Hydration Status and Repetitive Head Impacts: Neurophysiological Responses and Functional Implications

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Impact of COVID-19

This PhD was undertaken part-time and commenced in February 2019, approximately one year before the national lockdown was implemented in response to the COVID-19 pandemic. The original project plan involved initiating the thesis with a literature review, followed by an experimental study investigating the neurophysiological effects of hypo-hydration and rehydration, followed by a series of observational studies in various combat sport contexts, including a) sparring, b) fight preparation camps, then c) sanctioned competitions.

Following successful piloting of the initial experimental protocol (presented in Chapter 5), the national lockdown began in March 2020, halting participant recruitment and in-person data collection. To mitigate the disruption and optimise productivity during this period, the literature review component of the thesis was expanded in two key directions. First, a theoretical framework was developed to map the proposed mechanisms of neuromuscular impairment arising from different methods of hypo-hydration. Second, a quantitative survey was designed and implemented to explore the perceived link between hypo-hydration and increased susceptibility to brain trauma, a topic commonly discussed among combat sport athletes. These additions contributed to Chapter 2, Section 2.1 and Chapter 3 respectively.

Once public health restrictions eased and institutional research activities resumed, recruitment and data collection for the experimental study (Chapter 5) were restarted. This process required extensive adjustments to align with post-COVID protocols, including risk assessments, sanitation measures, and scheduling changes to ensure the safety of both participants and researchers, particularly due to the physical and invasive nature of the testing procedures.

Overall, while the pandemic caused substantial delays to experimental components of the project, the period was used constructively to broaden the theoretical and empirical scope of

the thesis. The adjusted timeline enabled a return to lab-based research with enhanced planning and precautionary protocols in place.

Summary of work

Combat sport athletes frequently experience two distinct yet potentially interacting physiological stressors: repetitive head impacts (RHIs) and rapid weight loss through hypo-hydration. While each has been studied independently, limited research has examined their combined neurophysiological consequences. This thesis aimed to investigate how RHIs and hypo-hydration influence neuromuscular and cortical function, using transcranial magnetic stimulation (TMS) and motor nerve stimulation (MNS) as assessment tools.

An initial survey of over 100 combat sport athletes highlighted widespread engagement in rapid weight loss practices, with many reporting overlapping symptoms of hypo-hydration and concussion. In addition, some athletes reported exacerbation of concussion symptoms after undergoing a weight-cut. A reliability study confirmed that TMS and MNS measures could be used with sufficient precision in ensuring robustness for subsequent investigations. In the first experimental study, exercise and heat-induced hypo-hydration impaired maximal strength and voluntary activation but did not significantly alter corticospinal excitability or inhibition, suggesting preserved central drive under fluid-restricted conditions.

In contrast, the following study examined responses to a single sparring session and found significant increases in cortical inhibition and motor thresholds, along with reduced maximal strength and voluntary activation, all of which resolved within 48 hours. A follow-up longitudinal study using instrumented mouthguards revealed that impact magnitude e.g., cumulative angular acceleration predicted the degree of increased cortical inhibition. Finally, a 10-week case series tracked three fighters through their fight camps. Voluntary activation and strength showed the most frequent meaningful changes, and over 200 RHIs were recorded in approximately 35% of their monitored sparring sessions.

In summary, repeated head impacts elevated cortical inhibitory activity and central fatigue, while heat-induced hypo-hydration produced a contrasting neurophysiological response. These opposing effects may suggest a complex physiological interplay if they occur concurrently, whereby head-impact-related neural inhibition could disrupt or override compensatory mechanisms associated with fluid loss, potentially increasing vulnerability to injury and impairing athletic performance.

Abstract

Combat sports such as boxing and mixed martial arts involve intentional head contact, making mild traumatic brain injuries especially prevalent. Repetitive head impacts are also common in training-based sparring, which often occurs without medical oversight and thus remains unreported. In parallel, combat sports enforce weight categories to promote fairness, but weight-making is typically achieved via acute body water losses. Recently, transcranial TMS, paired with MNS, has emerged as a tool to probe central and peripheral nervous system function. However, the neurophysiological consequences of repeated head trauma and hypo-hydration have largely been studied in isolation, with limited attention to their combined effect. This thesis aimed to investigate the neurophysiological impact of both hypo-hydration and repetitive brain trauma in combat athletes to inform potential safeguards for performance and brain health.

A preliminary survey identified a strong correlation between self-reported hypo-hydration and concussion, suggesting overlapping neurophysiological mechanisms that may obfuscate concussion diagnoses or exacerbate the risk. Following this, a between-day reliability study confirmed moderate to excellent reproducibility for all TMS and MNS measures, ensuring robustness for subsequent investigations.

In the first experimental study, low-intensity aerobic exercise and heat-induced hypo-hydration was investigated using TMS and PNS measures. Hypo-hydration resulted in a reduction in maximal voluntary strength, voluntary activation and peripheral excitability, but not corticospinal excitability or inhibition. Interestingly, cortical inhibition increased following exercise in the euhydrated condition only, suggesting a compensatory mechanism to sustain performance when fluid balance was preserved.

The second experimental study assessed the neurophysiological responses before, after and 48 hours after a single sparring session, demonstrating an increase in cortical inhibition and cortical excitability thresholds, as well as a reduction in maximal strength and voluntary activation. All measures returned to baseline within 48 hours, and no changes were observed in markers of peripheral fatigue, demonstrating an increase in central fatigue and cortical inhibition following a single sparring session.

In the third experimental study, the same measures were extended across multiple sparring sessions, in conjunction with instrumented mouthguards to monitor head impact frequency and magnitude. The findings also demonstrated a significant increase in cortical inhibition but also revealed that this was correlated and predicted by cumulative impact burden. Finally, in a case series of three athletes across a 10-week fight preparation camp, a typical error threshold was applied to detect meaningful individual change, demonstrating maximal strength and voluntary activation to be most meaningful, followed by cortical inhibition and half-relaxation time. In addition, over 200 impacts were observed in ~ 35% of their sparring sessions, establishing a frequency and cumulative head impact burden metric for combat sport athletes.

In conclusion, repeated head impacts elevate brain inhibitory activity and central fatigue, while hypo-hydration induces a contrasting response. These opposing effects suggest a complex physiological interplay, where brain trauma may override or disrupt compensatory mechanisms following fluid loss, ultimately increasing vulnerability to injury and impairing athletic performance.

“The brain immediately confronts us with its great complexity. The human brain weighs only three to four pounds but contains about 100 billion neurons. Although that extraordinary number is of the same order of magnitude as the number of stars in the Milky Way, it cannot account for the complexity of the brain. The liver probably contains 100 million cells, but 1,000 livers do not add up to a rich inner life.”

Gerald D. Fischbach (from *Scientific American*, September 1992)

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Chapter 6

compound muscle action potential *AMT* Active motor threshold *SICI* Short interval intracortical inhibition.

Chapter 7

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List of abbreviations

AMT	Active motor threshold
ANOVA	Analysis of variance
AQP4	Aquaporin-4
ATP	Adenosine triphosphate
BJJ	Brazilian jiu-jitsu
BM	Body mass
BMR	Body mass reduction
CNS	Central nervous system
CSE	Corticospinal excitability
cSP	Cortical silent period
ECC	Excitation-contraction coupling
EEG	Electroencephalography
EER	Estimated energy requirements
EIP	Experimentally induced pain
EMG	Electromyography
fMRI	Functional magnetic resonance imaging
GABA	γ-aminobutyric acid
GFAP	Glial fibrillary acidic protein
HIF	Head impact frequency
HRT	Half relaxation time
ICC	Intraclass correlation coefficients
ICF	Intracortical facilitation
IQR	Interquartile range
IMG	Instrumented mouthguard
KB/MT	Kickboxing/Muay-thai
LoA	Limits of Agreement

MEP_{RAW}	Motor evoked potential amplitude
MFVC	Muscle fibre conduction velocity
MMA	Mixed martial arts
M_{MAX}	Maximum compound muscle action potential
MNS	Motor nerve stimulation
MRS	Magnetic resonance spectroscopy
mTBI	Mild traumatic brain injury
MVC	Maximum voluntary contraction
PAA	Peak angular acceleration
PLA	Peak linear acceleration
PNS	Peripheral nervous system
RHI	Repetitive head impacts
RMT	Resting motor threshold
ROS	Reactive oxygen species
RWL	Rapid weight loss
SCAT	Sport concussion assessment tool
SDC	Smallest detectable change
SEM	Standard error of the mean
SERCA	Sarco/endoplasmic reticulum ATPase
SICI	Short interval intracortical inhibition
TE	Typical error
UCH-L1	Ubiquitin carboxyl-terminal hydrolase isoenzyme L1
USG	Urine specific gravity
U_{osm}	Urine osmolality
VA	Voluntary Activation
WC	Weight-cut
TMS	Transcranial magnetic stimulation

Publications arising from the thesis

Peer-reviewed publication

Uddin, N., Waldron, M., Patterson, S. D., Winter, S., & Tallent, J. (2022). A Survey of Combat Athletes' Rapid Weight Loss Practices and Evaluation of the Relationship With Concussion Symptom Recall. *Clinical journal of sport medicine : official journal of the Canadian Academy of Sport Medicine*, *32*(6), 580–587. https://doi.org/10.1097/JSM.0000000000001032

Uddin, N., Tallent, J., Patterson, S. D., Goodall, S., & Waldron, M. (2022). Corticospinal and peripheral responses to heat-induced hypo-hydration: potential physiological mechanisms and implications for neuromuscular function. *European journal of applied physiology*, *122*(8), 1797–1810. https://doi.org/10.1007/s00421-022-04937-z

Uddin, N., Scott, J., Nixon, J., Patterson, S. D., Kidgell, D., Pearce, A. J., Waldron, M., & Tallent, J. (2025). The effects of exercise, heat-induced hypo-hydration and rehydration on blood-brain-barrier permeability, corticospinal and peripheral excitability. *European journal of applied physiology*, *125*(2), 535–550. https://doi.org/10.1007/s00421-024-05616-x

Uddin, N., Waldron, M., Schoenmakers, P., Voyevoda, L., Adams, T., Jones, B., Patterson, S., Kidgell, D., Pearce, A., Tallent, J. Head impact frequency and magnitude are related to increased corticospinal inhibition after combat sports sparring. *Medicine in Sport and Exercise Science (Under Review).*

Book chapters arising from the thesis

Kirk, C., Follmer, B & Uddin, N. 'Brain trauma in combat sports: Can we better manage a necessary evil?' in Matthews, C. R., Black, A., Hardwick, J., Malcolm, D & Pearce, A. J. *The Routledge Handbook of Sport, Concussion and Brain Injuries (in press).*

Conference Presentations

Uddin, N., Waldron, M., Patterson, S. D., Winter, S., & Tallent, J. (2022). A survey of combat athletes' rapid weight loss practices and evaluation of the relationship with concussion symptom recall. *The Physiology of Exercise, London, 2022.*

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Uddin, N., Waldron, M., Schoenmakers, P., Voyevoda, L., Adams, T., Jones, B., Patterson, S., Kidgell, D., Pearce, A., Tallent, J. The neurophysiological responses to repetitive head impacts in combat sports. *UK Sensorimotor Conference, Oxford, 2025.*

Uddin, N., Waldron, M., Schoenmakers, P., Voyevoda, L., Adams, T., Jones, B., Patterson, S., Kidgell, D., Pearce, A., Tallent, J. The acute neurophysiological responses to repetitive head impacts in combat sports. *American College of Sports Medicine (ACSM) Combat Sport Special Interest Group UK Summit, Liverpool, 2026.*

Uddin, N., Waldron, M., Schoenmakers, P., Voyevoda, L., Adams, T., Jones, B., Patterson, S., Kidgell, D., Pearce, A., Tallent, J. Head impact frequency and magnitude are related to increased corticospinal inhibition after combat sports sparring. *European Congress of Sport Science, Lausanne, 2026.*

Uddin, N., Waldron, M., Schoenmakers, P., Voyevoda, L., Adams, T., Jones, B., Patterson, S., Kidgell, D., Pearce, A., Tallent, J. Head impact frequency and magnitude are related to increased corticospinal inhibition after combat sports training. *Brainbox Initiative, London, 2026.*

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To Yunus, if you ever read this one day, I hope the nap that follows is well worth it!

Author’s declaration

I declare that the work contained in this thesis has not been submitted in the past or is to be submitted at any other University for any other award and that it is all my own work. I also confirm that this work fully acknowledges opinions, ideas and contributions from the work of others.

The word count of this thesis is 41,030 words.

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Chapter 1:
Introduction

It is reported that there are 1.4 million annual attendances to emergency departments in England & Wales, following head trauma (1). Mild traumatic brain injuries (mTBI) represent approximately 1.2% of all emergency department presentations in England and Wales, however, the true incidence is likely to be considerably higher, as studies indicate that even when concussions are diagnosed or suspected, up to 90% are miscoded within hospital records, rendering health informatics data an unreliable estimate of prevalence (2). While mTBI constitutes a relatively small proportion of all emergency department attendances, sports-related concussions account for a notable fraction of cases, with recent epidemiological data suggesting that approximately 6% of mTBI presentations are attributable to sporting activities (3).

In sport, and particularly within combat sports, the risk of both concussive and sub-concussive head impacts is elevated due to the repetitive and intentional nature of head contact. A mTBI is caused by rapid acceleration–deceleration forces applied directly or indirectly to the head, resulting in transient functional disturbance of brain activity (4). Importantly, such injuries frequently occur in the absence of overt structural damage detectable via conventional neuroimaging (5). Despite this, evidence from non-invasive brain stimulation studies indicates that functional alterations within cortical circuitry can be detected following concussion, including increased cortical inhibition persisting up to 72 h post-injury (6,7). Similar neurophysiological changes have also been reported following repetitive sub-concussive exposures, such as sparring or ball heading, suggesting that functional impairment may occur even in the absence of clinically diagnosed concussion (8,9).

An unexplored possible risk factor for the above-mentioned cellular damage is the hydration status of an individual; athletes and soldiers often experience significant body water losses in both controlled (voluntary) and uncontrolled (environmental) conditions. Combat sports

present a unique context in which repetitive head impacts are often combined with additional physiological stressors. One such stressor is dehydration, most commonly arising from rapid weight-loss practices undertaken prior to competition. It is estimated that 60–80% of combat sport athletes engage in rapid weight-loss strategies, predominantly through acute dehydration achieved via fluid restriction and excessive sweating (10). These practices frequently result in body mass reductions exceeding 5% within 24 h of weigh-in, with extreme cases reporting losses of up to 10–15% of body mass (11). Although severe dehydration is associated with well-documented systemic health risks, including renal dysfunction (12-14), its potential influence on neurological vulnerability in the context of head impacts remains poorly understood.

Hypo-hydration has been shown to impair prolonged skeletal muscle performance; however, its effects on neuromuscular and neurophysiological function are inconsistent, and moderate body mass losses (4–6%) are not universally associated with reductions in muscle force, membrane excitability, or peak power output (15,16). Notably, dehydration has been shown to induce acute anatomical changes within the central nervous system, including reductions in brain and spinal cord volume and ventricular expansion (17-26). These findings raise important questions regarding the potential implications of dehydration for neural tissue during periods of mechanical stress, such as those experienced during repetitive head impacts. Concerns regarding cumulative neurological damage are particularly salient in combat sports, where long-term neurodegenerative conditions such as chronic traumatic encephalopathy (CTE) have been reported in retired boxers (27)

Despite growing concern among athletes and clinicians, it remains unclear whether dehydration may influence neurophysiological responses to head impacts or whether these stressors act on overlapping functional pathways within the central nervous system. Given the limitations of

conventional neuroimaging in detecting rapid or subtle functional disturbances, non-invasive brain stimulation techniques such as transcranial magnetic stimulation (TMS) provide a valuable tool for assessing the integrity of the corticospinal pathway in vivo. TMS has been widely applied across clinical neurology, exercise physiology, and sports science to detect central fatigue, characterise neuroplastic changes, and monitor recovery following brain injury (28-30).

Therefore, the overarching aim of this thesis was to examine the neurophysiological consequences of dehydration and repetitive head impacts, with particular relevance to contexts in which these stressors are prevalent. Specifically, this thesis aimed to:

a) explore the self-reported association between weight-cutting practices and the incidence of self-reported concussions in combat sports athletes;

b) investigate the independent neurophysiological effects of dehydration on corticospinal and peripheral excitability using non-invasive assessment techniques; and

c) examine the independent neurophysiological effects of repetitive head impacts on corticospinal and peripheral excitability, and to hypothesise points of conceptual convergence between dehydration and head impacts within the discussion.

These themes will be expanded across the chapters outlined in Figure 1.1.

