*] *shared Santos 2013a,b	Striatum [unclear – analysis conducted in one tissue slice]

Author Year	Type [n]	Intervention Description	Onset [Period]	Frequency	Duration (Session)	Intensity	Treatment Dose	Control, Housing [n]	Lesion Location [Volume]
Caliaperumal 2014[52]	ER [n=11]	EE housing + REACH [modified Montoya staircase]	7 days [14 days]	EE [1 continuous session daily] REACH [4 sessions daily with 2-hour interval, 5 days/week]	EE [15 hours] REACH [15 mins]	EE [15 hours/day] REACH [repetitions NR]	EE [150 hours] REACH [repetitions NR, 10 hours]	ICH, group housed [n=11]	Striatum [not assessed]
Tamakoshi 2014[15]*	AT [n=6]	Traverse 5 acrobatic courses [rope ladder, platform grid, single rope, parallel bars, series of barriers]	4 days [25 days]	AT [4 sessions daily]	AT [NR]	AT [5 courses/ session, 1m/ course]	AT [500 m (100 crosses/course), time NR]	ICH, housing unknown [n=8]	Striatum [unclear – likely reporting error]
Yong 2014a[62]*	REACH [n=NR]	REACH [single pellet task]	NR [unclear]	REACH [1 session daily, 6 days/week]	REACH [15 mins]	REACH [repetitions NR]	Cannot determine	ICH, housing unknown [n=NR]	Striatum [not assessed]
Yong 2014b[62]*	REACH [n=NR]	REACH [single pellet task]	NR [unclear]	REACH [1 session daily, 6 days/week]	REACH [15 mins]	REACH [repetitions NR]	Cannot determine	ICH, housing unknown [n=NR]	Striatum [not assessed]
Ishida 2015a[57]	FLU [n=8]	Unimpaired limb restrained	24 hours [7 days]	FLU [1 continuous session]	FLU [24 hours]	FLU [24 hours/day]	FLU [168 hours]	ICH, housing unknown [n=9]	Globus pallidus [8 mm^3, timing unclear]
Ishida 2015b[57]	FLU [n=6]	Unimpaired limb restrained	17 days [7 days]	FLU [1 continuous session]	FLU [24 hours]	FLU [24 hours/day]	FLU [168 hours]	ICH, housing unknown [n=9]	Globus pallidus [8 mm^3, timing unclear]

Author Year	Type [n]	Intervention Description	Onset [Period]	Frequency	Duration (Session)	Intensity	Treatment Dose	Control, Housing [n]	Lesion Location [Volume]
Ishida 2016[58]	FLU [n=7]	Unimpaired limb restrained	24 hours [7 days]	FLU [1 continuous session]	FLU [24 hours]	FLU [24 hours/day]	FLU [168 hours]	ICH, group housed [n=6]	Internal capsule [not assessed]
Takamatsu 2016[40]	AE [n=14]	Forced running [treadmill]	4 days [11 days]	AE [1 session daily]	AE [30 mins]	AE [9 m/min]	AE [2970 m, 5.5 hours]	ICH, housing unknown [n=14]	Striatum [not assessed]
Tamakoshi 2016[63]	AT [n=6]	Traverse 5 acrobatic courses [rope ladder, platform grid, single rope, parallel bars, series of barriers]	4 days [25 days]	AT [4 sessions daily]	AT [NR]	AT [5 courses/ session, 1m/ course]	AT [500 m (100 crosses/course), time NR]	ICH, group housed [n=7]	Striatum [not assessed]
Tamakoshi 2017[64]	AT [n=6]	Traverse 5 acrobatic courses [rope ladder, platform grid, single rope, parallel bars, series of barriers]	4 days [25 days]	AT [4 sessions daily]	AT [NR]	AT [5 courses/ session, 1m/ course]	AT [500 m (100 crosses/course), time NR]	ICH, housing unknown [n=6]	Striatum [assessed, NR]
Tamakoshi 2018a[41]*	AE [n=8]	Forced running [treadmill]	2 days [14 days]	AE [1 session daily]	AE [30 mins]	AE [9 m/min day 1, 11 m/min remainder]	AE [4560 m, 7 hours]	ICH, housing unknown [n=8*] *shared Tamakoshi 2018 a,b,c	Striatum [assessed, NR]

Author Year	Type [n]	Intervention Description	Onset [Period]	Frequency	Duration (Session)	Intensity	Treatment Dose	Control, Housing [n]	Lesion Location [Volume]
Tamakoshi 2018b[41]*	AE [n=6]	Forced running [treadmill]	2 days [7 days]	AE [1 session daily]	AE [30 mins]	AE [9 m/min day 1, 11 m/min remainder]	AE [2250 m, 3.5 hours]	ICH, housing unknown [n=8*] *shared Tamakoshi 2018 a,b,c	Striatum [assessed, NR]
Tamakoshi 2018c[41]*	AE [n=6]	Forced running [treadmill]	8 days [7 days]	AE [1 session daily]	AE [30 mins]	AE [9 m/min day 1, 11 m/min remainder]	AE [2250 m, 3.5 hours]	ICH, housing unknown [n=8*] *shared Tamakoshi 2018 a,b,c	Striatum [assessed, NR]
Sato 2020a[42]	AE [n=8]	Forced running [treadmill]	4 days [25 days]	AE [4 sessions daily with 60 min interval]	AE [30 mins]	AE [10 m/min]	AE [30 000 m, 50 hours]	ICH, housing unknown [n=10*] *shared Sato 2020a,b	Striatum [not assessed]
Sato 2020b[42]	AE [n=8]	Voluntary running [wheel in home cage]	4 days [25 days]	AE [1 continuous session]	AE [600 hours]	AE [Mean distance 1224 ± 86m/day]	AE [30 600 m, 600 hours]	ICH, housing unknown [n=10*] *shared Sato 2020a,b	Striatum [not assessed]
Tamakoshi 2020a[43]*	AE [n=23]	Forced running [treadmill]	2 days [7 days]	AE [1 session daily]	AE [60 mins]	AE [9 m/min day 1, 11 m/min remainder]	AE [4500 m, 7 hours]	ICH, group housed [n=24*] *shared Tamakoshi 2020a,b	Striatum [unclear – likely reporting error]

Author Year	Type [n]	Intervention Description	Onset [Period]	Frequency	Duration (Session)	Intensity	Treatment Dose	Control, Housing [n]	Lesion Location [Volume]
Tamakoshi 2020b[43]*	AE [n=22]	Forced running [treadmill]	9 days [7 days]	AE [1 session daily]	AE [60 mins]	AE [9 m/min day 1, 11 m/min remainder]	AE [4500 m, 7 hours]	ICH, group housed [n=24*] *shared Tamakoshi 2020a,b	Striatum [unclear – likely reporting error]
Xu 2020a[44]	AE [n=11]	Forced running [treadmill]	2 days [13 days]	AE [1 session daily]	AE [30 mins]	AE [16 m/min]	AE [6240 m, 6.5 hours]	ICH, housing unknown [n=11*] *shared Xu 2020a,b	Striatum [not assessed]
Xu 2020b[44]	AE [n=11]	Forced running [treadmill – fatigue controlled]	2 days [13 days]	AE [1 session daily]	AE [30 mins*] *if animal exceeded fatigue threshold, 3 min rest, then session continued (repeated until 30 mins running achieved)]	AE [16 m/min]	AE [6240 m, 6.5 hours]	ICH, housing unknown [n=11*] *shared Xu 2020a,b	Striatum [not assessed]
Tamakoshi 2021[45]	AE [n=13]	Forced running [treadmill]	6 hours [1 day]	AE [2 sessions in first 24 hours post-ICH (6 and 24 hours)]	AE [60 mins]	AE [9 m/min at 6 hours post-ICH, 11 m/min at 24 hours post-ICH]	AE [1200 m, 2 hours]	ICH, pair housed [n=14]	Striatum [hematoma ~12% of total brain volume at 27 hours]

Author Year	Type [n]	Intervention Description	Onset [Period]	Frequency	Duration (Session)	Intensity	Treatment Dose	Control, Housing [n]	Lesion Location [Volume]
Fedor 2022a[53]	ER [n=13]	EE housing + REACH [modified Montoya staircase] *all interventions in light phase of housing cycle	5 days [10 days]	EE [1 continuous session daily] REACH [4 sessions daily with 1.5-hour interval, days 5-12, 2 sessions daily with 1.5-hour interval days 13-14]	EE [6 hours] REACH [15 mins]	EE [6 hours/day] REACH [mean pellets retrieved 118/session]	EE [60 hours] REACH [mean pellets retrieved 4248, 9 hours]	ICH, group housed in light phase [n=9]	Striatum [hematoma volume 20 µL at 14 days]
Fedor 2022b[53]	ER [n=13]	EE housing + REACH [modified Montoya staircase] *all interventions in dark phase of housing cycle	5 days [10 days]	EE [1 continuous session daily] REACH [4 sessions daily with 1.5-hour interval, days 5-12, 2 sessions daily with 1.5-hour interval days 13-14]	EE [6 hours] REACH [15 mins]	EE [6 hours/day] REACH [mean pellets retrieved 107/session]	EE [60 hours] REACH [mean pellets retrieved 3852, 9 hours]	ICH, group housed in dark phase [n=11]	Striatum [hematoma volume 16 µL at 14 days]
Fedor 2022c[53]	ER [n=23]	EE housing + REACH [modified Montoya staircase] *all interventions in dark phase of housing cycle	5 days [10 days]	EE [1 continuous session daily] REACH [4 sessions daily with 1.5-hour interval]	EE [6 hours] REACH [15 mins]	EE [6 hours/day] REACH [mean pellets retrieved 110/session]	EE [60 hours] REACH [mean pellets retrieved 4400, 10 hours]	ICH, group housed in dark phase [n=23*] *shared Fedor 2022c,d	Striatum [38 mm^3 at 60 days]

Author Year	Type [n]	Intervention Description	Onset [Period]	Frequency	Duration (Session)	Intensity	Treatment Dose	Control, Housing [n]	Lesion Location [Volume]
Fedor 2022d[53]	ER [n=21]	EE housing + REACH [modified Montoya staircase] *all interventions in dark phase of housing cycle	5 days [24 days]	EE [1 continuous session daily] REACH [4 sessions daily with 1.5-hour interval days 5-14, 19-28]	EE [6 hours] REACH [15 mins]	EE [6 hours/day] REACH [mean pellets retrieved 121/session days 5-14, 134/session days 19-28]	EE [120 hours] REACH [mean pellets retrieved 10 200, 20 hours]	ICH, group housed in dark phase [n=23*] *shared Fedor 2022c,d	Striatum [38 mm^3 at 60 days]
Inoue 2022[46]	AE [n=8]	Forced running [treadmill]	7 days [21 days]	AE [1 session daily, 5 days/week]	AE [30 mins]	AE [12 m/min]	AE [5400 m, 7.5 hours]	ICH, housing unknown [n=8]	Internal capsule [14 mm^3 at 29 days]
Li 2022a[66*]	SWIM [n=10]	Continuous swimming	2 days [7 days]	SWIM [1 session daily]	SWIM [30 mins]	NR	SWIM [3.5 hours]	ICH, group housed [n=10]	Striatum [unclear – likely reporting error]
Li 2022b[66*]	SWIM [n=10]	Continuous swimming	2 days [14 days]	SWIM [1 session daily]	SWIM [30 mins]	NR	SWIM [7 hours]	ICH, group housed [n=10]	Striatum [unclear – likely reporting error]
Li 2022c[66*]	SWIM [n=12]	Continuous swimming	2 days [7 days]	SWIM [1 session daily]	SWIM [30 mins]	NR	SWIM [3.5 hours]	ICH, group housed [n=12]	Striatum [unclear – likely reporting error]
Tamakoshi 2022[47]	AE [n=14]	Forced running [treadmill]	6 hours [6 days]	AE [2 sessions in first 24 hours post-ICH (6 and 24 hours), 1 session daily days 2-6]	AE [60 mins]	AE [9 m/min at 6 hours post-ICH, 11 m/min for remainder]	AE [4500 m, 7 hours]	ICH, pair housed [n=16]	Striatum [~8% of total brain volume at 8 days]

[39]* group sizes for behavioural endpoints not reported
[60]* imprecise timeline, treatment dose could not be calculated
[15]* lesion data reported as volume of tissue lost, but appears to be volume of tissue remaining or based on an unspecified region of interest
[62]* group sizes not reported, total N listed as 30 in abstract and 20 in methods; imprecise timeline, treatment dose could not be calculated
[43]* lesion data is uninterpretable
[66]* calculation of hematoma volume appears to be off by a factor of 100-1000; unclear if hematoma volume is calculated as % brain or % hemisphere
Abbreviations: AE, aerobic exercise; AT, acrobatic training; CIMT, constraint-induced movement therapy; EE, environmental enrichment; ER, enriched rehabilitation; FLU, forced limb use; m, metres; MDS, motor deficit score; mNSS modified neurological severity score; NR, not reported; NDS, neurological deficit score; REACH, skilled reach training; REACH-ipsi, skilled reach training in unimpaired forelimb; SWIM, swim training; WALK, walk training

3.3.2 Study Quality

The CAMARADES checklist analysis revealed a wide range in scores (2-8), with a median score of 4/10, indicating low or unclear study quality (Figure 3-2c). Interrater reliability was high (weighted kappa=0.912), indicating almost perfect agreement between reviewers. All articles (30/30) scored a point for peer review and including a statement on compliance to animal welfare regulations. Temperature control during ICH induction was reported in 15/30 articles, with the remainder unclear. Use of randomization was reported in 21/30 articles. A conflict-of-interest statement was reported in 11/30 articles. Blinding was inconsistent and often vague or poorly described. Only 6/30 articles reported blinding to treatment allocation during stroke induction or that animals were randomized to treatment after stroke. Similarly, only 10/30 articles explicitly reported blinded assessment of subjective outcomes (e.g., neurological deficit assessments, walking errors, lesion volume); many articles were judged unclear, due to poor reporting and/or inconsistent use of blinding across endpoints. Only 1/30 articles included a population with comorbidity (ovariectomized female rats, menopause). Likewise, 1/30 articles described the use of a sample size calculation. No article used an anaesthetic without potential neuroprotective properties.[67] Individual article ratings are in Figure A-1 (Appendix A).

3.3.3 Risk of Bias

Analysis using the SYRCLE tool revealed unclear risk of bias in many articles (Figure 3-2d). Assessment of interrater reliability indicated substantial agreement between reviewers (weighted kappa=0.753). While most articles addressed one or more factors related to selection, performance, detection, attrition, or reporting bias,

information was often insufficient to determine how risk of bias was minimized (see Table 3-5 for common errors). Several articles reported manipulating housing conditions as part of treatment (e.g., EE housing). Based on SYRCLE guidelines, these articles received a high-risk rating for performance bias – however, we would argue that this is less indicative of a high risk of performance bias, but rather an intended treatment effect. Many articles failed to adequately address incomplete outcome data, resulting in our attrition bias assessment being approximately equal among each category of low-, unclear-, or high-risk of bias. These judgements were driven by unclear reporting of total N, group sizes, exclusions, and mortality, resulting in insufficient data to judge risk of bias. Approximately one third of articles were rated high risk of reporting bias due to selective outcome reporting. No articles reported the use of a pre-registered protocol (judged as unclear), whereas many failed to report data from some groups and/or specific endpoints or assessment times and were rated as high-risk. Other potential sources of bias identified included unit of analysis errors, improper use of statistical methods, and poor methodology. Individual article ratings are in Figure A-2 (Appendix A).

Table 3-5 Commonly Observed Errors in Preclinical ICH Rehabilitation Literature

Category	Incidence	Error
Reporting Errors	17%	Failure to report total number of animals used (N)
	40%	Failure to report housing conditions (i.e., solo, paired, group, EE)
	93%	Failure to describe the method of randomization/allocation to group/subgroup (e.g., random number generator)
	60%	Failure to explicitly report exclusions and mortality (including group identity and reason for exclusion)
	17-23%*	Failure to report group sizes (n) used in analysis after mortality/exclusion and division into subgroups/endpoints
	7-13%*	Methods describe endpoints not presented in the results (or vice versa)
	10%	Type of summary statistics not provided (e.g., mean ± SD)
Methodological Errors	97%	Group size not determined using a power calculation
	20-47%*	Total N reported is not equal to the total of n's reported in methods
	20-47%*	Addition of extra subjects (i.e., sum total n>N)
	57%	Baseline data not assessed and/or not reported
	23-30%*	Data not collected and/or presented for subjects/groups in one or more endpoint with no explanation
	20-23%*	Inappropriate methods of statistical analysis (i.e., using parametric tests on non-parametric data, only comparing to sham, ignoring significant baseline differences, handling of outliers)
	53-93%*	Unit of analysis errors related to housing (i.e., analyzing animals as independent samples instead of by cage)
	47%	Improper data presentation and/or reporting (i.e., figures uninterpretable, missing error terms, describing ordinal data with mean ± SD)

*lower value of range represents % of articles with confirmed error while upper value represents % of articles possibly containing error but with insufficient reporting to conclusively determine

3.3.4 Efficacy of Rehabilitation on Skilled Reaching

Twenty-four interventions assessed efficacy of rehabilitation on recovery of skilled reaching (Figure 3-3). Overall, rehabilitation improved skilled reaching [SMD 0.75 (95% CI 0.50-1.01), $p<0.01$] and heterogeneity was moderate ($I^2=45\%$, $p=0.01$). Subgroup analysis by rehabilitation type found a significant effect of CIMT+FLU [SMD

0.90 (95% CI 0.30-1.50), p<0.01], ER [SMD 0.69 (95% CI 0.35-1.03), p<0.01], and REACH [SMD 2.12 (95% CI 0.76-3.48), p<0.01]. Aerobic exercise failed to significantly improve outcome. Sensitivity analysis revealed a similar overall treatment effect on skilled reaching [SMD 0.67 (95% CI 0.42-0.91), p<0.01] with non-significant heterogeneity (I^2=30%, p=0.11). Interestingly, only ER and REACH subgroups remained significant in the sensitivity analysis (Figure A-3 Appendix A).

Funnel plot and Egger regression confirmed the presence of asymmetry in the dataset (p<0.01), therefore trim-and-fill analysis was completed (Figure A-4 Appendix A). Hypothetical data (n=5) added by trim-and-fill analysis revealed missing negative and null data, suggesting reporting or publication bias. Random-effects meta-analysis of the trim-and-fill model (n=29) produced a smaller treatment effect [SMD 0.59 (95% CI 0.32-0.87), p<0.01].

To conceptualize efficacy beyond statistical testing, we completed a post-hoc analysis using only interventions that reported the number of pellets retrieved in their respective skilled reaching tasks (Figure A-5 Appendix A). As before, rehabilitation improved skilled reaching success [MD 2.85 pellets retrieved (95% CI 1.97-3.74), p<0.01; SMD 0.82 (95% CI 0.51-1.13), p<0.01], which was comparable to the effect size observed in the full dataset.

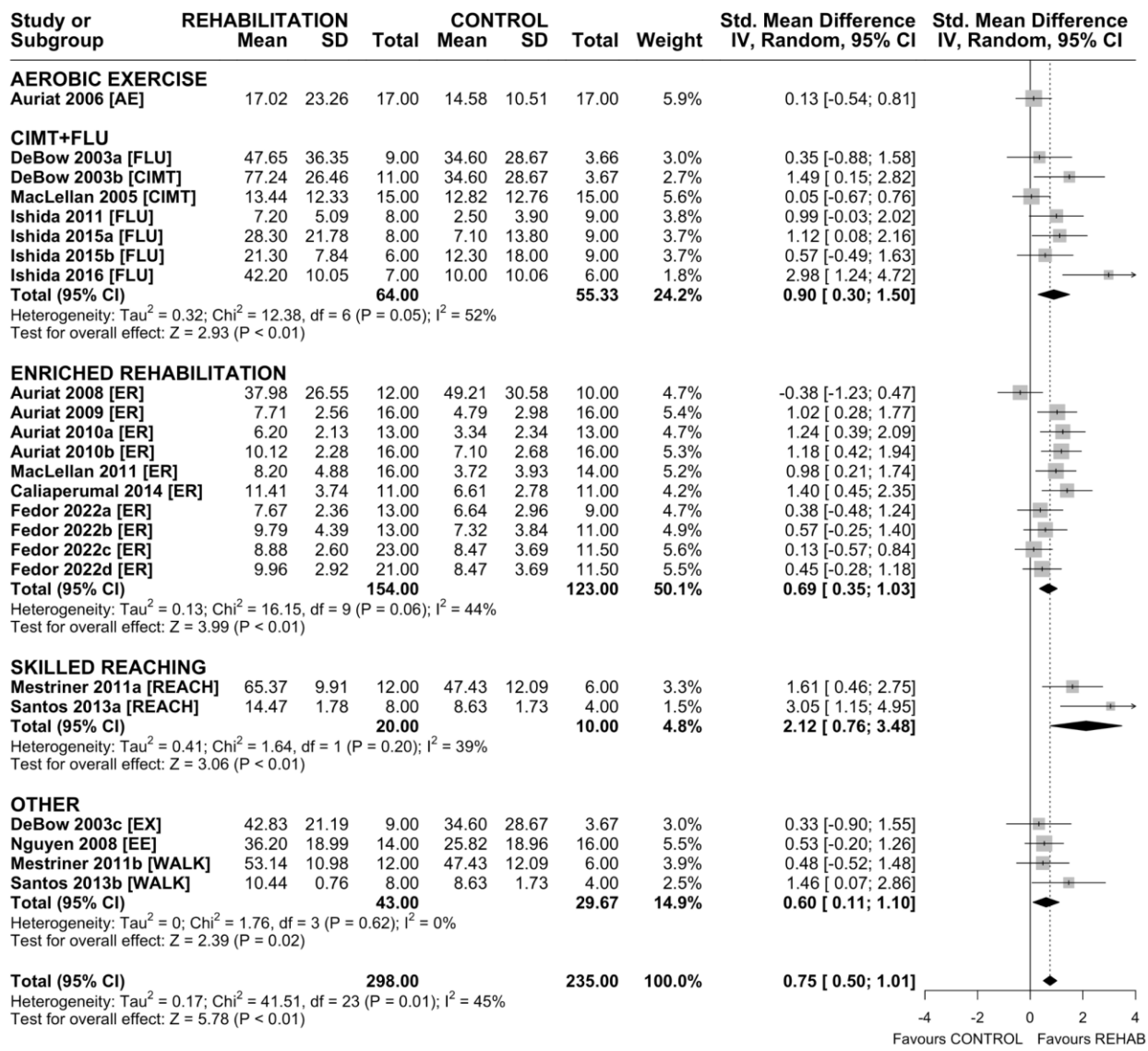

Figure 3-3 Forest plot of random-effects meta-analysis of post-ICH rehabilitation on performance in skilled reaching tasks (n=24). Rehabilitation significantly improved skilled reaching [SMD 0.75 (95% CI 0.50-1.01), p<0.01], with REACH and CIMT+FLU associated with the largest treatment effects. Egger regression indicated the presence of asymmetry in the dataset. Trim-and-fill analysis added 5 data points, all with SMD <0, suggesting null or negative data was missing in our original model. Follow-up random-effects meta-analysis of the trim-and-fill model (n=29) produced a noticeably smaller treatment effect [SMD 0.59 (95% CI 0.32-0.87), p<0.01]. Effect sizes presented as Hedge's *G* standardized mean difference (SMD) with 95% CI

3.3.5 Efficacy of Rehabilitation on Spontaneous Use of the Impaired Forelimb

Fourteen interventions assessed efficacy of rehabilitation on spontaneous impaired forelimb use in the cylinder task (Figure 3-4). Overall, rehabilitation increased use of the impaired forelimb [MD 6.36% increase in impaired forelimb use (95% CI 2.09-10.64), $p<0.01$], however heterogeneity was high ($I^2=67\%$, $p<0.01$). Subgroup analysis by rehabilitation type found a significant effect of CIMT+FLU [MD 7.55% increase in impaired forelimb use (95% CI 1.84-13.27), $p<0.01$] and REACH [MD: 14.30% increase in impaired forelimb use (95% CI 9.22-19.39), $p<0.01$]. Neither AE nor ER significantly increased impaired forelimb use, and interestingly, ER treated animals trended towards worse outcomes than non-treated controls. Sensitivity analysis revealed a similar overall treatment effect on impaired forelimb use [MD 7.49% increase in impaired forelimb use (95% CI 2.66-12.31), $p<0.01$], again with high heterogeneity ($I^2=67\%$, $p<0.01$). As before, CIMT+FLU and REACH improved spontaneous use of the impaired forelimb; ER did not, and again trended towards worse outcomes than non-treated controls (Figure A-6 Appendix A). Funnel plot and Egger regression did not reveal asymmetry in the dataset ($p>0.05$), therefore trim-and-fill analysis was not conducted (Figure A-7 Appendix A).

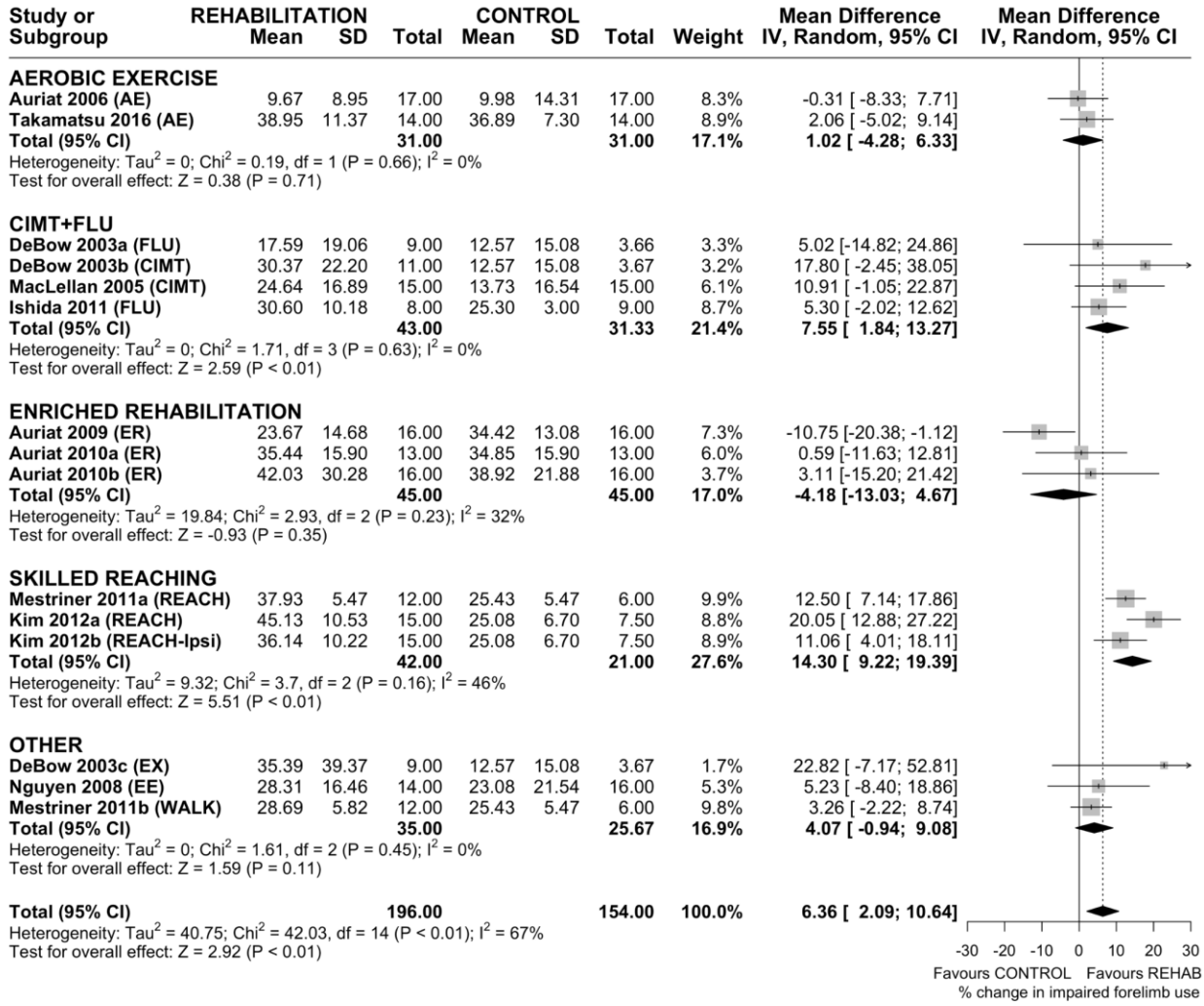

Figure 3-4 Forest plot of random-effects meta-analysis of post-ICH rehabilitation on spontaneous impaired forelimb use in the cylinder task. Overall, rehabilitation significantly increased impaired forelimb use [MD 6.36% improvement (95% CI 2.09-10.64), p<0.01], but only REACH and CIMT+FLU were associated with significant treatment effects. Egger regression did not indicate the presence of asymmetry in the dataset. Effect sizes presented as mean difference (MD), percent change in impaired forelimb use, with 95% CI

3.3.6 Efficacy of Rehabilitation on Locomotor Function

Thirty-one interventions assessed efficacy of rehabilitation on locomotor function in the ladder walking task (Figure 3-5). One intervention was unweighted in our analysis (variance of zero[53]), while 5 others were excluded as we did not receive a

response to our requests for clarification (1 did not report group sizes[62], in 4 we could not interpret the measure of central tendency or variability[43,45,47]). Overall, rehabilitation improved locomotor function [SMD 0.79, (95% CI 0.52-1.06), p<0.01] and heterogeneity was moderate (I^2=43%, p=0.01). Subgroup analysis by rehabilitation type found a significant effect of CIMT+FLU [SMD 0.92 (95% CI 0.33-1.52), p<0.01], ER [SMD 0.98 (95% CI 0.40-1.56), p<0.01], and REACH [SMD 1.31 (95% CI 0.71-1.92), p<0.01]. Neither AE nor AT significantly improved locomotor function. Sensitivity analysis revealed a similar overall effect size for locomotor function [SMD 0.83 (95% CI 0.52-1.15), p<0.01] and heterogeneity remained moderate (I^2=45%, p=0.03). As before, ER and REACH remained significant, whereas CIMT+FLU did not (Figure A-8 Appendix A). Funnel plot and accompanying Egger regression did not reveal asymmetry in the dataset (p>0.05), therefore trim-and-fill analysis was not conducted (Figure A-9 Appendix A).