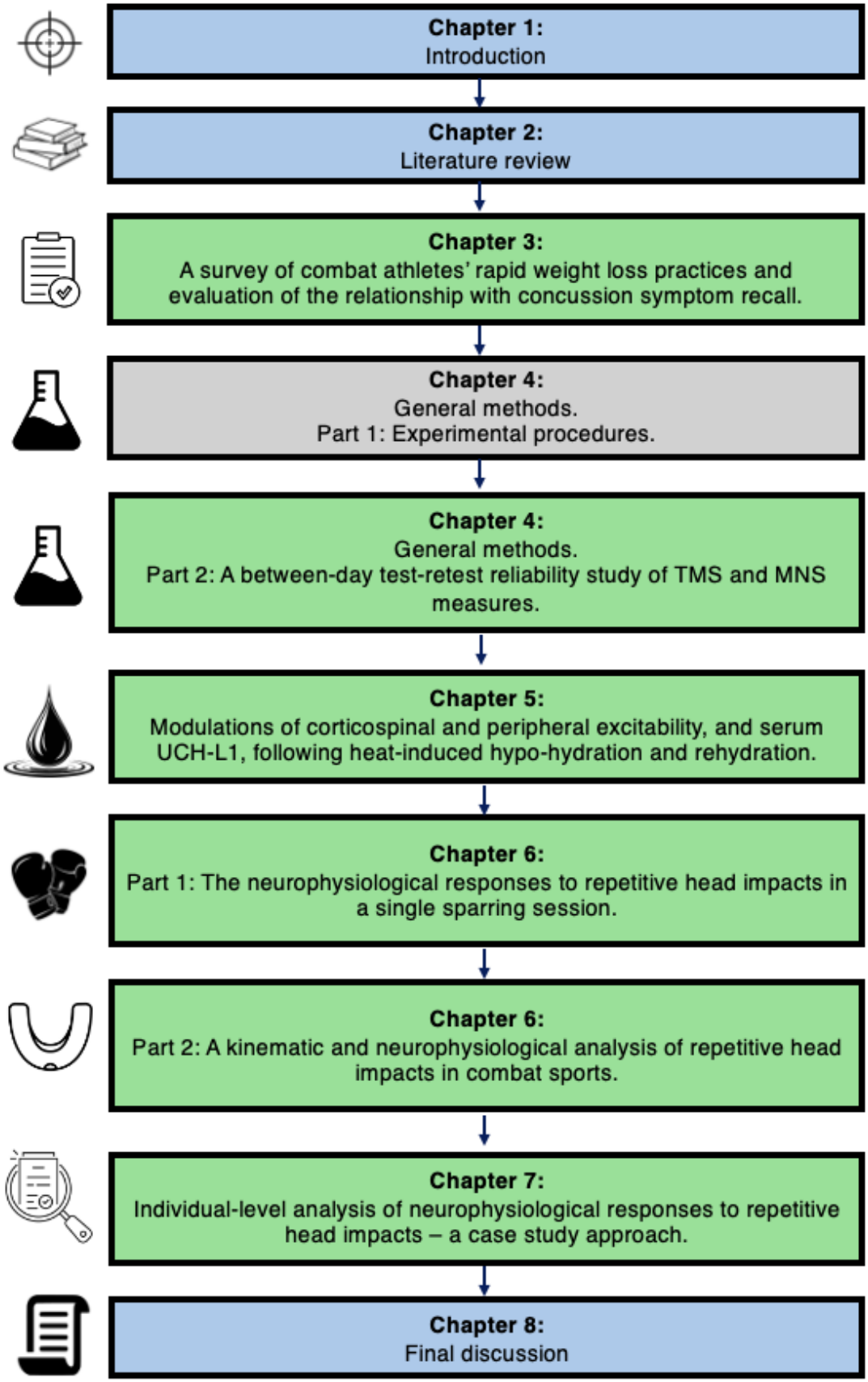

Figure 1.1: Flow chart of topics covered in the PhD thesis.

Chapter 2:
Literature review

Sections 2.1.1 & 2.1.2 published: Uddin, N., Tallent, J., Patterson, S. D., Goodall, S., & Waldron, M. (2022). Corticospinal and peripheral responses to heat-induced hypo-hydration: potential physiological mechanisms and implications for neuromuscular function. *European journal of applied physiology*, *122*(8), 1797–1810. https://doi.org/10.1007/s00421-022-04937-z

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Building on the rationale outlined in Chapter 1, this chapter provides a critical review of the current literature on the physiological and neurophysiological consequences of both hypo-hydration and brain trauma. Given the high prevalence of dehydration-induced weight-cutting in combat sport athletes and the growing evidence that repetitive head impacts can alter corticospinal excitability, this chapter explores how these two stressors might influence shared neural pathways and contribute to impaired motor function. Particular attention is given to studies employing transcranial magnetic stimulation (TMS) and motor nerve stimulation (MNS), as these techniques allow for real-time, non-invasive assessment of the brain-to-muscle pathway. The chapter is structured in four sections: the first two outline the physiological implications of repetitive head impacts (Section 2.1) and hypo-hydration (Section 2.2), the third introduces TMS and MNS as tools for assessing corticospinal and neuromuscular function (Section 2.2); and the fourth highlights the shared pathophysiology and lack of studies examining the combined effects of fluid deficits and brain trauma, thereby establishing the rationale for the experimental investigations that follow (Section 2.4).

2.1 Physiological implications of repetitive head impacts

2.1.1 Mild traumatic brain injuries (concussions)

Brain trauma in sports range from life-threatening acute injuries (e.g., subdural hematoma, epidural hematoma, and intracranial haemorrhage) to mild traumatic brain injuries (mTBIs), also synonymous with concussions. Such traumas result from blunt non-penetrating impacts, causing axonal stretching and tearing, known as diffuse axonal injury (31,32). A mTBI is defined as a transient neurological disturbance, induced by biomechanical forces (typically a blow or rapid acceleration to the head; 31,32). However, mTBIs are a complex and evolving construct, as they are not consistently associated with identifiable structural pathology using

conventional neuroimaging techniques, and its classification remains based largely on clinical presentation rather than objective biomarkers. As highlighted by Smith & Stewart (33), concussion is not currently recognised as a discrete pathological entity in the traditional sense, but rather as a functional disturbance characterised by a constellation of symptoms affecting cognition, balance, and neuromuscular control. Following mechanical insult, a complex neurochemical cascade often referred to as a "metabolic crisis" occurs (4). The initial biomechanical forces cause neuronal depolarisation**,** leading to an elevated release of excitatory neurotransmitters (glutamate)*,* resulting in widespread activation of ionotropic glutamate receptors, particularly NMDA receptors, causing an influx of Ca^{2+} and efflux of K^{+}**.** This ion imbalance increases demand for energy as Na^{+}/K^{+} ATPase pumps work to restore ionic homeostasis, leading to increased glucose metabolism (hyper-glycolysis)**.** However, this is soon followed by a period of metabolic depression, during which glucose utilisation drops despite ongoing energy demands. Simultaneously, the elevated intracellular calcium can impair mitochondrial function, worsen the energy crisis and increase oxidative stress. Excess Ca^{2+} also activates various enzymes that can damage cell membranes and cytoskeletal proteins. Additionally, axonal stretching can disrupt transport mechanisms and alter neuronal function (Figure 2.1; 4).

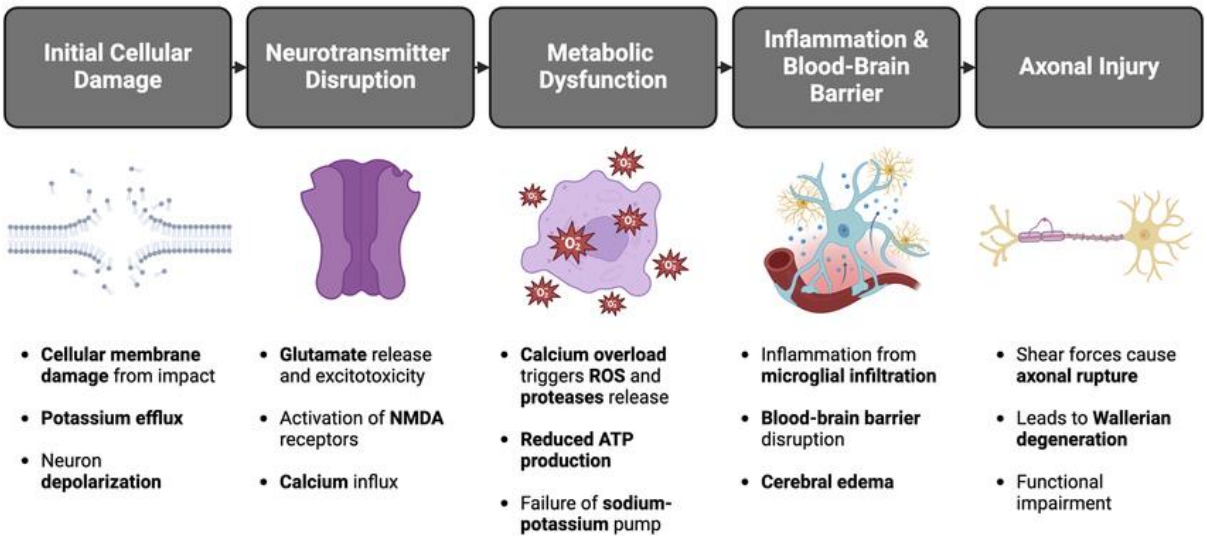

Figure 2.1: Summary of the pathophysiology and neurochemical cascade following an mTBI (Adapted from Chilmeran et al. [34]).

2.1.2 Non-concussive impacts

In addition to clinically diagnosed concussions, there is increasing interest in the potential cumulative effects of non-concussive head impacts, defined as head impacts that do not result in overt clinical symptoms (35). As discussed by Nowinski et al. (35) the use of the term "non-concussive" is preferred over "sub-concussive", as it avoids implying a linear relationship with concussion severity and instead reflects the absence of clinically recognised symptoms. This distinction between a concussive and non-concussive impacts is particularly relevant in combat sports, where repeated head impacts are an inherent component of participation. While the former may result in observable symptoms such as headache, dizziness, and confusion (36,37), the latter refers to cumulative damage from multiple non-concussive or concussive blows incurred across time (38,39). Emerging evidence suggests that repetitive exposure to such impacts may still result in measurable changes in brain function and structure over time (7,8,40,41).

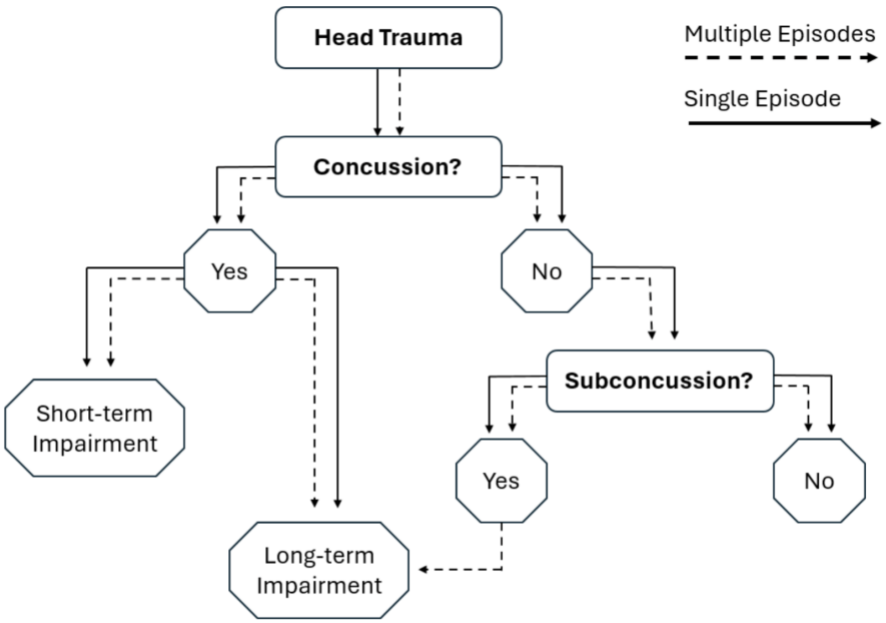

Figure 2.2: Differentiated classifications of head trauma (42).

While the literature surrounding brain trauma pathophysiology is extensive, the following subsection of this review will focus on the prevalence and physiology of brain trauma and hypo-hydration in combat sports, where these two factors are likely to converge.

2.1.3 Repetitive head impacts in combat sports

Combat sports involve two competitors using technical skill and physical force to achieve dominance across the bout duration, and render an opponent unable to continue (via knockout [KO], technical knockout [TKO], or submission). These sports are typically categorised into striking, grappling, or mixed disciplines depending on permitted techniques. Striking and mixed combat sports are distinct in that they explicitly aim to inflict intentional brain trauma through actions such as punches, kicks, elbows, and knees to the head (43), with scoring systems favouring techniques that produce greater visible impact or damage rather than mere control (43). Preparation for competition involves frequent training and sparring, during which

athletes both sustain and deliver repeated head impacts (44). Consequently, head trauma is not incidental but intrinsic to the structure and objectives of combat sports (45).

Competition-based concussion incidence rates are high – with estimates ranging from 16 to 25 per 100 exposures in boxing and MMA (46,47) while others have reported over 30% of surveyed professional MMA fighters had been diagnosed with at least one concussion (48). Though loss of consciousness is generally attributed to concussion, this is only known to occur in 10 – 20% of cases (4), thus potentially leaving athletes undiagnosed or unaware after brain trauma, resulting in a concussion prevalence amongst athletes that may be higher than suspected. In addition, training-based sparring also involves repetitive brain trauma that most often occur in the absence of medical staff and are therefore unaccounted.

In competition, knockout (KO) and technical knockout (TKO) rates are substantial, particularly in boxing and mixed martial arts (MMA), with reported KO/TKO rates of 26% in females and 36.5% in males in MMA, compared to 31% in females and 50% in males in boxing (49,50). MMA competitors may sustain an average of 6.2 KO/TKO losses across a career (51), and unlike boxing, are often subjected to additional strikes following a knockdown, receiving 2.6 ± 3 strikes after losing consciousness and approximately 18.5 head strikes in the 30 seconds preceding a TKO (47). Beyond bout outcomes, MMA athletes experience 2.41 “significant” head traumas per minute and 6.3 total head traumas per minute (49), with video analyses identifying suspected concussion rates of 0.085 concussions·min^{-1} in MMA and 0.047 concussions·min^{-1} in boxing (52). Other striking disciplines such as taekwondo also demonstrate high exposure, with over one-third of bouts involving at least one head kick and athletes experiencing 2.1 ± 1.3 (males) and 2.8 ± 1.3 (females) head traumas per bout, alongside 35 concussions per 1,000 athlete exposures (53). Grappling sports present lower but still

notable risks, with head trauma causing loss of consciousness accounting for 1.5% of judo injuries (54) and concussions representing 8.3% of wrestling injuries (55).

Importantly, the majority of head trauma occurs in training rather than competition due to the time athletes spend sparring and drilling (44). One-fifth of MMA athletes report training-related concussions (15 – 20.8%) and an average of 3.2 ± 4.9 training-related KOs (56), while concussions account for 7% of wrestling training injuries (55). Despite many impacts being lower in magnitude, athletes may experience hundreds of head traumas per month, reinforcing concerns around the cumulative effects of repetitive non-concussive impacts.

Concussion management in combat sports presents unique challenges because intentional head contact is not incidental to the sport, but a permitted and often strategically relevant method of winning through KO or TKO. The Association of Ringside Physicians consensus statement recommends that if a fighter displays signs or symptoms suggestive of concussion during a bout, including headache, confusion, blurred or double vision, nausea/vomiting or balance/gait disturbance, the bout should be stopped and the athlete evaluated by the ringside physician (57). The guidance also emphasises that concussion management should not focus solely on the losing athlete or the athlete sustaining a KO/TKO, as winners may also demonstrate delayed or evolving symptoms and should therefore be assessed both immediately ringside and later in a quieter controlled environment (57). For return-to-fight decisions, minimum suspensions of 30 days following TKO from head strikes, 60 days following KO without loss of consciousness, and 90 days following KO with loss of consciousness are recommended, with corresponding avoidance of sparring during these periods (57). Athletes should not return to contact activity while symptomatic, and return-to-fight progression should occur through staged phases beginning with general fitness, followed by non-contact combat sport activities, and finally contact sparring only after complete symptom resolution and, where indicated,

specialist medical clearance (57). However, the practical implementation of these recommendations remains difficult, as medical suspensions are often jurisdiction-dependent, baseline neuropsychological, vestibular/ocular and balance testing are not universally required, and suspensions may restrict competition without necessarily governing training or sparring exposure (57). This is particularly relevant to the present thesis because many repetitive head impacts occur during training environments where medical oversight is limited, making education, symptom recognition, athlete self-reporting and objective monitoring strategies especially important.

2.1.4 Methods of monitoring repetitive head impacts in combat sports

Monitoring approaches in repetitive head impacts in combat sports vary, but range from mounted accelerometers, physical modelling and traditional clinical measures e.g., neuroimaging and biomarkers. Instrumented mouthguards (IMGs) equipped with triaxial accelerometers allow for the measurement of linear and angular head accelerations during impacts (58). Linear accelerations, typically resulting from frontal strikes, produce translational brain movement associated with coup and contra-coup injuries, while angular accelerations (common in strikes to the chin or side of the head) induce rotational forces that can strain the brainstem (58,59). These biomechanical measurements enable estimation of forces transmitted to the brain, with concussed MMA athletes experiencing approximately 11.1 ± 4.1 kW of impact power compared to 4.5 ± 1.8 kW in non-concussed athletes (60). Although grappling sports involve more frequent but lower-magnitude impacts, striking sports typically generate higher acceleration events. Despite these advances, no definitive biomechanical threshold exists for predicting concussion or brain trauma.

Neuroimaging has been pivotal in advancing understanding of the consequences of repetitive head impacts, particularly in identifying both acute and chronic structural and functional brain

changes. Athletes with greater exposure to repetitive head trauma demonstrate reduced volumes in key brain regions, including the thalamus, hippocampus, corpus callosum, and cortical areas (61). Additional abnormalities, such as fluid-filled cavities, have been linked to reduced brain volume, slower processing speed, and impaired psychomotor performance (62). White matter connectivity disruptions are commonly observed in fighters with cognitive impairments, with alterations in regions such as the hippocampus, insula, and precuneus correlating with cognitive decline (63). Functional imaging further reveals long-range connectivity deficits in brain networks associated with executive and cognitive processes (64). Encouragingly, cessation of exposure appears to stabilise or partially reverse some of these changes, as retired athletes show improved cognitive performance, reduced biomarker concentrations, and stabilised cortical thickness (65). However, factors such as weight division may influence outcomes; for example, heavyweight MMA athletes are at a threefold greater risk of KO/TKO compared to lightweights (66), with differing patterns of brain volume change observed between divisions (67).

Alongside imaging, biomarkers such as glial fibrillary acidic protein (GFAP), S100 calcium-binding protein B (S100B), and ubiquitin carboxy-terminal hydrolase L1 (UCH-L1) have become increasingly valuable in detecting subtle neural injury and blood–brain barrier (BBB) disruption, particularly where conventional imaging lacks sensitivity. GFAP is a marker of astroglial injury, S100B reflects BBB permeability and astrocytic activation, and UCH-L1 is indicative of neuronal cell body damage, making them complementary indicators of brain injury pathophysiology. These biomarkers have gained clinical relevance, with GFAP and UCH-L1 incorporated into point-of-care diagnostic platforms approved for concussion assessment, aiding rapid decision-making regarding the need for further neuroimaging. In combat sports, elevations in these and related biomarkers have been observed not only following diagnosed concussions but also after non-concussive head impacts, including in

athletes without overt symptoms (32,68,69). Additionally, increased concentrations of inflammatory cytokines and matrix metalloproteinases suggest BBB disruption and neuroinflammatory responses even after ‘mild’ trauma (70). This is supported by findings linking BBB disruption with perivascular p-tau deposition, a hallmark of chronic traumatic encephalopathy (71), indicating that repetitive head impacts (irrespective of clinically diagnosed concussion) may contribute to progressive neurological damage detectable through biomarker analysis.

Complementary neurophysiological and biomechanical monitoring techniques further enhance understanding of the effects of repetitive head impacts. Non-invasive brain stimulation methods, such as transcranial magnetic stimulation, provide insight into corticomotor pathway integrity, with sparring shown to acutely increase corticomotor inhibition and alter motor unit recruitment (9,72). Longer-term alterations, including prolonged cortical silent periods and impaired intracortical inhibition, have also been observed years post-injury, suggesting persistent neurophysiological changes (286). Electroencephalography studies similarly report altered brain-wave activity in combat athletes following concussion, associated with deficits in stress regulation and focus (72-74). From a biomechanical perspective, rotational acceleration has been identified as a key predictor of mTBI risk and is typically higher in striking sports than in other contact sports (59). Emerging approaches combining IMGs with finite element modelling allow for real-time estimation of brain tissue deformation, providing a more detailed understanding of injury mechanisms (75). For instance, concussed MMA athletes exhibit substantially greater strain in the corpus callosum (87.9%) and increased shear stress (111.4%) compared to non-injured athletes (60). Additionally, monitoring studies highlight that sparring often results in a higher frequency of head impacts than competition, with most impacts falling within lower acceleration ranges ($< 50\ g$ and $< 4{,}000\ \text{rad}\cdot\text{s}^{-1}$) (60). Similar patterns are observed in taekwondo and Muay Thai, where impacts are predominantly low magnitude but highly

repetitive (53,76). Collectively, these approaches demonstrate that while individual impacts may be non-concussive, their cumulative effects represent a significant challenge for monitoring and underline the importance of integrating biomechanical, neurophysiological, and clinical assessments.

2.2 Physiological implications of hypo-hydration

Water plays a crucial role in cellular homeostasis, with transient loss of dissolved substances in body fluid leading to alterations in osmolality and, consequently, water distribution across neural and skeletal muscle cell membranes (77). Daily water balance is determined by the net difference between water gain and loss. Causes for loss may be respiratory, gastrointestinal, renal and/or from sweat, while gains due to metabolic water production, food and drink. As a result, daily fluctuations of total body water vary between 5 and 10% via essential fluid loss processes unrelated to exercise (78). Euhydration refers to a normal state of body water whereby fluctuations are corrected via diuresis and thirst response, whilst a state of sustained increase or decrease in body water, are known as hyper-hydration or hypo-hydration respectively (78). This is not necessarily the same as ‘dehydration’ whereby body water is in the process of being lost (79).

Increases in body temperature, incurred due to exercise-induced metabolic heat gain, or high ambient temperatures, triggers a thermo-effector sweating response (80). Typical thermoregulatory sweating, coupled with inadequate fluid intake, can result in hypotonic fluid losses from extracellular fluid in relation to blood plasma, leading to an osmotic gradient, thus facilitating transmembrane flow of fluid from the intracellular fluid space towards the extracellular fluid space (81,82). This process of fluid loss in intracellular fluid (and the hypertonic characteristics of the extracellular fluid) is referred to as hypertonic hypovolemia or intracellular dehydration (83-85) and has likely implications on neuromuscular function.

In addition to autonomic feedback loops regulating bodily fluid balance (86), it is thought that several complex regulatory mechanisms protect neuronal tissue from transient fluid-shifts. However, recent studies employing functional magnetic resonance imaging (fMRI) have demonstrated transient brain anatomical alterations, consistent with fluid loss (20-22) and increased neuronal activation to achieve a similar cognitive output (when euhydrated; 22). Furthermore, hypo-hydration results in a reduction of maximal isometric force (87,88), time to exhaustion during repeated submaximal contractions (15,18,89), and reductions in endurance performance (79,90-93). Interestingly, force decrements are observed despite reported increases in muscle excitability, unchanged corticospinal excitability (87), unchanged voluntary activation (16,94) or increased central activation (15). Though hypo-hydration notably reduces exercise performance via increased cardiovascular strain (95), reduced blood flow, aerobic metabolism (96) and thermoregulatory function (97), the neuromuscular responses to hypo-hydration are less well understood - in part, due to the combined effects of hyperthermia – and speculated to be a result of ionic imbalances (97,98), reduced muscle contractility and increased central fatigue (15). Whilst some work has reported EMG responses to hypo-hydration, the corticospinal, supraspinal, and morphological changes (in the CNS) observed following hypo-hydration have received less attention.

2.2.1 Central and peripheral responses to hypo-hydration

2.2.1.1 Brain and spinal cord-specific responses

Recent research investigating the effects of hydration status on brain and spinal cord tissue have observed transient anatomical alterations in moderately hypo-hydrated humans (17-25). Hypo-hydration is consistent with reductions in spinal cord cross-sectional area (25), brain volume (17-20), and brain ventricular expansion (proportionate to body mass loss; 21-23) indicating *in vivo* fluid losses from brain and spinal cord tissue. Therefore, hypo-hydration

leads to a reduction in brain and spinal cord volume and ventricular expansion, resulting in acute anatomical alterations. In addition, the increase in $PaCO_2$ secondary to hypo-hydration may lead to reductions in cerebral blood volume and flow during exercise (99,100), which in turn, increases oxygen extraction, suggesting a heightened cognitive effort to maintain physiological output (101,102). Indeed Kempton et al. (22) demonstrated hypo-hydration resulted in increased ventricular volume and neuronal activity in the fronto-parietal region (using blood-oxygen-dependent-level functional magnetic resonance imaging [fMRI] signal), during a cognitive task; however, the effect of acute anatomical alterations and reduced cerebral blood flow on neuromuscular function remains unknown. Furthermore, given the poor temporal resolution of MRI for rapid movement (103), it is possible that alternative techniques are required to measure rapid muscle contractions.

2.2.1.2 Muscle contractility-specific responses

As with the brain and spinal cord, morphological alterations are observed in skeletal muscle (reduced cross-sectional area and overall volume) during hypo-hydration (103-107), which could explain a reduction in maximum force production (107,108). Muscle contraction time and HRT reflect the rate of cross-bridge cycling and the re-uptake of Ca^{2+} from the sarcoplasmic reticulum (SR), respectively (109). In rats, 96 h of water deprivation led to increased tetanic tension relative to euhydrated rats, with no change in muscle contraction time and HRT, despite a 10% reduction of the soleus mass, indicating a compensatory pathway to preserve neuromuscular function (106). Additionally, VA is notably unaffected by hypo-hydration (2 – 5% BM; 16,87,94,110), therefore it is unlikely that fluid losses lead to a reduction in spinal motor neuron discharge (i.e., spinal fatigue). Minshull and James (111) reported a ~ 8% reduction in MVC force following 24 h fluid restriction, yet no changes in evoked force, rate

of force development, and electromechanical delay, indicating minimal changes to the ECC process. Interestingly, data are varied in humans, with reports of no changes (16,88,94,112,113) or reductions in peak strength and voluntary force production in response to hypo-hydration (15,87,114-119). Bowtell et al. (87) reported an increase in M_{MAX} during MVCs, yet there was a reduction in muscle torque and increased HRT. This indicates a disruption to the ECC process and efficiency of the release and reuptake of Ca^{2+} from the SR, irrespective of neural drive and a compensatory increase in muscle membrane excitability. A plausible mechanism for why muscle force is reduced, despite increased sarcolemma excitability, has not been proposed. However, this suggests that force production, despite increased neural drive and sarcolemma excitability after hypo-hydration, may be impaired at a contractile level.

2.2.1.3 Distinguishing between specific responses of hyperthermia and hypo-hydration

A methodological limitation of inducing intracellular dehydration is the use of heat stress and exercise, resulting in the possible effects of hypo-hydration being masked or exacerbated by that of hyperthermia and exercise-induced fatigue (117). This section will summarise the independent effects of hyperthermia and hypo-hydration on measures of neuromuscular function.

Cerebral neuronal activity can be ascertained from electroencephalography (EEG), which is notably distinguished from neural imaging techniques, such as MRI, due to superior resolutions in temporal neural networks (120). In clinical practice, cerebral activity obtained from EEG is subdivided into several bandwidths to signify the location of the acquired signal and brain state. Beta waves are predominantly located in the frontal region and represent a state of alertness and focus, whilst alpha waves are associated with relaxation and inhibition (121). Several studies have investigated the effects of hyperthermia with dehydration and exercise (122) and

without dehydration (123,124) on EEG activity, reporting an increased alpha and decreased beta power during prolonged exercise, potentially indicating increased inhibitory activity in pyramidal neurons. This agrees with van den Heuvel et al. (125), who investigated EEG changes after passive hyperthermia with and without dehydration, and found no independent effect of hypo-hydration on resting EEG, suggesting neural alterations to be related to thermoregulatory factors. In addition, Caputa et al (126) reported heightened hypothalamic temperatures (42 – 43°C) led to a reduction in exercise capacity in animals; however, trunk temperatures (below 43.5°C) were unrelated to exercise capacity, indicating a failure of central origin during hyperthermia. These data may partially explain the observations of supraspinal fatigue, after exercise in the heat (127-130). Collectively, passive and exercise-induced hyperthermia results in increased inhibitory brain activity during rest and prolonged exhaustive exercise. However, this is independent of hypo-hydration and might not reflect brain activity during brief and sustained MVCs. Further studies are required to elucidate brain activity during brief and sustained bouts of maximal strength, and to establish if there are differing mechanisms of hypo-hydration and hyperthermia which lead to force decrements.

Studies in which hyperthermia is induced either passively (128,131-133) or actively (93,127,129,134) without hypo-hydration, suggest a significant contribution of spinal and peripheral components to fatigue. Passive or active hyperthermia result in a reduction of MVCs, which is accompanied by reduced VA, H-reflex and M-wave amplitudes implicating altered supraspinal, spinal and peripheral excitatory output, respectively (for review, see 135). Therefore, it is likely that a reduction of VA is attributed to hyperthermia only; as evidenced by Morrison et al. (131), who demonstrated the restoration of VA to baseline values after cooling. In addition, despite a reduction of VA after hyperthermia and hypo-hydration, fluid restoration had no effect on VA (92). This agrees with various hypo-hydration studies (18,87,94) and suggests VA is unaffected by hypo-hydration (2 – 5% BM). Interestingly,

hyperthermia also leads to an increased muscle relaxation rate and decreased muscle HRT (128,129), yet it is reported that a centrally mediated rate of activation is sufficient to overcome the faster relaxation rate (130). Conversely, hypo-hydration leads to an unchanged half-relaxation time (16), muscle relaxation rate or increased half-relaxation time (87). In addition, Bowtell et al. (87) reported an increased M-wave amplitude and reduced corticospinal inhibition (relative to euhydrated participants) during an MVC after hypo-hydration, yet a deficit in muscle torque persisted, indicating an inadequate voluntary drive to activate sarcolemmal APs and the cross-bridge cycle as a potential site of contractile failure. While the reports of reduced maximal strength are varied, it is important to note that this is the result of a mixed body of work examining exercise performance, alongside factors which might mask, or exacerbate, the effects of hypo-hydration (e.g., ambient temperatures and caloric restriction; 117). When accounting for these factors, Judelsen et al. (117) concluded that hypo-hydration caused a 2 and 3% reduction in strength and power, respectively. These findings indicate distinctive mechanisms (related to contraction failure) when intracellular water has not been restored, which may differ from neural and contractile alterations during hyperthermia.

The next section summarises some of the proposed physiological mechanisms that explain the modulation of cortical circuitry and reduction of force after hypo-hydration.

2.2.2 Potential physiological mechanisms

2.2.2.1 Disrupted fibre conduction velocity

A reduction in muscle fibre conduction velocity (MFCV) indicates reduced membrane excitability and is attributed to blood flow reduction (136,137), reduced pH (138) and the simultaneous increase of extracellular K^+ and intracellular Na^+ (139,140). Therefore, reports of MFVC and membrane excitability in humans may vary with the use of a) resting membrane

potential (97), b) EMG spectral parameters or c) M-wave amplitude. Costill et al. (81) calculated the resting muscle membrane potential and reported no change in membrane excitability after dehydration of ~ 6% of body mass; however, these findings were taken from rested muscle. At warm (~ 37°C) muscle temperatures, the opening and closing of voltage-gated Na^+ channels is accelerated, which allows less Na^+ to enter the cell, leading to a more rapid onset depolarisation and faster MFVC (141). Hypo-hydration (independent of heat) is reported to reduce MFCV (15) as indicated by reductions in EMG mean power frequency (142). Conversely, Bowtell et al. (87) reported an increase in sarcolemma excitability in active muscles after hypo-hydration despite a reduction in HRT and peak force production. This was not observed in the euhydrated group and like Costill et al. (81), was not observed during rest, indicating increased MFCV to be an insufficient driver of force production during a MVC after hypo-hydration. Therefore, it is suggested that independent of heat, hypo-hydration may lead to an increased MFCV, yet despite this, muscle contractility is reduced.