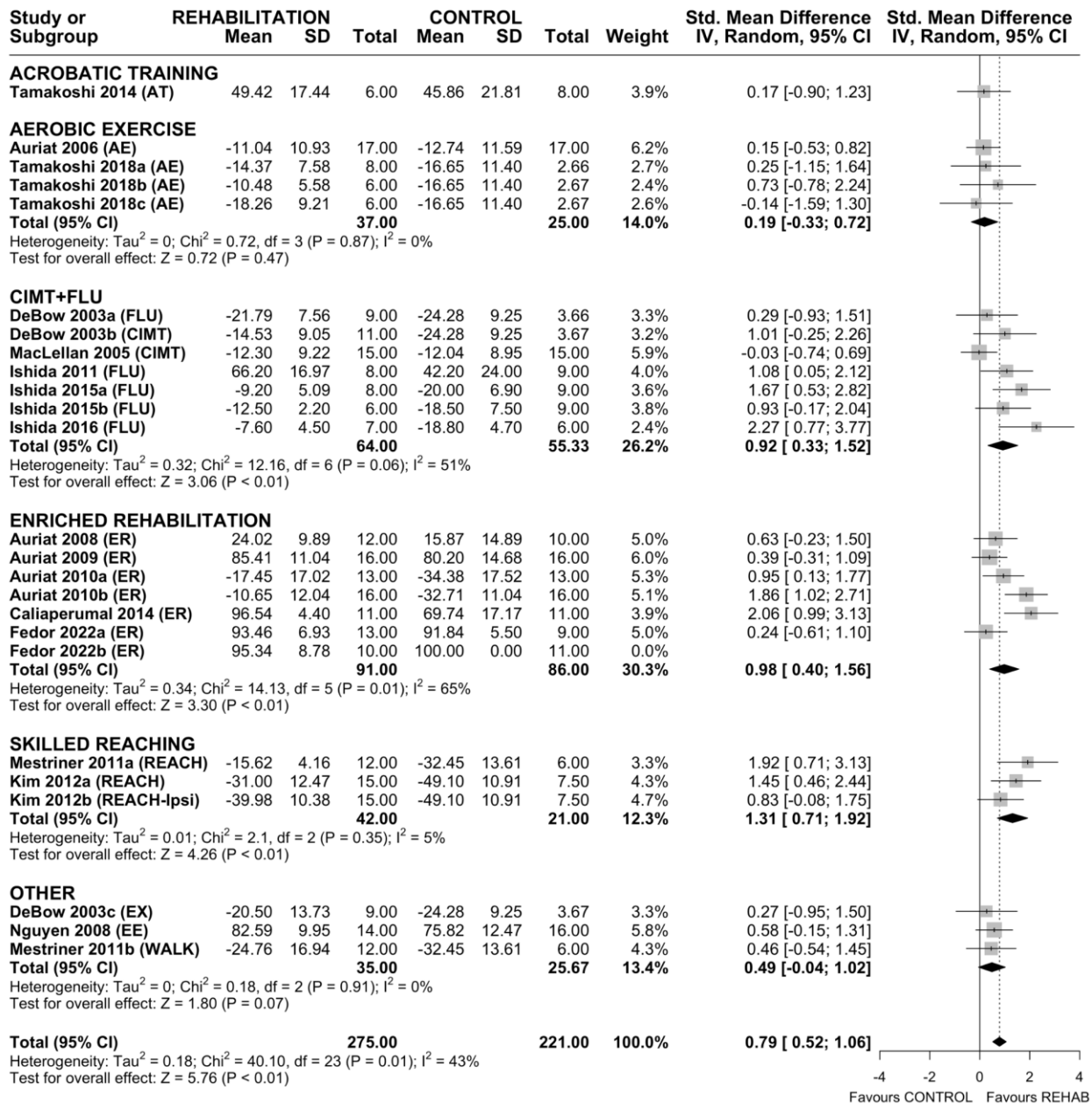

Figure 3-5 Forest plot of random-effects meta-analysis of post-ICH rehabilitation on locomotor function in the ladder walking task. Rehabilitation significantly improved locomotor function [SMD 0.79 (95% CI 0.52-1.06), p<0.01]. Egger regression did not indicate the presence of asymmetry in the dataset. Effect sizes presented as Hedge's *G* standardized mean difference (SMD) with 95% CI

3.3.7 Efficacy of Rehabilitation on Beam Walking and Composite Neurobehavioural Test Scores

Eight interventions assessed efficacy of rehabilitation on locomotor function in the beam walking task, while 13 used a composite neurobehavioural test (e.g., NDS) to assess global function. Unfortunately, we were unable to analyze these endpoints for reasons related to both reporting and principles of analysis. Beam walking and composite neurobehavioural test batteries rely on assessors to make a subjective judgement on behaviour or function; while this is not inherently a problem (assuming blinded assessors), the data for these results are non-parametric. As such, these tests should be analyzed using non-parametric methods with data reported as median ± IQR. Many articles made one or more of the following errors: use of parametric tests in analysis (i.e., ANOVA), reported mean ± SD or standard error (vs. median ± IQR), or failed to report a measure of variability. As such, it was inappropriate to analyze or draw conclusions from these data.

3.3.8 Impact of the Timing of Rehabilitation Onset on Efficacy

Based on intervention characteristics, timing of treatment onset grouped into 5 categories: 24-48 hours, 4-5 days, 7-8 days, ≥14 days, and UNCLEAR. Rehabilitation improved skilled reaching recovery with treatment onset 24-48 hours [SMD 1.48 (95% CI 0.48-2.48), $p<0.01$] and 7-8 days [SMD 1.03 (95% CI 0.75-1.30), $p<0.01$] after ICH, however treatment initiated at 4-5 or ≥14 days failed to significantly improve skilled reaching (Figure 3-6). The impact of treatment onset on improvement in spontaneous impaired forelimb use is unknown. While rehabilitation on the whole increased the use of the impaired forelimb, this effect was only significant in the UNCLEAR onset group

(Figure 3-7). Rehabilitation also improved locomotor function in the ladder walking task with treatment onset at 24-28 hours [SMD 1.21 (95% CI 0.58-1.84), p<0.01] and 7-8 days [SMD 0.90 (95% CI 0.47-1.32), p<0.01] after ICH. Again, treatment initiated at 4-5 days or ≥14 days failed to significantly improve locomotor function (Figure 3-8). Treatment onset within 24-48 hours or 7-8 days after ICH appear to be most efficacious.

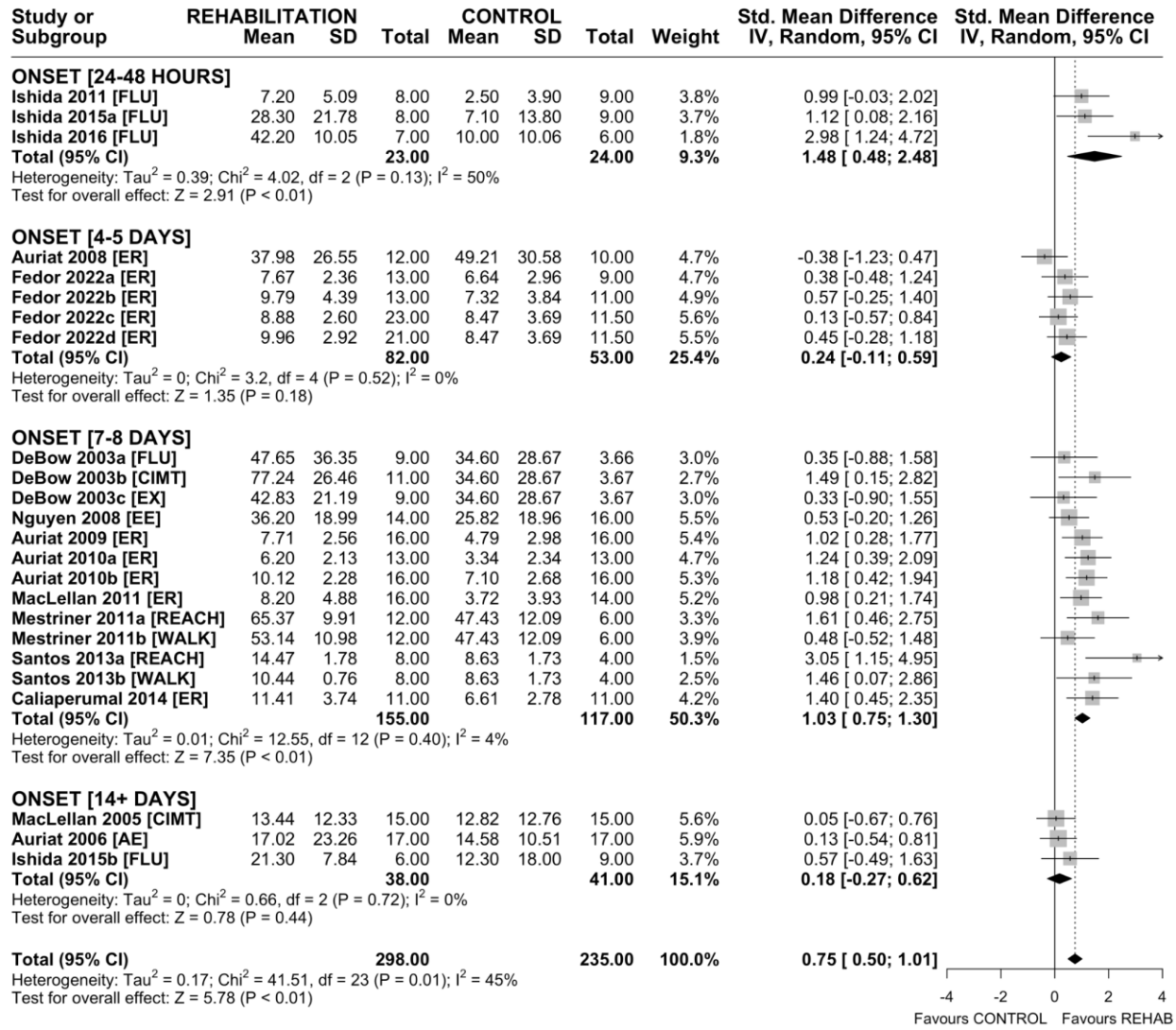

Figure 3-6 Forest plot of random-effects meta-analysis of skilled reaching performance grouped by timing of rehabilitation onset (days from ICH induction). Rehabilitation improved skilled reaching recovery with treatment onset of 24-48 hours [SMD 1.48 (95% CI 0.48-2.48), p<0.01] and 7-8 days [SMD 1.03 (95% CI 0.75-1.30), p<0.01] whereas treatment initiated at 4-5 or ≥14 days failed to significantly improve skilled reaching. Effect sizes presented as Hedge's *G* standardized mean difference (SMD) with 95% CI

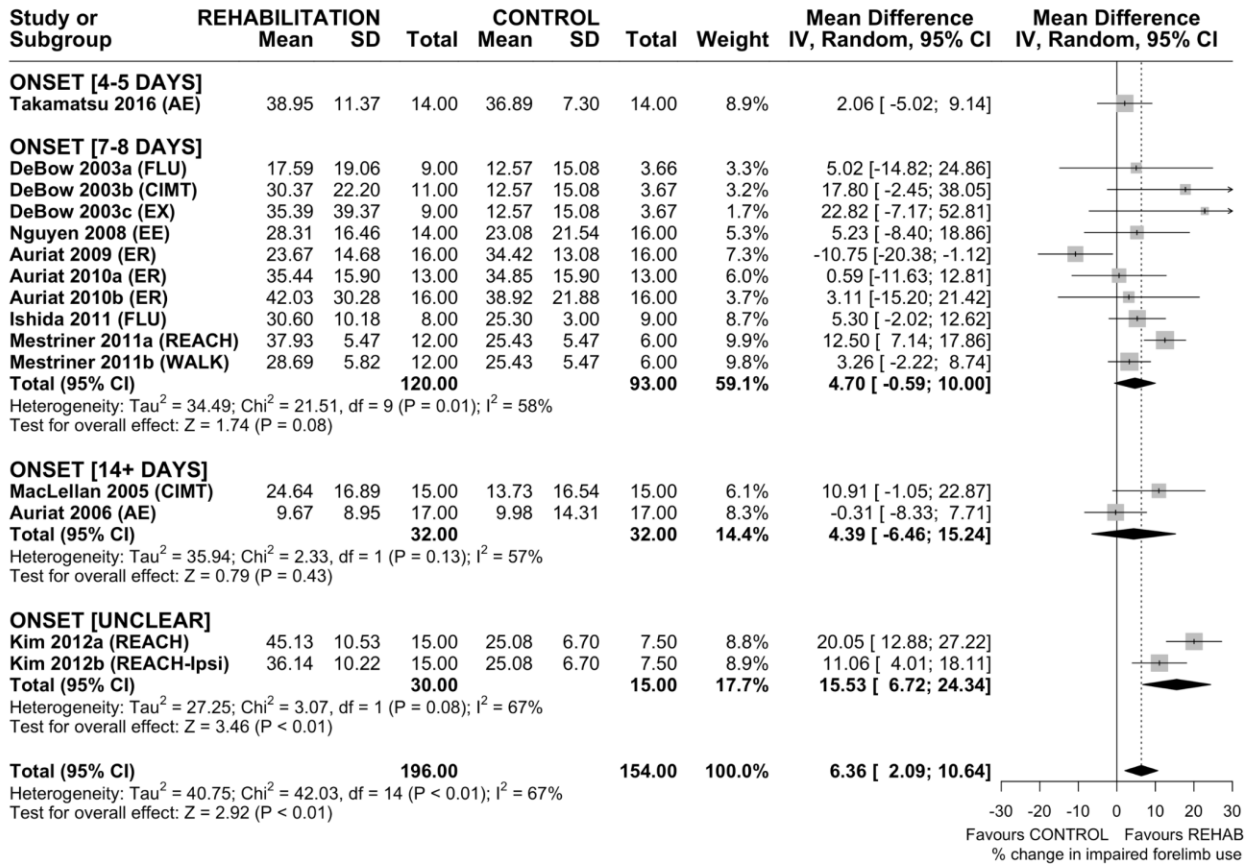

Figure 3-7 Forest plot of random effects meta-analysis of spontaneous impaired forelimb use grouped by timing of rehabilitation onset (days from ICH induction). Rehabilitation increased use of the impaired forelimb, however this effect was predominately driven by two interventions with unclear treatment onset (Kim 2012a, b). Effect sizes presented as mean difference (MD), percent change in impaired forelimb use, with 95% CI

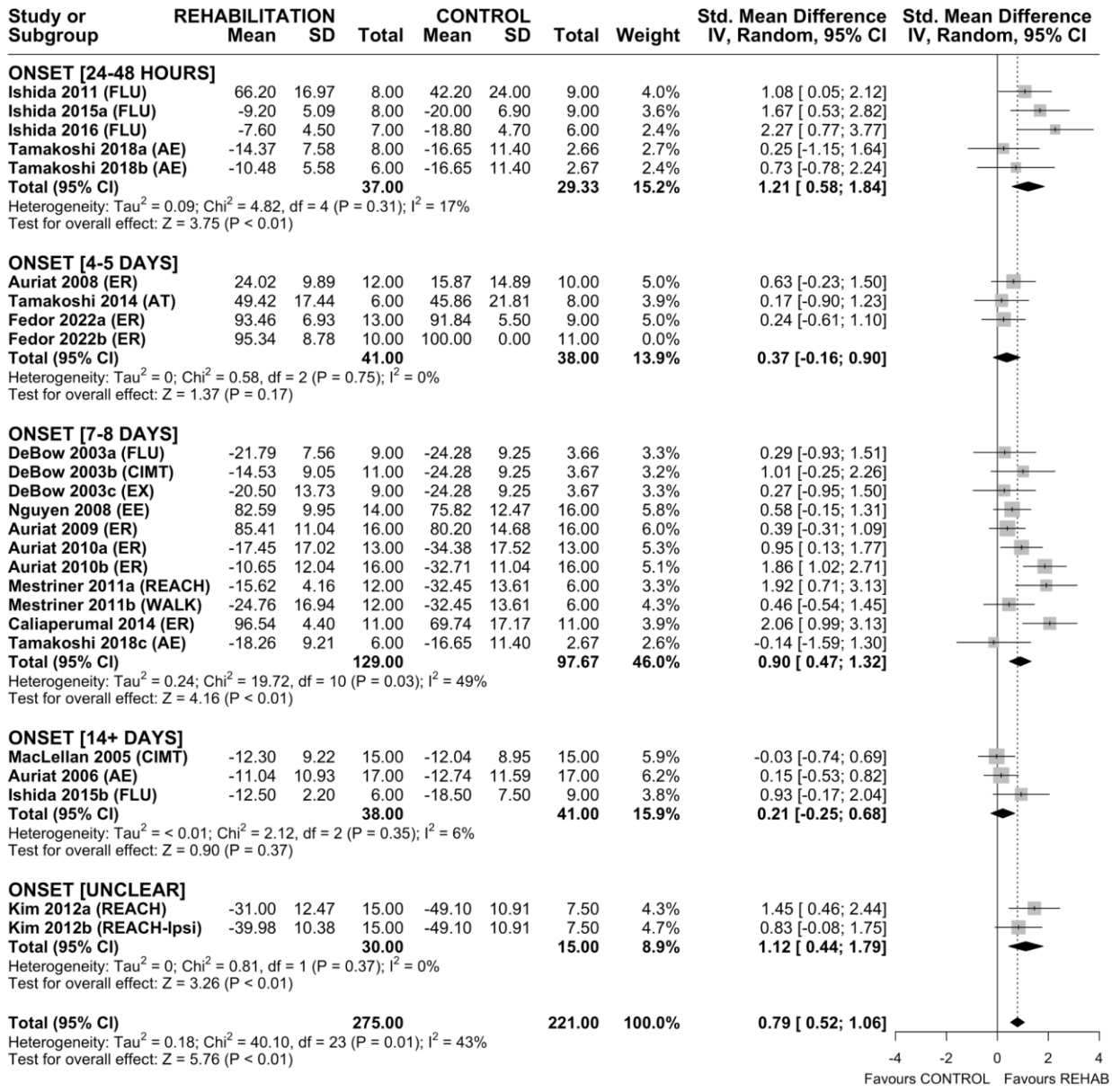

Figure 3-8 Forest plot of random-effects meta-analysis of recovery of locomotor function grouped by timing of rehabilitation onset (days from ICH induction). Rehabilitation improved locomotor function with treatment onset of 24-48 hours [SMD 1.21 (95% CI 0.58-1.84), p<0.01] and 7-8 days [SMD 0.90 (95% CI 0.47-1.32), p<0.01]; however, treatment initiated at 4-5 or ≥14 days failed to significantly improve locomotor function. Effect sizes presented as Hedge's *G* standardized mean difference (SMD) with 95% CI

3.3.9 Impact of Stroke Severity on Efficacy

To assess the impact of stroke severity on treatment efficacy, subgroups were identified using the mean lesion volume reported in the comparator group (untreated control). Severity was defined as mild (≤30 mm^3), moderate (31-60 mm^3), severe (≥61 mm^3), and UNCLEAR[68,69]. Rehabilitation improved skilled reaching in animals with mild [SMD 1.01 (95% CI 0.54-1.48), p<0.01] and moderate [SMD 0.54 (95% CI 0.14-0.94), p<0.01] but not severe ICH (Figure A-10 Appendix A). Rehabilitation did not improve impaired forelimb use when lesion severity was known (Figure A-11 Appendix A). Rehabilitation improved locomotor function in animals with mild [SMD 1.45 (95% CI 0.94-1.95), p<0.01] and moderate [SMD 0.63 (95% CI 0.25-1.01), p<0.01] but not severe ICH (Figure A-12 Appendix A). Consistent with clinical data, the most severe strokes were associated with limited treatment efficacy.

3.3.10 Impact of Rehabilitation Dose by Treatment Type

Owing to substantial heterogeneity among rehabilitation interventions, impact of dose was assessed by treatment type. Analysis was only conducted if ≥3 intervention groups and ≥2 doses were present for an endpoint. Subgroups for treatment dose were identified by natural differences observed within each dataset.

CIMT+FLU was divided into three treatment doses: FLU (56 hours), CIMT (FLU 56 hours + EX 7 hours), and FLU (168 hours) (Figure A-13 Appendix A). While CIMT+FLU significantly improved skilled reaching, only FLU (168 hours) was significantly associated with improved recovery [SMD 1.21 (95% CI 0.42-2.00), p<0.01]. CIMT+FLU improved spontaneous use of the impaired forelimb, however only CIMT had a significant treatment effect [MD 12.69% (95% CI 2.39-22.99), p=0.02].

CIMT+FLU significantly improved locomotor function; similar to skilled reaching, only FLU (168 hours) significantly improved recovery [SMD 1.37 (95% CI 0.79-1.95), $p<0.01$].

Aerobic exercise was divided into two doses: 0-2500 metres and 2501-5000 metres (Figure A-14 Appendix A). Ladder walking was the only endpoint with ≥3 intervention groups and ≥2 treatment doses; however, AE did not improve locomotor recovery in the ladder walking task.

Enriched rehabilitation was divided into four doses based on time in EE and REACH: EE (50-100 hours) + REACH (9-10 hours), EE (100-150 hours) + REACH (10 hours), EE (100-150 hours) + REACH (20 hours), and EE (600 hours) + EX (1.5 hours) (Figure A-15 Appendix A). Enriched rehabilitation as a whole significantly improved skilled reaching, however EE (100-150 hours) + REACH (10 hours) was the only protocol to confer significant benefit [SMD 1.14 (95% CI 0.78-1.50), $p<0.01$]. Similarly, ER significantly improved locomotor function in the ladder walking task, and only EE (100-150 hours) + REACH (10 hours) conferred significant benefit [SMD 1.26 (95% CI 0.48-2.04), $p<0.01$].

3.4 Discussion

Rehabilitation improved motor recovery in skilled reaching, spontaneous impaired forelimb use, and locomotor function. Unsurprisingly, there was substantial variation in the quality of reporting and risk of bias among reviewed articles. Both CIMT+FLU and REACH improved function across all endpoints, whereas ER only improved skilled reaching and locomotor function, and AE failed to improve recovery in any endpoint. Treatment dose did not influence recovery equally across rehabilitation

types, and greater treatment dose did not consistently improve recovery. Treatments initiated 24-48 hours and 7-8 days after ICH improved skilled reaching and ladder walking, whereas treatments initiated 4-5 days or ≥14 days after ICH did not facilitate recovery. Animals with smaller lesions (≤30 mm^3, ~3.7% hemisphere volume) showed the greatest recovery in skilled reaching and locomotor function, whereas those with moderate lesions recovered to a lesser extent. We found limited treatment efficacy in animals that had severe ICH (≥61 mm^3, ~7.5% hemisphere volume), and this was true across all functional domains. These findings are consistent with clinical data, and represent a translationally relevant range in injury relative to the average ICH size in patients (~27 cm^3, or ~4.5% hemisphere volume).[69,70] However, this encompasses a wider range of injury than the mild and often narrow ranges reported in recent clinical trials of mobilization[71] and rehabilitation[72] after hemorrhagic stroke (1.1-1.6% and ~2% hemisphere volume, respectively).

Only CIMT+FLU and REACH treatments reliably improved recovery across all three functional domains. Treatment effects were greatest in the REACH group across all endpoints, followed by CIMT+FLU, suggesting that functional gains transferred to non-trained skills. Interestingly, AE failed to improve recovery after ICH. While it is unclear why, perhaps these interventions used very severe ICH; however only one reported severity (91 mm^3).[38] Our findings differ from those arising from a meta-analysis of preclinical rehabilitation after ischemia, where rehabilitation improved running ability but not impaired forelimb function.[26] Additionally, forced running (AE) was effective in improving motor recovery after ischemia but CIMT was not,[26] suggesting subtype specific rehabilitative efficacy. Owing to the small number of interventions included in each subgroup, it is unclear what underlies these differences.

Factors such as injury type (ischemia vs. ICH), location (cortical vs. subcortical), stroke size (mild vs. severe), treatment type and intensity, and timing of intervention may play a role.

The impact of treatment dose varied by treatment type, with greater dosages not consistently improving efficacy, suggesting a non-linear relationship between dose and recovery. In our CIMT+FLU analysis, only high dose FLU (168 hours) improved skilled reaching and ladder walking, whereas only CIMT (FLU 56 hours + EX 7 hours) improved impaired forelimb use. In contrast, only moderate dose ER (EE 100-150 hours + REACH 10 hours) improved skilled reaching and ladder walking. Complicating our interpretation of dose, outside of AE interventions, few articles reported sufficient detail to assess total treatment dose, for example only one article using REACH or ER reported the number of repetitions completed.[53] Consequently, and similar to most clinical trials, dose was assessed as time in treatment (CIMT+FLU, ER, REACH), which may not reflect the true extent of participation or whether intensity varied among interventions, thereby impacting efficacy.[73] Given the importance of dosage, and the limited and somewhat confusing findings here, it is clear that additional dose-response work is needed.

Rehabilitation initiated 24-48 hours or 7-8 days after ICH was most beneficial, yet why treatment initiated 4-5 days after ICH did not provide benefit is unclear. Further assessment of interventions delivered ≤5 days after ICH identified FLU initiated 24 hours after a small capsular hemorrhage (7-8 mm^3) as the only intervention to provide significant functional benefit.[56–58] Treatments initiated at 48 hours (AE, striatal ICH, severity unknown),[41] 4 days (AE, striatal ICH, ~60% striatal damage; AT, striatal ICH, severity unknown),[15,40] and 5 days (ER, moderate striatal ICH, 38-40 mm^3)[48,53] did

not provide benefit. Furthermore, of the interventions excluded from locomotor function analysis, 4 used an onset ≤48 hours after ICH and were interpreted to suggest mixed effects of early AE intervention on both behaviour and inflammation.[43,45,47] While earlier intervention in the subacute versus chronic phase of recovery is generally linked with greater benefits, clinical investigations into the safety and utility of interventions in the hyper-acute (0-24 hours) and acute (1-7 days) phases of recovery[74] have yielded mixed results.[71,75–77] Notably, the AVERT trial found that frequent, high dose, early out of bed mobilization was associated with decreased odds of favourable outcome at three months post-stroke,[75] and that increased intensity (i.e., greater time out of bed), but not increased frequency of mobilization was associated with less favourable outcomes.[76] Similarly, studies in experimental models of brain injury have demonstrated that early and intense rehabilitation can exacerbate injury and worsen functional outcomes.[21,22] Based on our findings, FLU (restraint, no training, standard laboratory housing) initiated 24 hours after capsular hemorrhage may be beneficial, however further exploration is required. One might hypothesize that FLU was of lower intensity and less stressful than AE and ER, with the latter negatively impacting recovery at sensitive times (e.g., nearing the peak of edema and secondary cell death after ICH).[78–80] It becomes plausible then, that intervention induced stress responses interact with endogenous injury and repair processes in a complex manner, such as exacerbating inflammation and/or supressing mechanisms thought to mediate recovery after ICH (e.g., activation of M2-type microglia and hematoma clearance), and that this may vary by lesion location or severity.

Study quality and risk of bias assessments showed pervasive reporting and methodological issues and potential publication bias among the reviewed articles.

Common reporting errors (Table 3-5) increase the risk of bias, while incomplete and unclear reporting impairs our ability to draw conclusions about data validity and generalizability. Furthermore, small sample sizes with low statistical power often overinflate effect sizes, issues thoroughly discussed elsewhere.[81–83] We found a considerable range in group sizes used for behavioural analysis (n=5 to 23) with analyzed group sizes often much smaller than initially reported, particularly in long-term survival and time course studies. Relatedly, the role of laboratory housing conditions on stroke recovery and treatment efficacy was often overlooked. A recent meta-analysis found conventional laboratory housing (vs. enriched housing) significantly compromises rodent health and likely increases severity of several diseases.[84] As conventional and solo housing are frequently used in preclinical rehabilitation for control conditions, many studies may have exaggerated effect sizes due to housing related worsening of health status in untreated (impoverished) controls relative to human control groups that still receive conventional therapies. Together, these quality issues may lead to widespread overestimation of effect sizes, a trend we observed in our skilled reaching analysis (Figure A-16 Appendix A).

No single experimental model or animal population perfectly replicates the complexity of the human brain,[85] the pathological features of spontaneous ICH,[79] or the timing of injury and recovery processes.[18] Thus, diversity in models, settings, and endpoints is recommended. Unfortunately, all articles in our review modeled sub-cortical ICH in rodents and 29/30 used the collagenase model of ICH, raising concerns about translation (e.g., to other injury locations or populations). Many of the interventions we reviewed lacked clinical relevance. For example, running >1 kilometre within the first day after ICH is unlikely to be used in clinical settings.[45,47] Similarly,

completing thousands of task repetitions during a 1-2 week training period is unlikely to be achieved in clinical practice.[53] Although some patients may achieve >200 repetitions/day, the average number of task repetitions completed in upper limb clinical rehabilitation trials is 23-32 repetitions/session, and decreases as impairment increases.[86] Despite the clinical importance, only ~1/3 of articles systematically compared factors such as treatment onset,[41,43,57] type,[42,44,54,59–61] or dose.[41,53,62,66] Mixed results were often reported, underscoring the need for additional confirmatory-type studies in ICH that systematically manipulate and directly compare the impact of treatment parameters. Although useful, meta-analysis will never replace the need for high quality, original research. At this time, we think it prudent to interpret our findings as strongly supporting the need for additional experimentation specifically varying intervention time or dose while also considering potential interactions with mechanisms of injury and repair (e.g., inflammatory responses). Should such studies confirm complex intervention-delay or dosage effects, then clinical studies will have to evaluate such hypotheses and determine a way to optimize intervention timing (and dosage), such as with biomarkers.

Preclinical interventions were typically characterized by a “one-size-fits-all” approach, which fails to replicate the individual and impairment specific, goal-oriented approach used by clinical rehabilitation professionals. Although skill transference may be expected from some interventions, many studies often selected endpoints that were unlikely to be improved by their chosen therapy without task specific training (e.g., using skilled reaching to measure AE efficacy) or only assessed gross impairment (e.g., NDS). Many of these endpoints fail to distinguish true recovery from compensation, a limitation relevant to our findings. Here, we refer to improvement in function as

recovery of function, however we cannot rule out that treatment effects could be a combination of recovery and compensation, or compensation alone. Although the statistical effects observed in this meta-analysis are considered large – they do not directly show how much rehabilitation improves functional recovery. Clinical trials frequently report treatment effects relative to the minimum clinically importance difference (MCID),[87] however few preclinical studies report an equivalent to the MCID or conceptualize efficacy beyond statistical testing, making interpretation of functional effect sizes challenging. In our review, only one article proposed an MCID-like threshold,[53] arguing that a 3-pellet increase in reaching success (i.e., 1 level in the 7 level, 21-pellet staircase task) represents a meaningful difference in function. Thus, we analyzed the subset of interventions that reported the number of pellets retrieved in skilled reaching assessment. Rehabilitation improved reaching success by an average of 2.85 pellets but failed to exceed the 3-pellet threshold; by this measure, the treatment effect is likely of limited functional impact.

Numerous resources and guidelines have been developed to improve the quality of stroke research and translational success,[29,88–94] yet for reasons unknown, adherence to these guidelines remains far from universal.[67] Preclinical successes will continue to fail to translate if we do not disrupt this norm. Table 3-6 provides a roadmap for improving quality and translational potential in preclinical rehabilitation research. With the goal of increasing transparency and replicability, we identify actions to be taken at each step of the scientific process, identify relevant resources, and outline how each action contributes to the goal of improving scientific and translational rigor. In the spirit of transparency, we encourage researchers to make raw data available as supplementary files upon publication or available in a discipline specific open data repository (for an

example see the Open Data Commons for Spinal Cord Injury[95]). Similarly, we strongly encourage journals to require authors to submit raw data as part of the peer review and publication process. We believe that incorporating these actions from the outset of experimental design provides a framework to reduce bias and improve methodological rigour and transparency in reporting. However, poor reporting quality is not the root of the problem – it is a consequence of a larger systemic issue. New trainees, established researchers, and peer reviewers alike must be provided access to adequate training and resources if we want to improve research quality and reproducibility. We encourage research groups to include formal training on best practices in research and statistics when onboarding new members. Familiarization with best practice guidelines (i.e., ARRIVE,[89,92,93] RIGOR[90]), how compliance to these guidelines is assessed (i.e., CAMARADES,[31] SYRCLE[32]), and what these guidelines look like in practice are equally important skills to preclinical researchers as learning animal husbandry or basic surgical techniques.