A higher MFVC is associated with higher ATP hydrolysis by myofibrillar ATPase at the myosin heads (143). In addition, an increase in AP propagation results in the efflux of extracellular K^+ (98). Therefore, there is an increased demand for ATP hydrolysis for the ECC process, as well facilitating the Na^+ K^+ adenosine triphosphatase (Na^+/K^+/ATPase) pump, to restore ionic balance. The combination of cellular shrinkage, increased need for ATP hydrolysis and extracellular K^+ accumulation may explain a reduction in contractility, through a reduced cross-bridge cycle function and ability to repolarise and hyperpolarise the cell membrane in time to propagate further APs (144). Perhaps, a slower Ca^{2+} reuptake and longer repolarisation times (as indicated through prolonged HRT [87]), is a result of reduced capacity or slower activation of Na^+/K^+/ATPase pumps to defend intracellular water volume (Figure 2.3A & B). In summary, maintaining adequate force production after hypo-hydration, may rely on higher ATP hydrolysis, which may be limited as a result of protecting intracellular water.

2.2.2.2 Impaired Ca^{2+} reuptake and excitation-contraction-coupling

Since the lengthening of muscle relaxation time is related to reduced Ca^{2+} re-uptake (145), it is of interest that the production of reactive oxygen species (ROS) inhibits SERCA pump activity, subsequently reducing Ca^{2+} reuptake into the SR (146). Indeed, hyperthermia and hypo-hydration are reported to increase ROS production via various mechanisms, such as increased blood viscosity and endothelial shear stress (147-151). In addition, sweat losses, fluid shifts and increased blood osmolality leads to a change haemoconcentration and viscosity (152), resulting in shear stress along the vascular walls and the subsequent release of nitric oxide (153) and ROS (154). Irrespective of an increase in peripheral and corticospinal excitability, it is hypothesised that the muscle contractile units are unable to utilise the neural drive, owing to reduced intra-cellular Ca^{2+} reuptake (Figure 2.3C). This would result in decreased force production and increased muscle HRT (87) or accelerated fatigue during repeated contractions (15).

An alternative hypothesis related to reduced Ca^{2+} handling consists of specialised water channels in skeletal muscle (aquaporins). Aquaporin-4 (AQP4) is a crucial water channel of the neuromuscular system, particularly found in the sarcolemma of fast twitch fibres and determines muscle permeability (155,156). Farhat et al. (106) observed more than a 50% decline in AQP4 expression in rodent fast-twitch fibres after 96 h water deprivation. In animals, the absence of AQP4 channels in muscle fibres has been reported to alter protein expression related to Ca^{2+} handing, buffering and glycolytic metabolism (157), resulting in impaired voluntary exercise (158). In addition, Gulati and Babu (159) observed a reduction in maximal isometric force after exposing frog muscle fibres to a hypertonic solution; this was associated with reduced fibre width and altered lattice spacing of thick and thin filaments in the sarcolemma. Lattice spacing is a crucial regulator of force generation via the muscle length-

tension relationship (160). It is hypothesised that AQP4 may determine muscle-specific responsiveness to hyperosmolality, thus reducing cross-sectional area and altering lattice spacing in fast-twitch muscle fibres, subsequently reducing muscle force (168; Figure 2.3D). However, further research is required on human muscle fibres to determine the effects of *in vivo* hypo-hydration.

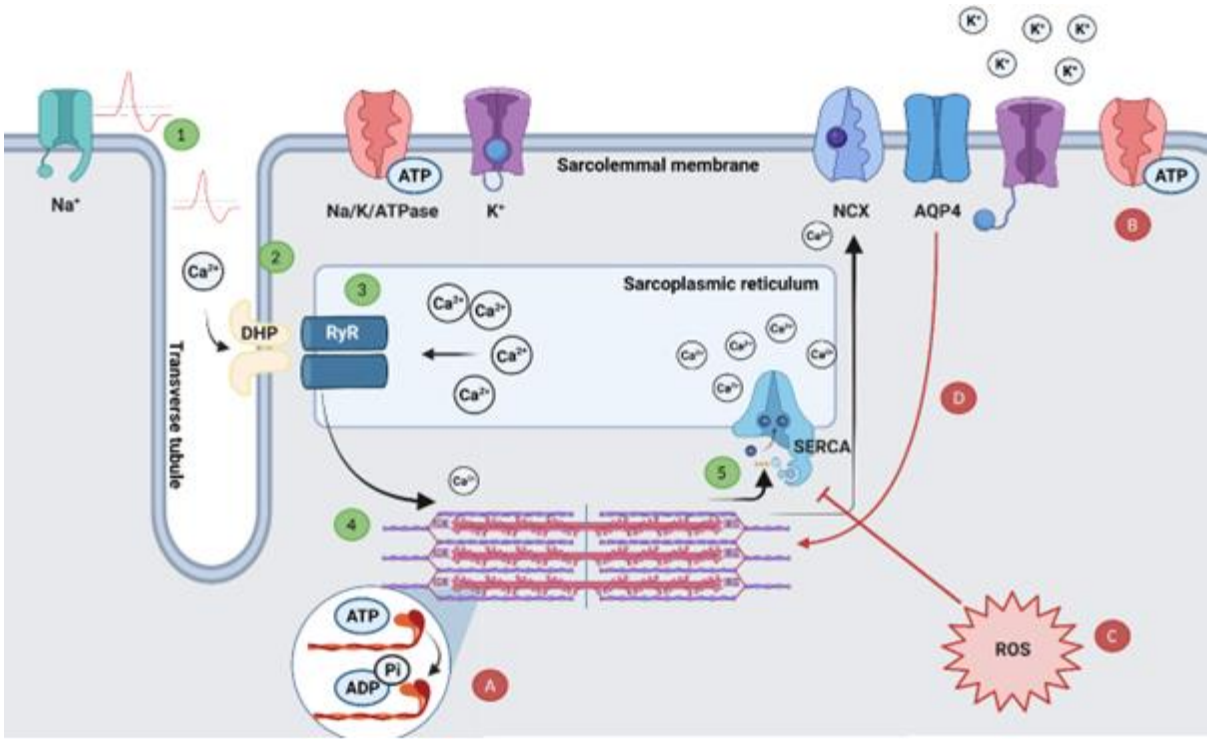

Figure 2.3: Typical release and re-uptake of Ca^{2+} in the sarcolemma (1-5), and proposed mechanisms of impaired contractility (A-D). 1) Action potential propagates down the transverse tubule. 2) DHP/LTCC senses membrane depolarization and activates RyR on SR. 3) RyR briefly opens to release a pulse of Ca^{2+}. 4) Ca^{2+} bonds to troponin, activating cross-bridge cycle. 5) During relaxation, SERCA pump remove Ca^{2+} from the myofilaments to restore SR Ca^{2+} levels, some may enter into mitochondria or be removed by NCX. A) & B) Combination of cellular shrinkage, increased need for ATP hydrolysis at myosin heads and Na^{+}/K^{+}/ATPase pump, and extracellular K^{+} accumulation might reduce contractility, through impaired cross-bridge cycle function and ability to repolarise and hyperpolarise the cell membrane in time to propagate further action potentials. C) The increase in ROS from increased blood viscosity and shear stress inhibits SERCA activity, thus reducing Ca^{2+} reuptake into the SR (146,153,154). D) A reduction in AQP4 channels may alter lattice spacing of myofilaments and alter protein expression related to Ca^{2+} reuptake (106,158). Abbreviations: ADP, adenosine di-phosphate; AQP4, aquaporin 4; ATP, adenosine triphosphate; DHP/LTCC, dihydropyridine/L-type calcium channel; NCX, Na^{+}/ Ca^{2+} exchanger; RyR, ryanodine receptor; SR, sarcoplasmic reticulum; SERCA, sarcoplasmic/endoplasmic reticulum calcium ATPase; ROS, reactive oxygen species.

2.2.2.3 Altered neural drive and contraction-specific fatigue

It is also possible that the effect of hypo-hydration on skeletal muscle is dependent on contraction and/or fibre type, which further rely on glycogen breakdown or a sustained Ca^{2+} re-uptake in the SR (106). Hypo-hydration has not been shown to reduce muscle strength, nor alter phosphocreatine recovery or H^{+} concentration (88); though, it could feasibly increase phosphocreatine and muscle glycogen utilisation (88,161). However, reductions are notably observed during the performance of repeated, strength-endurance protocols (17,87,88) and high-intensity endurance performance (117). Approximately 2.7 g of water are bound to 1 g of glycogen (162); therefore, muscle contractions relying on glycogenolysis will facilitate the movement of water molecules from the intra to extracellular space (163). A reduction in AQP4 channels could present a challenge for muscle fibres that rely on rapid and efficient water and Ca^{2+} turnover (see Section 2.1.3.2 & Figure 2.1), thus reducing force output and time to fatigue during repeated contractions. In addition, hypo-hydration may influence specific contraction types, potentially indicating distinct locations and mechanisms of failure. Lawrence and Hayes (118) investigated the dose response of hypo-hydration on muscle performance and reported a reduction in isometric force after one exposure (1% BMR), yet isokinetic force was either unchanged or reduced after three exposures or more. It was suggested that concentric contractions at a high velocity may not be as susceptible to hypo-hydration-induced decrements as slow isokinetic or isometric contractions (118). Similarly, Bowtell et al. (87) reported the reduction of peak isometric and eccentric, but not concentric torque (87). Since eccentric and isometric contractions are less reliant on motor unit activation and energy expenditure (164-166), this indicates performance decrements during brief eccentric contractions to be a result of contractile failure, as opposed to a reduction in central drive or substrate depletion. Therefore, it is likely that hypo-hydration modulates force production according to the type of

contraction; a supposition further supported by the selective responsiveness in fast-twitch muscle fibres and alterations to contractile elements.

The vast majority of studies investigating neuromuscular function utilise isometric contractions, therefore it is important to note that isometric exercise involves the occlusion of blood flow to active muscle, depending on the intensity of contraction (167,168). The metabolic and resultant ischemic environment increases local muscle temperature and stimulates chemo- and mechanoreceptor activity (169,170), resulting in afferent stimulation of sympathetic nervous activity (171). The combination is thought to depress motor unit firing rates (172,173), thereby modifying the relationship between central neural drive and motor unit recruitment (174,175). Motor unit discharge rates are proportionate to the synaptic input they receive (176), but in addition to ionotropic input, rate coding may be influenced by neuromodulatory input (e.g., noradrenaline) to the motor neuron pool via persistent inward currents (175-177). However, noradrenaline has not been associated with changes in sarcolemma excitability nor motor neuron discharge activity (178-183). Therefore, an alternative theory related to the reduction in force despite reduced cortical inhibition and unaltered corticospinal excitability after hypo-hydration (87), is attributed to the increase in sympathetic nerve activity (in order to preserve vasomotor function; 184). In summary, sympathetic nerve activity could result in altered neural drive (i.e., reduced cortical inhibition or unaltered corticospinal excitability) as observed after hypo-hydration (87), yet has no effect on muscle function.

During an MVC, the reported effects of hypo-hydration are extremely varied; however, when analysing the specific role of the CNS & PNS, some have reported a reduced cSP duration, increased sarcolemma excitability (87), unchanged (18,94) or increased central motor drive (15), and higher mean power frequency (185) relative to euhydrated controls. Despite this,

force reductions continue to persist. Interestingly, Periard et al. (94) and Barley et al. (16) reported a decline in force production during repeated MVCs, not associated with VA, indicating a loss of force to be unrelated to voluntary central drive and more likely to be a result of alterations to the peripheral musculature. Therefore, an alternative view of heat-induced hypo-hydration is proposed as: a) central drive may be enhanced via reduced cortical inhibition or increased cortical facilitation, in an attempt to compensate for potential force decrements when hypo-hydrated but, b) this may not be sufficient, particularly during sustained and repeated voluntary contractions where contractile function is impaired (128). This may explain why heat-induced hypo-hydration is notably reported to have 'no effect' on brief measures of power and strength (88,104,112,113,186-190) but consistently impairs performance during repeated or sustained contractions (15,94,104,105,107,109,190-192). Further studies are required to elucidate the facilitatory and inhibitory responses (in the corticospinal pathway) to hypo-hydration.

2.2.2.4 Supraspinal fatigue, increased perception of effort and activation of pain-related networks

Supraspinal fatigue is defined as loss of force caused by suboptimal output from the primary motor cortex (193). Hypo-hydration also notably increases perceptions of fatigue, tension, and anxiety (194-197). Conscious signals originating from both central and peripheral afferent pathways could mediate behaviour and reduce motivation to minimise discomfort (198). Heat-induced hypo-hydration resulting in a 4% BMR resulted in no change of muscle strength, despite a 15 % reduction in time to fatigue. Interestingly, hypo-hydration did not exacerbate muscle pH, H^+ and inorganic phosphate accumulation during the fatiguing task, thus it was proposed that hypo-hydration may result in an inability or unwillingness to sustain force production, despite adequate muscle strength (131). Furthermore, the negative psychological associations attributable to thirst may act as a signalling mechanism to promote a greater

conscious perception of effort thus, invoking a behavioural change to reduce physical effort (199). Alternatively, force may be maintained but only at the expenditure of higher metabolic cost, as seen in increased blood-oxygen-dependant-level activation (using fMRI) of the fronto-parietal brain region during a cognitive task (22). Therefore, it is suggested that hypo-hydration may negatively affect motivation and increase effort perception, resulting in reduced central motor drive during exercise.

Hypo-hydration has also been shown to enhance activation of pain-related brain networks (200) and increase pain perception (201-203). The cold pressor test is commonly used to assess autonomic outflow to the extremities (204) and involves the immersion of a limb in cold water, thus inducing high levels of pain (205,206). Perry et al. (202) reported a modified cerebrovascular response to the cold pressor test in hypo-hydrated subjects due to increased pain perception. Furthermore, Ogino and colleagues (200) observed the effects of a 12 h fasting and 40 min exercise protocol, resulting in increased activation of the anterior cingulate cortex, insula, and thalamus, alongside increased thirst, and reduced pain threshold during the cold pressor test. Interestingly, Farrell and colleagues (207) found similar brain areas were activated after inducing pain and thirst via noxious pressure and infused hypertonic saline respectively, but activation of the pregenual cingulate and orbitofrontal cortices occurred in the combined presence of thirst and pain, suggesting an integrative role of thirst and pain sensation. Minor discomfort is also sensed at the onset of a contraction, developing into severe discomfort and pain over time that alters the perception of sensations in the contracting musculature (208). Experimentally induced pain (EIP) via intramuscular injections of hypertonic saline, is proposed to invoke similar nociceptive pathways of exercise-induced pain (209,210). Current evidence suggests EIPs to reduce muscle strength (211-213) and submaximal force steadiness (214,215) indicating increased nociceptive activity to be a cause of force decrements. Interestingly, Graven-Nielsen and colleagues (216) demonstrated that EIP reduced maximal

voluntary torque, despite an unaffected twitch torque, implying that performance decrements were due to mechanisms residing in the CNS rather than the peripheral musculature (216). Indeed, EIP is shown to modify corticospinal and intracortical excitability (217,218), emphasising the strong relationship between the nociceptive and motor systems; however, the relationship with hypo-hydration is yet to be explored. A summary of all the proposed mechanisms can be found in Figure 2.4.