Table 3-6 Roadmap to Improving the Quality of Preclinical Rehabilitation Research

Element	Action	Goal
Scientific Rigor	Use the RIGOR,[90] ARRIVE, [89,93] SYRCLE,[32] and CAMARADES[31] guidelines and checklists to inform decision making during experimental design. Consult the CONSIGN tool[94] to ensure selection of appropriate control groups [Phase: experimental planning]	Improve methodological rigor, reduce risk of bias through *a priori* study design
	Implement recommendations from RIGOR and ARRIVE during data collection – this includes (but is not limited to): use of power calculations to determine group size, collecting baseline data for ALL experimental groups, blinded induction of ICH or randomizing animals to treatment group AFTER induction, blinding researchers to treatment identity for assessment and analysis (particularly for subjective measures) [Phase: data collection]	Improve quality of data collected, reduce risk of bias introduced by researchers
	Analyze data using appropriate statistical methods (i.e., parametric tests for parametric data, non-parametric tests for non-parametric data, ensure data does not violate test assumptions), analyze and present appropriate summary statistics (e.g., mean ± SD or 95% CI for continuous data, median ± IQR for ordinal data), report exact summary data values and error terms, and use scatterplots to represent data whenever possible [Phase: data analysis]	Improve statistical reporting and interpretation of treatment effects
	Report methods and data as outlined by the RIGOR and ARRIVE guidelines – this includes (but is not limited to): explicit reporting of total N, inclusion and exclusion criteria, mortality and exclusions (with group identity; if none this should also be stated), n/group analyzed for EACH endpoint, types and timing of assessments used etc. [Phase: manuscript preparation]	Improve transparency and reproducibility of methods and findings
	Review final manuscript for compliance with RIGOR, ARRIVE, SYRLCE, and CAMARADES guidelines and checklists; address any deficiencies prior to submission for peer review [Phase: peer review and publication]	Improve reporting quality
	Make raw data and relevant analysis code available via supplemental data files or data repository [Phase: peer review and publication]	Improve data sharing and research transparency

Translational Rigor	Select translationally relevant endpoints and interventions (i.e., assessment type, timing, and dosage that mimic clinical elements) and include functional endpoints in ALL studies of rehabilitation. The goal of rehabilitation is to improve function and independence – endpoints used in preclinical work must assess impairments that are similar to those observed clinically [Phase: experimental planning]	Ensure preclinical treatments assess endpoints relevant to human disease/disorder
	Conduct multiple functional assessments (baseline, post-stroke and pre-treatment, and follow up) and prioritize long-term functional assessment (>28 days) in study design. Whenever possible, ALL treated subjects should complete ALL functional endpoints to prevent loss of statistical power [Phase: experimental planning, data collection]	Ensure treatment effects are not due to baseline differences; provide insight into stability of treatment effects
	Include a histological assessment of stroke severity (i.e., hematoma or lesion volume) and report total volume of injury (i.e., not injury in a single slice or small region). Use comorbidities relevant to ICH (e.g., hypertension, old age, diabetes) [Phase: experimental planning, data collection, data analysis, manuscript preparation]	Provide context to interpret what population the treatment may benefit (or harm)
Rehabilitation Dosage	Define and describe the following treatment parameters: type, timing (onset, hours/days from stroke), treatment period (first day of treatment – last day of treatment), duration of single treatment session (mins/hours/days), frequency of treatment sessions [Phase: experimental planning, data analysis, manuscript preparation]	Improve reporting quality of treatment protocols; increase replicability
	Operationally define and report treatment intensity (e.g., repetitions, m/min, time in restraint); calculate and report total treatment dosage (i.e., time in treatment, total repetitions, distance run, etc.) [Phase: experimental planning, data collection, data analysis, manuscript preparation]	Provide insight into how intensity may impact recovery
	Adopt a standardized approach to reporting timing of intervention and assessments: stroke=day 0 should be used as a reference point [Phase: experimental planning, data analysis, manuscript preparation]	Standardize how data is discussed in the context of time since stroke
	Include experimental timeline figure indicating the timing for all periods of training, treatment, and assessment [Phase: manuscript preparation]	Clarify experimental design; improve replicability

3.5 Conclusion

Our systematic review and meta-analysis show that rehabilitation improves skilled reaching, spontaneous impaired forelimb use, and locomotor function after ICH. CIMT+FLU and REACH were the only therapies to improve motor recovery across all three domains, whereas ER improved skilled reaching and locomotor function, and AE did not provide benefit in any domain. Acknowledging the limited quality and scope of articles included in our analysis, these findings provide strong evidence for a statistically significant effect of rehabilitation after ICH, but one of unclear functional meaning. While earlier intervention was generally better than delayed intervention (i.e., onset at 7-8 days versus ≥14 days), efficacy of rehabilitation delivered <7 days after ICH is unclear. In alignment with clinical findings, rehabilitation was most effective following mild-moderate ICH, but of limited benefit after severe ICH. As others have called for, our analysis of key issues in scientific rigour and translational relevance highlights the need for continuing improvements in ICH rehabilitation research, and a clear need for additional work on dosage, timing, and other parameters. Without these improvements, future studies risk using rehabilitative interventions with limited functional efficacy or clinical relevance, potentially wasting limited financial resources and time pursuing lines of inquiry propped up by poor data. As we move into the era of precision medicine, identifying characteristics that pinpoint who best benefits from a treatment and what factors impact efficacy is essential to increasing translational success, optimizing rehabilitation, and ultimately, improving patient outcomes.

3.6 References

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Chapter 4 | An exploratory analysis of predictors of motor recovery after preclinical intracerebral hemorrhage

4.1 Introduction

In the aftermath of stroke, ~80% of survivors will experience some level of temporary or permanent paresis in one or more limbs.[1] Independence in activities of daily living is often diminished, as up 50-60% of survivors will live with persistent impairment or disability.[2,3] Owing to the heterogenous nature of stroke, no two injuries are the same. Therefore, understanding what factors predict patient recovery or response to treatment is of critical clinical importance and essential to clinicians, family, and patients in making informed choices regarding care.[4]

In the hours, days, weeks, and months after stroke, endogenous systems of repair lead to spontaneous and rehabilitation-facilitated recovery of neurological and behavioural function.[5] However, this recovery is often incomplete, and in some cases, extremely limited. In 2008, Prabhakaran et al.[6] were the first to report evidence indicating a proportional relationship between initial impairment and recovery after stroke. In an analysis of upper limb recovery measured as change in the Fugl-Meyer Upper Extremity score (FM-UE) in ischemic stroke patients, they reported that when outliers were removed (i.e., patients with severe initial impairment that achieved limited to no recovery), >80% of patients recovered ~70% of their early impairment.[6] Subsequent studies of recovery in the upper[7,8] and lower limbs,[9,10] aphasia,[11] and neglect[12] have supported this initial finding, reporting mean recovery of initial impairment ranging from 63-97% in the majority of patients (deemed “fitters”). Together, these results have been interpreted to suggest the possibility of an underlying biological factor or mechanism of recovery,[13] leading stroke researchers to term this phenomenon the proportional recovery rule. However, up to 30% of patients will not achieve proportional recovery – these “non-fitters” show more modest improvement or

worsening over time.[6–8,10,12,13] As not all patients achieve proportional recovery, and tools that exclusively use clinical information to predict outcome struggle to accurately predict for those with moderate-severe initial impairments,[14] detecting biological characteristics (i.e., biomarkers) to identify who recovers and why has become an important topic of research.

Indeed, biomarkers capable of predicting long-term outcomes and potential for recovery after stroke have been identified as a key priority for moving stroke recovery and rehabilitation research forward.[15] Integrity of the corticospinal tract (CST), a white matter tract that passes through the internal capsule, has been identified as a reliable predictor of motor recovery due to its role in voluntary movement.[13,16] Clinically, CST integrity can be assessed both functionally, through neurophysiological assessment of motor evoked potential (MEP) status, and structurally, using neuroimaging to assess CST injury (i.e., CST lesion load)[16] or fractional anisotropy (FA) asymmetry in the posterior internal capsule.[14] Good prognosis is typically associated with the ability to elicit MEPs,[17] greater hemispheric symmetry, limited CST injury (i.e., <63% loss of CST),[18] and less FA asymmetry in the posterior internal capsule.

Animal models are frequently used to explore underlying mechanisms of injury and recovery after stroke, with the goal of translating research findings to clinical practice. Yet despite numerous promising preclinical findings, very few results successfully replicate, and even fewer survive the "valley of death" as they move from bench to bedside.[19] Many factors have been attributed to these failures (e.g., experimental models, design, and methodology; statistical errors; lack of transparency),[20,21] and several guidelines have been developed to help researchers improve translation, often by addressing concerns related to internal validity.[22–25]

However, these guidelines often provide limited direction in addressing factors related to external validity, ensuring that questions persist surrounding the generalizability of preclinical research results.[26] One way to improve the likelihood of successful translation is to ensure that our experimental models reflect not only the pathological features of our condition of interest (i.e., mode and timing of cell injury), but also the variability of the condition in the human population.[26] As such, understanding whether proportional recovery exists in preclinical models of stroke is important to evaluating the suitability of our experimental models and populations and validating whether similar biomarkers exist between clinical and preclinical populations.

There is some evidence in translational models of stroke to support that proportional recovery is a phenomenon conserved across species. In a retrospective analysis of recovery after ischemic stroke induced by endothelin-1 (ET-1), Jeffers and colleagues found evidence for proportional recovery in rats (62-70% recovery of initial impairment), a range overlapping with those reported clinically.[27] However, only ~30% of rats were considered "fitters" of the rule, compared to ≥70% of individuals in clinical reports.[6–13] Interestingly, ~60% of rats showed no or very limited recovery after ischemic stroke ("non-fitters"), while ~10% showed worsening over time ("decliners"); though why is unclear, it may be a result of including a wider range in injury severity compared to the clinical literature.[27] Similar to clinical findings, biomarkers for rats that fit the proportional recovery rule included less initial impairment, smaller lesion size, and less striatal injury.[27]

There is insufficient work as to whether patterns of recovery differ by stroke subtype. Nevertheless, and despite a growing interest in proportional recovery, most studies have used either exclusively ischemic populations, or a heterogenous population

with <15% hemorrhagic stroke patients.[8] Although some have reported no subtype specific differences in impairment at time of admission or discharge from rehabilitation,[28,29] others in both clinical[30–33] and preclinical populations[34] have reported differing patterns of functional recovery after ischemic and hemorrhagic stroke. While intracerebral hemorrhage (ICH) is typically associated with greater functional and cognitive impairments at time of admission to inpatient rehabilitation, ICH patients make greater gains during treatment and are discharged with comparable impairment to those admitted following ischemic stroke.[31–33] Furthermore, when matched for stroke severity, ICH has been associated with better neurological[30] and functional prognosis.[30,34] The mechanisms underlying these findings are unclear, however some have hypothesized they are related to the resolution of the hematoma, edema, and mass effect after ICH.[30,31,35] Although ischemic and hemorrhagic stroke share many pathological features and mechanisms of injury (discussed in 1.6.2 Secondary Injury), several distinct processes occur that may alter the timeline of cell death, injury resolution, and response to treatment (e.g., triggering of the ischemic cascade vs. cytotoxic effects of hematoma breakdown).[36] As preclinical studies are habitually used to explore mechanisms of injury and repair and how they contribute to resolution of impairment, characterizing recovery in preclinical models is an important step towards understanding and improving translation.

Here, we provide a retrospective, post-hoc exploratory analysis of recovery after hemorrhagic stroke in a preclinical rat model of striatal ICH[37] and present the first known analysis of proportional recovery in an exclusively hemorrhagic stroke population. The primary aims of this analysis were to 1) determine whether recovery in skilled reaching is proportional to initial impairment after experimentally induced

striatal ICH, and if so, to what extent, and 2) to evaluate whether clinically relevant factors such as severity, lesion size, and internal capsule damage predict recovery after ICH.

4.2 Methods

4.2.1 Dataset

Data from the 60-day survival study described in Chapter 2 (Experiment 2)[37] was compiled for retrospective analysis. All rats in this dataset received a striatal ICH in the hemisphere contralateral to their preferred paw (1.2 μL type IV-S bacterial collagenase; injected AP +0.5, ML ±3.5, DV -6.5 from Bregma). Rats were eligible for inclusion in this analysis if the following data were available:

1. pellets retrieved in Montoya staircase pre-stroke ($Pellets_{pre}$),
2. pellets retrieved in initial post-stroke staircase assessment ($Pellets_{pi}$)
    a. significant impairment: $Pellets_{pi}$ outside the rat's 95% confidence interval (CI) of $Pellets_{pre}$,[27]
3. pellets retrieved in final post-stroke staircase assessment ($Pellets_{pf}$),
4. total volume of tissue loss (mm^3),
5. average pellets retrieved in rehabilitation daily ($Rehab_{int}$),
6. total pellets retrieved in rehabilitation ($Rehab_{dose}$).

Sixty-four rats met the inclusion criteria and were eligible for analysis.

4.2.2 Internal Capsule Damage

Damage to the white matter passing through the internal capsule was not quantified in our original experiment, therefore a secondary series of slides were retrieved for each animal and stained with Luxol Fast Blue to visualize myelin.[38] Brightfield microscopy was then used to explore damage to the white matter in the internal capsule between 0 to -3 mm from Bregma with landmarks identified using the WHS rat brain atlas (v1.01, RRID: SCR_017124).[39] Owing to inconsistent stain deposition, injury to the internal capsule could not be directly quantified and was therefore rated as present (lesion overlapping tract), adjacent (lesion <50 μm from tract), or absent (lesion >50 μm from tract or not present). Brain sections were inspected under the microscope to evaluate the presence or absence of injury in the internal capsule for each 500 μm interval between 0 to -3 mm from Bregma. Each interval was scored as 2 (present), 1 (adjacent), or 0 (absent) and then summed to create the IC_{damage} score (0-12), where a score of 0 represented no damage and 12 represented damage to the internal capsule across all analyzed intervals.

4.2.3 Statistical Analysis

Initial impairment ($Pellets_{ii}$) in skilled reaching was calculated as the change in pellets retrieved prior to ICH ($Pellets_{pre}$) and in the initial post-ICH, pre-treatment testing session ($Pellets_{pi}$) for each animal as:

$$Pellets_{ii} = \#\ Pellets_{pre} - \#\ Pellets_{pi},$$

where greater values of $Pellets_{ii}$ represent greater impairment.

Observed recovery in skilled reaching (ΔPellets) was calculated as the change in pellets retrieved between the initial post-ICH assessment ($Pellets_{pi}$) and the final post-ICH skilled reaching assessment ($Pellets_{pf}$) for each animal as:

$$\Delta Pellets = \#\,Pellets_{pf} - \#\,Pellets_{pi},$$

where greater values of ΔPellets represent greater recovery.

Percent recovery of initial impairment ($R_{\%}$) was then calculated as the observed recovery in skilled reaching (ΔPellets) divided by initial impairment ($Pellets_{ii}$) for each animal as:

$$R_{\%} = \frac{\Delta Pellets}{Pellets_{ii}}\ x\ 100\%.$$

As done by others,[27] agglomerative hierarchical cluster analysis using Ward's minimum variance method was performed on the $R_{\%}$ data to identify unique recovery groups, using R (v.4.3.0; R Core Team, 2023) and RStudio (v.2023.3.1.446; Posit Team, 2023) with the tidyverse,[40] cluster,[41] and nbclust[42] packages. Assessment of optimal number of clusters was completed using the elbow and average silhouette methods. Mean $R_{\%}$ and 95% CI were then calculated for each cluster; if the 95% CI of the cluster's $R_{\%}$ overlapped with a clinically reported CI of proportional recovery (i.e., ≥47%, the lowest 95% CI boundary reported)[7], the group was considered likely to fit the proportional recovery rule.

Descriptive characteristics were then analyzed for each group. Parametric data was analyzed in GraphPad Prism (version 10.0.2 for Mac, GraphPad Software, San Diego, California) using one-way ANOVA with Tukey's multiple comparisons test; if

data failed normality, the Kruskal-Wallis test with Dunn's multiple comparison test was used. Owing to the small size of some of the groups identified in the dataset, non-parametric (categorical data) was analyzed in R using the Fisher's Exact test. Data are presented as mean and 95% CI for parametric data and median ± IQR for non-parametric data in figures; for the purpose of comparing to others,[27,43] descriptive characteristics for recovery groups are presented as mean ± standard deviation (SD) in Tables 4-1 and 4-2 and when referred to in text.

Next, simple linear regression was performed to explore whether the observed recovery (ΔPellets) in skilled reaching was directly proportional to initial impairment ($Pellets_{ii}$) and determine whether the groups identified in the cluster analysis were distinct. To assess how well proportional recovery was predicted by hierarchical clustering, simple linear regression was performed between observed recovery (ΔPellets) and predicted recovery ($\Delta Pellets_{predict}$) using $R_{\%}$, the average percent recovery of impairment, in the moderate-recoverers in each clustering model:

$$\Delta Pellets_{predict} = (Pellets_{ii})\ x\ R_{\%}$$

Based on previous clinical and preclinical analyses, data for initial impairment ($Pellets_{ii}$),[6–13,27] volume of tissue loss,[6,27] CST injury (IC_{damage}),[8,13] and rehabilitation ($Rehab_{dose}$)[8,9,13,43] were selected as candidate variables for evaluating predictors of recovery. Multiple linear regression was performed using a backwards stepwise elimination procedure, where non-significant predictors were removed in a stepwise fashion and regression was repeated.[6,7,43] Owing to the exploratory nature of this analysis, the elimination procedure was repeated until only significant predictors remained ($p<0.05$) or removal of an unsignificant predictor worsened the goodness-of-

fit of the model (i.e., decreased adj-R^2 and increased residual standard error).[6] Finally, to assess whether the best multiple regression model (i.e., highest adj-R^2 and lowest residual standard error) was superior at predicting observed recovery (ΔPellets) than initial impairment alone ($Pellets_{ii}$), a partial F-test was conducted.

4.3 Results

4.3.1 Identification of Recovery Clusters

Hierarchical cluster analysis of $R_{\%}$ identified clusters with statistically different average $R_{\%}$ ($p<0.001$ in all comparisons). However, assessment of the optimal number of clusters lacked consensus and suggested recovery may be best characterized by 3 or 4 groups (elbow method vs. average silhouette method). As such, both the 3-cluster and 4-cluster models of recovery were characterized (Table 4-1).

Table 4-1 Characteristics of recovery of impairment ($R_{\%}$) in recovery models

Model	Cluster 1	Cluster 2	Cluster 3	Cluster 4	p-value
Full dataset	Mean $R_{\%}$: 30.2% Median $R_{\%}$: 29.0 95% CI: 21.8, 38.7 Range: -69.6, 114.6 n=64				
3-cluster model	Mean $R_{\%}$: -48.6%^{p,m} Median $R_{\%}$: -46.8% 95% CI: -64.8, -32.3 Range: -69.6, -36.7 n=5/64, 7.8% Group: decliners	Mean $R_{\%}$: 17.6%^{d,m} Median $R_{\%}$: 19.1% 95% CI: 14.2, 21.1 Range: -2.5, 31.55 n=31/64, 48.4% Group: poor-recoverers	Mean $R_{\%}$: 58.3%^{d,p} Median $R_{\%}$: 52.1% 95% CI: 50.5, 66.1 Range: 35.2, 114.6 n=28/64, 43.8% Group: moderate-recoverers		<0.0001 [1]
4-cluster model	Mean $R_{\%}$: -48.6%^{p,m,h} Median $R_{\%}$: -46.8% 95% CI: -64.8, -32.3 Range: -69.6, -36.7 n=5/64, 7.8% Group: decliners	Mean $R_{\%}$: 17.6%^{d,m,h} Median $R_{\%}$: 19.1% 95% CI: 14.2, 21.1 Range: -2.5, 31.55 n=31/64, 48.5% Group: poor-recoverers	Mean $R_{\%}$: 48.5%^{d,p,h} Median $R_{\%}$: 46.6% 95% CI: 44.4, 52.5 Range: 35.2, 66.3 n=21/64, 32.8% Group: moderate-recoverers	Mean $R_{\%}$: 87.8%^{d,p,m} Median $R_{\%}$: 79.4% 95% CI: 75.1, 101 Range: 77.4, 115 n=7/64, 10.9% Group: high-recoverers	<0.0001 [2]

Legend: Significant differences among groups ($p<0.001$) represented by superscript lettering ^{d} (decliners), ^{p} (poor-recoverers), ^{m} (moderate-recoverers), and ^{h} (high-recoverers). [1] Result of one-way ANOVA with Welch's correction and Dunnett's multiple comparisons test. [2] Result of one-way ANOVA with Tukey's multiple comparisons test

Both models shared two identical recovery clusters, “decliners” (mean R%=-48.6%, 7.8% of dataset) and “poor-recoverers” (mean R%=17.6%, 48.4% of dataset). The remaining rats were identified as “moderate-recoverers” (mean R%=58.3%, 43.8% of dataset) in the 3-cluster recovery model (Figure4-1A); this group was further subdivided in the 4-cluster recovery model as it identified a unique group of rats that achieved near complete recovery (Figure4-1B). As such, the remaining two groups in the 4-cluster model were “moderate-recoverers” (mean R%=48.5%, 32.8% of dataset) and “high-recoverers” (mean R%=87.8%, 10.9% of dataset).

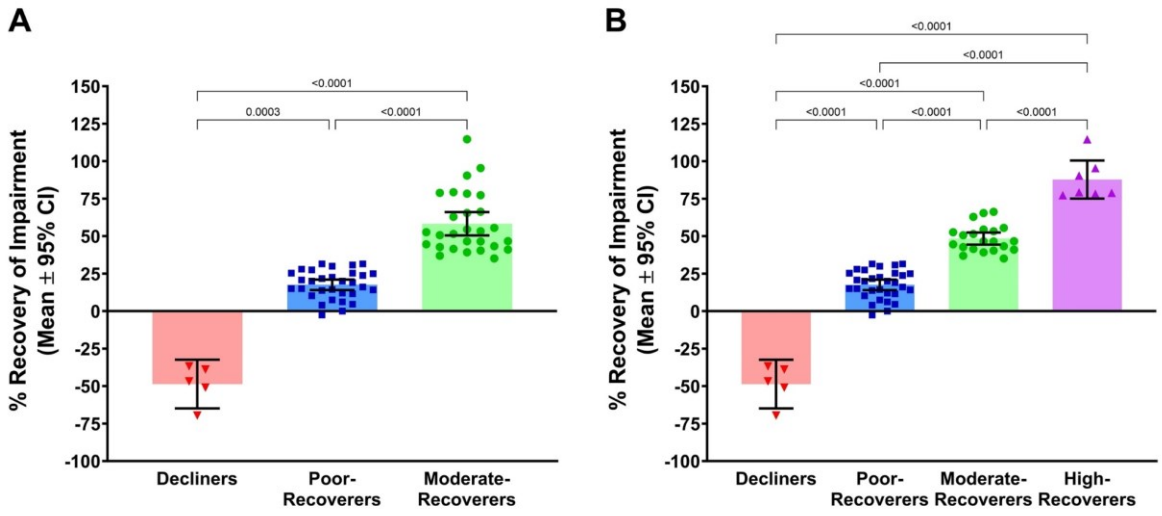

Figure 4-1 Recovery of impairment (R%) in the recovery groups identified in the **A** 3-cluster and **B** 4-cluster models. Both models identified statistically distinct recovery groups ($p<0.001$) and generated identical clusters for decliners and poor-recoverers. In the 4-cluster model, the moderate-recoverers were subdivided, with rats displaying greatest recovery (>75%) identified as a unique subgroup termed high-recoverers. Data presented as scatterplots (individual rats) superimposed over bar graphs (group mean) with 95% CI

4.3.2 Characteristics of Recovery Groups in 3-Cluster Model

There were no significant differences in pre-stroke performance in skilled reaching ($Pellets_{pre}$) among recovery groups (p=0.204) identified in the 3-cluster model. Initial impairment ($Pellets_{ii}$, Figure 4-2A) was not significantly different among groups (p=0.071), however there was a significant difference (p=0.016) in pellets retrieved in the initial post-ICH skilled reaching assessment ($Pellets_{pi}$, Figure 4-2B), with decliners retrieving significantly more pellets than both poor- and moderate-recoverers (p<0.05). There was a significant difference (p<0.001) in pellet retrieval in the final post-ICH skilled reaching assessment ($Pellets_{pf}$, Figure 4-2C) and the moderate-recoverers retrieved more pellets than both decliners and poor-recoverers (p<0.01). Similarly, observed recovery (ΔPellets, Figure 4-2D) significantly differed among groups (p<0.001), with moderate-recoverers showing superior recovery to both decliners and poor-recoverers (p<0.001), and poor-recoverers showing superior recovery to decliners (p<0.001). Total volume of tissue loss (Figure 4-2E) and damage in the internal capsule (IC_{damage}, Figure 4-2F) did not differ among recovery groups (p=0.143 and p=0.343, respectively). Similarly, the number of rats with or without rehabilitation (Figure 4-3A) did not differ among groups (p=0.375); intervention characteristics of dose ($Rehab_{dose}$, Figure 4-3C) and intensity ($Rehab_{int}$, Figure 4-3E) were also not significantly different (p≥0.249) in the subsets of rehabilitation treated rats. Descriptive statistics and comparisons for the 3-cluster model are described in Table 4-2.

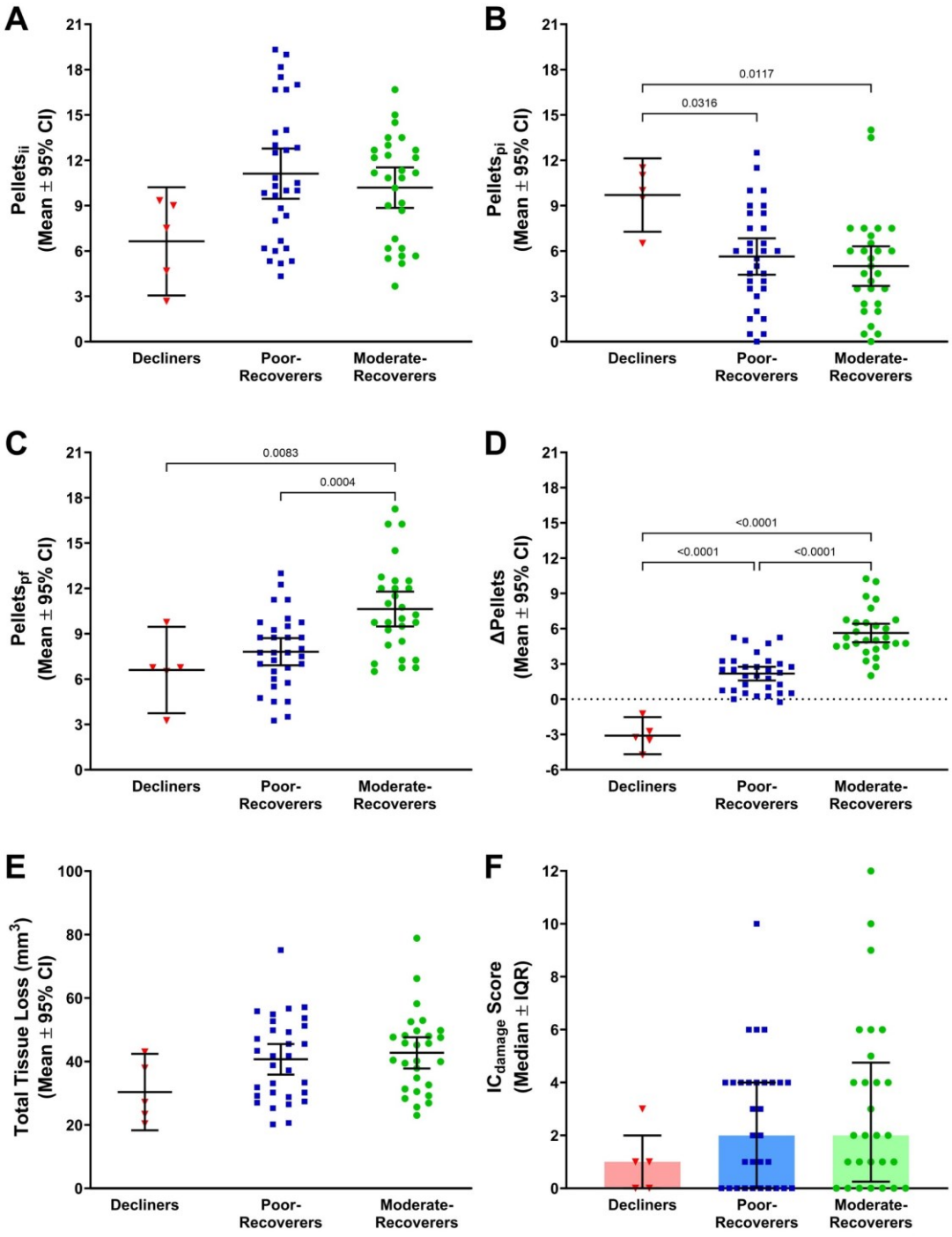

Figure 4-2 Functional and injury characteristics of recovery groups in the 3-cluster recovery model. **A** Initial impairment post-ICH (Pellets$_{ii}$); higher scores represent worse impairment. **B** Number of pellets retrieved in initial post-ICH skilled reaching assessment (Pellets$_{pi}$). **C** Number of pellets retrieved in final post-ICH skilled reaching assessment (Pellets$_{pf}$). **D** Recovery of skilled reaching function post-ICH (ΔPellets); higher scores represent greater recovery. **E** Total volume of tissue loss (mm^3). **F** Internal capsule damage score

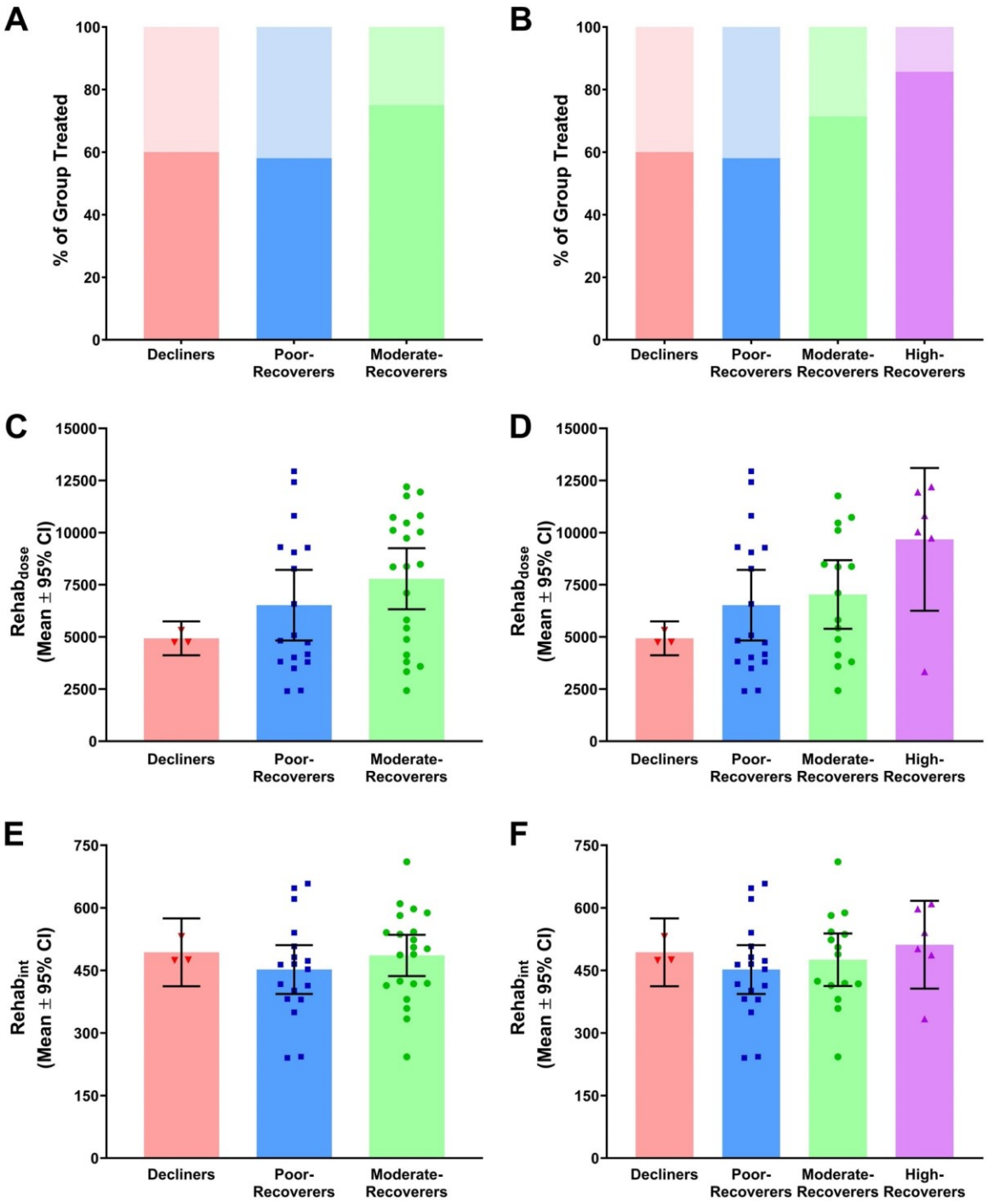

Figure 4-3 Rehabilitation characteristics of recovery groups. **A, B** Percentage of group that received rehabilitation; darkened bar represents % treated. **C, D** Total number of pellets retrieved in rehabilitation (dose). **E, F** Average number of pellets retrieved daily in rehabilitation (intensity). **C-F** 3-cluster model: decliners (n=3), poor-recoverers (n=18), moderate-recoverers (n=21); 4-cluster model: decliners (n=3), poor-recoverers (n=18), moderate-recoverers (n=15), high-recoverers (n=6)

Table 4-2 Descriptive statistics of 3-cluster model of recovery groupings

	Decliners (n=5)	**Poor-recoverers (n=31)**	**Moderate-recoverers (n=28)**	**p-value**
Functional Characteristics				
$Pellets_{pre}$	16.3±3.9	16.8±2.9^{m}	15.2±3.5	0.204 [1]
$Pellets_{pi}$	9.7±2.0^{p,m}	5.6±3.3^{d}	5.0±3.4^{d}	0.016 [2]
$Pellets_{pf}$	6.6±2.3^{f}	7.8±2.4^{m}	10.6±3.0^{d,p}	<0.001 [2]
$Pellets_{ii}$	6.6±2.9	11.1±4.5	10.2±3.5	0.071 [2]
ΔPellets	-3.1±1.3^{p,m}	2.2±1.6^{d,m}	5.6±2.0^{d,p}	<0.001 [2]
Injury Characteristics				
Tissue loss (mm^3)	30.3±9.7	40.7±13.1	42.7±12.7	0.143 [2]
IC_{damage} (median ± IQR)	1 (0, 2)	2 (0, 4)	2 (0.25, 4.75)	0.343 [1]
0 to -1 from Bregma (#yes/adjacent/no)	1/2/2	17/4/10	15/6/7	0.441 [3]
-1 to -2 from Bregma (#yes/adjacent/no)	0/0/5	4/1/26	6/1/21	0.783 [3]
-2 to -3 from Bregma (#yes/adjacent/no)	0/0/5	1/0/30	3/0/25	0.525 [3]
Rehabilitation Characteristics				
Rehabilitation (yes/no, % yes)	3/2, 60%	18/13, 58.1%	21/7, 75%	0.375 [3]
Days of treatment (#10 days/20 days)	3/0	11/7	9/12	0.196 [3]
$Rehab_{dose}$	4933±327	6523±3403	7788±3215	0.249 [2]
$Rehab_{int}$	493±32.7	452±118	485±109	0.601 [2]

Legend: Data presented as mean±SD except when noted otherwise. Significant differences among groups ($p<0.05$) represented by superscript lettering ^{d} (decliners), ^{p} (poor-recoverers), and ^{m} (moderate-recoverers). [1] Result of Kruskal-Wallis test with Dunn's multiple comparison test. [2] Result of one-way ANOVA with Tukey's multiple comparisons test. [3] Result of Fisher's Exact Test

4.3.3 Characteristics of Recovery Groups in 4-Cluster Model

There were no significant differences in pre-stroke performance in skilled reaching ($Pellets_{pre}$) among recovery groups (p=0.074) in the 4-cluster model. As before, initial impairment ($Pellets_{ii}$, Figure 4-4A) was not significantly different among groups (p=0.053), however there was a significant difference (p=0.042) in pellets retrieved in the initial post-ICH skilled reaching assessment ($Pellets_{pi}$, Figure 4-4B), with decliners retrieving significantly more pellets than moderate-recoverers (p<0.05). There was a significant difference (p<0.001) in pellet retrieval in the final post-ICH skilled reaching assessment ($Pellets_{pf}$, Figure 4-4C), with both moderate- and high-recoverers performing superiorly to decliners and poor-recoverers (p<0.05); moderate- and high-recoverers were not significantly different from one another (p=0.426). Similarly, observed recovery (ΔPellets, Figure 4-4D) significantly differed among groups (p<0.001), with moderate- and high-recoverers showing superior recovery to both decliners and poor-recoverers (p<0.001); again, moderate- and high-recoverers were not significantly different from one another (p=0.069). As in the 3-cluster model, total volume of tissue loss (Figure 4-4E) and damage in the internal capsule (IC_{damage}, Figure 4-4F) did not differ among recovery groups (p=0.224 and p=0.132, respectively). Similarly, the number of rats with or without rehabilitation (Figure 4-3B) did not differ among groups (p=0.502); intervention characteristics of dose ($Rehab_{dose}$, Figure 4-3D) and intensity ($Rehab_{int}$, Figure 4-3F) were also not significantly different (p≥0.126) in the subsets of rehabilitation treated rats. Descriptive statistics and comparisons are described in Table 4-3.

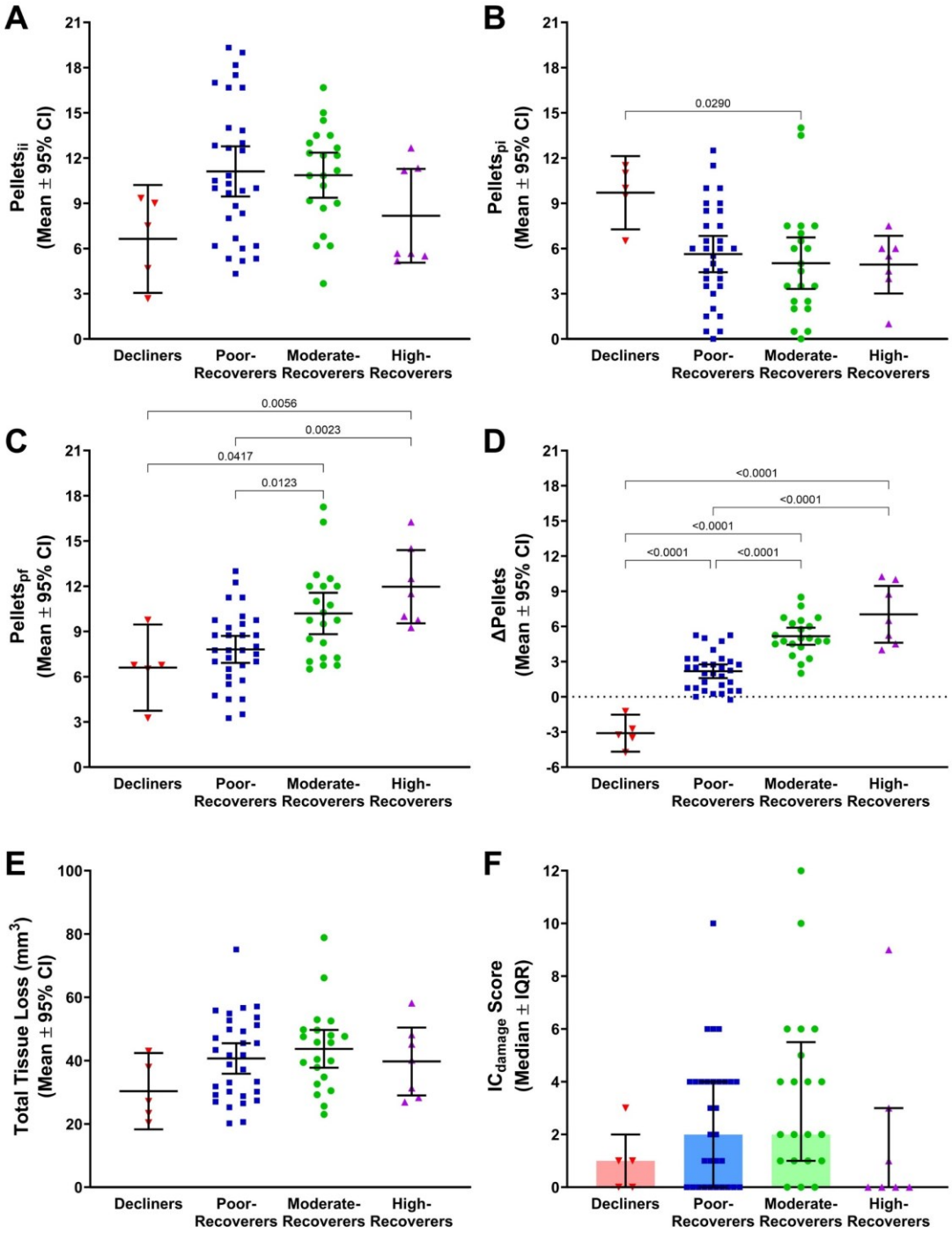

Figure 4-4 Functional and injury characteristics of recovery groups in the 4-cluster recovery model. **A** Initial impairment post-ICH (Pellets$_{ii}$); higher scores represent worse impairment. **B** Number of pellets retrieved in initial post-ICH skilled reaching assessment (Pellets$_{pi}$). **C** Number of pellets retrieved in final post-ICH skilled reaching assessment (Pellets$_{pf}$). **D** Recovery of skilled reaching function post-ICH (ΔPellets); higher scores represent greater recovery. **E** Total volume of tissue loss (mm^3). **F** Internal capsule damage score

Table 4-3 Descriptive statistics of 4-cluster model of recovery groupings

	Decliners (n=5)	**Poor-recoverers (n=31)**	**Moderate-recoverers (n=21)**	**High-recoverers (n=7)**	**p-value**
Functional Characteristics					
$Pellets_{pre}$	16.3±3.9	16.8±2.9^{h}	15.9±3.4	13.1±3.0	0.074 [1]
$Pellets_{pi}$	9.7±2.0^{m}	5.6±3.3	5.0±3.8^{d}	4.9±2.0	0.042 [2]
$Pellets_{pf}$	6.6±2.3^{m,h}	7.8±2.4^{m,h}	10.2±3.0^{d,p}	12.0±2.6^{d,p}	<0.001 [2]
$Pellets_{ii}$	6.6±2.9	11.1±4.5	10.9±3.3	8.2 ±3.4	0.053 [2]
ΔPellets	-3.1±1.3^{d,p,m}	2.2±1.6^{d,m,h}	5.2±1.6^{d,p}	7.0±2.6^{d,p}	<0.001 [2]
Injury Characteristics					
Tissue Loss (mm^3)	30.3±9.7	40.7±13.1	43.7±13.1	39.7±11.6	0.224 [2]
IC_{Damage} (median±IQR)	1 [0, 2]	2 [0, 4]	2 [1, 5.5]	0 [0, 3]	0.132 [1]
0 to -1 (#yes/adjacent/no)	1/2/2	17/4/10	13/5/3	2/1/4	0.176 [3]
-1 to -2 (#yes/adjacent/no)	0/0/5	4/1/26	5/1/15	1/0/6	0.845 [3]
-2 to -3 (#yes/adjacent/no)	0/0/5	1/0/30	2/0/19	1/0/6	0.513 [3]
Rehabilitation Characteristics					
Rehabilitation (yes/no, % yes)	3/2, 60%	18/13, 58.1%	15/6, 71.4%	6/1, 85.7%	0.502 [3]
Days of treatment (#10 days/20 days)	3/0	11/7	8/7	1/5	0.116 [3]
$Rehab_{dose}$	4933±327	6523±3403	7032±2971	9678±3260	0.126 [2]
$Rehab_{int}$	493±32.7	452±118	476±114	512±100	0.691 [2]

Legend: Data presented as mean±SD except when noted otherwise. Significant differences among groups ($p<0.05$) represented by superscript lettering ^{d} (decliners), ^{p} (poor-recoverers), ^{m} (moderate-recoverers), and ^{h} (high-recoverers). [1] Result of Kruskal-Wallis test with Dunn's multiple comparison test. [2] Result of one-way ANOVA with Tukey's multiple comparisons test. [3] Result of Fisher's Exact Test

4.3.4 Exploring Proportional Recovery

On average, rats recovered ~30% of initial impairment ($R_{\%}$). Observed recovery in skilled reaching (ΔPellets) was considered to be directly proportional to initial impairment ($Pellets_{ii}$) in the full dataset ($Y=0.34*X-0.24$, $R^2=0.21$, $F(1,62)=16.1$, $p<0.001$, Figure 4-5A,B) as both x- and y-intercepts were ≈0, with 0 falling in the middle of their respective 95% CIs. Despite the identification of statistically distinct groups in the hierarchical cluster analysis, only the decliner group was visually identifiable as its own distinct cluster.

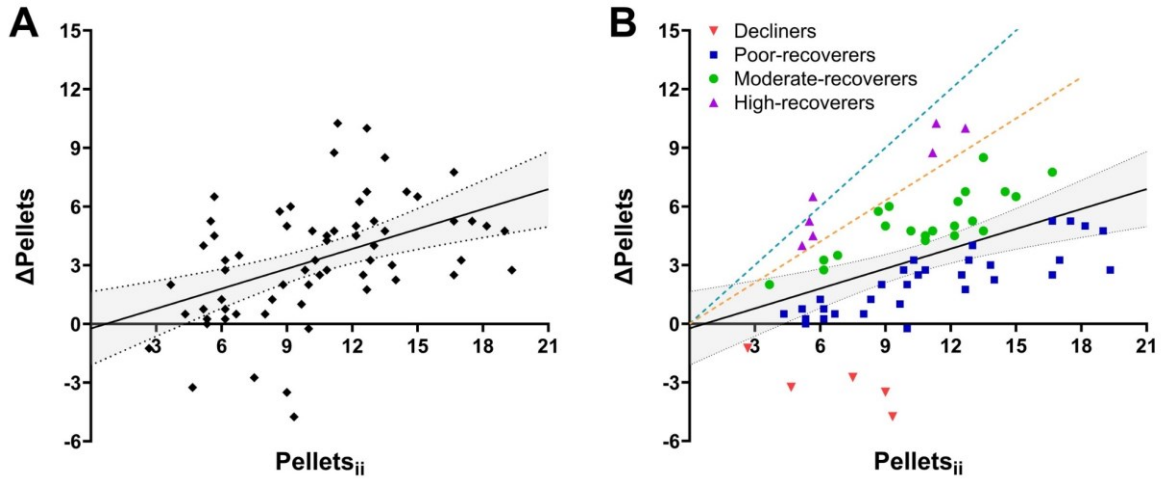

Figure 4-5 Exploring proportional recovery. **A** Relationship between initial impairment ($Pellets_{ii}$) and observed recovery (ΔPellets) in the full dataset (regression line: $Y=0.34*X-0.24$, $R^2=0.21$, $F(1,62)=16.1$, $p<0.001$; n=64). Although hierarchical clustering identified statistically different recovery groupings, only the decliner group (i.e., ΔPellets<0) was identifiable. Most rats showed some degree of recovery; there was no obvious delineation between clusters identified as poor-, moderate-, or high-recoverers. **B** Full dataset visualized with group identities from the 4-cluster model; black line represents regression line, dotted teal line represents threshold of 100% recovery, and dotted orange line represents threshold of 70% recovery

To assess how well proportional recovery was predicted by hierarchical clustering, simple linear regression was performed between observed recovery (ΔPellets) and predicted recovery ($\Delta Pellets_{predict}$) using $R_{\%}$=58.3%, the average percent recovery of impairment in the moderate-recoverers in the 3-cluster model (Figure 4-6A). Approximately 44% of rats (n=28) achieved the predicted ~58% recovery of impairment; while linear, this relationship was not directly proportional ($Y=0.339*X+2.18$, $R^2=0.333$, $F(1, 26)=13.0$, $p=0.001$), and several datapoints from the poor-recoverer group (~19%) fell within the 95% CI. Furthermore, the poor-recoverers still displayed a linear relationship between observed and predicted recovery, albeit to a lesser extent ($Y=0.293*X-1.08$, $R^2=0.694$, $F(1, 29)=65.72$, $p<0.001$).

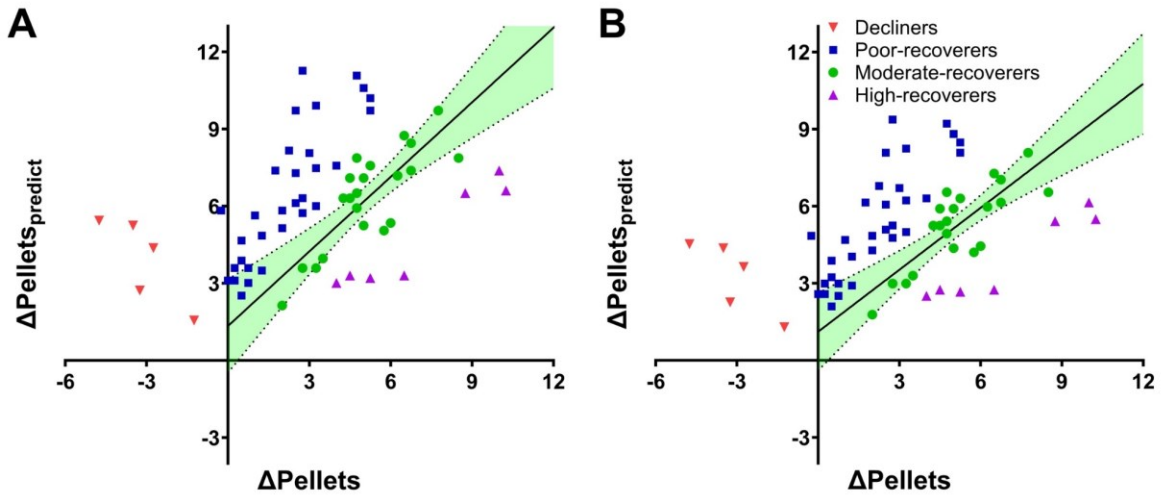

Figure 4-6 Relationship between observed recovery (ΔPellets) and predicted recovery ($\Delta Pellets_{predict}$). Note the absence of datapoints clustered around the y-axis, indicating a lack of a distinct group of non-recoverers as well as the overlap of several poor-recoverers inside the 95% CI of moderate-recoverers. **A** Relationship between observed recovery and predicted recovery in the 3-cluster model where $R_{\%}$=58.3% (regression line: $Y=0.339*X+2.18$, $R^2=0.333$, $F(1, 26)=13.0$, $p<0.001$). **B** Relationship between observed recovery and predicted recovery in the 4-cluster model where $R_{\%}$=48.5% (regression line: $Y=0.804*X+1.12$, $R^2=0.656$, $F(1,19)=36.21$, $p<0.001$)

We then assessed whether treating the high-recoverers as overperformers (i.e., outliers) improved predictive ability. Simple linear regression was again performed, this time using $R_{\%}$=48.5%, the average percent recovery of impairment in the moderate-recoverers in the 4-cluster model (Figure4-6B). Approximately 34% of rats (n=21) achieved the predicted ~49% recovery of impairment ($Y=0.804*X+1.12$, $R^2=0.656$, $F(1,19)=36.21$, $p<0.001$); although the line of best fit did not pass through the origin, the relationship may be directly proportional as the 95% CI of the x- and y- intercepts both contained 0. Despite adjusting predicted recovery to ~49%, the number of poor-recoverers that fell within the 95% CI of the moderate-recoverers remained unchanged.

4.3.5 Predictors of Recovery

Together, initial impairment ($Pellets_{ii}$) and rehabilitation dose ($Rehab_{dose}$) were superior at predicting recovery in the full dataset compared to initial impairment alone (partial F-test p=0.0132) and accounted for greater variance ($R^2=0.260$ and $R^2=0.207$ respectively). Regression coefficients are described in Table 4-4; correlational data between observed recovery ($\Delta Pellets$) and initial impairment ($Pellets_{ii}$), internal capsule damage (IC_{damage}), rehabilitation dose ($Rehab_{dose}$), and rehabilitation intensity ($Rehab_{int}$) are in Figure B-1 and Table B-1 (Appendix B).

Table 4-4 Multiple linear regression statistics for predicting ΔPellets

Full dataset						
Predictor	***β***	***95% CI***	***β****	***t-value***	***p-value***	***Goodness-of-Fit***
Intercept	-1.40	[-3.42, 0.619]	-	-1.39	0.171	adj-R^2=0.260 RMSE=2.63 p<0.0001
$Pellets_{ii}$	0.362	[0.200, 0.525]	0.486	4.45	<0.0001	
$Rehab_{dose}$	0.000199	[-0.0000431, 0.000356]	0.278	2.55	0.0132	
Intercept	-0.237	[-2.12, 1.64]	-	-0.252	0.802	R^2= 0.207 RMSE=2.75 p<0.001
$Pellets_{ii}$	0.339	[0.170, 0.508]	0.454	4.02	<0.0001	

Partial F-Test: p=0.0132, models are significantly different

Legend: β*(standardized β)

4.4 Discussion

This exploratory post-hoc analysis suggests that proportional recovery occurs in preclinical models of striatal ICH, albeit to a much lesser extent (~30%) than what has been reported elsewhere (63-97% clinically,[6–13] ~66% preclinical ischemia[27]). Interestingly, hierarchical cluster analysis proposed that recovery following preclinical ICH may be characterized by four recovery groups (decliners, poor-recoverers, moderate-recoverers, and high-recoverers), rather than two[7,8,10,12,13] or three[27] as others have previously described. Nevertheless, although many rats achieved limited recovery, there was no evidence of a distinct group of non-recoverers (i.e., "non-fitters") outside of the 5 rats whose performance worsened over time (decliners).

Hierarchical cluster analysis could not definitively determine an optimal clustering model; therefore, both the 3-cluster and 4-cluster models were characterized. Both models shared two identical recovery groups, decliners ($R_{\%}$=-48.6%) and poor-recoverers ($R_{\%}$=17.6%), however they differed in how they grouped the remaining rats; the 3-cluster model left these rats as one group (moderate-recoverers, $R_{\%}$=58.3%),

whereas the 4-cluster model split this group into moderate-recoverers ($R_{\%}$=48.5%) and high-recoverers ($R_{\%}$=87.8%).

While recovery of impairment differed among groups (i.e., ΔPellets and $R_{\%}$), these differences could not be linked to the proposed biomarkers of lesion severity, internal capsule damage, or initial impairment. The number of pellets retrieved in the initial post-stroke reaching assessment ($Pellets_{pi}$) was nearly identical among poor-, moderate-, and high-recoverer groups; while initial impairment ($Pellets_{ii}$) was somewhat greater in poor- and moderate-recoverers compared to high-recoverers owing to pre-stroke differences, this difference was not significant. Interestingly, rats in the decliner group had the least initial impairment ($Pellets_{ii}$) and showed the greatest performance in the initial post-stroke skilled reaching assessment ($Pellets_{pi}$), however they did not achieve any recovery and worsened over time. It is unclear what factors may have contributed to this worsening, however a similar pattern has also been observed after preclinical ischemia[27] and in clinical populations.[8,10,13] As bleeding is largely complete within the first 12 hours of collagenase ICH,[44] it is unlikely hematoma expansion is the direct cause of the worsening observed here ($Pellets_{pi}$ was measured 4 days after ICH). Perhaps this small subset of rats experienced a more delayed edema response which resulted in less initial impairment or experienced late re-bleeding leading to worsened function. Although previous work from our lab has found no evidence for cerebral microbleeds or hematoma expansion 3 days after ICH, we cannot definitively rule out the possibility that late re-bleeding may occur after this time.[45]

Differing from the findings of Jeffers et al., in preclinical ischemia,[27,43] injury characteristics were not significantly different among recovery groups. While greater infarct volume and >3mm^3 striatal injury was associated with worse outcomes following

ischemic stroke (i.e., identification as non-fitter or decliner),[27] total volume of tissue loss did not significantly differ among groups after ICH. Average volume of tissue loss after striatal ICH ranged from ~30 to 44 mm^3, or approximately 3.7% to 5.4% of the ipsilesional hemisphere. As the average clinical ICH is reported to be ~27 cm^3 (~4.5% hemisphere volume),[46,47] these groups represent a translationally relevant range in stroke size, although greater severity than reported in recent clinical trials of mobilization and rehabilitation after hemorrhagic stroke (1.1-1.6 and 2% hemisphere volume, respectively).[48,49] Unfortunately, this work cannot be easily compared to the one clinical study that described proportional recovery in a mixed stroke population, as lesion/hematoma volume was not reported.[8] Similarly, it is unclear to what extent proportional recovery exists after hemorrhagic stroke as this clinical subgroup also contained patients with previous stroke;[8] it is entirely possible that there may be confounds in these findings due to pre-existing impairment from previous stroke (i.e., FM-UA <66).

Perplexingly, damage to the internal capsule was relatively limited, was not associated with worsened outcomes, and in some instances was associated with greater recovery. While the reasons for this finding are unclear, two likely possibilities arise. Internal capsule damage in this dataset was predominately localized to the anterior limb (i.e., between 0 to -1 from Bregma),[39] largely sparing corticospinal and rubrospinal tract fibres in the posterior limb. This lack of lesion overlap with the posterior internal capsule may account for why we did not observe a distinct group of non-recoverers in this dataset. Indeed, clinical studies report worsened motor function with lesions to the posterior limb of the internal capsule,[50] and biomarker related assessment of CST integrity after stroke is routinely conducted in the posterior limb of the internal

capsule.[13,51] Alternatively, the IC_{damage} score may have limited validity and not accurately represent the extent of injury in this region; further validation of this scale or use of techniques such as longitudinal imaging or electrophysiological assessment of CST are needed.

Proportion of rehabilitation treated rats did not significantly vary by group nor was there a relationship between observed recovery (ΔPellets) and daily rehabilitation intensity ($Rehab_{int}$) or total rehabilitation dose ($Rehab_{dose}$). However, regression analysis found dose (i.e., total number of pellets successfully retrieved during the rehabilitation period) was a significant predictor of observed recovery (ΔPellets). Similarly, Jeffers et al. found that whether an animal received rehabilitation did not distinguish if it achieved proportional. recovery;[27] however rehabilitation intensity was non-linearly related to recovery,[43] once again supporting the idea that a threshold of intensity must be met to induce recovery.[52] These preclinical findings are at odds with clinical studies of proportional recovery which have all reported that rehabilitation dose is not predictive of recovery.[8,9,13] A few possibilities arise that may account for the discrepancy between these findings. In clinical settings, rehabilitation is part of standard/usual care for all patients; comparatively, in preclinical studies rehabilitation is typically only delivered to the treatment group, with the control remaining in conventional laboratory housing. A recent meta-analysis reported that conventional laboratory housing significantly compromised rodent health, likely increases the severity of several diseases, and is associated with worsened functional outcomes after stroke when compared to animals housed in enrichment.[53] It therefore becomes plausible that the effect of rehabilitation in preclinical studies is magnified, due to the negative effect of conventional housing on control animals. Perhaps the largest benefit

of rehabilitation in preclinical research comes from preventing worsening of health status, which leads to an exaggerated interpretation of treatment efficacy. Another possibility for this discrepancy arises from the reporting of the rehabilitation data itself; whereas most clinical trials report dose as time spent in treatment, both preclinical analyses used a more precise estimate of dose (total number of pellets retrieved) and intensity (daily number of pellets retrieved). As time in treatment does not provide an indication of participation (i.e., intensity or number of repetitions), it is possible that an undetectable dose- or intensity-response relationship exists in the clinical populations.

While average recovery of impairment ($R_{\%}$) for the clusters identified within each model were statistically different from one another and reflected clinically relevant ranges of recovery, injury and rehabilitation characteristics did not significantly differ among groups. In their prospective experiment on predictors of recovery, Jeffers et al. suggest that an improvement of at least 2.2 pellets in the skilled reaching task should be a significant improvement in function as this threshold represents the upper limit (95% CI) of recovery in rats that do not receive rehabilitation.[43] Interestingly, while the non-fitters in their retrospective analysis of preclinical ischemia displayed very minimal recovery (1±1.8 pellets; ~5% improvement),[27] the poor-recoverers in our ICH analysis improved by roughly double (2.2±1.6 pellets; ~10% improvement); by this metric, even though the poor-recoverers display less recovery, it is likely meaningful, although less than the 3-pellet cut-off we have previously used.[37,54] Here, most rats showed some degree of recovery when the relationship between initial impairment ($Pellets_{ii}$) and observed recovery ($\Delta Pellets$) was plotted; only the decliner group (i.e., $\Delta Pellets<0$) presented as an obvious cluster. There was no clear delineation between clusters identified as poor-, moderate-, or high-recoverers. Had there been a distinctive group of

non-recoverers, we would expect to observe datapoints clustered along the x-axis (i.e., ΔPellets≈0); similarly, had a distinct high-recoverer group been present we would expect a well-defined grouping above the regression line. Altogether, and based on the data presented in Figure 4-5 and the frequency distribution of $R_{\%}$ (Figure B-2 Appendix B), it is unlikely that the clusters identified through hierarchical clustering represent distinct recovery groups in our ICH dataset.