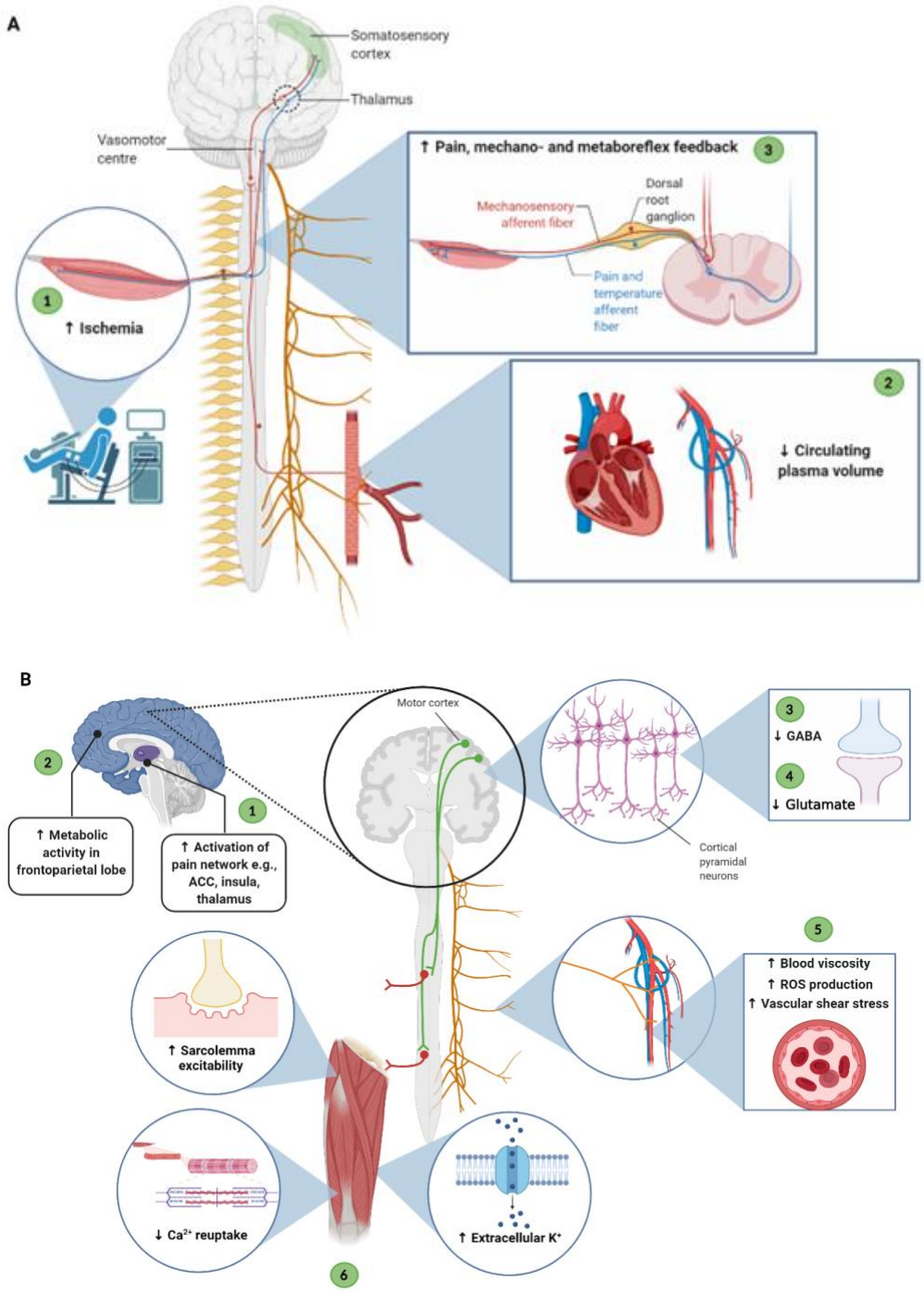

Figure 2.4: Summary of proposed afferent (A) and efferent (B) responses to heat-induced hypo-hydration during an MVC. A) afferent responses: 1. Ischemia as a result of increased/prolonged contractions. 2. Reduced plasma volume due to water losses trigger a vasomotor response. 3. Upon an MVC, there is an increase in pain, mechano- and metaboreflex feedback sent to the thalamus and somatosensory cortex to alter behaviour and central motor drive. B) efferent responses: 1. Increased activation of pain network due to reduced pain threshold (200). 2. Increased metabolic activity in other brain regions (e.g., frontoparietal lobe) due to increased effort perception (22). 3. Reduced GABA to compensate for force losses in contractile units (87). 4. Reduced glutamate and central motor drive in conscious reduction of effort (loss in motivation or increased pain) (219). 5. Volume changes result in increased blood

viscosity, vascular shear stress and ROS production (147-154). 6. Impaired contractile function (contraction-dependent) due to increased need for ATP hydrolysis and reduced Ca^{2+} reuptake in SR (see Figure 2.3). Abbreviations: GABA, γ-aminobutyric acid; ROS, reactive oxygen species.

2.2.3 Hypo-hydration in combat sports

Hypo-hydration is highly prevalent in combat sports due to the competitive drive to qualify at lower weight classes (220). Surveys indicate that 60 – 90% of fighters engage in rapid weight loss (RWL) before competitions, primarily through fluid restriction and sweating tactics (221). While most athletes reduce 2 – 5% of body mass, many lose 5 – 10% (222) and reductions exceeding 10% are not uncommon (223). In a recent case study by Kasper et al (11), it was reported that one professional MMA athlete reduced 18% of their initial body mass prior to competition, equating to a loss of 14.5 kg in under 8 weeks. These practices can carry acute health risks, as evidenced by reports of young combatants suffering dehydration-induced kidney failure, cardiac arrest and death while attempting drastic weight cuts (224-226). Despite these dangers, weight-cutting remains deeply ingrained in combat sport culture as a perceived competitive necessity (227).

It is also important to recognise that RWL may not reflect dehydration in isolation. In practice, combat sport athletes often combine fluid restriction and sweating strategies with reduced energy intake, carbohydrate restriction, fasting, low-residue diets and increased exercise volume (10,221,228). These strategies may induce a transient shift towards greater fat oxidation and ketone body availability, particularly β-hydroxybutyrate, depending on the severity and duration of carbohydrate and energy restriction. This may be relevant to brain health because β-hydroxybutyrate has been implicated in neuroprotective signalling pathways, including modulation of oxidative stress, inflammation, mitochondrial metabolism and brain-derived neurotrophic factor (BDNF) expression (229-232). In experimental models of brain

injury, ketogenic diets, fasting or exogenous ketone provision have been associated with improved neurological outcomes, although most evidence remains preclinical and cannot be directly extrapolated to acutely dehydrated combat sport athletes (231,233,234). Therefore, while acute RWL is commonly discussed in terms of physiological risk, it may also involve competing metabolic adaptations that could theoretically modulate neural vulnerability. Importantly, the extent to which combat athletes enter a meaningful ketotic state during typical weight-making practices, and whether this alters responses to repetitive head impacts, remains unknown.

2.2.4 Monitoring hypo-hydration in combat sports

Monitoring hydration status in athletes remains a complex and contested area of research, with no single gold standard method universally accepted for assessing whole-body hydration (78,235,236). This complexity arises from the dynamic nature of body water distribution across intracellular and extracellular compartments, as well as the wide range of available assessment techniques that vary in validity, reliability, and practicality (78,235). In combat sports, these challenges are further exacerbated by the rapid and often extreme weight-cutting practices employed prior to competition, which can involve substantial acute reductions in body mass through dehydration (10,221,228).

Urinary indices, particularly urine specific gravity (USG) and urine osmolality, are among the most commonly used tools in both research and applied settings due to their relative ease of collection and low cost (77,236,237). However, these measures reflect renal responses to fluid balance rather than real-time hydration status and are subject to numerous confounding factors, including recent fluid intake, dietary composition, and timing of measurement (77,236,237). This limitation is particularly relevant in combat sports, where rapid rehydration following

weigh-in may obscure true hydration status, reducing the utility of urinary measures in isolation (237,238).

Changes in body mass are also frequently used as a gross indicator of hydration status, based on the assumption that acute fluctuations in mass primarily reflect changes in total body water. Under controlled conditions, body mass changes can provide a useful estimate of fluid loss over short time periods; however, this method is influenced by factors such as food intake, substrate utilisation, respiratory water loss, and gastrointestinal contents (77,78,236). In combat sports, where athletes may engage in strategies such as water loading, sauna use, and dietary manipulation, these confounders are amplified, limiting the precision of body mass as a standalone measure (16,228,238).

Recent work examining hydration testing specifically in combat sport contexts has further highlighted the limitations of commonly used biomarkers. Zubac et al. (237) noted that although USG is commonly used to detect fluid deficits in combat sport athletes, its interpretation requires caution due to methodological and physiological limitations. Similarly, a systematic review and meta-analysis in Olympic combat sport athletes questioned the utility of urinary hydration indices in both laboratory and field settings (238). More recently, Doherty et al. (239) examined body mass loss and changes in serum osmolality, urine osmolality, tear osmolarity, haematocrit and USG following passive dehydration in combat sport athletes, reporting substantial variability in biomarker responses and poor-to-moderate reliability for several commonly used measures. These findings suggest that commonly employed hydration markers may lack the sensitivity and specificity required for accurate classification of hydration status, particularly in regulatory settings where decisions regarding competition eligibility may be made.

Given these limitations, current recommendations emphasise the use of multiple complementary measures to improve the accuracy and validity of hydration assessment (77,78,235). Combining markers from different physiological compartments, such as body mass change, urinary indices and, where feasible, blood-based measures such as plasma or serum osmolality, may provide a more comprehensive assessment of hydration status than any single measure alone (78,235,236). However, in applied combat sport settings, the feasibility, cost, invasiveness and timing of these measures must also be considered, particularly when hydration testing is used around weigh-in, training or competition.

Given the possible overlap of the occurrences of hypo-hydration and repeated head impacts in combat sports, there is a need for precise, non-invasive tools that can detect subtle changes in cortical and neuromuscular function under real-world training conditions. To address this, the following section outlines the use of TMS and MNS for assessing corticospinal and peripheral function after hypo-hydration and repeated head impacts across the thesis.

2.3 Tools for investigating corticospinal and neuromuscular function

2.3.1 Transcranial magnetic stimulation

Transcranial magnetic stimulation is a non-invasive technique relying on electromagnetic induction, whereby a high-current pulse through a scalp-held coil generates a magnetic field that penetrates the skull and induces an electric field in the underlying cortex (Figure 2.5; 240). If sufficiently strong and correctly oriented, this electric field alters ion flow, depolarising neurons and triggering action potentials. The depth and precision of stimulation depend on coil shape; figure-of-eight coils provide more focal stimulation compared to circular coils, with postero-anterior-induced currents optimally evoking motor evoked potentials (MEPs) due to efficient trans-synaptic indirect (I) wave recruitment (Figure 2.5; 241-243). These descending

volleys comprise direct (D) waves and I-waves, with the latter originating from cortical layer II/III projections to layer V pyramidal neurons (244,245). TMS thus preferentially activates axons or interneurons, initiating cascades of cortical and corticospinal excitation that result in muscle activation, measurable as an MEP (Figure 2.6; 240).

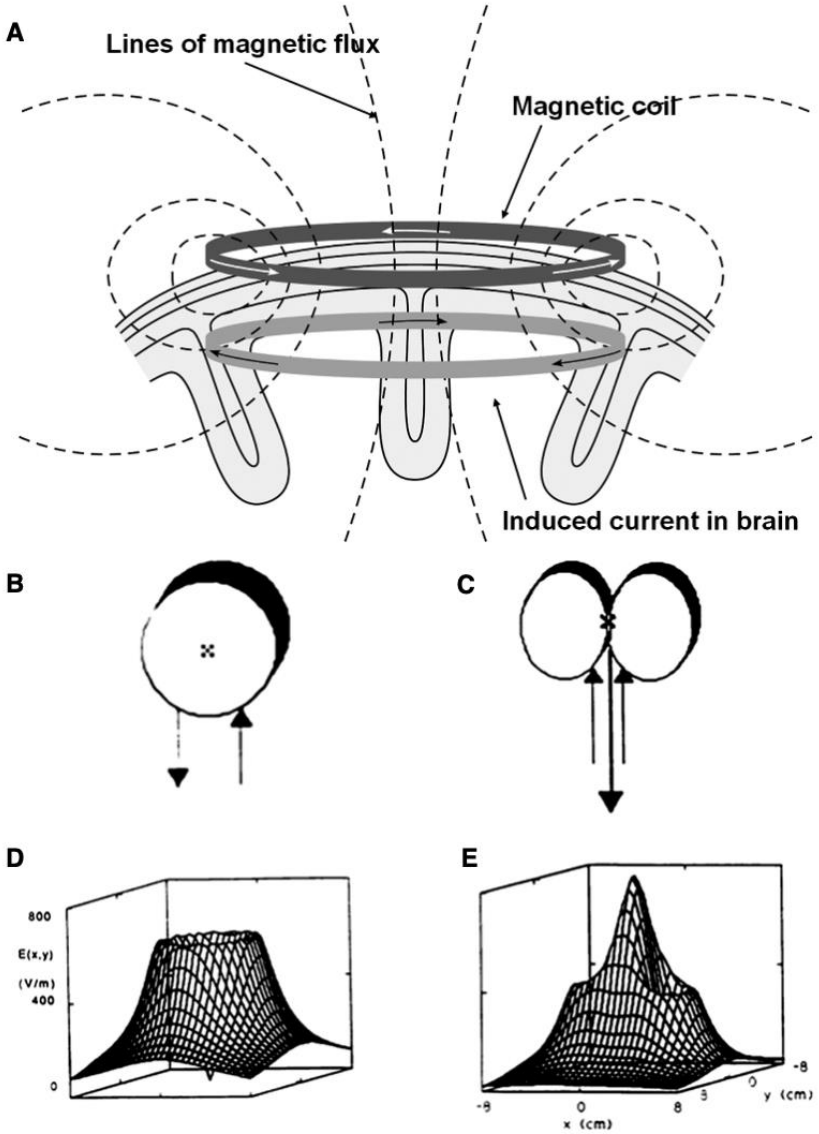

Figure 2.5: A. For magnetic stimulation, a brief, high-current pulse is produced in a coil of wire, called the magnetic coil. A magnetic field is produced with lines of flux passing perpendicularly to the plane of the coil, which ordinarily is placed tangential to the scalp. B-E. Magnetic Coil Shape Determines the Pattern of the Electric Field. (Adapted from Hallet [246]).

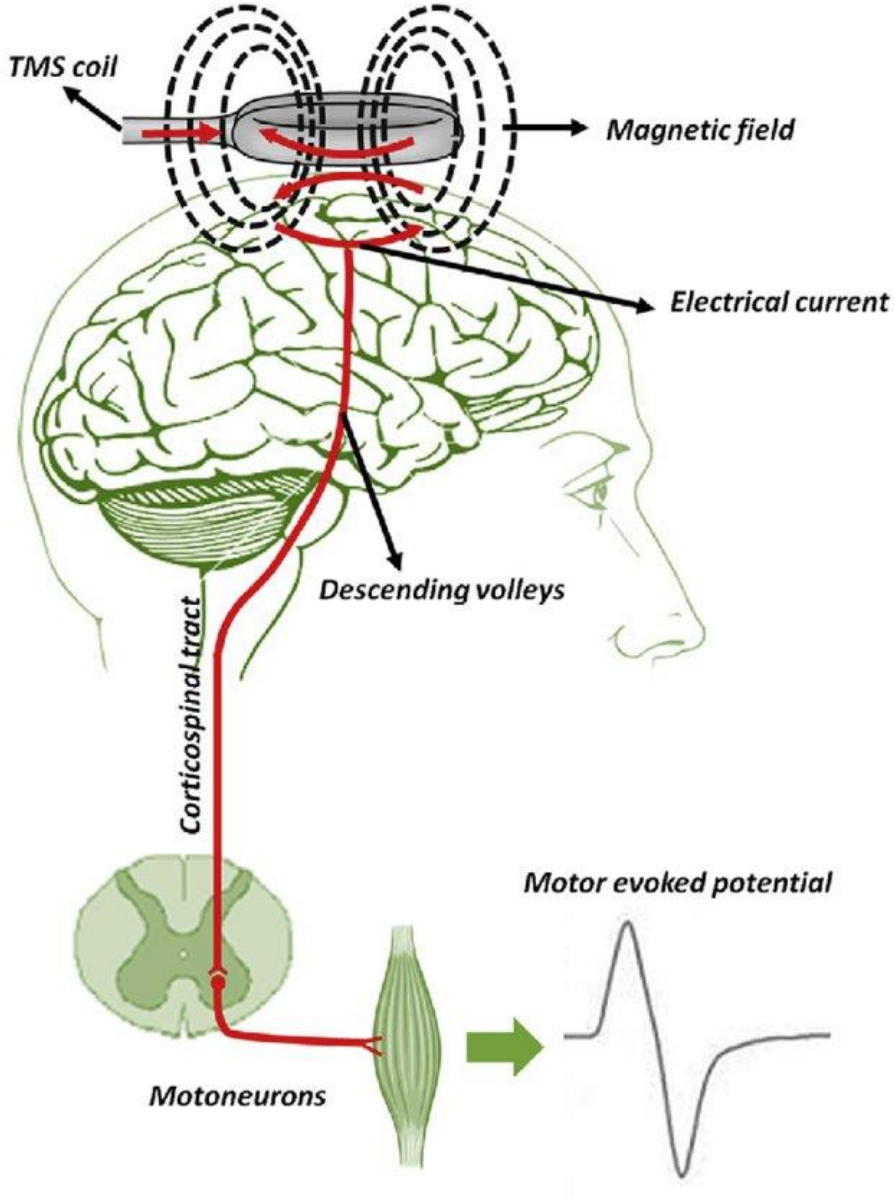

Figure 2.6: Schematic representation of transcranial magnetic stimulation (TMS; 247).