Several factors are likely to contribute to these findings. The most parsimonious explanation for why nearly all rats experience some degree of proportional recovery is the resolution of the hematoma, edema, and mass effect. [30,31,35] Indeed, resolution of these factors has been proposed for why ICH is associated with greater functional and cognitive impairments at time of admission to inpatient rehabilitation, but greater gains over the course of treatment.[31–33] As to why proportional recovery occurred to a lesser extent than other reports is less clear. It is unlikely that stroke type alone is responsible for this lessened recovery. While the lesions observed in this study were all induced in the same location (striatum), there was still considerable variability in lesion shape and size, as is common in this model. Subcortical striatal and combined cortical-subcortical injury have been associated with worse functional outcomes than cortical injury alone in both clinical[55] and preclinical populations.[56] While the average volume of tissue loss after striatal ICH here ranged from ~30 to 44 mm^3, this range was ~2-4x greater than the volume of striatal injury observed in the recovery groups reported by Jeffers et al.,[27,43] which may account for some of the differences observed between our analyses. As this region plays an essential role in facilitating voluntary movement, it is possible that subcortical lesions over a certain size threshold disrupt corticostriatal connections thereby altering the capacity for network reorganization and resulting in less

recovery.[55,57] While it is unclear what this threshold may be in rodents, there is a clear interaction between the effect of hemorrhage location and size on clinical outcomes after ICH.[58]

This exploratory analysis is based on a moderate-sized, somewhat homogenous dataset, which may limit the generalizability of these findings. As well, the Montoya staircase task does not fully capture the same nuance in recovery as the clinical FM-UE; while we assess recovery of impairment as a change in the ability to successfully retrieve pellets, this more accurately describes recovery at the level of activity rather than impairment.[59] As such, it is difficult to distinguish what is resolution of impairment or simply compensation, and rats with striatal ICH injury do show compensatory movements in skilled reaching.[60] Comparatively, clinical measures such as the FM-UE assess recovery of impairment through the resolution of motor synergies, reflexes, coordination, and sensation;[61] partial resolution of function can be captured across these domains, whereas the staircase task is more of an all-or-nothing approach. Future studies of proportional recovery in preclinical models should consider using a battery of assessments and include kinematic analysis to better gauge recovery of impairment.[62,63] Unlike clinical reports of proportional recovery which assume pre-stroke function to be the maximum achievable FM-UE score (66), preclinical studies have the benefit of directly assessing pre-stroke function. However, performance in the staircase test can have significant interindividual variability, and not all rats will achieve a maximal score (21) after several weeks of training. This creates a unique yet interesting problem as it allows for recovery to exceed 100%, as observed in one rat in this analysis. Lastly, it is possible that due to the small and variable sample sizes of the groups identified (n=5-31), the statistical analyses presented here are more sensitive to extreme values (i.e., rats

with PR>100%) and variability than models with a greater number of observations. Similarly, though exploring predictors of recovery in the groups identified by hierarchical clustering could be valuable, doing so would be statistically dubious owing to the group sizes, variability, and number of predictor variables.[64] Expansion of this analysis to a larger dataset (i.e., retrospective analysis of other ICH studies) or a prospective comparison of recovery after equivalent subcortical hemorrhagic and ischemic stroke (i.e., ICH and ET-1) would both be very interesting avenues for future investigation.

4.5 Conclusion

These results provide further support that proportional recovery is a biologically conserved phenomenon after stroke, albeit to a much lesser extent than what has been observed clinically. Although observed recovery and initial impairment were proportional, initial impairment alone only weakly explained observed recovery of skilled reaching (R^2=0.21) while initial impairment and rehabilitation dose somewhat improved this prediction, together they still only accounted for a small amount of the variability in observed recovery (R^2=0.26). Nearly all rats showed at least some recovery of impairment after ICH, however no characteristic reliably identified whether an animal would achieve poor-, moderate-, or high recovery. As such, these results highlight the need to do more research identifying biomarkers capable of predicting outcome after ICH, as initial impairment alone is insufficient.

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Chapter 5 | General Discussion

5.1. Summary of Findings

The overarching goal of this thesis was to characterize and assess the efficacy of rehabilitation in translational models of intracerebral hemorrhage (ICH). As clinical trials have yet to identify a treatment that consistently lessens injury and impairment after ICH,[1,2] even with advances in acute care (e.g., surgery), treatment for hemorrhagic stroke remains limited to medical management and rehabilitation.[3] Yet despite the limited treatment options available following ICH, and the disproportionate burden of disease related to hemorrhagic stroke (8.1-12.6 disability adjusted life years for ICH, 4.6-5.9 for ischemia),[4,5] most preclinical studies use experimental models of ischemia when exploring how and why rehabilitation contributes to recovery after stroke. Consequently, much of our understanding of recovery after ICH is predicated on the assumption that the spatiotemporal dynamics of the mechanisms of injury and recovery after ischemic and hemorrhagic stroke are similar. However, there is increasing evidence to suggest that this assumption is unfounded.[6,7] Here, I provide a summary of our findings related to rehabilitation and motor recovery in translational models of ICH, discuss the limitations of these findings, and recommend several avenues of investigation that should be pursued in the future.

5.1.1 Early, Intense Rehabilitation after ICH

To explore how altering treatment parameters impacts mechanistic responses and functional outcomes, we conducted two experiments assessing the efficacy of enriched rehabilitation (ER) initiated 5 days after ICH (Chapter 2).[8] Our lab has repeatedly shown behavioural benefit with ER beginning at 7 days after ICH,[9–12] although evidence supporting the neuroprotective effect of rehabilitation has been

inconsistent, perhaps related to key model differences.[9,12] In 2017 Williamson et al. found that ER initiated 7 days after ICH accelerated hematoma clearance and limited both ionic dyshomeostasis and oxidative stress, proposing that these mechanisms may contribute to neuroprotection and functional recovery.[13] However, this study did not assess behavioural function or overall neuroprotection. As others have observed treatment efficacy with ER initiated 5 days after ischemic stroke,[14] we hypothesized that ER initiated 5 days after moderate striatal ICH would accelerate hematoma clearance and improve functional recovery, which would be associated with a reduction in chronic cell death similar to or greater than observed when therapy is initiated 7 days post-ICH. We used high-intensity ER protocols initiated 5 days after ICH and assessed hematoma clearance in the sub-acute phase of recovery (14 days) and neuroprotection in the chronic phase (60 days), both acceptable survival times for these endpoints. Contrary to both our hypothesis and previous findings,[11,13] we did not detect a significant difference in hematoma clearance, lesion volume, or recovery in skilled reaching compared to untreated controls.

These are unlikely to be spurious findings (i.e., false negatives). We used treatment protocols with similar or greater intensity as reported beneficial in ischemic stroke,[15,16] similar treatment complexity as others in ICH,[9–12,14,17] *a priori* planning to reduce bias,[18] group sizes up to 3 times larger than commonly used in preclinical ICH research,[18] and high statistical power in our experiments[19] (e.g., 99% power to detect 3-pellet treatment effect in pooled analysis). We assessed whether intervention intensity differed when delivered in the light or dark phase of the housing cycle and if it impacted efficacy[15] (it did not); all groups performed >400 pellet retrievals/day, however intervention in the dark trended towards greater improvement. We also assessed

whether longer treatment duration (i.e., 10 or 20 days) altered efficacy and whether later functional assessment (i.e., 31 days) would detect benefit. Again, it did not. While Williamson et al. reported accelerated hematoma clearance with ER beginning 7 days after ICH, neither treatment intensity nor functional measures were reported, so it is unclear if this finding is associated with improved behaviour.[13] Perhaps acceleration of hematoma clearance must occur in order to see significant therapeutic benefit. If so, maybe our therapy, either due to timing or intensity, failed to accelerate hematoma clearance and this accounts for the lack of functional or neuroprotective benefit observed in our work.

In our original analysis we could not rule out the possibility that our ICH model had caused significant damage to the internal capsule, thereby limiting potential for recovery (as seen in humans).[20] In our exploratory analysis of predictors of recovery we stained and analyzed a secondary set of tissue with the myelin stain Luxol Fast Blue,[21] and we were able to address this concern. Owing to the age and quality of the tissue when it was stained, we were only able to grossly characterize injury in this region which is unlikely to have adequately captured sub-lethal structural and functional damage. However, direct damage to the internal capsule was generally quite limited, predominately localized to the anterior limb when present, and well below the threshold of corticospinal tract (CST) injury associated with non-responder status in patients (i.e., >63% CST injury);[20] furthermore, increased internal capsule damage did not correlate with worsened recovery.

While small inter-study differences in treatment protocols and injury may be responsible for why we failed to see benefit with our ER interventions, perhaps the most parsimonious explanation involves the timing of intervention onset. Results of our

meta-analysis align with this explanation – no treatment delivered with an onset between 48 hours to 5 days after ICH provided significant functional benefit.[22] Together, these findings suggest that <7 days after ICH (e.g., near the peaks of intracranial pressure, edema, and secondary cell death)[23–25] may represent a delicate window in post-ICH recovery where intervention may interact with endogenous mechanisms of injury and repair to accelerate, delay, or impede recovery.

5.1.2 Exploring Rehabilitation Efficacy in Translational Models of ICH

The challenges we faced in extending the findings of Williamson et al.[13] led us to question the overall efficacy of motor rehabilitation in translational models of ICH. Our systematic review and meta-analysis (Chapter 3)[22] aimed to characterize common motor interventions used after preclinical ICH, assess the quality of this research, and provide insight into the efficacy of post-ICH rehabilitation. Overall, study populations were largely homogenous (i.e., young, male rodents with collagenase induced subcortical ICH), however the type of rehabilitation intervention and associated parameters (i.e., timing, period, duration, frequency, intensity, and dose) ranged considerably. Aerobic exercise (AE), enriched rehabilitation (ER), and constraint-induced movement therapy (CIMT)/forced limb use (FLU) accounted for ~70% of the rehabilitation interventions identified in our review. Unsurprisingly, and in agreement with other reviews of translational stroke research,[18,26] quality of reporting and risk of bias varied considerably, and many articles had one or more methodological or reporting error considered to be significant (e.g., inappropriate statistical methods, unclear timing of treatment onset or duration, unclear group sizes, etc.). Similarly, many failed to consider critical translational concerns and used interventions that are extremely

unlikely to be used clinically (e.g., running >1km within 24 hours of ICH)[27,28] or included so little detail about the intervention as to make it impossible to determine intensity or dose.

Overall, rehabilitation modestly improved motor recovery in skilled reaching, spontaneous impaired forelimb use, and locomotor function, but only CIMT+FLU and skilled reaching (REACH) interventions improved recovery across all three endpoints. Aligning with clinical findings, rehabilitation efficacy was greatest in animals with mild ICH (i.e., lesions ≤30 mm^3, ~3.7% hemisphere volume), and very limited in those with the most severe strokes. Perhaps most intriguingly, we observed a complex relationship between intervention timing and treatment efficacy, as only treatments initiated 24-48 hours or 7-8 days after ICH conferred significant benefit. Upon closer examination, only FLU initiated 24 hours after small capsular ICH was beneficial, whereas all other interventions delivered between 48 hours-5 days post-ICH were not. This is in direct contrast to findings from a meta-analysis of preclinical rehabilitation in ischemia by Schmidt and colleagues, which found intervention timing of 1-5 days after stroke to be most efficacious.[29] Furthermore, they found an overall neuroprotective effect of rehabilitation on infarct volume (14% reduction, 95% CI 2-25%); while not formally quantified in our analysis, only 2/37 interventions that assessed lesion or hematoma volume after ICH reported a significant neuroprotective effect of rehabilitation.[11,30] In combination with the wide range in study quality we observed, these results highlight the necessity of future studies that systematically manipulate treatment parameters, most notably timing and dosage, and the need to conduct direct comparisons of efficacy between stroke subtypes.

5.1.3 Predicting Recovery

Finally, we conducted a retrospective, exploratory post-hoc analysis to assess whether the clinically documented phenomenon of proportional recovery[31–38] occurs in preclinical models of subcortical ICH (Chapter 4). Noting that not every patient will benefit from every therapy, identifying biomarkers to predict recovery and capacity for rehabilitation facilitated recovery is essential to understanding who may benefit from a given therapy and why.[39,40] Translational studies play a critical role in our understanding of the mechanisms of injury and disease, yet many promising preclinical results are not reproducible and/or fail to translate into viable clinical treatments.[41] Therefore, ensuring that preclinical studies adequately model the pathogenesis and variability of clinical conditions is essential to improving the odds of successful translation, particularly when using mechanistic approaches to exploring treatment efficacy.

The results of our exploratory analysis provide the first characterization of proportional recovery in an exclusively hemorrhagic stroke population and lend support to the idea that proportional recovery after stroke is a biologically conserved phenomenon across species, a claim first made by Jeffers and colleagues in a retrospective analysis of proportional recovery after endothelin-1 (ET-1) induced ischemic stroke.[42] However, our analysis showed that on average, ~30% of impairment is recovered in a proportional manner after striatal ICH in rats, a much lesser extent than others have reported (63-97% clinically,[31–38] ~66% preclinical ischemia[42]). Plotting the relationship between initial impairment and observed recovery did not provide clear evidence for multiple distinct recovery groups; even rats identified as non-recoverers showed some degree of proportional recovery. Furthermore, initial impairment alone

poorly explained observed recovery of skilled reaching (R^2=0.21). Though hierarchical clustering identified statistically different recovery subgroups, differences in recovery could not be attributed to differences in the proposed biomarkers of lesion severity, internal capsule damage, or initial impairment. These findings were surprising as the retrospective analysis conducted by Jeffers et al. found that rats that fit the proportional recovery rule had less initial impairment, smaller lesion size, and less striatal injury,[42] and CST integrity is considered to be a reliable biomarker for recovery after stroke.[33,38]

It is likely that resolution of the hematoma, edema, and mass effect[43–45] play a role in why nearly all rats experience some degree of recovery; however why recovery occurs to a lesser extent than reported elsewhere is unclear. Though the strokes assessed in this dataset were by no means severe, they represent damage equivalent to ~3.7-5.4% of the hemisphere, and this damage was predominately localized to the striatum. As subcortical injury is associated with worse functional outcomes,[46–48] it is likely that lesion size, location (striatum), and stroke subtype all contribute to the pattern of recovery observed in this analysis. To definitively determine the extent to which stroke subtype contributes to these findings, a direct comparison of recovery between subcortical striatal ischemic and hemorrhagic stroke is required.

5.2 Limitations

Despite our efforts to use translationally relevant experimental designs and *a priori* planning whenever possible, there are several interrelated factors that may limit the generalizability of the findings of our experimental work, as we only explored the efficacy of early, intense ER in the context of moderate severity striatal ICH. As outcomes are influenced by both stroke size and location,[48,49] it is possible that ER

beginning 5 days after ICH may be beneficial in smaller strokes or strokes in other regions of the brain. Furthermore, while Williamson et al. reported accelerated hematoma clearance with ER beginning 7 days after ICH, neither treatment intensity nor functional measures were reported.[13] It may be the case that in order to see significant therapeutic benefit hematoma clearance must be accelerated – if so, perhaps our intervention failed to accelerate hematoma clearance, maybe due to intensity or timing, and this accounts for the lack of functional or neuroprotective benefit observed in our work.

As intensity and dose are often poorly described or defined in both clinical and preclinical literature, there is limited data available regarding optimal rehabilitation intensity or dose. Preclinical studies in ischemia support the idea that a threshold of rehabilitation intensity must be met to induce recovery,[15,16] though it is unclear whether there is a limit that produces diminishing returns or even harm. However, there is some clinical evidence that supports both of these ideas – a recent analysis reported diminishing returns after patients achieved 95 min therapy/day,[50] and a clinical trial found that delivery of high-intensity CIMT early after stroke resulted in less improvement in functional outcomes than lower-intensity CIMT or standard care, which suggests an inverse dose-response relationship.[51] While there is no other ER intensity data available in preclinical ICH, studies in ischemia have reported therapeutic benefit with ~200-250 successful pellet retrievals/day following large middle cerebral artery occlusion (MCAo)[15] and combined cortical-subcortical stroke;[52] in our work, rats completed >400 pellet retrievals/day, which may simply be too intense, or too intense too early after ICH.

Though it is generally accepted that earlier intervention provides greater benefit (i.e., subacute phase vs. chronic phase), clinical interventions delivered in the hyper-acute (0-24 hours) and acute (1-7 days) phases of recovery[53] have produced mixed results.[54–57] The AVERT clinical trial found that frequent, high dose, early out of bed mobilization was associated with decreased odds of favourable outcome at 3 months after stroke.[55] Further analysis found that when time out of bed was kept constant, increased intensity (i.e., greater time out of bed), but not increased frequency of mobilization was associated with less favourable outcomes.[56] While underpowered to detect a significant treatment effect, subgroup analysis also suggested reduced odds of favourable outcome with very early mobilization in both ICH and severe stroke.[55] Similarly, experimental studies in non-ICH models of brain injury have also demonstrated that early and intense rehabilitation can worsen functional outcomes and exacerbate brain injury in a use-dependent manner.[58,59] Though there is some investigation of early, intense intervention <7 days after ICH in preclinical models that suggests both benefit and harm,[27,28] our understanding of these treatment effects is poor due to limited resolution of time dependent effects (i.e., interventions/endpoints only assessed at 1-2 times with no long term measures) and numerous methodological and reporting errors in this work.

While our meta-analysis provides some insight into how treatment parameters impact efficacy, it is limited by the small number of studies eligible for many of the subgroup analyses. Similarly, differing methods of analysis between our work and the meta-analysis of treatment efficacy in preclinical models of ischemia conducted by Schmidt et al.[29] do not allow us to directly compare how treatment efficacy differs by treatment type or modifiable parameter (i.e., timing, dose) between ischemia and ICH.

Although meta-analysis can provide excellent insight into therapeutic efficacy, it does not replace the necessity of completing high quality, original research as both heterogeneity and quality of included studies influence the quality of the meta-analysis and its results.[60] Experimental design choices such as the use of conventional housing for control animals[61] or use of assessments that cannot definitively distinguish between compensation and true recovery may lead to overestimated effect sizes in original research and subsequent meta-analysis. Many of the articles we analyzed contained one or more methodological errors (see Chapter 3, Table 3-5); while one error may not have a major effect on a single treatment estimate, the cumulative effect of errors across interventions could have a substantial impact in our overall estimate of treatment efficacy. Finally, our trim and fill analysis of the skilled reaching data revealed missing negative and null data, suggesting likely reporting and/or publication bias. This is unsurprising, as dark data, work that is unpublished or otherwise inaccessible,[62] is a common concern across many disciplines of translational neuroscience and believed to make up nearly 50% of research output.[63]

While using highly controlled, simplified models of disease may increase the internal validity of our research, this may have the unintended consequence of sacrificing external validity.[64] Although women account for ~50% of all stroke cases, very few translational stroke studies use female subjects.[18,26] In the experimental studies described in Chapter 2 we only used young, healthy male rats with no relevant comorbidities (e.g., hypertension, diabetes).[8] Similarly, only 1/48 intervention groups evaluated in our meta-analysis (Chapter 3) reported the use of female subjects.[65] Many researchers justify the use of exclusively male populations when studying potential neuroprotectants as 17-β estradiol has been shown to be neuroprotective and alter

inflammatory responses after brain injury.[66] While this may present as a confound if not appropriately controlled for, this does not negate the necessity of researching treatments in female cohorts. Furthermore, many argue that the rapid cycling of the estrous cycle in rats and related effects of female sex hormones introduces excess variability into research that is avoidable by using male subjects.[26] However, this is simply not true – females are not inherently more variable than males.[67,68] Critically, accounting for different stages of the estrous cycle (i.e., high-estradiol during proestrus, low-estradiol during diestrus) provides greater insight into both mechanistic and behavioural sex differences, which may actually improve translational success.[69] For example, increases in estradiol are associated with increased expression of brain derived neurotrophic factor (BDNF),[70] which plays a critical role in post-stroke neuroplasticity. Exercise is well documented to upregulate BDNF,[71] and there is evidence to suggest that a critical threshold of BDNF expression must be met to induce functional recovery after stroke.[15] Relatedly, there is some evidence to suggest that exercise induced BDNF expression differs by sex, with females showing greater upregulation of BDNF than males when compared to their respective controls.[72]

Another limitation to this thesis is the lack of aged animals used in both our experimental work and the interventions analyzed in our meta-analysis. The risk of stroke varies by both sex and age across the lifespan,[73] with advanced age associated with both increased risk[74] and worsened outcomes.[75] Aging induces many structural and physiological changes to the brain, cerebrovascular system, and immune system.[75–77] Of particular relevance to rehabilitation and recovery after ICH are the age related changes observed in microglia, such as alterations in resting state phenotype and increased proinflammatory function.[78] As microglia play a critical role in the clearance of debris

after brain injury (i.e., hematoma clearance),[79] these age related changes likely contribute to worsened recovery. Indeed, some have documented less early neuronal death and microglial activation 1 day after ICH in aged rats (i.e., >18 months),[80] but by day 3 aged rats display greater edema[75,81] and a substantially greater number of activated microglia in the ipsilesional hemisphere than their younger counterparts.[75,81,82] Some have also reported delayed hematoma resolution in aged animals, with impaired microglial function hypothesized to contribute to this effect.[80,82] Interestingly, aerobic exercise has been shown to reduce microglia proliferation and expression of proinflammatory phenotypes in naïve aged mice,[83] however there is limited research on how exercise (i.e., rehabilitation) alters microglial function after ICH.[27,28]

A final consideration in interpreting the work presented in this thesis surrounds the choice of experimental stroke models used in our work and the broader ICH rehabilitation literature. As animal models are used to explore underlying mechanisms of injury and recovery after stroke, the generalizability of our findings is reliant on the ability of our chosen models to mimic the pathophysiology of ICH. However, modelling a spontaneous phenomenon such as ICH is particularly challenging, and no model perfectly mimics the clinical pathophysiology of ICH.[24,84,85] Despite multiple experimental models of ICH, the collagenase (COL) model is used in >50% of all ICH studies[18] and was used in 97% of the ICH rehabilitation articles included in our meta-analysis.[22] While the COL model results in a period of bleeding not entirely dissimilar to that seen in humans, injection of an exogenous substance such as collagenase may exacerbate inflammation[86] and blood brain barrier dysfunction,[24] complicating our understanding of the spatiotemporal dynamics of the neuroinflammatory response after

stroke and how it may be impacted by various interventions. Although the autologous whole blood (AWB) model largely avoids these concerns, it is characterized by one large bleed and is not caused by spontaneous vascular rupture, making it inappropriate for exploring hematoma expansion or therapies that may impact ongoing bleeding.[24] It is still somewhat unclear which model more accurately mimics the course of cell death and hematoma resolution after ICH, in part due to the differing time course of injury between humans and preclinical populations (i.e., rodents). The COL model is associated with a period of ongoing cell death as tissue loss is observed to increase for up to 4 weeks after ICH, whereas tissue loss is largely stabilized within one week of AWB ICH.[24] Our knowledge of the timing and pattern of cell death and hematoma resolution after human ICH and how it compares to preclinical models is somewhat incomplete; though many clinical trials use serial neuroimaging, few collect images with a similar frequency to preclinical studies to establish a complete understanding of the temporal evolution of injury after human ICH. Similar to cell death, patterns of recovery differ between COL and AWB ICH; recovery is typically more rapid and complete after AWB ICH, whereas recovery after COL is characterized by slower, incomplete resolution of impairment.[24] As the majority of humans stroke survivors will experience some degree of incomplete resolution of impairment,[74,87] use of the AWB model for the purpose of assessing long-term functional change may be somewhat dubious. As no single model fully captures the features of spontaneous clinical ICH, therapies shown to be successful in one model of ICH should be assessed in a second model before proceeding to the next phase of the translational process.[24]

5.3 Future Directions

The findings from our experimental work and meta-analysis suggest a complex relationship between timing of intervention onset, intensity, and treatment efficacy after ICH. Comparing the results of our meta-analysis in ICH with the meta-analysis in ischemia conducted by Schmidt et al.[29] suggests the possibility of differing responses to rehabilitation after ischemic and hemorrhagic stroke. Similarly, our analysis of predictors of recovery suggests that there may be subtype specific differences in recovery. Together, these findings provide a starting point for multiple avenues of investigation in future stroke recovery research. Subsequent work should aim to address whether manipulating intervention timing and intensity during the acute phase of recovery after ICH (i.e., <7 days) alters outcomes, if rehabilitation efficacy varies by stroke subtype, whether ~30% proportional recovery is a characteristic of all hemorrhagic stroke or only striatal hemorrhage, and finally, whether these findings differ by sex and age.

To better understand how treatment may alter mechanisms of injury and recovery early after ICH, future studies should include assessment of markers of inflammation, cell stress, and dysfunction. While exercise is associated with a number of health benefits, intense, exhaustive exercise has been shown to produce reactive oxygen species (ROS) in both brain and muscle at a rate that may overwhelm cellular antioxidant capacities and lead to cell dysfunction or death.[88,89] Oxidative stress is a critical threat to cell health after ICH, due to increased ROS as a result of degradation of the hematoma (i.e., hemoglobin breakdown)[90,91] and phagocytic activity of microglia and macrophages[92,93] (see Section 1.6.2, Secondary Injury); understanding whether rehabilitation initiated early after ICH exacerbates to oxidative stress and tips the

balance in favour of cell health, dysfunction, or death is likely a fundamental piece of the puzzle in determining the optimal timing of intervention after ICH.

To further evaluate predictors of recovery, characterization of injury must be improved. While lesion volume is useful for assessing overall neuroprotective effects, it does not provide sufficient detail to fully assess possible biomarkers of recovery. Future work would benefit from the use of techniques such as serial MRI neuroimaging,[24] which would provide repeated measures, within-subject information about hematoma size and resolution. Similarly, a number of methods could be used to comprehensively assess structure and function (or dysfunction) in the CST such as serial MRI neuroimaging,[24] injecting tracers into cortical layer V and assessing descending CST fibre density,[94] using electrophysiology to measure presence or absence of motor evoked potentials (MEPs),[95] or simply using better immunohistochemical targets to identify axonal modeling and repair in the CST (e.g., protein kinase C γ).[96] In addition to providing greater insight into injury, the aforementioned techniques would help to validate whether clinically accepted biomarkers of recovery, such as CST function, also predict recovery in common experimental stroke models.

Demonstrating recovery at the level of impairment, not simply activity, must be improved in subsequent preclinical work. Future studies need to include a combination of functional assessments (e.g., test battery consisting of skilled reaching, walking, neurological deficit score, grasping tasks, etc.) and kinematic movement analysis in evaluations of recovery and treatment efficacy.[97] When used in combination, these assessments will provide insight into recovery at both the level of impairment and activity,[98] and deliver a more comprehensive understanding of recovery in preclinical

models that more closely reflects how impairment and recovery are measured by clinical tools (e.g., Fugl-Meyer).

Future studies must also include the use of translationally relevant populations that consider the factors of sex and age. As ~50% of stroke patients are female, understanding if sex differences exist in response to rehabilitation interventions should be a critical component of future research. Since there appears to be sex differences in response to exercise in naïve animals (e.g., increased BDNF expression in females),[72] it is entirely possible that efficacy of rehabilitation may differ between sexes or that optimal treatment parameters may not be identical. While we failed to see benefit of early, intense ER in our experimental work and only modest benefit across all therapies in our meta-analysis, it is possible that studies conducted in females may have different results. From a purely technical standpoint, use of female rats would likely improve the ability to collect long term behavioural data; female rats are generally smaller than males and should not outgrow testing apparatus as quickly (an issue we grappled with in our experimental work). Although there are many challenges associated with conducting studies in aged rats (e.g., costs, loss of subjects, greater mortality), future rehabilitation studies must consider exploring rehabilitation interventions in aged populations. As there are known temporal differences in immune cell activation after ICH in aged animals, [75,80–82] it is entirely possible that optimal timing of intervention onset and intensity may differ between young and aged subjects. Furthermore, as the risk of stroke increases after menopause,[73] these age studies must include both male and female subjects.

Finally, future research must directly address the overarching question of whether recovery and response to rehabilitation differs by stroke subtype. In order to

answer whether the proportional recovery we observed after ICH is a result of stroke subtype or simply striatal injury requires direct comparison; an experiment of recovery after subcortical striatal ischemic (i.e., ET-1 induced) and hemorrhagic (i.e., COL induced) stroke is warranted. Ultimately, completion of an updated, meta-analysis exploring efficacy of motor rehabilitation in translational models of ischemia using the same methodology, endpoints, and subgroups as described in our meta-analysis is needed.

Experiments designed to explore these fundamental questions of how timing, intensity, and stroke subtype influence recovery after stroke must be guided by evidence, rigorously designed, and carefully executed using translationally relevant endpoints and assessment times. To ensure scientific and translational rigour, researchers must adhere to best practices as outlined in the and RIGOR[99] and ARRIVE[100–102] guidelines; we also recommend consulting the roadmap to improving the quality of preclinical rehabilitation research outlined in our meta-analysis (Chapter 3, Table 3-6).

As it will take substantial time and resources to complete this work, a multi-laboratory approach such as that used by the Stroke Preclinical Assessment Network (SPAN)[103,104] should be used to address these questions. Collaboration will be essential, as tackling these questions will be an incredibly complex task requiring a series of experiments that systematically manipulate various experimental factors. An example of this complexity can be found in the sheer volume of treatment groups that would be needed to address various elements of interest: intervention timing (e.g., ER initiated at 3 days, 5 days, and 7 days), intensity (e.g., low, moderate, high intensity), stroke subtype (e.g., ischemic vs. hemorrhagic), stroke location (e.g., cortical, striatal, combined), sex

(i.e., female, male), and age (i.e., young, middle aged, old). In order to relate how these factors effect mechanisms of neurological and behavioural recovery, a combination of behavioural (e.g., skilled reaching, locomotor function, kinematic movement analysis), histological (e.g., hematoma clearance, lesion volume, CST injury, dendritic branching), and mechanistic endpoints (e.g., markers of oxidative stress, microglia activation, inflammation, etc.) would need to be assessed. To provide sufficient temporal resolution to these processes, endpoints would have to be evaluated at multiple times in each group in each of the acute (<7 days), sub-acute (<30 days), and chronic phases (>30 days) of recovery. Completing this work would likely require >1000 experimental subjects to ensure sufficient statistical power in primary endpoints, appropriate use of control groups, and account for endpoints that that are incompatible with each other (e.g., microglia activity at day 3 vs. hematoma clearance at day 14 vs. lesion volume at day 60). It quickly becomes clear that to address these questions would be onerous, costly, and technically demanding; nevertheless, answering how timing, intensity, and stroke subtype influence recovery after stroke would provide invaluable insight to preclinical researchers and clinicians alike.

5.4 Conclusion

The work presented in this thesis evaluated the efficacy of rehabilitation in translational models of ICH and delivers a critical review of the current state of translational rehabilitation research. While safety and efficacy of very early intervention (i.e., ≤24 hours) has been explored in both clinical and preclinical settings, there is a marked gap in our understanding of the optimal window for intervention within the first week of stroke. Here, we investigated whether initiating ER 5 days after ICH would

accelerate hematoma clearance, improve behavioural function, and lessen chronic cell death. We hypothesized that shortening the delay between injury and intervention onset from 7 to 5 days would provide similar or greater benefit than that reported by Williamson et al. in 2017.[13] Surprisingly, our intervention did not provide benefit. Next, we completed a systematic review and meta-analysis of motor rehabilitation after preclinical ICH, providing a critical review of the current state of translational rehabilitation research and offering insight into how altering intervention parameters, such as timing, influences efficacy. Our analysis suggested a complex relationship between intervention onset and treatment efficacy after ICH, and that this is likely influenced by treatment type and/or intensity. Future research that systematically manipulates intervention onset and directly compares the effect on efficacy must be completed; as such, we have provided a roadmap to ensure that these next critical studies are designed to avoid the errors of the past. Finally, we explored whether the phenomenon of proportional recovery exists in preclinical ICH. We provide further support that proportional recovery is a biologically conserved phenomenon after stroke, however it occurs to a much lesser extent in preclinical models of striatal ICH (~30%).

While at times this thesis offers a bleak view of the past and present state of translational stroke rehabilitation research, it is not written without hope for the future. The scientific community is on the precipice of major systemic change as many researchers embrace the ideas of open science and data sharing. Similarly, the acknowledgement that data should be FAIR (findable, accessible, interoperable, reusable)[105] has led to recent policy changes where funding agencies now require researchers to implement data management and sharing policies (e.g., NIH). While data sharing should improve transparency and replicability,[106] its greatest utility will likely lie

in its ability to enable future researchers to conduct individual subject data meta-analysis,[107] ultimately leading to a more precise understanding of rehabilitation efficacy after stroke and the factors that influence it.

5.5 References

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Chapter 3

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Chapter 4

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Chapter 5

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Appendix A | Supplement to Chapter 3

Table A-1 Experimental Design Characteristics

Article ID	Experimental Design					Behavioural Endpoints				
First Author Year [Ref]	**Species, Strain [Sex]**	**ICH Model**	**Survival**	**Stroke Size Assessment**	**Rehab-ilitation**	**Forelimb Function [Test]**	**Locomotor Function [Test]**	**Neuro-behavioural Battery**	**Other [Test]**	**Latest Assess-ment**
DeBow 2003a[1]	Rat, Sprague-Dawley [M]	COL	60 days	Lesion volume	FLU	Skilled reaching [Montoya staircase], Spontaneous impaired forelimb use [cylinder]	Walking [horizontal ladder]		Elevated body swing test	60 days
DeBow 2003b[1]	Rat, Sprague-Dawley [M]	COL	60 days	Lesion volume	CIMT	Skilled reaching [Montoya staircase], Spontaneous impaired forelimb use [cylinder]	Walking [horizontal ladder]			60 days
DeBow 2003c[1]	Rat, Sprague-Dawley [M]	COL	60 days	Lesion volume	EX	Skilled reaching [Montoya staircase], Spontaneous impaired forelimb use [cylinder]	Walking [horizontal ladder]			60 days
MacLellan 2005[2]	Rat, Sprague-Dawley [M]	COL	60 days	Lesion volume	CIMT	Skilled reaching [Montoya staircase], Spontaneous impaired forelimb use [cylinder]	Walking [horizontal ladder]			56 days
Auriat 2006[3]	Rat, Long Evans [M]	COL	49 days	Lesion volume	AE	Skilled reaching [Montoya staircase], Spontaneous impaired forelimb use [cylinder]	Walking [horizontal ladder]			46 days

Article ID	Experimental Design					Behavioural Endpoints				
First Author Year [Ref]	Species, Strain [Sex]	ICH Model	Survival	Stroke Size Assessment	Rehab-ilitation	Forelimb Function [Test]	Locomotor Function [Test]	Neuro-behavioural Battery	Other [Test]	Latest Assess-ment
Auriat 2008[4]	Rat, Sprague-Dawley [M]	COL	30 days	Lesion volume	ER	Skilled reaching [Montoya staircase, tray task], Spontaneous impaired forelimb use [cylinder]	Walking [horizontal ladder, elevated beam]	NDS		28 days
Nguyen 2008[5]	Rat, Sprague-Dawley [F]	COL	56 days	Lesion volume	EE	Skilled reaching [tray task], Spontaneous impaired forelimb use [cylinder]	Walking [horizontal ladder, elevated beam]			56 days
Auriat 2009[6]	Rat, Sprague-Dawley [M]	COL	46 days	Lesion volume	ER	Skilled reaching [Montoya staircase], Spontaneous impaired forelimb use [cylinder]	Walking [horizontal ladder]			46 days
Auriat 2010a[7]	Rat, Sprague-Dawley [M]	COL	39 days	Not assessed	ER	Skilled reaching [Montoya staircase], Spontaneous impaired forelimb use [cylinder]	Walking [horizontal ladder]			39 days
Auriat 2010b[7]	Rat, Sprague-Dawley [M]	COL	32 days	Lesion volume	ER	Skilled reaching [Montoya staircase, tray task], Spontaneous impaired forelimb use [cylinder]	Walking [horizontal ladder]			32 days
Takamatsu 2010[8]	Rat, Wistar [M]	COL	15 days	Lesion volume	AE			MDS		15 days

Article ID	Experimental Design					Behavioural Endpoints				
First Author Year [Ref]	**Species, Strain [Sex]**	**ICH Model**	**Survival**	**Stroke Size Assessment**	**Rehab-ilitation**	**Forelimb Function [Test]**	**Locomotor Function [Test]**	**Neuro-behavioural Battery**	**Other [Test]**	**Latest Assess-ment**
Ishida 2011[9]	Rat, Wistar [M]	COL	37 days	Lesion volume	FLU	Skilled reaching [single pellet task], Spontaneous impaired forelimb use [cylinder]	Walking [horizontal ladder]	MDS	Sensori-motor [forelimb contact placing response], Kinematic analysis [forelimb]	28 days
MacLellan 2011[10]	Rat, Sprague-Dawley [M]	AWB	49 days	Lesion volume	ER	Skilled reaching [single pellet task]				46 days
Mestriner 2011a[11]	Rat, Wistar [M]	COL	33-34 days	Lesion volume	REACH	Skilled reaching [Montoya staircase], Spontaneous impaired forelimb use [cylinder]	Walking [horizontal ladder]			33-34 days
Mestriner 2011b[11]	Rat, Wistar [M]	COL	33-34 days	Lesion volume	WALK	Skilled reaching [Montoya staircase], Spontaneous impaired forelimb use [cylinder]	Walking [horizontal ladder]			33-34 days
Kim 2012a[12]	Rat, Sprague-Dawley [M]	COL	Unclear, imprecise timeline	Lesion volume	REACH	Spontaneous impaired forelimb use [cylinder]	Walking [horizontal ladder]			Unclear, imprecise timeline
Kim 2012b[12]	Rat, Sprague-Dawley [M]	COL	Unclear, imprecise timeline	Lesion volume	REACH-ipsi	Spontaneous impaired forelimb use [cylinder]	Walking [horizontal ladder]			Unclear, imprecise timeline

Article ID	Experimental Design					Behavioural Endpoints				
First Author Year [Ref]	Species, Strain [Sex]	ICH Model	Survival	Stroke Size Assessment	Rehab-ilitation	Forelimb Function [Test]	Locomotor Function [Test]	Neuro-behavioural Battery	Other [Test]	Latest Assess-ment
Santos 2013a[13]	Rat, Wistar [M]	COL	33-34 days	Lesion volume* (conducted in one tissue slice)	REACH	Skilled reaching [Montoya staircase], Spontaneous impaired forelimb use [cylinder]				33-34 days
Santos 2013b[13]	Rat, Wistar [M]	COL	33-34 days	Lesion volume* (conducted in one tissue slice)	WALK	Skilled reaching [Montoya staircase], Spontaneous impaired forelimb use [cylinder]				33-34 days
Caliaper-umal 2014[14]	Rat, Sprague-Dawley [M]	COL	32 days	Not assessed	ER	Skilled reaching [Montoya staircase]	Walking [horizontal ladder]			32 days
Tamakoshi 2014[15]	Rat, Wistar [M]	COL	29 days	Lesion volume	AT		Walking [horizontal ladder, elevated beam]	MDS		28 days
Yong 2014a[16]	Rat, Sprague-Dawley [M]	COL	7 days	Not assessed	REACH		Walking [horizontal ladder]			Unclear, imprecise timeline
Yong 2014b[16]	Rat, Sprague-Dawley [M]	COL	28 days	Not assessed	REACH		Walking [horizontal ladder]			Unclear, imprecise timeline
Ishida 2015a[17]	Rat, Wistar [M]	COL	14 days	Lesion volume	FLU	Skilled reaching [single pellet task]	Walking [horizontal ladder]			12 days

Article ID	Experimental Design					Behavioural Endpoints				
First Author Year [Ref]	Species, Strain [Sex]	ICH Model	Survival	Stroke Size Assessment	Rehab-ilitation	Forelimb Function [Test]	Locomotor Function [Test]	Neuro-behavioural Battery	Other [Test]	Latest Assess-ment
Ishida 2015b[17]	Rat, Wistar [M]	COL	30 days	Lesion volume	FLU	Skilled reaching [single pellet task]	Walking [horizontal ladder]			28 days
Ishida 2016[18]	Rat, Wistar [M]	COL	60 days	Not assessed	FLU	Skilled reaching [single pellet task]	Walking [horizontal ladder]	MDS		28 days
Takamatsu 2016[19]	Rat, Wistar [M]	COL	15 days	Not assessed	AE	Spontaneous impaired forelimb use [cylinder]	Walking [elevated beam] *hindlimb	MDS		15 days
Tamakoshi 2016[20]	Rat, Wistar [M]	COL	29 days	Not assessed	AT				Sensori-motor [forepaw grasping, modified forelimb placing test, postural instability]	28 days
Tamakoshi 2017[21]	Rat, Wistar [M]	COL	29 days	Lesion volume* (assessed, not reported)	AT				Sensori-motor [modified forelimb placing test]	28 days
Tamakoshi 2018a[22]	Rat, Wistar [M]	COL	15 days	Unclear* (lesion volume mentioned, methods/data not reported)	AE		Walking [horizontal ladder]		Sensori-motor [forelimb contact placing response]	15 days

Article ID	Experimental Design					Behavioural Endpoints				
First Author Year [Ref]	Species, Strain [Sex]	ICH Model	Survival	Stroke Size Assessment	Rehab-ilitation	Forelimb Function [Test]	Locomotor Function [Test]	Neuro-behavioural Battery	Other [Test]	Latest Assess-ment
Tamakoshi 2018b[22]	Rat, Wistar [M]	COL	15 days	Unclear* (lesion volume mentioned, methods/data not reported)	AE		Walking [horizontal ladder]		Sensori-motor [forelimb contact placing response]	15 days
Tamakoshi 2018c[22]	Rat, Wistar [M]	COL	15 days	Unclear* (lesion volume mentioned, methods/data not reported)	AE		Walking [horizontal ladder]		Sensori-motor [forelimb contact placing response]	15 days
Sato 2020a[23]	Rat, Sprague-Dawley [M]	COL	28 days	Not assessed	AE		Walking [elevated beams - narrow, wide]	MDS		28 days
Sato 2020b[23]	Rat, Sprague-Dawley [M]	COL	28 days	Not assessed	AE		Walking [elevated beams - narrow, wide]	MDS		28 days
Tamaokshi 2020a[24]	Rat, Wistar [M]	COL	16 days	Lesion volume	AE		Walking [horizontal ladder]		Sensori-motor [forelimb contact placing response], Balance/ coordin-ation [rotarod]	15 days

Article ID	Experimental Design					Behavioural Endpoints				
First Author Year [Ref]	Species, Strain [Sex]	ICH Model	Survival	Stroke Size Assessment	Rehab-ilitation	Forelimb Function [Test]	Locomotor Function [Test]	Neuro-behavioural Battery	Other [Test]	Latest Assess-ment
Tamaokshi 2020b[24]	Rat, Wistar [M]	COL	16 days	Lesion volume	AE		Walking [horizontal ladder]		Sensori-motor [forelimb contact placing response], Balance/ coordin-ation [rotarod]	15 days
Xu 2020a[25]	Rat, Sprague-Dawley [NR]	COL	14 days	Not assessed	AE			mNSS		14 days
Xu 2020b[25]	Rat, Sprague-Dawley [NR]	COL	14 days	Not assessed	AE			mNSS		14 days
Tamakoshi 2021[26]	Rat, Wistar [M]	COL	27 hours	Hematoma volume	AE		Walking [horizontal ladder]		Balance/ coordin-ation [rotarod]	25 hours
Fedor 2022a[27]	Rat, Sprague-Dawley [M]	COL	14 days	Hematoma volume	ER	Skilled reaching [Montoya staircase]	Walking [horizontal ladder, elevated beam]			14 days
Fedor 2022b[27]	Rat, Sprague-Dawley [M]	COL	14 days	Hematoma volume	ER	Skilled reaching [Montoya staircase]	Walking [horizontal ladder, elevated beam]			14 days

Article ID	Experimental Design					Behavioural Endpoints				
First Author Year [Ref]	Species, Strain [Sex]	ICH Model	Survival	Stroke Size Assessment	Rehab-ilitation	Forelimb Function [Test]	Locomotor Function [Test]	Neuro-behavioural Battery	Other [Test]	Latest Assess-ment
Fedor 2022c[27]	Rat, Sprague-Dawley [M]	COL	60 days	Lesion volume	ER	Skilled reaching [Montoya staircase]				31 days
Fedor 2022d[27]	Rat, Sprague-Dawley [M]	COL	60 days	Lesion volume	ER	Skilled reaching [Montoya staircase]				31 days
Inoue 2022[28]	Rat, Wistar [M]	COL	29 days	Lesion volume	AE				Sensori-motor [tape removal task]	28 days
Li 2022a[29]	Mouse, C57BL/6J [M]	COL	Unclear, imprecise timeline	Hematoma volume	SWIM			mNSS		14 days
Li 2022b[29]	Mouse, C57BL/6J [M]	COL	Unclear, imprecise timeline	Hematoma volume	SWIM			mNSS		14 days
Li 2022c[29]	Mouse, C57BL/6J [M]	COL	Unclear, imprecise timeline	Hematoma volume	SWIM			mNSS		7 days
Tamakoshi 2022[30]	Rat, Wistar [M]	COL	8 days	Lesion volume	AE		Walking [horizontal ladder]		Balance/ coordin-ation [rotarod]	7 days

Abbreviations: AE, aerobic exercise; AT, acrobatic training; AWB, autologous whole blood; CIMT, constraint-induced movement therapy; COL, collagenase model; EE, environmental enrichment; ER, enriched rehabilitation; F, female; FLU, forced limb use; M, male; MDS, motor deficit score; mNSS modified neurological severity score; NR, not reported; NDS, neurological deficit score; REACH, skilled reach training; REACH-ipsi, skilled reach training in unimpaired forelimb; SWIM, swim training; WALK, walk training

	CAMARADES Checklist Item									
Article	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
DeBow 2003	+	+	+	−	+	×	×	×	+	×
MacLellan 2005	+	+	−	−	+	×	×	×	+	×
Auriat 2006	+	+	−	−	−	×	×	×	+	×
Auriat 2008	+	+	+	−	−	×	×	×	+	×
Nguyen 2008	+	+	+	+	+	×	+	×	+	×
Auriat 2009	+	+	+	+	+	×	×	×	+	×
Auriat 2010	+	+	+	−	−	×	×	×	+	×
Takamatsu 2010	+	−	+	−	−	×	×	×	+	×
Ishida 2011	+	+	+	−	−	×	×	×	+	×
MacLellan 2011	+	+	+	−	+	×	×	×	+	+
Mestriner 2011	+	+	×	−	+	−	×	×	+	×
Kim 2012	+	−	+	−	−	×	×	×	+	+
Santos 2013	+	+	×	−	−	−	×	×	+	×
Caliaperumal 2014	+	+	+	+	+	×	×	×	+	+
Tamakoshi 2014	+	−	−	+	−	×	×	×	+	×
Yong 2014	+	−	+	−	−	×	×	×	+	×
Ishida 2015	+	−	−	−	−	×	×	×	+	×
Ishida 2016	+	+	−	−	−	×	×	×	+	×
Takamatsu 2016	+	−	+	−	×	×	×	×	+	×
Tamakoshi 2016	+	−	+	−	−	×	×	×	+	×
Tamakoshi 2017	+	−	+	−	−	×	×	×	+	×
Tamakoshi 2018	+	−	+	−	−	×	×	×	+	×
Sato 2020	+	−	−	−	−	−	×	×	+	+
Tamakoshi 2020	+	−	+	−	−	×	×	×	+	+
Xu 2020	+	+	+	+	+	−	×	×	+	+
Tamakoshi 2021	+	−	+	−	+	×	×	×	+	+
Fedor 2022	+	+	+	+	+	×	×	+	+	+
Inoue 2022	+	−	−	−	−	−	×	×	+	+
Li 2022	+	−	+	−	−	×	×	×	+	+
Tamakoshi 2022	+	−	+	−	−	×	×	×	+	+

CAMARADES Checklist Item

1: Peer reviewed
2: Temperature control during ICH surgery
3: Reported random allocation to groups
4: Blinded ICH induction OR post-ICH randomization
5: Blinded outcome assessments
6: Used anaesthetic(s) without intrinsic neuroprotective effects
7: Included comorbidities relevant to ICH
8: Described sample size calculation
9: Statement on compliance with animal welfare regulations
10: Explicit conflict of interest statement

Rating

× No
− Unclear
+ Yes

Figure A-1 Individual article ratings for assessment of compliance with 10 item CAMARADES checklist. Articles were rated as yes, unclear, or no for their compliance on each item. A rating of unclear was given when reviewers deemed there was insufficient and/or inconsistent reporting of detail to accurately judge compliance with the checklist item. Article quality ranged considerably (scores of 2-8), with a median score of 4

	SYRCLE Risk of Bias Domain									
Article	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
DeBow 2003	−	−	−	−	−	+	+	+	−	+
MacLellan 2005	−	−	−	−	−	+	+	+	−	−
Auriat 2006	−	+	−	−	−	+	−	−	−	+
Auriat 2008	−	−	−	×	−	+	−	+	−	×
Nguyen 2008	−	+	−	×	−	+	+	−	−	×
Auriat 2009	−	+	−	×	−	+	+	+	−	×
Auriat 2010	−	+	−	×	−	−	−	×	−	×
Takamatsu 2010	−	−	−	−	−	−	−	−	−	−
Ishida 2011	−	−	−	−	−	−	−	−	−	×
MacLellan 2011	−	+	−	×	−	−	+	−	−	×
Mestriner 2011	×	+	×	−	−	+	+	−	−	×
Kim 2012	−	−	−	−	−	−	−	−	×	−
Santos 2013	×	+	−	−	−	+	−	+	−	×
Caliaperumal 2014	−	+	−	×	−	+	+	+	−	×
Tamakoshi 2014	−	−	−	−	−	−	−	−	×	×
Yong 2014	−	−	−	−	−	−	−	−	−	−
Ishida 2015	−	+	−	−	−	−	−	×	−	×
Ishida 2016	−	−	−	−	−	−	−	−	×	−
Takamatsu 2016	−	−	−	−	−	−	×	×	−	×
Tamakoshi 2016	−	−	−	−	−	−	−	−	×	×
Tamakoshi 2017	−	−	−	−	−	−	−	×	×	×
Tamakoshi 2018	−	×	−	−	−	−	−	−	×	−
Sato 2020	−	−	−	×	−	−	−	×	−	×
Tamakoshi 2020	−	−	−	−	−	−	−	×	×	×
Xu 2020	−	×	−	−	−	−	−	+	×	−
Tamakoshi 2021	−	−	−	−	−	−	+	−	−	×
Fedor 2022	+	+	−	×	−	+	+	+	−	×
Inoue 2022	−	−	−	−	−	+	−	+	−	−
Li 2022	−	×	−	−	−	−	−	×	×	×
Tamakoshi 2022	−	−	−	−	−	−	−	×	×	×

SYRCLE Risk of Bias Domain

1: Sequence generation (Selection bias)
2: Baseline characteristics (Selection bias)
3: Allocation concealment (Selection bias)
4: Random housing (Performance bias)
5: Caregiver blinding (Performance bias)
6: Random outcome assessment (Detection bias)
7: Blinding (Detection bias)
8: Incomplete outcome assessment (Attrition bias)
9: Selective outcome reporting (Reporting bias)
10: Other sources of bias (Other)

Rating

× High
− Unclear
+ Low
− Not rated

Figure A-2 Individual article ratings for SYRCLE Risk of Bias tool. Articles were rated for each domain as low-, unclear-, or high risk. Caregiver blinding (performance bias) was not rated, as it is near impossible for preclinical researchers to be blinded to rehabilitation delivery. A rating of unclear was given when reviewers deemed there was insufficient and/or inconsistent reporting of detail to accurately judge compliance with the signalling questions for a domain. Risk of bias was predominately unclear, as articles often lacked sufficient detail to determine how/if risk of bias was minimized

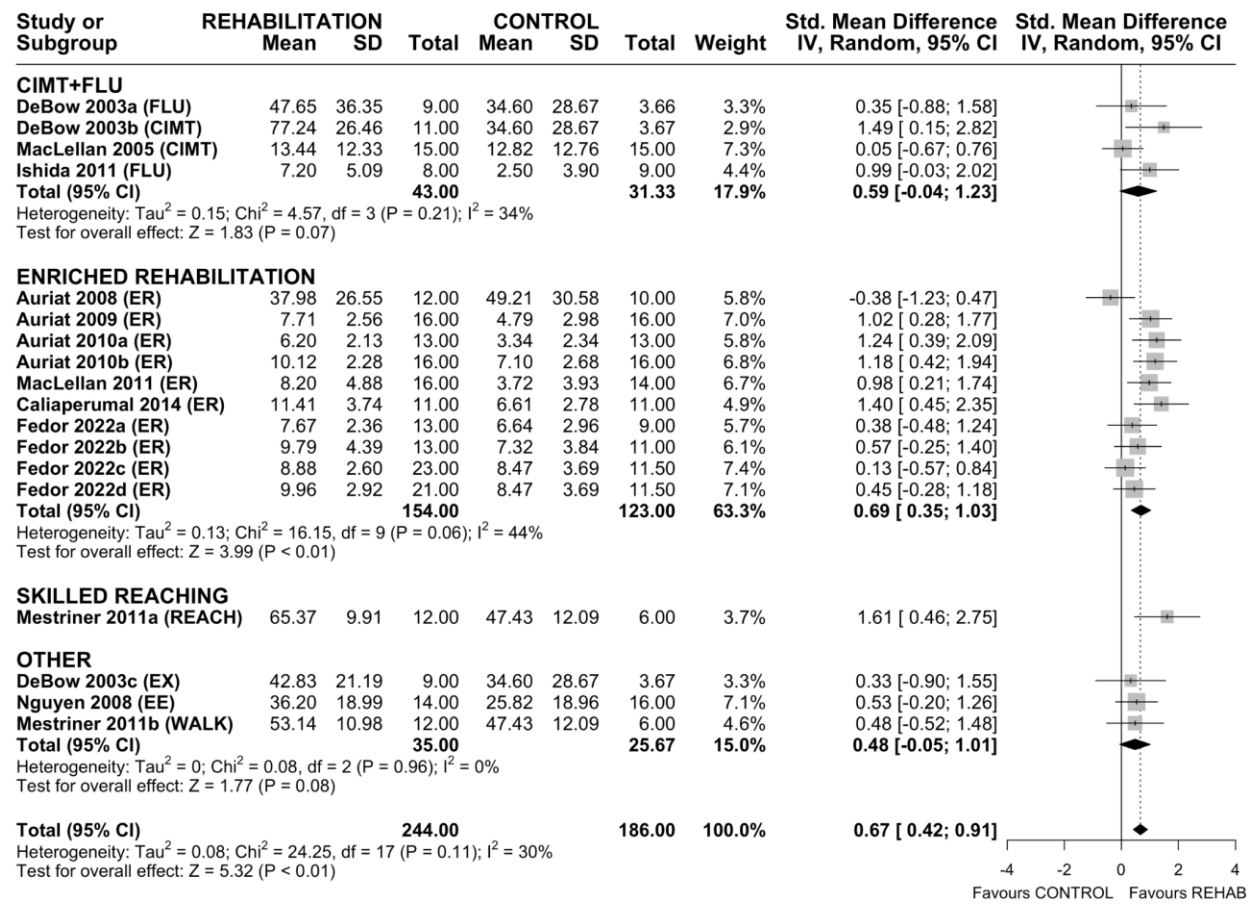


Figure A-3 Forest plot of the sensitivity analysis conducted to evaluate the impact of research quality on recovery of skilled reaching. Interventions from articles with a CAMARADES score of 0-3 were removed (n=6) and random-effects meta-analysis was conducted (n=18). We observed a similar overall treatment effect [SMD 0.67 (95% CI 0.42-0.91), p<0.01] to our original model and found removing low quality studies significantly reduced heterogeneity (I^2=30%, p=0.11). Effect sizes presented as Hedge's *G* standardized mean difference (SMD) with 95% CI

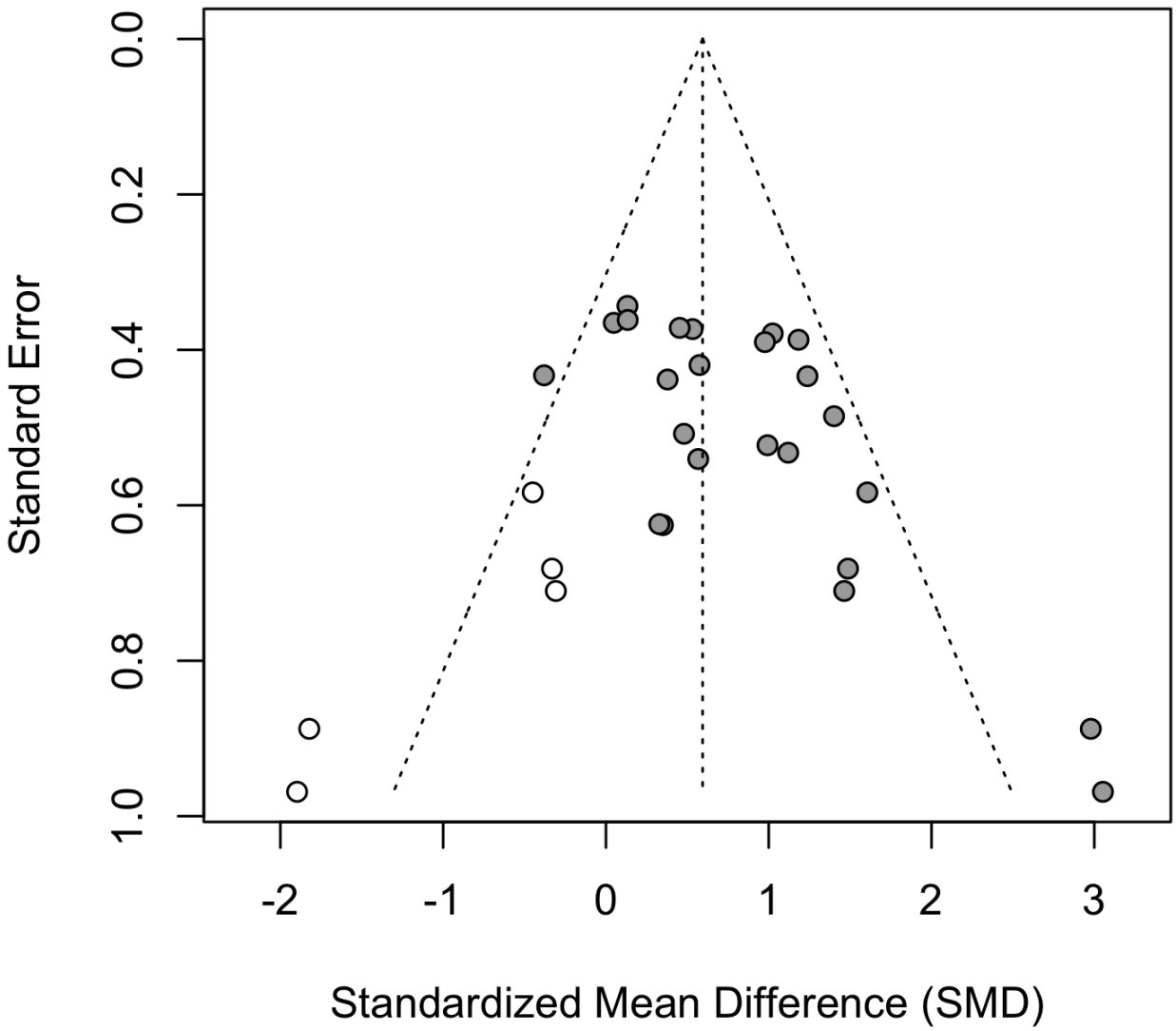


Figure A-4 Funnel plot of skilled reaching data (Figure 3-3). Egger regression indicated the presence of asymmetry in the dataset ($p<0.01$); therefore, trim-and-fill analysis was conducted. Filled circles represent real data (n=24), open circles represent hypothetical data added through trim-and-fill analysis (n=5). All additional data points were added in the bottom left quadrant, suggesting null or negative data may be absent in our original model, likely due to reporting and/or publication bias. Random-effects meta-analysis of the trim-and-fill model (n=29) produced a smaller treatment effect [SMD 0.59 (95% CI 0.32-0.87), $p<0.01$] than our original model [SMD 0.75 (95% CI 0.50-1.00)]. Effect sizes presented as Hedge's *G* standardized mean difference (SMD)

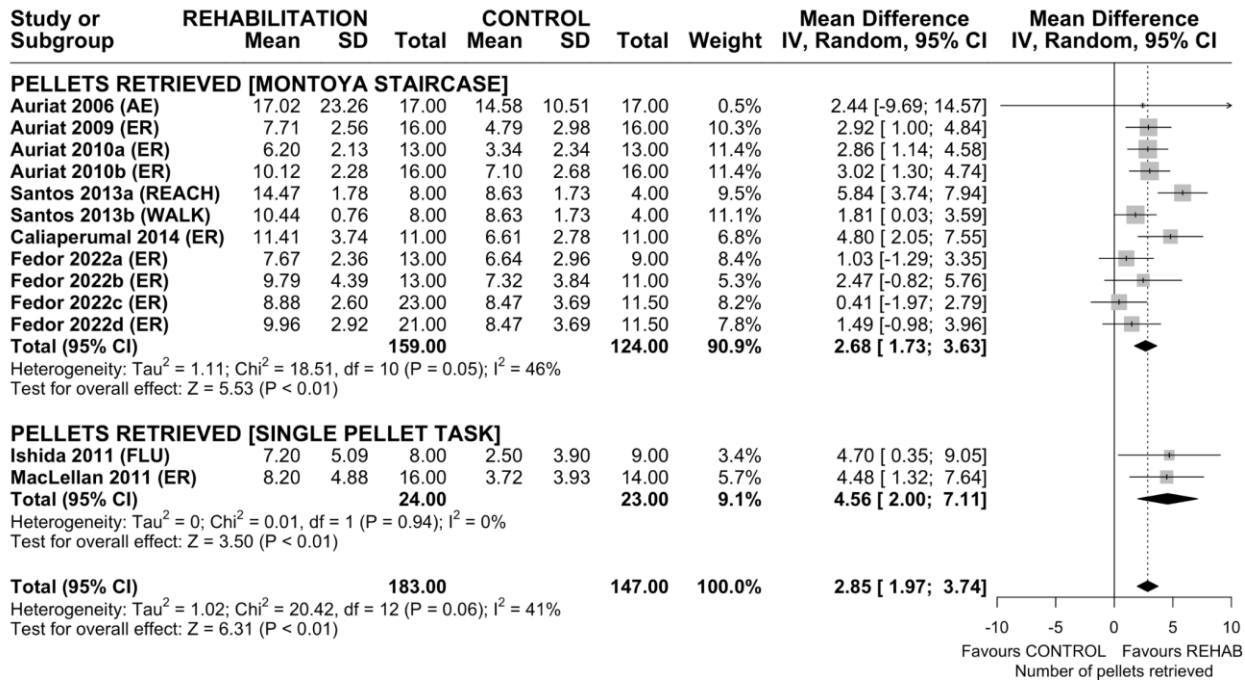


Figure A-5 Forest plot of the post-hoc random-effects meta-analysis of interventions that reported the number of pellets retrieved in their respective skilled reaching endpoints (n=13). Rehabilitation improved skilled reaching success [MD 2.85 pellets retrieved (95% CI 1.97-3.74), p<0.01; SMD 0.82 (95% CI 0.51-1.13), p<0.01] to a similar extent as observed in our full analysis [SMD 0.75 (95% CI 0.50-1.01), p<0.01]. While an overall treatment effect is evident, the mean difference in pellets retrieved between treated and untreated animals fails to reach or exceed the 3-pellet threshold we have argued to be of functional importance (i.e., one level of Montoya staircase). Effect sizes presented as mean difference (MD), number of pellets retrieved, with 95% CI