2.3.1.1 Single pulse vs. paired pulse stimulation

TMS can be delivered as single or paired pulses to assess different aspects of motor cortex excitability. Single-pulse TMS, typically used to evoke MEPs, maps corticospinal excitability, motor thresholds, and conduction times (240). Paired-pulse TMS involves two closely spaced stimuli to the same motor region and is used to probe intracortical circuits. Early work showed that applying a second stimulus to distant or ipsilateral brain regions could modulate MEP

amplitude (248,249), with Kujirai et al. (250) later demonstrating that intracortical circuits within the same hemisphere could produce long/short interval intracortical inhibition (LICI/SICI) or intracortical facilitation (ICF), depending on the interstimulus interval. SICI occurs when a subthreshold pulse suppresses the MEP from a subsequent suprathreshold pulse (1–5 ms interval), reflecting GABAergic inhibition, while ICF appears at longer intervals (8–15 ms), indicating excitatory interneuronal facilitation (240,251). These protocols help differentiate the balance of inhibition and excitation in the primary motor cortex.

2.3.1.2 Active motor threshold

Motor threshold, a core TMS parameter, represents the minimum stimulus intensity needed to evoke a motor response, thereby reflecting primary motor cortex excitability (252). Two primary types are measured: the Resting Motor Threshold (RMT), defined as the lowest intensity eliciting a ≥50 μV MEP in at least 5 of 10 trials when the target muscle is relaxed, and the Active Motor Threshold (AMT), assessed during a light voluntary contraction (~ 10 – 20% of Maximum voluntary contraction [MVC]) and typically requiring a ≥ 200 μV MEP in 5 of 10 trials (253). AMT is generally lower than RMT because volitional drive pre-depolarises motor neurons, reducing the extra input required to reach threshold. The RMT is sensitive to changes in membrane excitability and synaptic efficacy, while AMT reflects facilitation during active states (254). The difference between these thresholds offers insight into cortical responsiveness, and AMT is frequently used to calibrate inhibitory protocols like SICI, which were originally developed using active muscle states (250).

2.3.1.3 Motor evoked potentials

MEPs are muscle responses elicited by stimulation of the primary motor cortex via TMS, recorded using surface EMG as a burst of activity characterized by its amplitude (in mV) and

latency (in ms; 255). They reflect activation of the corticospinal tract, resulting in involuntary muscle contraction. MEP amplitude is a widely used index of corticospinal excitability where larger amplitudes (at a given stimulus intensity) indicate greater responsiveness of cortical and spinal neurons (Figure 2.7; 256). Factors influencing MEPs include stimulus intensity, muscle pre-contraction, and modulations in cortical or spinal excitability (257). While MEPs encompass the entire motor pathway, under controlled conditions they are primarily interpreted as markers of motor cortical excitability (240), especially when spinal contributions are accounted for via complementary measures like the H-reflex or F-wave (258).

2.3.1.4 Corticospinal silent period

The corticospinal silent period (cSP) refers to the transient suppression of EMG activity following a TMS-evoked MEP during active muscle contraction and is primarily a marker of cortical inhibition (Figure 2.7; 240). Its duration, measured from MEP onset to electromyography (EMG) resumption, includes early spinal contributions (~ 50 – 60 ms) but is predominantly cortical beyond ~ 100 ms (259). Pharmacological evidence, such as baclofen-induced prolongation of cSP, supports the role of $GABA_B$-mediated inhibition in its later phase (260). Thus, cSP duration is commonly used as an index of transient $GABA_B$-related cortical inhibition, with shorter durations indicating reduced inhibitory function, as seen in disorders like dystonia (261).

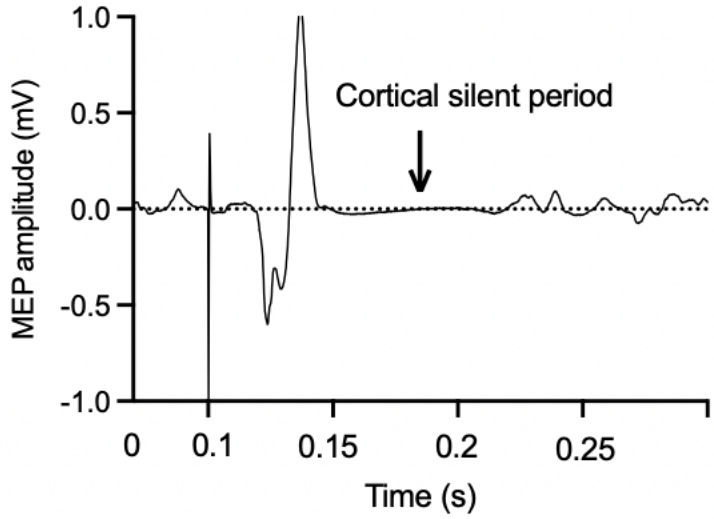

Figure 2.7: An example trace of a motor evoked potential (MEP) and the cortical silent period (cSP) derived from surface EMG recordings of the rectus femoris.

2.3.1.5 Short interval intracortical inhibition

SICI is a TMS paradigm that assesses the activity of inhibitory interneurons in the primary motor cortex, particularly those mediated by $GABA_A$ receptors. As introduced earlier, SICI is measured using a paired-pulse TMS protocol: a subthreshold conditioning stimulus is delivered shortly (1 – 5 ms) before a suprathreshold test stimulus. The effect is that the MEP from the test stimulus is suppressed compared to its unconditioned size (240). Kujirai et al. (250) first characterised this phenomenon, showing that a 3 ms interstimulus interval drastically reduces the MEP amplitude, indicative of strong intracortical inhibition triggered by the first pulse (Figure 2.8).

It is postulated that the mechanism of suppressed MEPs following a sub-threshold conditioning pulse is not due to refractoriness of the corticospinal tract (axonal conduction; 244), but due to a trans-synaptic GABA mediated inhibition, as observed by a suppression in late I waves, but not early I1 waves (262). This is further supported by pharmacological studies which have shown enhancement of SICI with benzodiazepines which exert their effects through $GABA_A$

receptors (263), therefore, SICI is widely used as a biomarker of $GABA_A$ mediated inhibition (264).

SICI is distinct from the silent period though both reflect inhibition. SICI is a very short-lasting inhibition affecting an upcoming test pulse, mediated by $GABA_A$ fast synaptic action (240). The cSP mainly reflects $GABA_B$ (longer, slower inhibitory signals). Thus, the combination of measures may provide insight into which inhibitory system and neurons are affected.

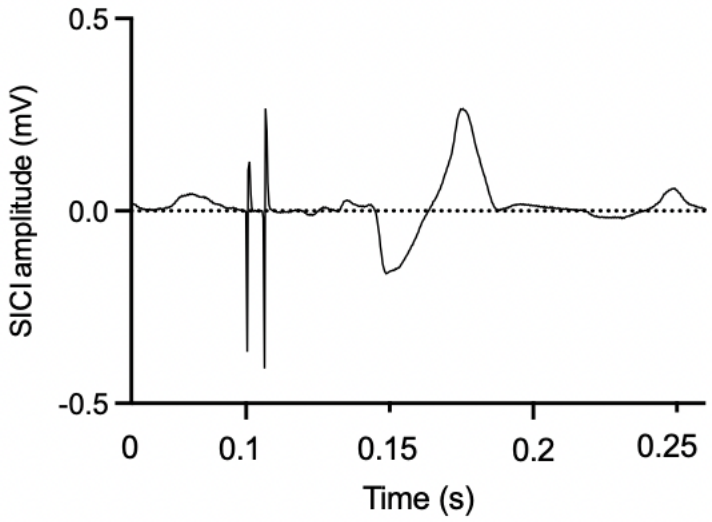

Figure 2.8: An example trace of Short Interval Intracortical Inhibition (SICI) elicited by paired-pulse stimulation of the rectus femoris at 80 and 130% of AMT.

2.3.2 Motor nerve stimulation

A brief square-wave electrical pulse (typically 0.1 – 1 ms in duration) delivered via surface or needle electrodes can depolarise peripheral motor nerve axons by bringing their membranes to threshold, thus initiating an action potential (AP; 265). The induced AP propagates distally along the motor nerve into the muscle fibres, triggering contraction; a single stimulus evokes an isolated muscle twitch, whereas a train of stimuli at sufficient frequency produces summation of twitches and can induce a sustained contraction (tetanus; 266). The muscle's

electrical response to nerve stimulation is recorded as a compound muscle action potential or M-wave, which represents the summated APs of all the activated muscle fibres. The amplitude and area of the M-wave thus depend on the number and size of recruited motor units (265), and this provides an electrophysiological index of motor unit recruitment. Notably, the recruitment order with electrical stimulation differs from voluntary activation: under normal physiological conditions (Henneman's size principle; 266), small, low-threshold motor units are recruited first and larger units last (267), whereas an externally applied stimulus preferentially activates large-diameter motor fibres first because their lower resistance and higher membrane excitability yield a lower activation threshold (268). As the stimulus intensity is increased, progressively more motor axons are excited, recruiting additional motor units and enlarging the M-wave, until a point of maximal recruitment is reached. Beyond this "supramaximal" stimulus level i.e., where all motor units in the nerve are activated, further increases in current produce no further increase in muscle response (265).

2.3.2.1 M-wave

The M-wave, or compound muscle action potential is the summated electrical response of muscle fibres following direct motor nerve stimulation, reflecting near-synchronous activation of motor units (269). As stimulus intensity increases, more motor units are recruited until a maximal response (M_{MAX}) is reached, indicating full peripheral activation (270,271). The M_{MAX} serves as a physiological benchmark for neuromuscular excitability and is frequently used to normalise evoked responses. Under stable conditions, M_{MAX} should remain consistent; however, reductions (e.g., during fatigue) may signal diminished muscle membrane excitability (Figure 2.9; 272).

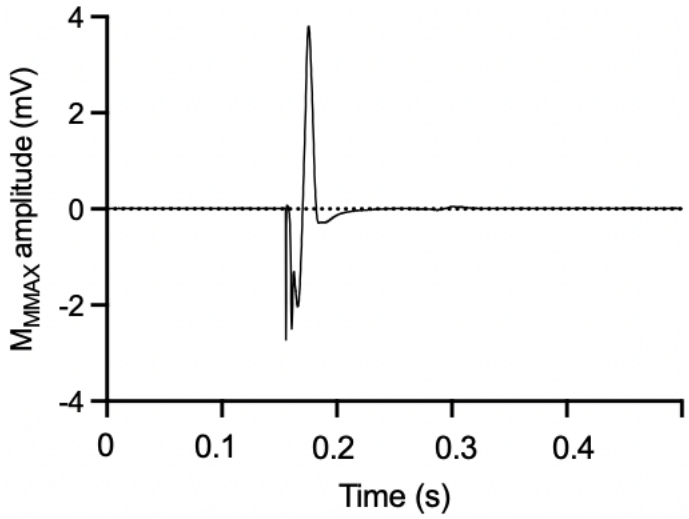

Figure 2.9: An example trace of the maximal M-wave amplitude of the rectus femoris, known as the maximum compound muscle action potential (M_{MAX}).

2.3.2.2 Voluntary activation and twitch parameters

Voluntary activation (VA) refers to the proportion of a muscle's maximal force-generating capacity that is achieved through central neural drive during a voluntary contraction. It is commonly assessed using the interpolated twitch technique, first described by Merton (273), whereby a supramaximal electrical stimulus is applied to a motor nerve during an MVC. If no additional force is produced, the muscle is considered fully activated; however, if a superimposed twitch occurs (Figure 2.10), it indicates incomplete central drive. The size of this twitch, relative to a resting twitch, provides a quantifiable index of VA. A reduction in VA following fatiguing contractions reflects central fatigue, as shown by Todd, Taylor & Gandevia (274), who demonstrated that both TMS and peripheral nerve stimulation revealed impaired VA contributing to force loss. TMS-based approaches allow assessment of supraspinal contributions to fatigue by stimulating the primary motor cortex directly; a superimposed twitch under this condition suggests a failure in cortical drive.

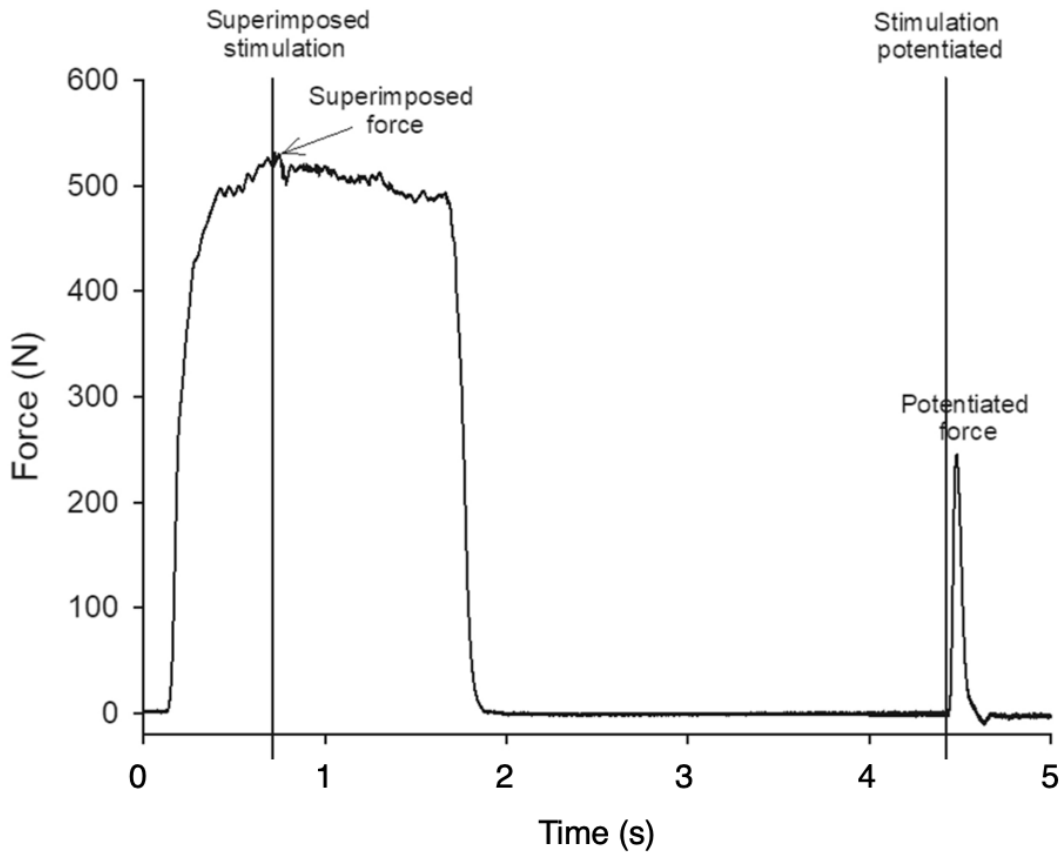

Figure 2.10: Visual representation of superimposed force representing submaximal voluntary activation, and potentiated force representing maximal contractile capacity (Adapted from Ce et al [275]).

A resting twitch represents the mechanical response of a relaxed muscle to electrical stimulation and provides a measure of peripheral contractile function. Following prior activation, this twitch becomes potentiated due to post-activation potentiation, where twitch force is enhanced through molecular changes such as phosphorylation of myosin regulatory light chains, increasing calcium sensitivity (276,277). The potentiated twitch is often used as a reference for the muscle's maximal contractile capacity. Another important twitch-derived measure is half-relaxation time (HRT), defined as the time for twitch force to decline from peak to 50% during the relaxation phase (Figure 2.11). HRT reflects calcium reuptake dynamics via sarco/endoplasmic reticulum ATPase (SERCA) pumps and cross-bridge detachment kinetics. Fatigue can impair these processes due to metabolic by-products such as

Pi, ADP, and H^+, leading to prolonged HRT, resulting in an increase from ~ 50 ms to 80 ms post-exercise (274). This slowing of relaxation is a sensitive indicator of peripheral fatigue and altered Ca^{2+} handling.

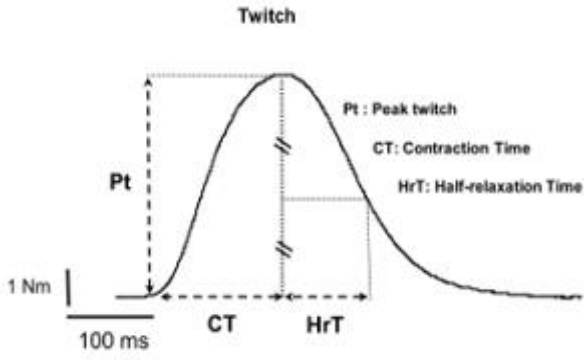

Figure 2.11: Muscle half relaxation time calculated from the potentiated twitch force (Adapted from Rozand et al [278]).