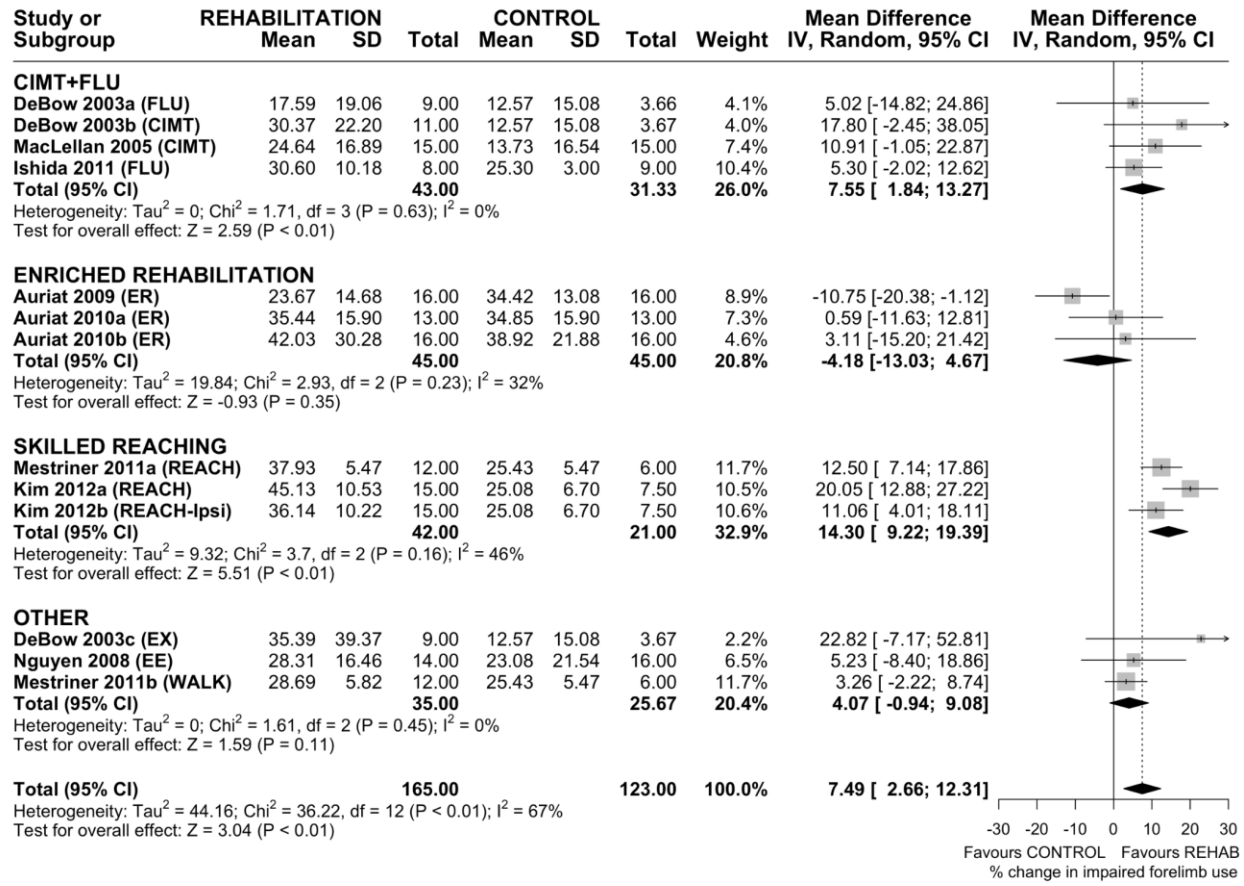


Figure A-6 Forest plot of the sensitivity analysis conducted to evaluate the impact of research quality on recovery of spontaneous impaired forelimb use. Interventions from articles with a CAMARADES score of 0-3 were removed (n=2) and random-effects meta-analysis was conducted (n=13). We observed a similar overall treatment effect [MD 7.49% increase in impaired forelimb use (95% CI 2.66-12.31), p<0.01] to our original model; removing low quality studies did not improve heterogeneity (I^2=67%, p<0.01). Effect sizes presented as mean difference (MD), percent change in impaired forelimb use, with 95% CI

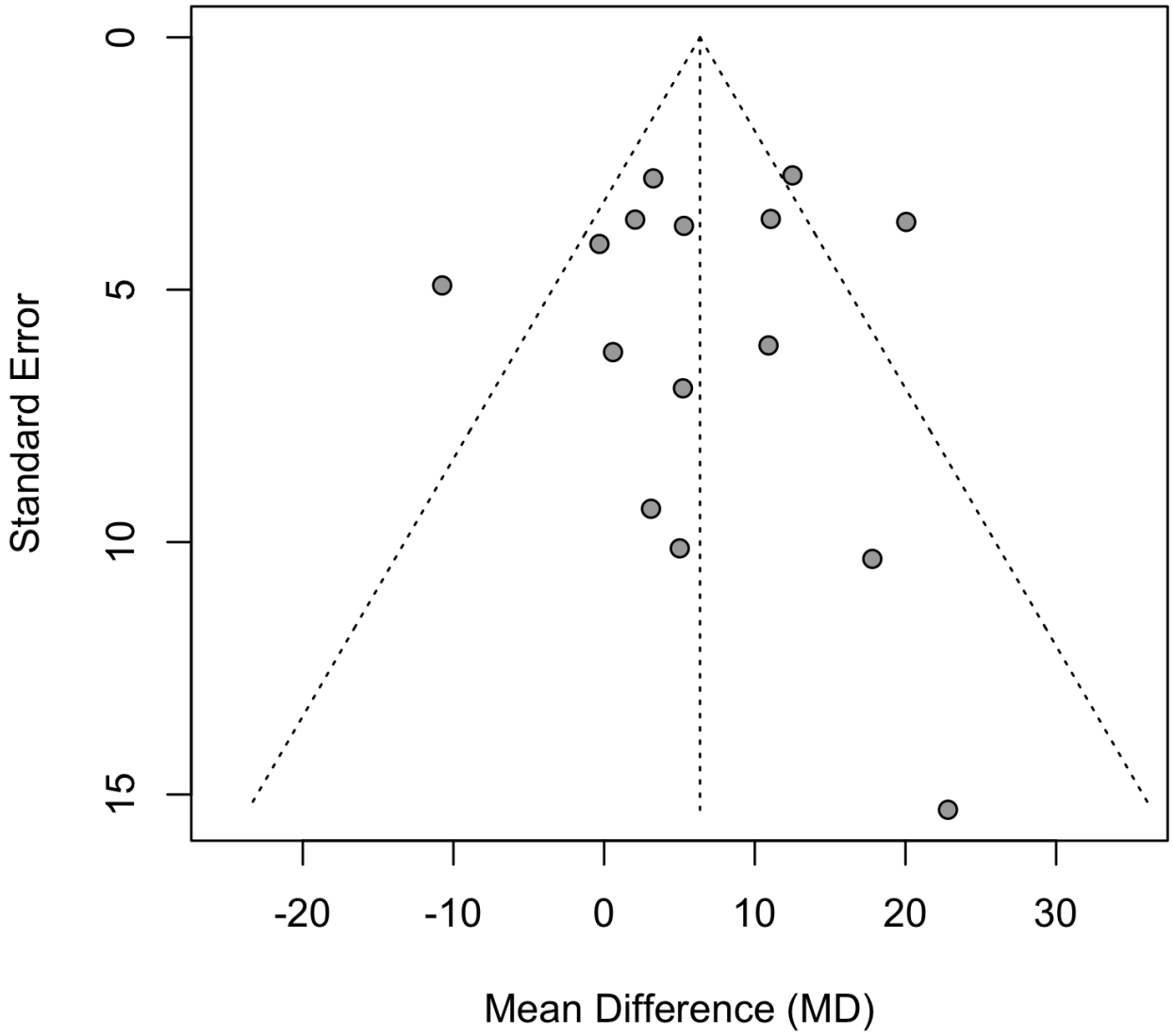


Figure A-7 Funnel plot of spontaneous impaired forelimb use data (Figure 3-4). Egger regression did not indicate the presence of asymmetry in the dataset ($p>0.05$), therefore trim-and-fill analysis was not completed. Effect sizes presented as mean difference (MD), percent change in impaired forelimb use

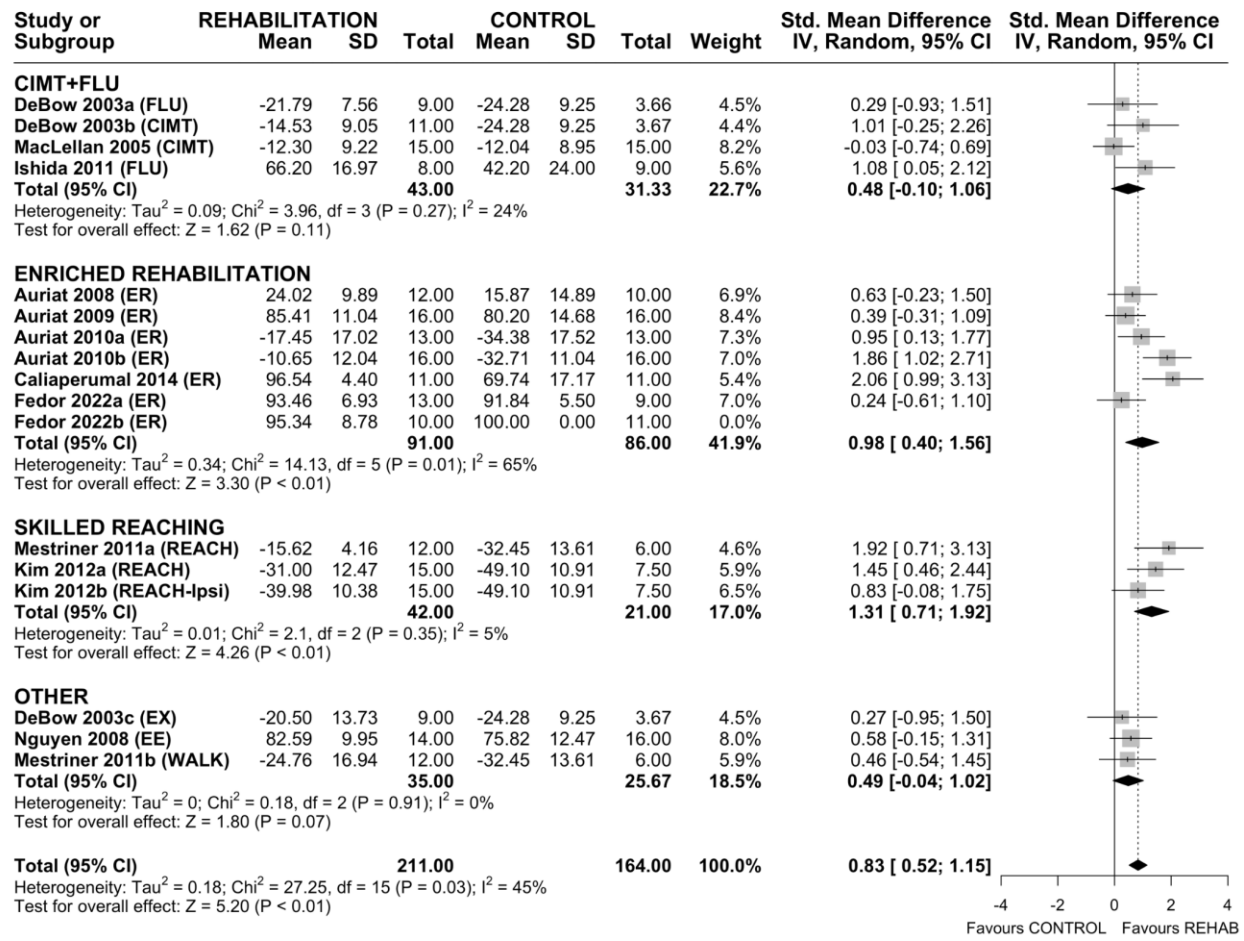


Figure A-8 Forest plot of the sensitivity analysis conducted to evaluate the impact of research quality on recovery of locomotor function. Interventions from articles with a CAMARADES score of 0-3 were removed (n=10) and random-effects meta-analysis was conducted (n=16). We observed a similar overall treatment effect [SMD 0.83 (95% CI 0.52-1.15), p<0.01] to our original model and found removing low quality studies somewhat reduced heterogeneity (I^2=45%, p=0.03). Effect sizes presented as Hedge's *G* standardized mean difference (SMD) with 95% CI

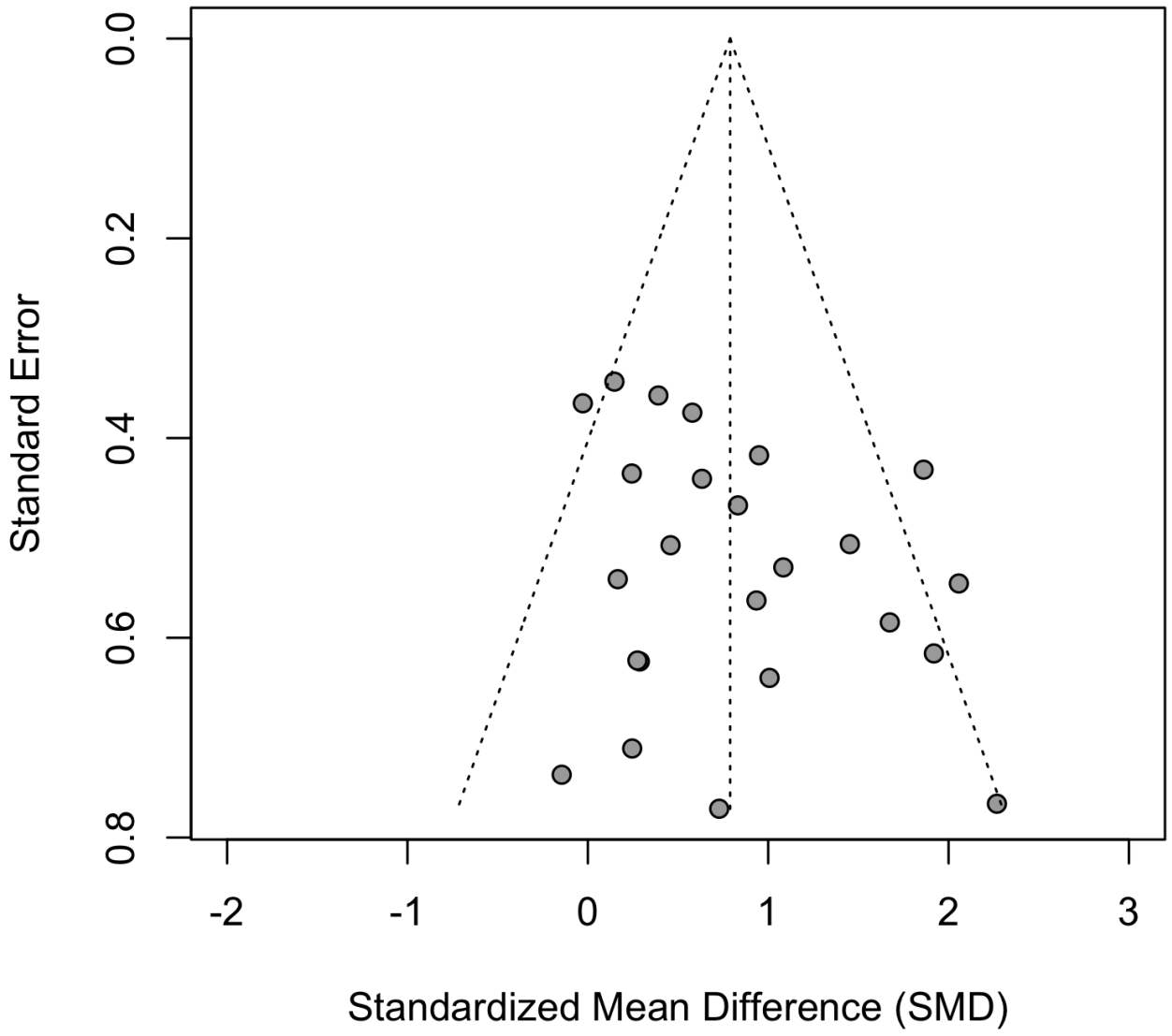


Figure A-9 Funnel plot of locomotor function data (Figure 3-5). Egger regression did not indicate the presence of asymmetry in the dataset ($p>0.05$), therefore trim-and-fill analysis was not completed. Effect sizes presented as Hedge's *G* standardized mean difference (SMD)

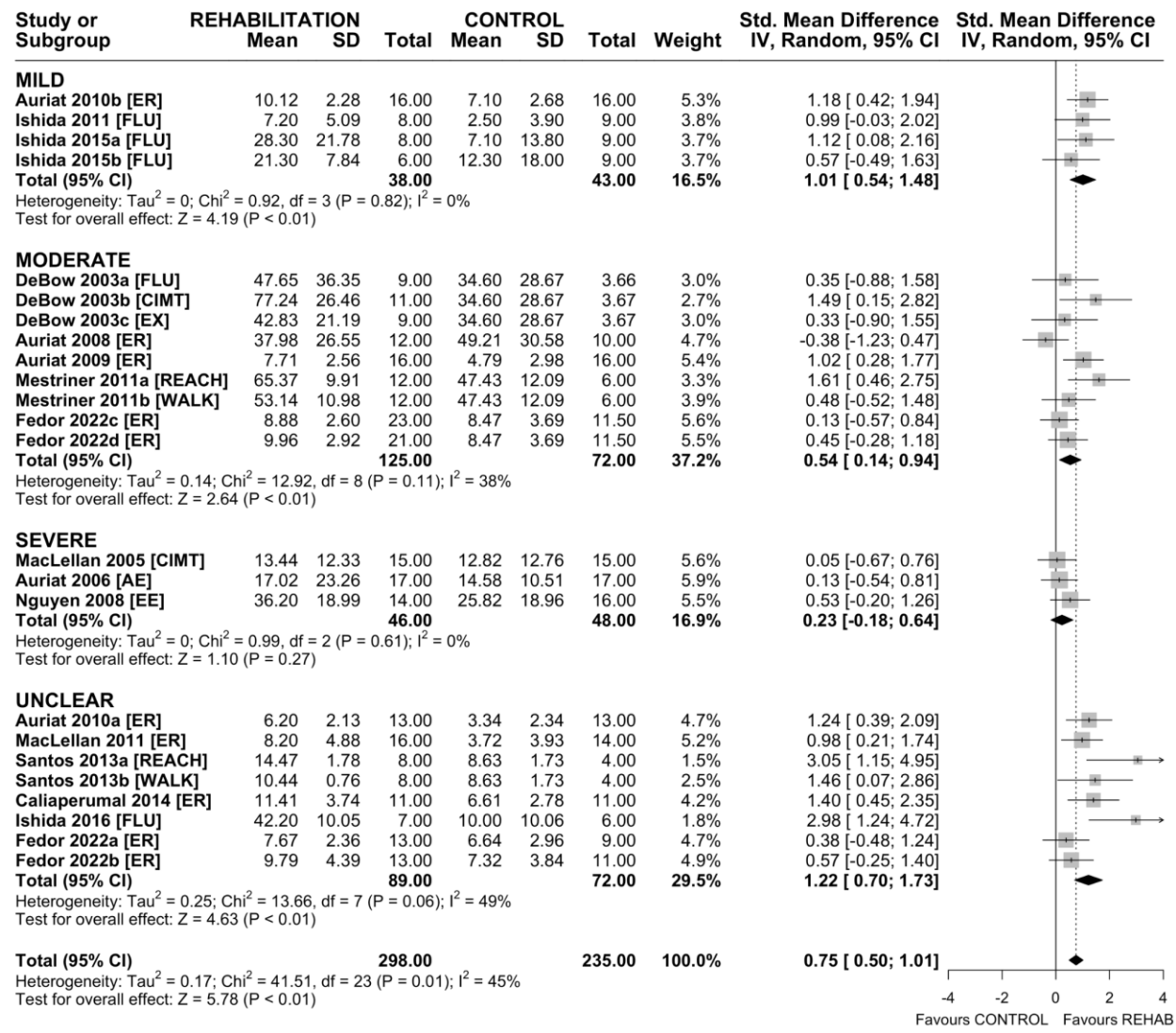


Figure A-10 Forest plot of random-effects meta-analysis of skilled reaching recovery grouped by lesion size reported in the study's untreated control group. Severity was grouped into mild [≤30 mm^3], moderate [31-60 mm^3], severe [≥61 mm^3], and UNCLEAR. Rehabilitation improved skilled reaching recovery in animals with mild and moderate, but not severe ICH. Effect sizes presented as Hedge's *G* standardized mean difference (SMD) with 95% CI

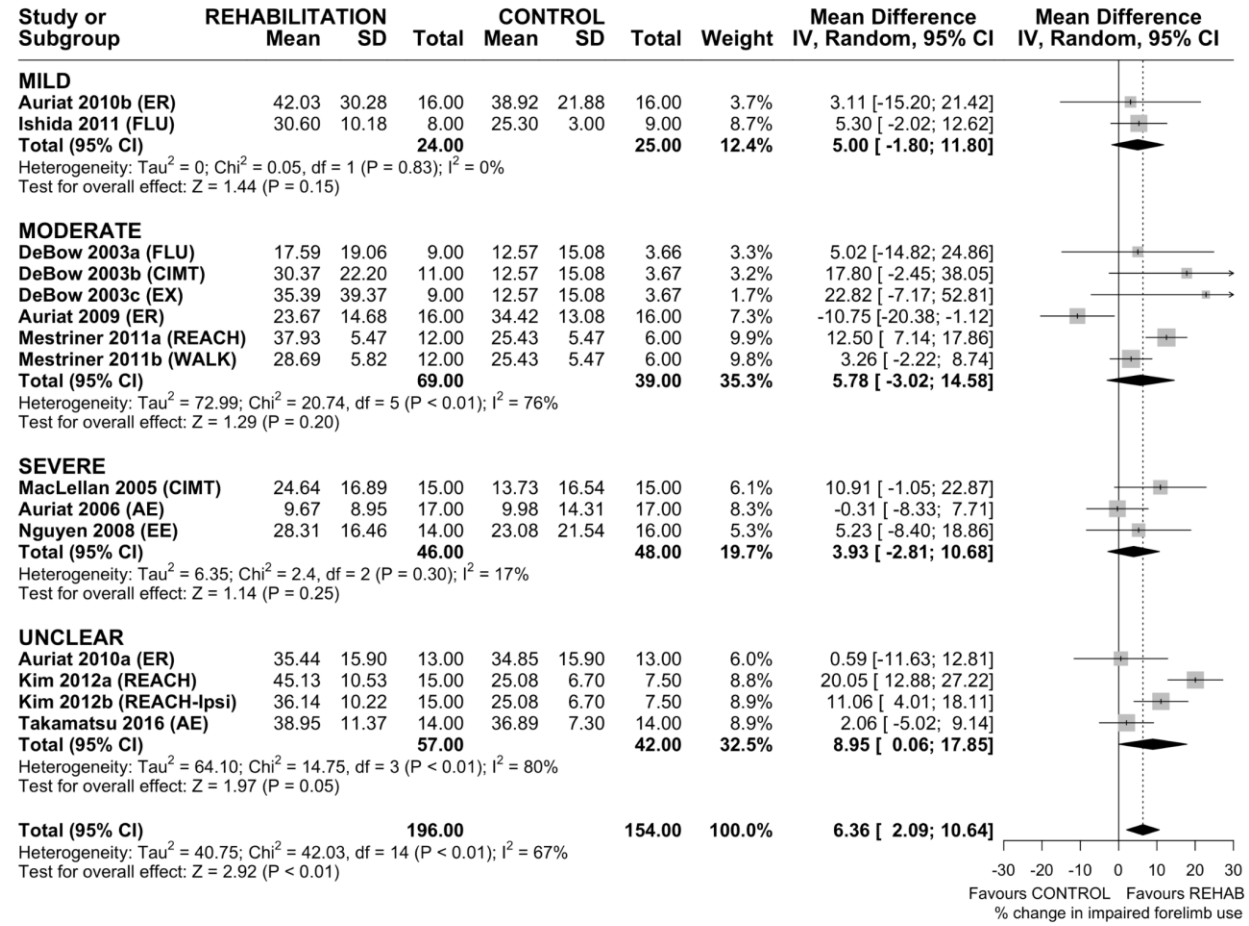


Figure A-11 Forest plot of random effects meta-analysis of recovery of spontaneous impaired forelimb use grouped by lesion size reported in the study's untreated control group. Severity was grouped into mild [≤30 mm^3], moderate [31-60 mm^3], severe [≥61 mm^3], and UNCLEAR. While rehabilitation increased spontaneous impaired forelimb use, there was no discernable effect of rehabilitation in groups with known severity. Effect sizes presented as mean difference (MD), percent change in impaired forelimb use, with 95% CI

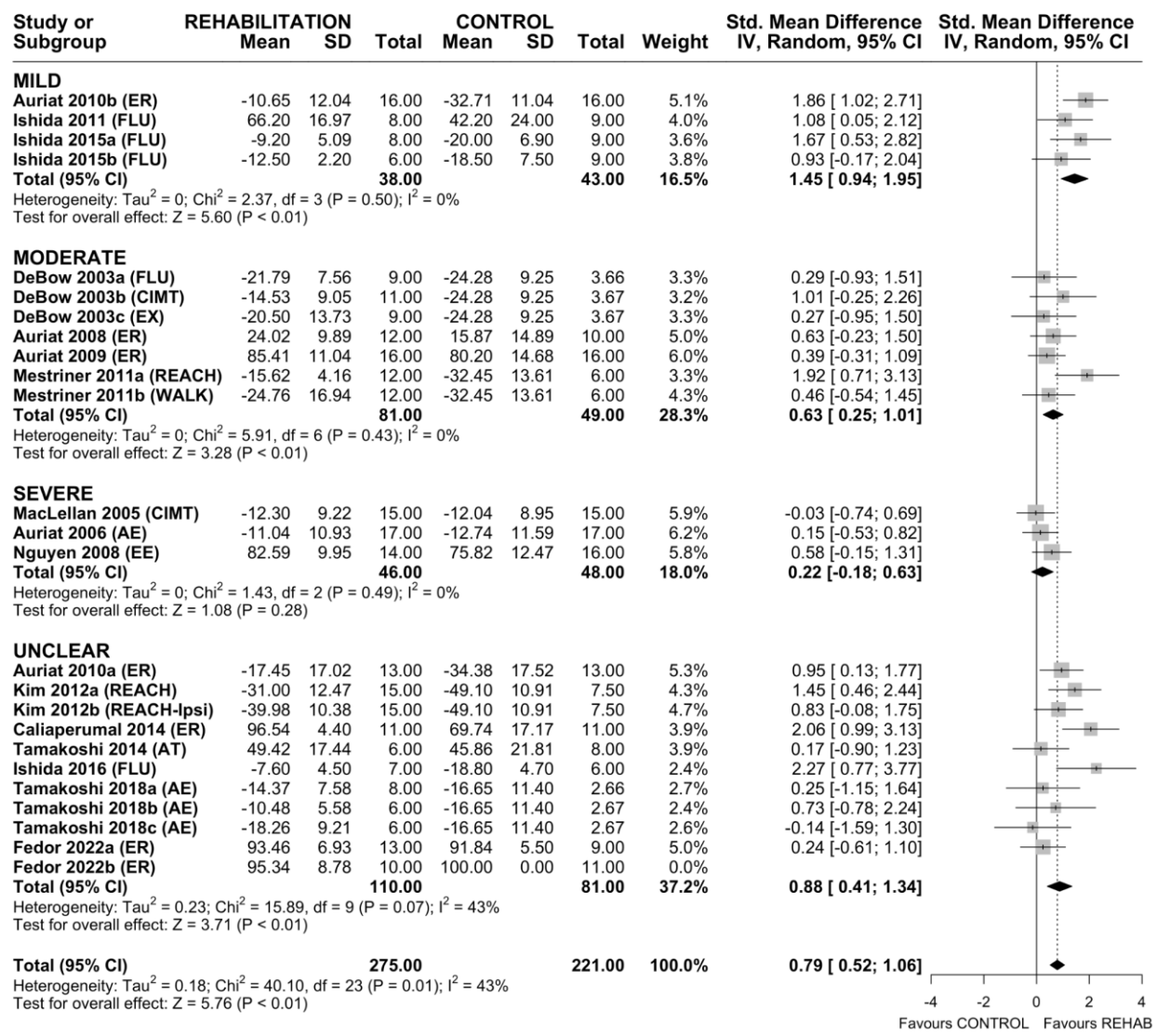
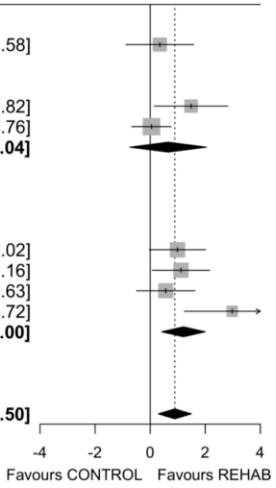


Figure A-12 Forest plot of random-effects meta-analysis of locomotor recovery grouped by lesion size reported in the study's untreated control group. Severity was grouped into mild [≤30 mm^3], moderate [31-60 mm^3], severe [≥61 mm^3], and UNCLEAR. Rehabilitation improved locomotor recovery in animals with mild and moderate, but not severe ICH. Effect sizes presented as Hedge's *G* standardized mean difference (SMD) with 95% CI

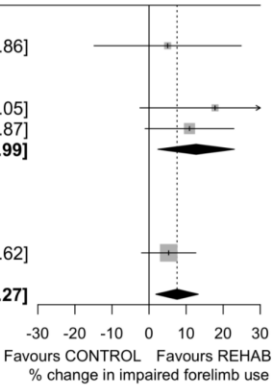
a

Study or Subgroup	REHABILITATION Mean	SD	Total	CONTROL Mean	SD	Total	Weight	Std. Mean Difference IV, Random, 95% CI
FLU [56 HOURS]								
DeBow 2003a [FLU]	47.65	36.35	9.00	34.60	28.67	3.66	13.1%	0.35 [-0.88; 1.58]
CIMT [FLU 56 HOURS + EX 7 HOURS]								
DeBow 2003b [CIMT]	77.24	26.46	11.00	34.60	28.67	3.67	11.9%	1.49 [0.15; 2.82]
MacLellan 2005 [CIMT]	13.44	12.33	15.00	12.82	12.76	15.00	20.4%	0.05 [-0.67; 0.76]
Total (95% CI)			**26.00**			**18.67**	**32.3%**	**0.65 [-0.74; 2.04]**
Heterogeneity: $Tau^2 = 0.74$; $Chi^2 = 3.46$, df = 1 (P = 0.06); $I^2 = 71\%$								
Test for overall effect: Z = 0.92 (P = 0.36)								
FLU [168 HOURS]								
Ishida 2011 [FLU]	7.20	5.09	8.00	2.50	3.90	9.00	15.7%	0.99 [-0.03; 2.02]
Ishida 2015a [FLU]	28.30	21.78	8.00	7.10	13.80	9.00	15.4%	1.12 [0.08; 2.16]
Ishida 2015b [FLU]	21.30	7.84	6.00	12.30	18.00	9.00	15.2%	0.57 [-0.49; 1.63]
Ishida 2016 [FLU]	42.20	10.05	7.00	10.00	10.06	6.00	8.4%	2.98 [1.24; 4.72]
Total (95% CI)			**29.00**			**33.00**	**54.6%**	**1.21 [0.42; 2.00]**
Heterogeneity: $Tau^2 = 0.29$; $Chi^2 = 5.49$, df = 3 (P = 0.14); $I^2 = 45\%$								
Test for overall effect: Z = 3.00 (P < 0.01)								
Total (95% CI)			**64.00**			**55.33**	**100.0%**	**0.90 [0.30; 1.50]**
Heterogeneity: $Tau^2 = 0.32$; $Chi^2 = 12.38$, df = 6 (P = 0.05); $I^2 = 52\%$								
Test for overall effect: Z = 2.93 (P < 0.01)								



b

Study or Subgroup	REHABILITATION Mean	SD	Total	CONTROL Mean	SD	Total	Weight	Mean Difference IV, Random, 95% CI
FLU [56 HOURS]								
DeBow 2003a (FLU)	17.59	19.06	9.00	12.57	15.08	3.66	8.3%	5.02 [-14.82; 24.86]
CIMT [FLU 56 HOURS + EX 7 HOURS]								
DeBow 2003b (CIMT)	30.37	22.20	11.00	12.57	15.08	3.67	8.0%	17.80 [-2.45; 38.05]
MacLellan 2005 (CIMT)	24.64	16.89	15.00	13.73	16.54	15.00	22.8%	10.91 [-1.05; 22.87]
Total (95% CI)			**26.00**			**18.67**	**30.8%**	**12.69 [2.39; 22.99]**
Heterogeneity: $Tau^2 = 0$; $Chi^2 = 0.33$, df = 1 (P = 0.57); $I^2 = 0\%$								
Test for overall effect: Z = 2.42 (P = 0.02)								
FLU [168 HOURS]								
Ishida 2011 (FLU)	30.60	10.18	8.00	25.30	3.00	9.00	60.9%	5.30 [-2.02; 12.62]
Total (95% CI)			**43.00**			**31.33**	**100.0%**	**7.55 [1.84; 13.27]**
Heterogeneity: $Tau^2 = 0$; $Chi^2 = 1.71$, df = 3 (P = 0.63); $I^2 = 0\%$								
Test for overall effect: Z = 2.59 (P < 0.01)								



c

Study or Subgroup	REHABILITATION Mean	SD	Total	CONTROL Mean	SD	Total	Weight	Std. Mean Difference IV, Random, 95% CI
FLU [56 HOURS]								
DeBow 2003a (FLU)	-21.79	7.56	9.00	-24.28	9.25	3.66	13.0%	0.29 [-0.93; 1.51]
CIMT [FLU 56 HOURS + EX 7 HOURS]								
DeBow 2003b (CIMT)	-14.53	9.05	11.00	-24.28	9.25	3.67	12.6%	1.01 [-0.25; 2.26]
MacLellan 2005 (CIMT)	-12.30	9.22	15.00	-12.04	8.95	15.00	20.4%	-0.03 [-0.74; 0.69]
Total (95% CI)			**26.00**			**18.67**	**33.0%**	**0.36 [-0.62; 1.34]**
Heterogeneity: $Tau^2 = 0.26$; $Chi^2 = 1.97$, df = 1 (P = 0.16); $I^2 = 49\%$								
Test for overall effect: Z = 0.71 (P = 0.48)								
FLU [168 HOURS]								
Ishida 2011 (FLU)	66.20	16.97	8.00	42.20	24.00	9.00	15.4%	1.08 [0.05; 2.12]
Ishida 2015a (FLU)	-9.20	5.09	8.00	-20.00	6.90	9.00	13.9%	1.67 [0.53; 2.82]
Ishida 2015b (FLU)	-12.50	2.20	6.00	-18.50	7.50	9.00	14.5%	0.93 [-0.17; 2.04]
Ishida 2016 (FLU)	-7.60	4.50	7.00	-18.80	4.70	6.00	10.1%	2.27 [0.77; 3.77]
Total (95% CI)			**29.00**			**33.00**	**54.0%**	**1.37 [0.79; 1.95]**
Heterogeneity: $Tau^2 = 0$; $Chi^2 = 2.54$, df = 3 (P = 0.47); $I^2 = 0\%$								
Test for overall effect: Z = 4.62 (P < 0.01)								
Total (95% CI)			**64.00**			**55.33**	**100.0%**	**0.92 [0.33; 1.52]**
Heterogeneity: $Tau^2 = 0.32$; $Chi^2 = 12.16$, df = 6 (P = 0.06); $I^2 = 51\%$								
Test for overall effect: Z = 3.06 (P < 0.01)								

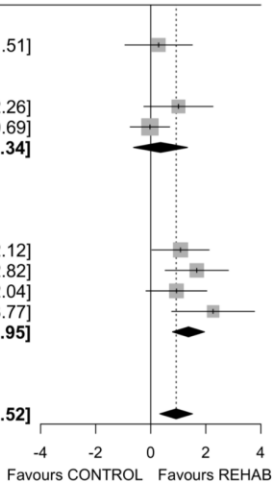


Figure A-13 Impact of treatment dose on recovery in CIMT+FLU interventions. CIMT+FLU interventions were divided into three treatment doses: FLU (56 hours), CIMT (FLU 56 hours + EX 7 hours), and FLU (168 hours). **A** Forest plot of random-effects meta-analysis of skilled reaching recovery grouped by treatment dose; greater time in restraint [FLU (168 hours)] significantly improved skilled reaching. **B** Forest plot of random-effects meta-analysis of recovery of spontaneous impaired forelimb use grouped by treatment dose; CIMT (FLU 56 hours + EX 7 hours) significantly increased spontaneous impaired forelimb use. **C** Forest plot of random-effects meta-analysis of locomotor recovery grouped by treatment dose; again, greater time in restraint [FLU (168 hours)] significantly improved locomotor function. Effect sizes in **A, C** presented as Hedge's *G* standardized mean difference (SMD) with 95% CI; effect sizes in **B** presented as mean difference (MD), percent change in impaired forelimb use, with 95% CI

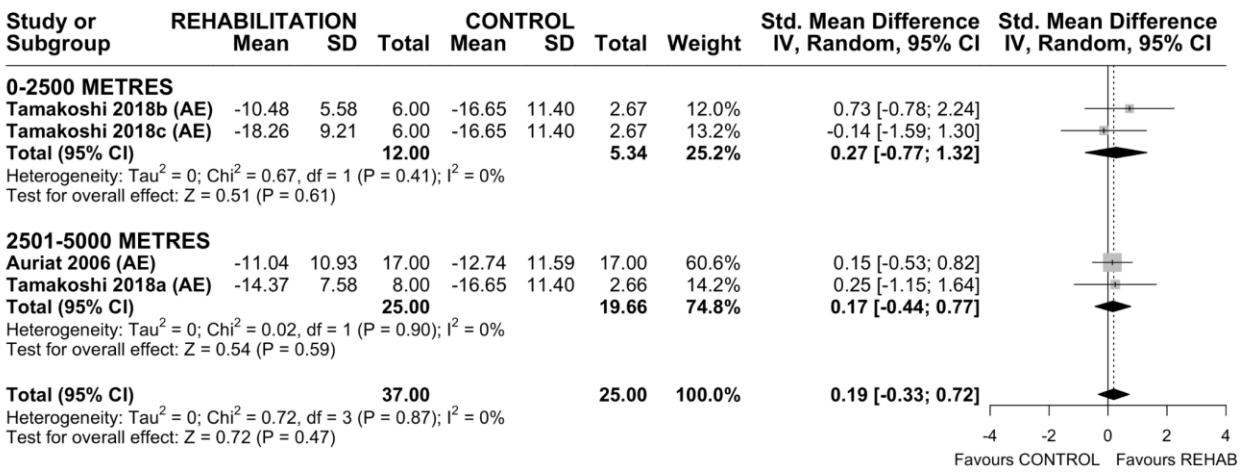


Figure A-14 Impact of treatment dose on recovery in AE interventions. AE interventions were divided into two treatment doses: 0-2500 metres and 2501-5000 metres. AE did not improve locomotor recovery. Effect sizes presented as Hedge's *G* standardized mean difference (SMD) with 95% CI

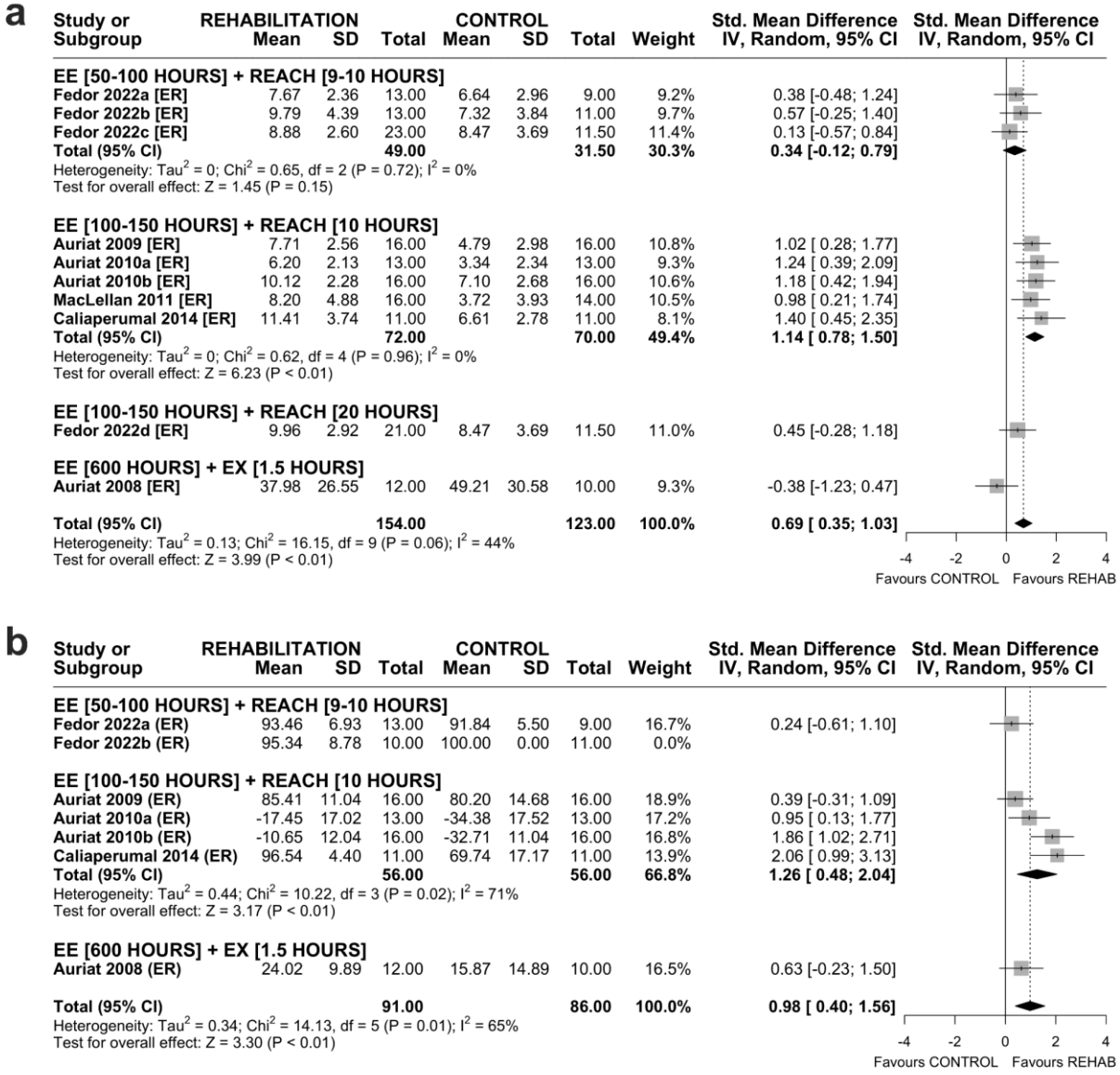


Figure A-15 Impact of treatment dose on recovery in ER interventions. ER interventions were divided into four treatment doses based on time in EE and REACH: EE (50-100 hours) + REACH (9-10 hours), EE (100-150 hours) + REACH (10 hours), EE (100-150 hours) + REACH (20 hours), and EE (600 hours) + EX (1.5 hours). **A** Forest plot of random-effects meta-analysis of skilled reaching recovery grouped by treatment dose; only the moderate dose group [EE (100-150 hours) + REACH (10 hours)] significantly improved skilled reaching. **B** Forest plot of random-effects meta-analysis of locomotor recovery grouped by treatment dose; again, only the moderate dose group [EE (100-150 hours) + REACH (10 hours)] significantly improved locomotor recovery. Effect sizes presented as Hedge's *G* standardized mean difference (SMD) with 95% CI

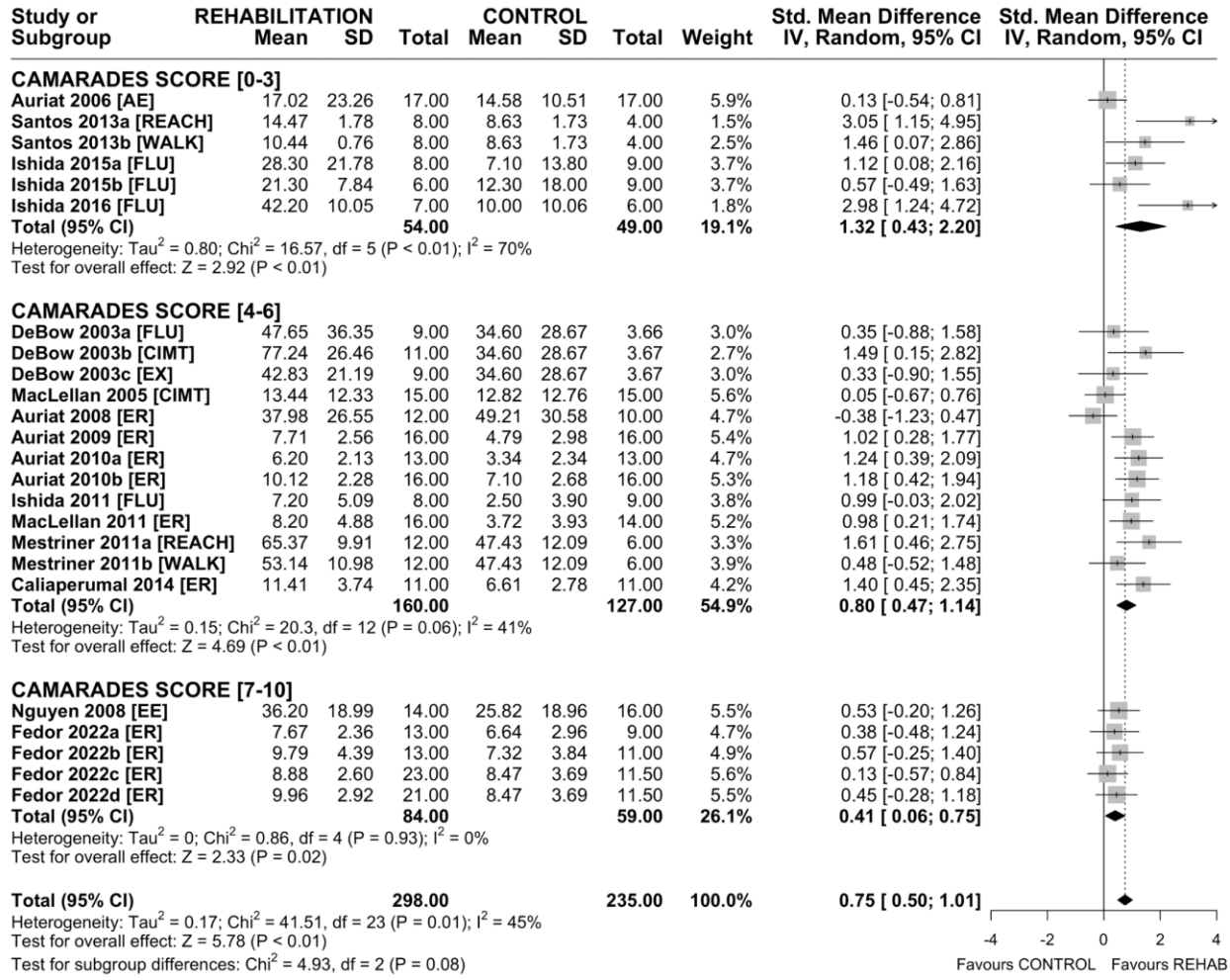


Figure A-16 Impact of study quality (CAMARADES score) on effect sizes reported in skilled reaching assessments. We observed a trend that as study quality increased, effect sizes decreased, and 95% CIs narrowed (test for subgroup differences p=0.08)

Search Strategy

Database: Academic Search Complete

TI = title search, AB = abstract search, SU = subject search, KW = keyword search

Rehab Terms (S1)

rehabilitation OR rehab OR exercise OR motor-therapy OR physical-therap* OR physiotherap* OR aerobic-training OR running OR walking OR treadmill* OR constraint-induced-movement-therapy OR mobilization OR mobilisation OR forced-use-therapy OR enrichment OR environmental-enrichment OR enriched-rehabilitation OR training OR reach* OR grasp*

S1: (TI (rehab terms)) OR (AB (rehab terms)) OR (SU (rehab terms)) OR (KW (rehab terms))

Stroke Terms (S2)

cerebral-hemorrhage* OR cerebral-haemorrhage* OR intracerebral-hemorrhage* OR intracerebral-haemorrhage* OR intracranial-hemorrhage* OR intracranial-haemorrhage* OR intracerebral-bleed OR cerebral-hematoma* OR hemorrhagic-stroke* OR haemorrhagic-stroke*

S2: (TI (stroke terms)) OR (AB (stroke terms)) OR (SU (stroke terms)) OR (KW (stroke terms))

Population Terms (S3)

rat OR rats OR mouse OR mice OR rodent* OR primate OR canine OR murine OR non-human OR animal-model

S3: ((TI (population terms)) OR (AB (population terms)) OR (SU (population terms)) OR (KW (population terms))

Search: S1 AND S2 AND S3

Search Strategy

Database: Medline

mp = title, abstract, original title, name of substance word, subject heading word, floating sub-heading word, keyword heading word, organism supplementary concept word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms

Rehab Terms (S1)

rehabilitation OR rehab OR exercise OR motor-therapy OR physical-therap* OR physiotherap* OR aerobic-training OR running OR walking OR treadmill* OR constraint-induced-movement-therapy OR mobilization OR mobilisation OR forced-use-therapy OR enrichment OR environmental-enrichment OR enriched-rehabilitation OR training OR reach* OR grasp*

S1: (rehab terms).mp.

Stroke Terms (S2)

cerebral-hemorrhage* OR cerebral-haemorrhage* OR intracerebral-hemorrhage* OR intracerebral-haemorrhage* OR intracranial-hemorrhage* OR intracranial-haemorrhage* OR intracerebral-bleed OR cerebral-hematoma* OR hemorrhagic-stroke* OR haemorrhagic-stroke*

S2: (stroke terms).mp.

Population Terms (S3)

rat OR rats OR mouse OR mice OR rodent* OR primate OR canine OR murine OR non-human OR animal-model

S3: (population terms).mp.

Search: S1 AND S2 AND S3

Search Strategy

Database: EMBASE

mp = title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword, floating subheading word, candidate term word

Rehab Terms (S1)

rehabilitation OR rehab OR exercise OR motor-therapy OR physical-therap* OR physiotherap* OR aerobic-training OR running OR walking OR treadmill* OR constraint-induced-movement-therapy OR mobilization OR mobilisation OR forced-use-therapy OR enrichment OR environmental-enrichment OR enriched-rehabilitation OR training OR reach* OR grasp*

S1: (rehab terms).mp.

Stroke Terms (S2)

cerebral-hemorrhage* OR cerebral-haemorrhage* OR intracerebral-hemorrhage* OR intracerebral-haemorrhage* OR intracranial-hemorrhage* OR intracranial-haemorrhage* OR intracerebral-bleed OR cerebral-hematoma* OR hemorrhagic-stroke* OR haemorrhagic-stroke*

S2: (stroke terms).mp.

Population Terms (S3)

rat OR rats OR mouse OR mice OR rodent* OR primate OR canine OR murine OR non-human OR animal-model

S3: (population terms).mp.

Search: S1 AND S2 AND S3

Search Strategy

Database: CINAHL

TI = title search, AB = abstract search, SU = subject search

Rehab Terms (S1)

rehabilitation OR rehab OR exercise OR motor-therapy OR physical-therap* OR physiotherap* OR aerobic-training OR running OR walking OR treadmill* OR constraint-induced-movement-therapy OR mobilization OR mobilisation OR forced-use-therapy OR enrichment OR environmental-enrichment OR enriched-rehabilitation OR training OR reach* OR grasp*

S1: TI (rehab terms) OR AB (rehab terms) OR SU (rehab terms)

Stroke Terms (S2)

cerebral-hemorrhage* OR cerebral-haemorrhage* OR intracerebral-hemorrhage* OR intracerebral-haemorrhage* OR intracranial-hemorrhage* OR intracranial-haemorrhage* OR intracerebral-bleed OR cerebral-hematoma* OR hemorrhagic-stroke* OR haemorrhagic-stroke*

S2: TI (stroke terms) OR AB (stroke terms) OR SU (stroke terms)

Population Terms (S3)

rat OR rats OR mouse OR mice OR rodent* OR primate OR canine OR murine OR non-human OR animal-model

S3: TI (population terms) OR AB (population terms) OR SU (population terms)

Search: S1 AND S2 AND S3

Search Strategy

Database: PMC

TI = title search, AB = abstract search

Rehab Terms (S1)

rehabilitation OR rehab OR exercise OR motor-therapy OR physical-therap* OR physiotherap* OR aerobic-training OR running OR walking OR treadmill* OR constraint-induced-movement-therapy OR mobilization OR mobilisation OR forced-use-therapy OR enrichment OR environmental-enrichment OR enriched-rehabilitation OR training OR reach* OR grasp*

S1: TI (rehab terms) OR AB (rehab terms)

Stroke Terms (S2)

cerebral-hemorrhage* OR cerebral-haemorrhage* OR intracerebral-hemorrhage* OR intracerebral-haemorrhage* OR intracranial-hemorrhage* OR intracranial-haemorrhage* OR intracerebral-bleed OR cerebral-hematoma* OR hemorrhagic-stroke* OR haemorrhagic-stroke*

S2: TI (stroke terms) OR AB (stroke terms)

Population Terms (S3)

rat OR rats OR mouse OR mice OR rodent* OR primate OR canine OR murine OR non-human OR animal-model

S3: TI (population terms) OR AB (population terms)

Search: S1 AND S2 AND S3

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Appendix B | Supplement to Chapter 4

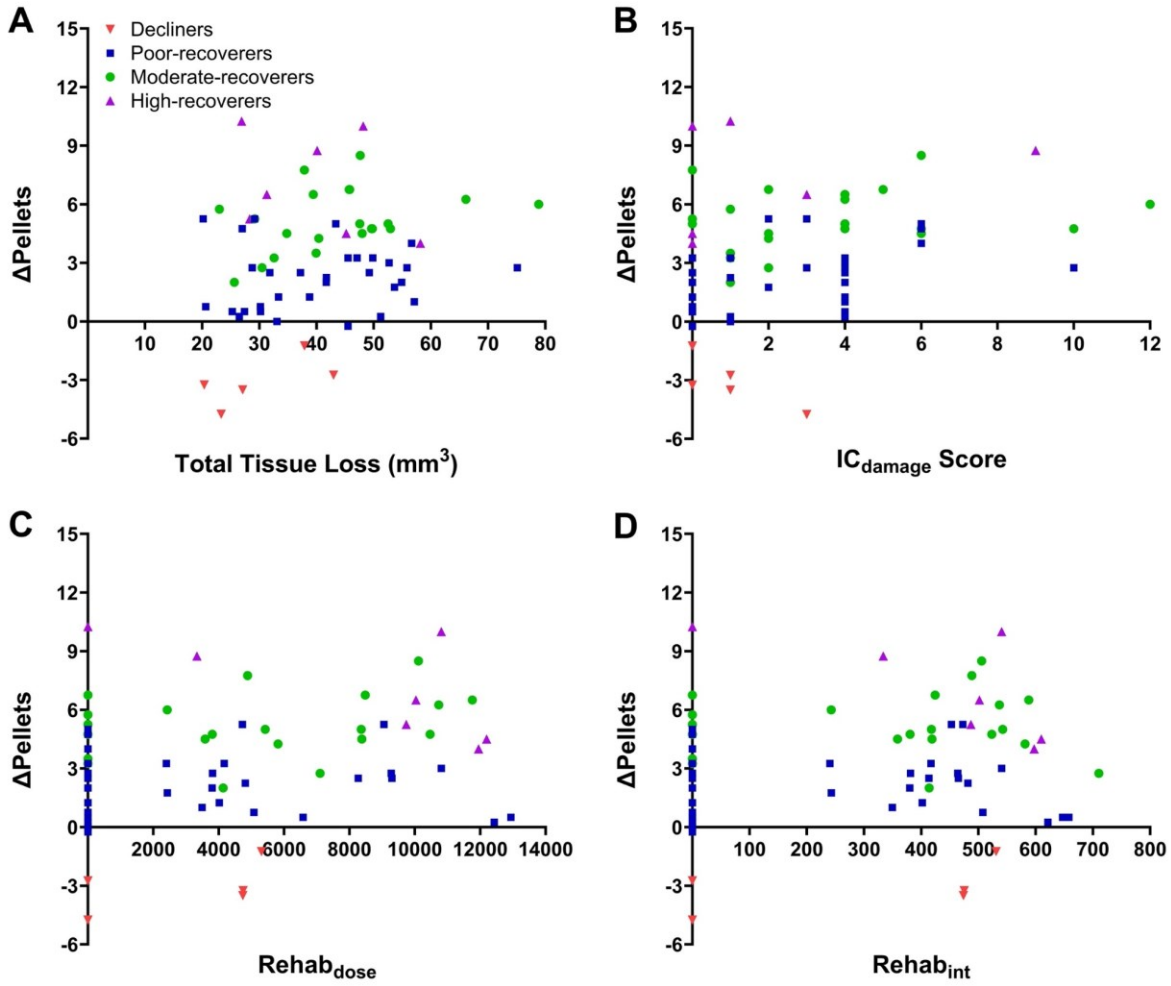

Figure B-1 Variables entered into multiple linear regression analysis to predict recovery of impairment (ΔPellets). **A** Total volume of tissue loss (mm^3) did not significantly correlate with recovery of skilled reaching in the full dataset (p=0.0525) or any subgroup (p≥0.110). **B** Increased internal capsule damage was weakly correlated with increased recovery of skilled reaching in the full dataset (r=0.296, p=0.0175); however, subgroup analysis found only a moderate correlation between increased internal capsule damage and greater recovery in the poor-recoverers (r=0.416, p=0.0198). **C** Rehabilitation dose did not significantly correlate with recovery of skilled reaching in the full dataset (p=0.0750) or any subgroup (p≥0.0773). **D** Rehabilitation intensity did not significantly correlate with recovery of skilled reaching in the full dataset (p=0.370) or any subgroup (p≥0.0856). Correlation values for each predictor and (ΔPellets) are found in Table B-1

Table B-1 Correlation data for predictor variables and observed recovery (ΔPellets)

Predictor: Tissue Loss			
Group	***r***	***R²***	***p-value***
Full dataset	0.244	0.0593	0.0525
Decliners	0.665	0.442	0.221
Poor-recoverers	0.0918	0.00845	0.623
Moderate-recoverers	0.359	0.129	0.110
High-recoverers	-0.332	0.110	0.468
Predictor: IC Damage*			
Group	**r**	***R²***	***p-value***
Full dataset	0.296		0.0175
Decliners	-0.738		0.200
Poor-recoverers	0.416		0.0198
Moderate-recoverers	0.189		0.412
High-recoverers	0.473		0.286
Predictor: Rehab Dose			
Group	***r***	***R²***	***p-value***
Full dataset	0.224	0.0502	0.0750
Decliners	0.524	0.275	0.365
Poor-recoverers	0.00855	<0.001	0.964
Moderate-recoverers	0.284	0.0805	0.213
High-recoverers	-0.704	0.496	0.0773
Predictor: Rehab Intensity			
Group	***r***	***R²***	***p-value***
Full dataset	0.114	0.0130	0.370
Decliners	0.524	0.275	0.365
Poor-recoverers	-0.0233	0.0005	0.9009
Moderate-recoverers	0.0743	0.00552	0.749
High-recoverers	-0.691	0.478	0.0856

Legend: *Spearman correlation

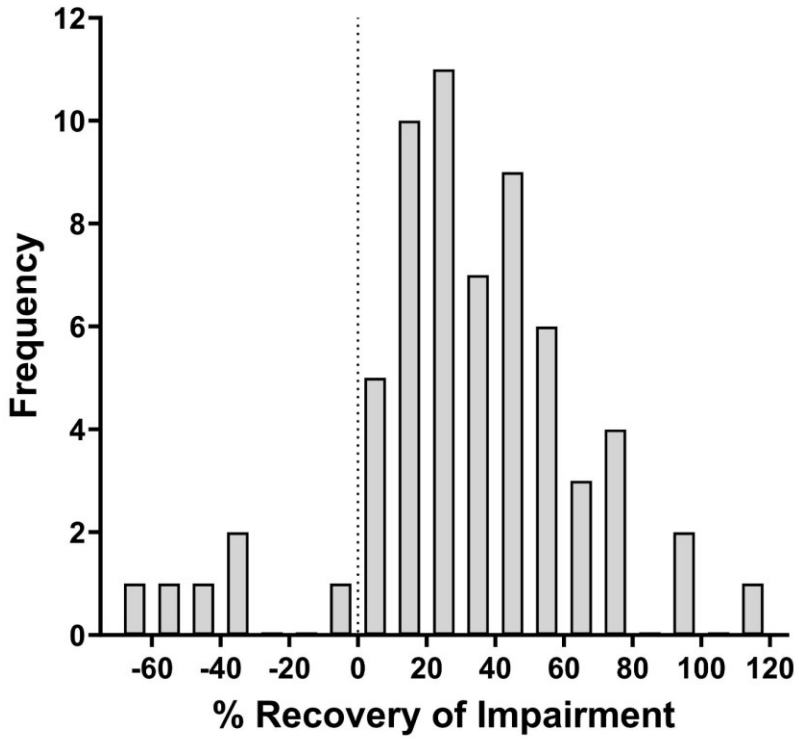

Figure B-2 Frequency distribution of recovery of impairment (R%). Frequency represents number of rats within each bin; bin width=10%