2.4 Gaps in the literature and the rationale for the present work

2.4.1 Monitoring hypo-hydration and repetitive head impacts with TMS

Brain activation (in the context of skeletal muscle function) has been investigated by measures of corticospinal excitability utilising TMS; however, little is known of corticomotor activity (elicited through TMS) after hypo-hydration. To date, only one study (87) investigated the effects of exercise-induced hypo-hydration and euhydration (after exercise in the heat) on corticomotor output. No changes were observed in VA (elicited by TMS), cortical excitability and cSP among hypo-hydrated subjects despite a reduction in force; however, the cSP was lengthened in euhydrated subjects, indicating reduced corticospinal inhibition after hypo-hydration, despite reduced muscle function. In addition, Critzer and colleagues (279) reported minimal changes to TMS measures after a 12 h fluid restriction protocol. Collectively, it can be suggested that hypo-hydration does not elicit any changes to motor cortical output but could

reduce cortical inhibition during active muscle contractions; however, the reasons for this are unclear.

In contrast, TMS has been widely used to characterise the neurophysiological consequences of concussion and repetitive head trauma. Across acute, subacute, and chronic stages of injury, a consistent finding is a prolongation of cSP, reflecting increased cortical inhibition mediated through GABAergic pathways (280-287). These inhibitory changes are often accompanied by elevated motor thresholds and reduced MEP amplitudes, suggesting a broader pattern of cortical hypo-excitability (6,288,289). While some heterogeneity exists between studies, particularly in the variability of SICI and LICI responses, the prevailing consensus is that concussion shifts the excitatory–inhibitory balance of the primary motor cortex towards suppression of corticospinal output (6). The underlying neurophysiological explanation for this is likely to be the post-traumatic neurochemical cascade: the acute phase of an mTBI involves ionic fluxes (influx of Ca^{2+} and efflux of K^{+}) and energy deficits (excitotoxicity and hyper-glycolysis) that can transiently suppress neuronal firing, followed by longer-term increased GABA release or receptor sensitivity, as the brain attempts to restore homeostasis (4). This framework is reinforced by evidence that abnormalities in cSP and related measures can persist long after clinical recovery, suggesting that TMS is sensitive to subclinical disruption of cortical function that may not be captured by symptom reports or conventional imaging (280-287). Such findings strengthen the rationale for using TMS to investigate the neurophysiological sequelae of concussion in both research and applied sport settings.

Beyond diagnosed concussions, TMS has also been applied to the study of non-concussive impacts. Soccer heading has repeatedly been associated with transient increases in cortical inhibition and decrements in cognitive function within 24 hours (8). This reinforces the sensitivity of TMS to RHIs below the threshold of clinical concussion. However, a critical gap

remains in the literature: despite combat sports being the most direct context in which the head is a legitimate target, relatively few TMS studies have examined this population in depth. As argued by Follmer and colleagues (45), combat sports should be considered “ground zero” for understanding brain trauma, yet systematic neurophysiological monitoring of fighters is still rare. This gap limits the ability to fully contextualise how repetitive sparring and competition shape brain function across both acute and cumulative timescales. Taken together, the available evidence converges on the view that concussive and non-concussive impacts induce heightened GABAergic inhibition and reduced cortical excitability, a neurophysiological signature of trauma that carries implications for both motor control and cognition. But to date, there are no combined real-world observations of how hypo-hydration might influence the neurophysiological outcomes of RHIs. In the following subsections, the review will summarise and hypothesise possible risk factors for fluid deficits and exacerbated risk of brain trauma.

2.4.2 Hypo-hydration-related risk factors for brain trauma

In addition to the risk of brain trauma, combat sports are also regulated by weight class to promote fair competition; however, this is typically achieved through fluid restriction (290). To date, fluid deficits and brain trauma in combat sports have been observed as separate entities. In order to determine an intervention to safeguard combat athletes with regards to brain health, the mechanisms underpinning the implications of hypo-hydration and RHIs must be studied individually at a neurophysiological level. The following sections summarise the literature and rationale for investigating the link between hypo-hydration and exacerbated risk of brain trauma.

2.4.2.1 Reduced Cerebrospinal Fluid Volume

CSF surrounds the brain and spinal cord, providing a fluid cushion that buffers the brain against impact – akin to a shock absorber. The adult cranium is a fixed-size "vault," within which intracranial components (brain tissue, blood, and CSF) exist in a volume equilibrium (the Monro–Kellie doctrine; 23), therefore, changes in one component must be compensated by another, which will be further discussed in the following subsections. Typically, CSF circulates the CNS from the choroid plexus through the cortex into the subarachnoid space and spinal cord, ensuring the meningeal layers are not in direct contact, while the brain and spinal cord are floating intact amongst the surrounding bony structures. It is assumed that the density of the CSF acts as a medium to keep the cerebrum buoyant within the cranium, while the viscosity simultaneously functions as a method of reducing impulse moments during rapid acceleration and deceleration movements associated with sport (23,291). Changes of CSF directly alter the functions of tissues, serving as a layer of protection to the cerebral cortex and the neurons themselves. This reduces the tissue's ability to resist shear and torsional force occurring during any head impact and the resultant transmission of energy throughout the cerebral tissues, causing diffuse axonal injuries (292-296). Hypo-hydration perturbs CSF volume via reduced total blood volume and cortical volume (brain shrinkage), which is further discussed below. Furthermore, hypo-hydration causes loss of plasma volume and total blood volume (78), and given that CSF is a product of blood filtration (via highly specialised cells of the choroid plexus), in a hypovolemic state, cerebral perfusion can decrease and the choroid plexus may produce less CSF. Hypo-hydration (~ 2% BMR) has been noted to significantly reduce CSF volume (23), demonstrating that with a reduction in circulating blood, intracranial blood volume will drop, resulting in lower CSF volume and, therefore, potentially less fluid cushion around the brain at baseline. Additionally, reduced blood volume might lead to brain tissue shrinkage (due to lowered capillary pressure and extracellular volume), which in turn, leads to

ventricular expansion (22), creating extra space within the skull that CSF normally occupies (297,298).

High brain water content (~ 75% of brain mass) is maintained for optimal function (299), but even short-term water deficits cause brain water reduction. MRI studies show that prolonged hypo-hydration increases CSF space and decreases brain tissue volume/density (297). For example, 36 h of fluid deprivation led to higher CSF density and a widespread reduction in grey and white matter density, which was reversed after 1.5 h upon rehydration (297). Another study with exercise-induced dehydration (~ 2% BMR) found a strong correlation between the percentage of hypo-hydration and expansion of the cerebral ventricles (23). In a similar manner to reduced CSF, the fluid-filled ventricles enlarged to fill the void of a smaller brain volume, permitting the brain to move more freely upon impact, potentially increasing shear forces during a head acceleration (2306).

2.4.2.2 Tissue Dehydration

Water is integral to the mechanical properties of cells and their substructures; and therefore, beyond gross volume changes, cellular-level dehydration can render brain tissue more vulnerable to injury (299). Hypo-hydration can lead to increased solute concentration and alter lipid packing in cell membranes. Biophysical studies of model membranes have shown that removing water causes membranes to transition to a more rigid, “gel-like” phase (300). In a hypo-hydrated state, the neuronal membranes may become less flexible and more brittle. This raises the likelihood of structural failure under stress. For instance, when a concussive force causes rapid tissue distortion, a well-hydrated brain cell membrane might stretch and deform, but a hypo-hydrated, stiff membrane could tear, leading to breaches in the cell’s integrity or dysfunctional ion channel activity. Similarly, the myelin sheaths that insulate axons could be compromised; myelin has a high-water content; hypo-hydration could disturb its lamellar

structure and protein alignment, reducing its capacity to absorb mechanical shock. A commentary on brain injury mechanisms proposed that the extent of diffuse axonal injury could be directly linked to the tissue's hydration status (299) i.e., drier axons are more prone to shearing.

2.4.2.3 Ion Balance

Hypo-hydration disturbs the body's electrolyte balance and osmotic environment, which can influence neurological function and injury cascades. Electrolytes are critical in nerve impulse conduction and in the molecular events during a concussion. Hypo-hydration can contribute to mTBI risk through several ionic mechanisms.

2.4.2.3.1 Hypernatremia and cellular excitability

Clinically, hypernatremia is known to injure the brain via cellular shrinkage and damage of the myelin sheaths (301). For example, in severe TBI patients, elevated serum Na^+ correlates with worse outcomes, and experimentally, hypernatremia can cause additional axonal and myelin damage by increasing neuron dehydration (302). Consequently, a hypo-hydrated state accompanied by elevated extracellular Na^+, may place neurons under heightened osmotic and physiological strain, potentially impairing membrane function and reducing resilience to subsequent stressors (303,304). When a concussive impact occurs, the typically described ionic flux (K^+ efflux and Ca^{2+} influx; 3) might be exacerbated, triggering an acute metabolic crisis or cell death pathways more readily than if they were well-hydrated. In summary, the ionic imbalance associated with hypo-hydration could amplify the ionic perturbation of concussion, worsening the energy crisis in neurons (since the Na^+/K^+ pumps must work harder in a disturbed ionic environment to restore equilibrium).

2.4.2.3.2 Impaired Buffering of K^+ and Neurotransmitters

During a concussion, extracellular K^+ can rise to dangerous levels, and excitatory neurotransmitters i.e., glutamate are released, leading to excitotoxicity (4). Adequate hydration is required for glial cells i.e., astrocytes to buffer excess K^+ and take up glutamate efficiently (305). If astrocytes are shrunken and electrolytes imbalanced, their ability to clear K^+ may be reduced, prolonging the depolarisation of neurons. Similarly, Ca^{2+} handling might be affected by hypo-hydration as hyperosmolar states can alter Ca^{2+} influx pathways. Although direct research is limited, it's plausible that hypo-hydration lengthens the time for ionic homeostasis to be restored after brain trauma, thereby extending the window of vulnerability when the brain is in an energy-deprived, hyper-excitable state. Studies using indirect methods of assessing neurotransmitter activity such as TMS and Magnetic Resonance Spectroscopy (MRS) have reported heightened intracortical inhibition and reduced cortical excitability shortly after mTBI. Yasen et al. (306) conducted a longitudinal study assessing glutamate and GABA levels in the dorsolateral prefrontal cortex and primary motor cortex using MRS. They found that, glutamate concentrations were significantly reduced in the dorsolateral prefrontal cortex (72 h post-mTBI), and GABA levels were lower at both 72 h and two weeks post-injury. These findings suggest region-specific and time-dependent neurochemical disruptions following mTBI. Similarly, Miller et al. (285) observed increased cSP durations within 72 h post-injury, persisting up to two months, suggesting enhanced $GABA_B$-mediated inhibition without significant changes in MEPs or RMTs.

It is notable that Yasen et al. (289) found prolonged cSP durations in individuals with mTBI, indicating increased cortical inhibition; however, this heightened inhibition did not correlate with cognitive or motor function recovery, suggesting that while mTBI affects cortical inhibition, it may not directly influence functional outcomes. In addition, Di Virgilio et al. (9)

investigated the effects of repetitive non-concussive head impacts in amateur boxers and reported increased cortical inhibition post-training, highlighting that even in the absence of concussion, repetitive brain trauma can acutely alter cortical function and neurotransmitter activity.

2.4.2.3.3 Concurrent Symptom Exacerbation

Even without a head impact, hypo-hydration itself can cause symptoms that overlap with concussion. A study by Patel et al. (307) demonstrated that hypo-hydration (~ 2.5% BMR) in healthy athletes led to significantly higher self-reported symptoms like headache, tiredness, and difficulty concentrating (307), which are notable concussion symptoms. No differences were found in objective balance or cognitive tests in that study, but hypo-hydrated participants felt worse and had lower visual memory scores (307). This suggests that hypo-hydration can independently produce symptoms associated with concussion (e.g., headaches; 308,309); and therefore, separating hypo-hydration effects from concussion effects is difficult, and importantly, the combined effect could be additive.

In summary, hypo-hydration skews the ionic environment of the brain in a way that might lower the threshold for mTBI diagnosis. It introduces a state of pre-existing electrolyte stress (e.g., hypernatremia, intracellular K^+ deficits, etc.) that can intensify the ionic flux and metabolic disturbance caused by brain trauma. It also can mask or mimic concussion symptoms, confounding clinical assessment (307), although it is unknown if this is experienced amongst combat sport athletes.

2.4.2.4 Physical function

There is some evidence to support the notion that hypo-hydration can modulate CNS activity, with studies using TMS demonstrating changes in the brain-to-muscle pathway in hypo-

hydrated humans (87). For instance, some findings suggest a reduction in inhibitory signalling to motor neurons when hypo-hydrated, perhaps as a compensatory mechanism to offset force decrements as a result of hypo-hydration (87). Hypo-hydration also increases perceptions of fatigue, pain and degrades neuromuscular control and cognitive-motor function: studies have found slower reaction times, impaired coordination, and increased perceived effort when athletes are hypo-hydrated (194-197,201-203,310). Such deficits in neuromotor response speed and precision can hinder complex sport skills and may elevate injury risk, as athletes react more slowly to hazards or execute movements less accurately. Therefore, in a combat sporting context, a hypo-hydrated athlete may not only experience diminished performance but also face an elevated risk of sustaining musculoskeletal injuries or brain trauma due to compromised motor control. A recent longitudinal study of college wrestlers reported athletes who cut more weight (via dehydration) had higher injury rates (including concussions), than those who cut less (310). While this is correlative, it underscores that the magnitude of hypo-hydration can impair physical function to the point of causing more frequent trauma.

2.5 Summary

In summary, while hypo-hydration and mTBIs have traditionally been studied in isolation, emerging evidence suggests they may influence similar neurophysiological pathways (Figure 2.12). For instance, dehydration-induced changes in CSF volume or ionic concentration could plausibly alter brain volume or neuronal excitability, thereby modifying neuronal vulnerability to mechanical insult. However, this relationship remains largely theoretical and underexplored in human populations. Critically, there is a paucity of research examining whether hypo-hydration may lower the threshold for injury or exacerbate the acute neurophysiological consequences of head impacts. The literature also lacks clarity on whether these two stressors interact temporally during combat sport training or whether their effects are independent but

overlapping. This knowledge gap provides a strong rationale for the present thesis, which aims to investigate the independent effects of hypo-hydration and RHIs on neuromuscular and cortical function, as well as the potential for additive or compounding effects when both stressors are experienced in close proximity. The following chapters address this gap by integrating athlete perceptions, laboratory-based assessments, and field-based monitoring tools to evaluate how fluid balance and RHIs interact with corticomotor function in combat sport athletes. The overarching hypothesis of this thesis is that RHIs will lead to heightened corticospinal inhibition, while hypo-hydration will modulate excitatory and inhibitory dynamics in a manner that prolongs recovery or exacerbates inhibition.

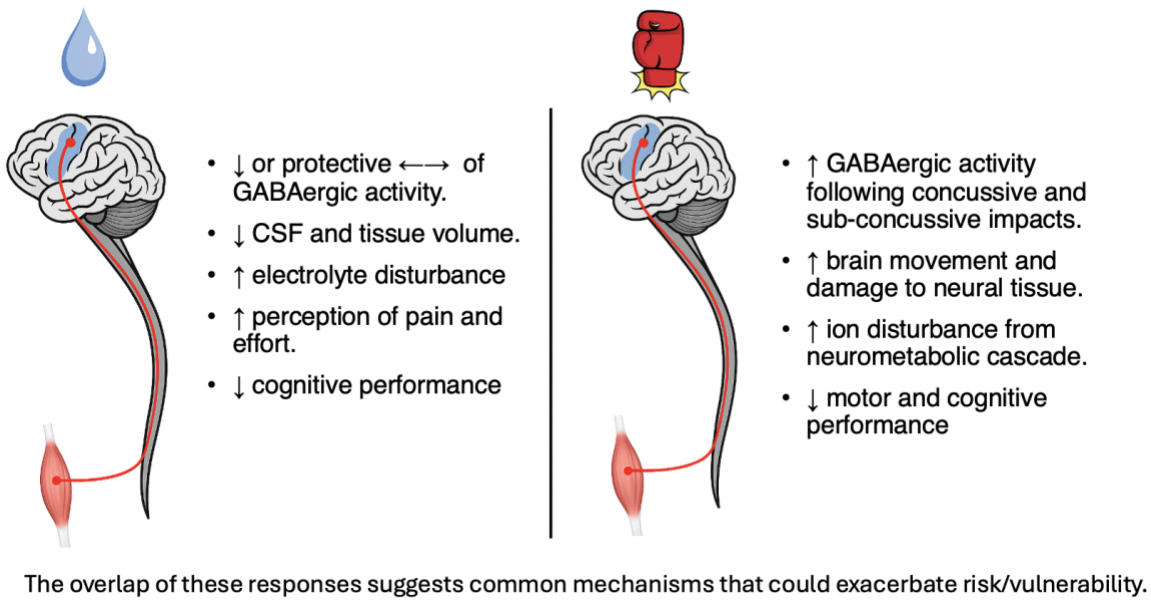

Figure 2.12: Summary of the unique physiological responses that present a potential mechanistic overlap of neurophysiological pathways of hypo-hydration and brain trauma.

Chapter 3:

A survey of combat athletes' rapid weight loss practices and evaluation of the relationship with concussion symptom recall.

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Note on Terminology: While the term *mTBI* is used throughout this thesis, the term *concussion* is used predominantly within Chapter 3. This decision was made to ensure clarity and accessibility when circulating the survey to athletes, for whom '*concussion'* is a more familiar and digestible term. As such, readers should note that for the purpose of this chapter, *concussion* is often used interchangeably with *mTBI*.

3.1 Introduction

Concussions are mTBIs, generally experienced in sport from direct or indirect contact to the head, leading to linear and rotational acceleration of the brain (311). Concussions occur commonly in combat sports, such as MMA, boxing, and kickboxing/Muay-Thai (KB/MT) due to head impacts in training and competition (46). Within boxing and MMA, concussion rates range between 16 and 25 per 100 athletic exposures (46,312,313), but this may be underestimated due to the absence of medical personnel in a large proportion of training time. With evidence of both short- and long-term health consequences as a result of repetitive head trauma (314,315), there is an emerging concern for the welfare of combat sport athletes. Therefore, determining the factors that contribute to concussion risk has become a priority.

Combat sports are categorised by weight divisions to ensure fair bouts between opponents and reducing potential injuries that might occur as a result of major weight differences (316). Typically, athletes aim to lose body mass (via water) in the shortest time possible, utilising RWL methods to obtain a perceived advantage of competing against a lighter, or weaker opponent (317). Common forms of RWL are fluid restriction, dehydration by sweating, diuretics, laxatives, and 'water-loading' (283,284). These strategies can lead to hypo-hydration and, subsequently, alterations to renal function (11), immunoendocrine status (318), brain ventricular volume, and metabolic activity (22).

Both cellular dehydration, induced by hypo-hydration or 'water-cutting', and concussive events have been reported to impair CNS function (22,276). While the mechanistic basis of these effects is currently uncertain and meaningful links are unestablished, disruption of fluid or ion homeostasis have been commonly reported among hypo-hydrated subjects and those experiencing concussions (24,317). In addition, hypo-hydration (~ 2% BMR) has been noted to significantly reduce CSF volume (23), demonstrating that with a reduction in circulating

blood, intracranial blood volume will drop, resulting in lower CSF volume and, therefore, potentially less fluid cushion around the brain at baseline. Additionally, reduced blood volume might lead to brain tissue shrinkage (due to lowered capillary pressure and extracellular volume), which in turn, leads to ventricular expansion (22), creating extra space within the skull that CSF normally occupies (297). Hypo-hydration is also reported to lead to cognitive and neuromuscular deficits (16,319), which could theoretically lead to reduced performance and a heightened risk of concussion. Patel et al. (307) investigated the symptomology of hypo-hydrated, non-concussed subjects, and found an increase in concussion-related symptoms including fatigue, headache, dizziness, balance disturbances, difficulty concentrating, and feelings of being slowed down, alongside impairments in visual memory, indicating that hypo-hydration and concussion may elicit similar effects. Indeed, any synergistic effects caused by hypo-hydration or concussive events could exacerbate the reported symptoms. Whether such physiological and physical alterations lead to combat athletes competing or training with a higher risk (or perceived risk) of brain trauma is unknown, though this has been anecdotally reported by mainstream media outlets (320). It is also unclear if certain combat sports place athletes at greater risk, particularly among striking-dominant disciplines or those with stringent weight classifications. In addition to clinically diagnosed concussions, the potential cumulative effects of non-concussive head impacts may further complicate interpretation. The distinction between concussive and non-concussive impacts is particularly relevant in combat sports, where repetitive head impacts (RHIs) are inherent to participation. While concussive impacts may result in observable symptoms such as headache, dizziness, and confusion (31,32), non-concussive impacts are typically asymptomatic in isolation but may contribute to cumulative neural alterations when sustained repeatedly over time (35). This distinction is important when considering the overlap in symptoms associated with hypo-hydration and concussion, as diagnostic criteria for both conditions (and their apparent severity) are often dependent on

subjective symptom reporting. Consequently, the presence of both hypo-hydration and repetitive head impact exposure may increase the risk of misattributing symptoms, particularly in the absence of overt concussion, which has important implications for athlete safety and clinical decision-making around competition.

Studies investigating concussion symptoms, in isolation, have frequently reported neck pain, fatigue or low energy, trouble falling asleep, and headaches (56), with 60% of MMA athletes returning to sparring within two days or less of their first experienced symptom (321). Symptoms pertaining to heat stress and hypo-hydration are also reported to include headaches, dizziness, and increased perception of fatigue (33,307). Despite the incidence of concussions and abundant use of RWL among combat sport athletes (10,322), to date, there has been no study of both self-reported concussion and RWL symptoms in combat sport athletes or an evaluation of their inter-relationships. As outlined in Chapter 2, the rationale for examining RWL and concussion-related symptomology together is not that hypo-hydration has been shown to directly worsen concussion pathophysiology, but rather that both stressors may produce overlapping clinical presentations and may influence aspects of CNS function relevant to athlete safety. RWL practices in combat sports can produce symptoms such as headache, dizziness, fatigue, impaired concentration and perceived weakness (16,221,228) and these symptoms overlap with those commonly assessed in sport-related concussion frameworks, where symptom reporting remains a central component of clinical recognition and removal decisions (323,324). Therefore, in a population where both weight-cutting and RHIs are common, understanding athlete-reported symptom overlap is an important first step before experimental work can examine neurophysiological mechanisms more directly. Consequently, the aims of this survey were to; i) investigate the differences in RWL and concussion symptom-onset and recovery between combat sports and ii) evaluate the relationships between

concussion and RWL symptoms among combat sport athletes who have previously suffered from a concussion and undergone RWL prior to competition.

3.2 Methods

3.2.1 Participants

Following institutional ethical approval, participants were recruited via websites and social media, with informed consent obtained in accordance with the Declaration of Helsinki (2013). Participants were required to be over the age of 18 years, competed (at least once) in a combat sport, and had 'cut weight' prior to a competition. A 'weight-cut' (WC) was defined as any method leading to the loss of water, including (but not limited to) sauna, fluid restriction, diuretics, sweating gels, hot water baths, laxatives, fat burners, vomiting, and 'water-loading'. A total sample size of 132 (115 male, 17 female) combat athletes (age: 29 ± 8 years, BM: 77.0 ± 12.9 kg, training experience: 13 ± 6 years) completed the survey. Sporting modalities reported were MMA (31%), KB/MT (20%), boxing (22%), wrestling (5%), Brazilian Jiu-Jitsu (BJJ; 10%), judo (8%) and 'others' (6%). Of the total sample, 70% of athletes were actively competitive, with 50% of male athletes and 29% of female athletes competing professionally.

3.2.2 Survey instrument

The survey combined two validated questionnaires: the Mixed Martial Arts Weight-Cutting Questionnaire (MAWC-Q; 322) and the Sport Concussion Assessment Tool 5 (SCAT-5; 323). Participants were asked preliminary questions regarding demographic, sporting, and medical history, after which the questionnaire was split into three sections, which aimed to address 1) WC practices, 2) WC symptoms, and 3) concussion history and symptoms, in which they were asked to confirm number of concussions and how many of these were medically diagnosed.

The survey was piloted through completion by 15 combat sport athletes, a wrestling coach, and two experts (academic in the field of nutrition and a ringside medic). Written feedback was provided on the suitability and clarity of questions and amendments were made before the finalised version was released online (Joint Information Systems Committee Online surveys; Appendix 2).

3.2.3 MAWC-Q

The MAWC-Q consisted of 26 questions, aiming to establish the prevalence and magnitude of RWL methods, specifically amongst MMA athletes. Modifications were made to the MAWC-Q, as follows: a) re-phrasing of questions to ensure appropriateness to all combat athletes, b) omission of questions regarding age of first WC, specific method of weight loss and symptoms, c) addition of questions related to cumulative number of WCs and time in between a WC, and d) the addition of a question, asking if a WC had '*not gone according to plan*' and a series of resultant scenarios. This was to establish the frequency and experiences of worst-case scenarios in WC practices.

3.2.4 SCAT-5 symptom checklist

Symptom profiles for concussion and WCs were assessed using a 22-point symptom checklist from the SCAT-5. Each symptom was assessed using a Likert scale of severity, ranging from 0 (none) to 6 (severe +), resulting in an overall symptom score (for each selected symptom) out of 22 and a symptom severity score (based on the sum of all Likert scale selections) out of 132. Modifications were made to the symptom checklist through a) the addition of two physical symptoms '*physically weak*' and '*unable to train*', leading to a symptom score of 24 and a symptom severity of 144, and b) six additional questions related to the relationship between

selected symptoms and fluid consumption or head impacts. This led to a third measure: an 'adjusted symptom score' of 57, accounting for the six additional questions.

3.2.5 Statistical analysis

Statistical analyses were completed using SPSS (v22, IBM Corp., Armonk, NY). Data were presented as means ± standard deviations (SD), frequency of responses to each item (n), range (where appropriate), or median and interquartile range (IQR) if non-normally distributed. Assumptions of normality and equality of variance were assessed via Shapiro–Wilk and Levene's tests, respectively. For parametric (WC symptom variables) data, a one-way analysis of variance (ANOVA) was used to assess the difference between each sport (MMA, Boxing, KB/MT, Judo, Wrestling, & BJJ). For non-parametric data, a Kruskal-Wallis test was used to establish differences in concussion symptom variables between each sport. Where there was a significant main effect, pairwise comparisons were examined by a Tukey (parametric) or Dunn-Bonferroni *post-hoc* (non-parametric) to account for the homogeneity of variance. A Spearman's Rho correlation was used to assess the relationship between concussion and WC symptom variables (symptom severity, symptom score, adjusted symptom score). An alpha level of ≤ 0.05 was set for all analyses.

3.3 Results

3.3.1 Modified MAWC-Q

The majority of athletes (83%) reported using a fight preparation camp, with the highest reported WCs being 10 kg (10 days), 8.6 kg (7 days), 7 kg (3 days), and 5 kg (24 h). Median WC was 6.6% of body mass (Table 3.1). Most athletes (86%) reported a WC to '*not going according to plan*', with the most frequent consequences being a lack of energy (83%), lack of

strength/power (70%), and suboptimal coordination and reaction time (55%). Responses included in the 'other' section were: '*collapsed*' & '*cramping*' (*n* = 2), and '*coughing blood and unable to function*', '*projectile vomiting*', '*was asked to keep weight off for an extra 36 hours*', '*felt cold and lips became blue during fight*', '*menopause*', '*fluid ascites*', and '*process was more gruelling due to period making me retain more water*' (*n* = 1).

Table 3.1: Participant preparation camp and weight-cutting characteristics (average) (A), WC characteristics (frequency) (B). *BM* body mass *WC* weight-cut

A

	Mean ± SD / Median (IQR)	**Range**
Average camp length (weeks)	7 ± 3	4 - 12
Shortest camp length (weeks)	3 ± 2	1 - 5
General 'walking' weight (kg)	76.3 ± 13.0	47 - 105
Usual WC (kg)	5 (5)	0 - 22
Usual BM regain (kg)	3 (3.5)	0 - 10
Shortest WC timing (days)	3 (6)	0 - 15
BM lost (kg)	5 (4)	0 - 13
BM loss in 24 h before weigh-in (kg)	2 (2)	0 - 5
Total no. of weight cuts in career	15 (20)	1 - 147

B

	n	**%**
Time between weigh-in and bout (h)		
24 – 36	75	56.8
12 – 23	14	10.6
6 – 12	10	7.6
2 – 5	20	15.2
< 2	6	4.5
Time between each WC (months)		
< 1	76	57.6
1 – 2	31	23.5
3 – 4	18	13.6
≥ 5	7	5.3
WC not according to plan (Y/N)	Y = 86	65.1
Felt a lack of energy	71	82.6
Felt a lack of strength/power	60	69.8
Coordination & reaction time felt suboptimal	47	54.7
Felt dehydrated during the fight	41	47.7
Felt easier to get 'rocked' during the fight	33	38.4
Initially failed to make weight	30	34.9
Fell ill in the lead up to the fight	27	31.4
Felt too bloated going into the fight	20	23.3
*Other**	11	12.8

3.3.2 WC and concussion symptom profiles

There was a significant relationship between WC and concussion symptom severity (r_s = 0.68, 95% CI [0.54, 0.74], $p < 0.001$), symptom scores (r_s = 0.60, 95% CI [0.44, 0.72], $p <$ 0.001) and adjusted symptom scores (r_s = 0.72, 95% CI [0.59, 0.81], $p < 0.001$; Figure 3.1).

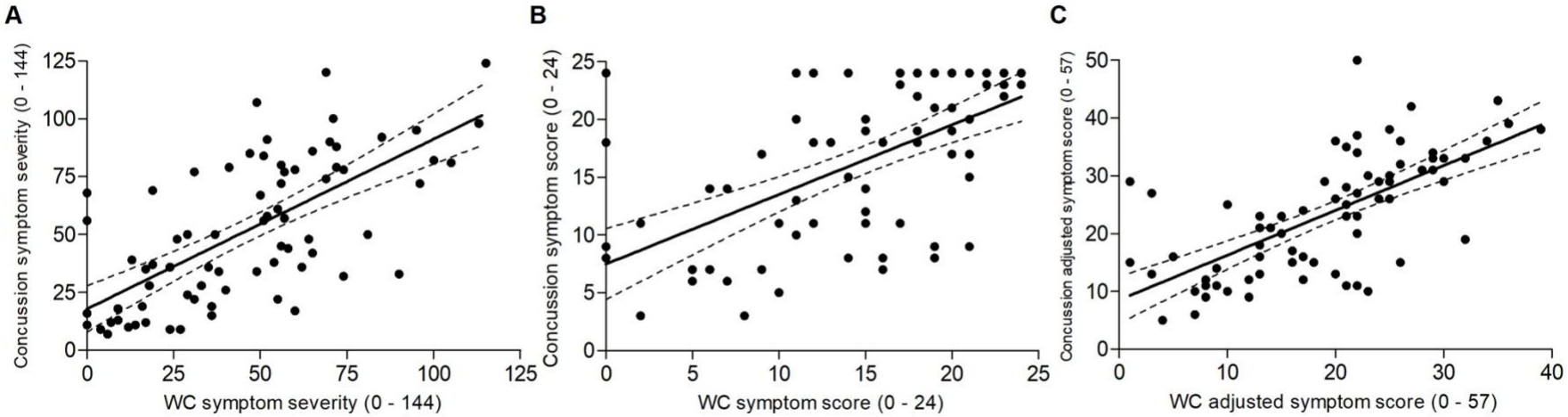

Figure 3.1: Significant correlations between concussion and weight-cutting with 95% confidence intervals. (A) symptom severity (r_s = 0.68), (B) symptom scores (r_s = 0.60) and (C) adjusted symptom scores, (r_s = 0.72). (n = 80).

Mean scores for WC symptom severity, symptom score, and adjusted score were 49 ± 30 out of 144, 15 ± 7 out of 24, and 19 ± 9 out of 57, respectively. A significant main effect was observed in WC symptom severity ($F_{(5, 118)} = 5.464, p < 0.001$), with differences between MMA and KB/MT (59.2 *vs.* 36.5, $p = 0.01$), MMA and boxing (59.2 *vs.* 38.0, $p = 0.02$), judo and KB/MT (67.5 *vs.* 36.5, $p = 0.02$), judo and boxing (67.5 *vs.* 38.0, $p = 0.03$), judo and BJJ (67.5 *vs.* 31.9, $p = 0.04$). A significant main effect was also observed in adjusted symptom scores ($F_{(5, 118)} = 3.213, p = 0.009$), with a difference between MMA and KB/MT (22.0 *vs.* 15.6, $p = 0.03$) (Figure 3.2A & B).

Median (IQR) scores for concussion symptom severity, symptom score, and adjusted score were 48 (57) out of 144, 18 (14) out of 24 and 26 (19) out 57, respectively. A significant main effect was observed in concussion symptom severity ($X^2_{(5)} = 14.142, p = 0.015$), with a difference between MMA and boxing (56.0 *vs.* 31.9, $p = 0.023$). A main effect was also observed in adjusted symptom scores ($X^2_{(5)} = 13.348, p = 0.02$), with pairwise comparisons showing a significant difference between MMA and boxing (55.5 *vs.* 32.8, $p = 0.04$) (Figure 3.2C & D).

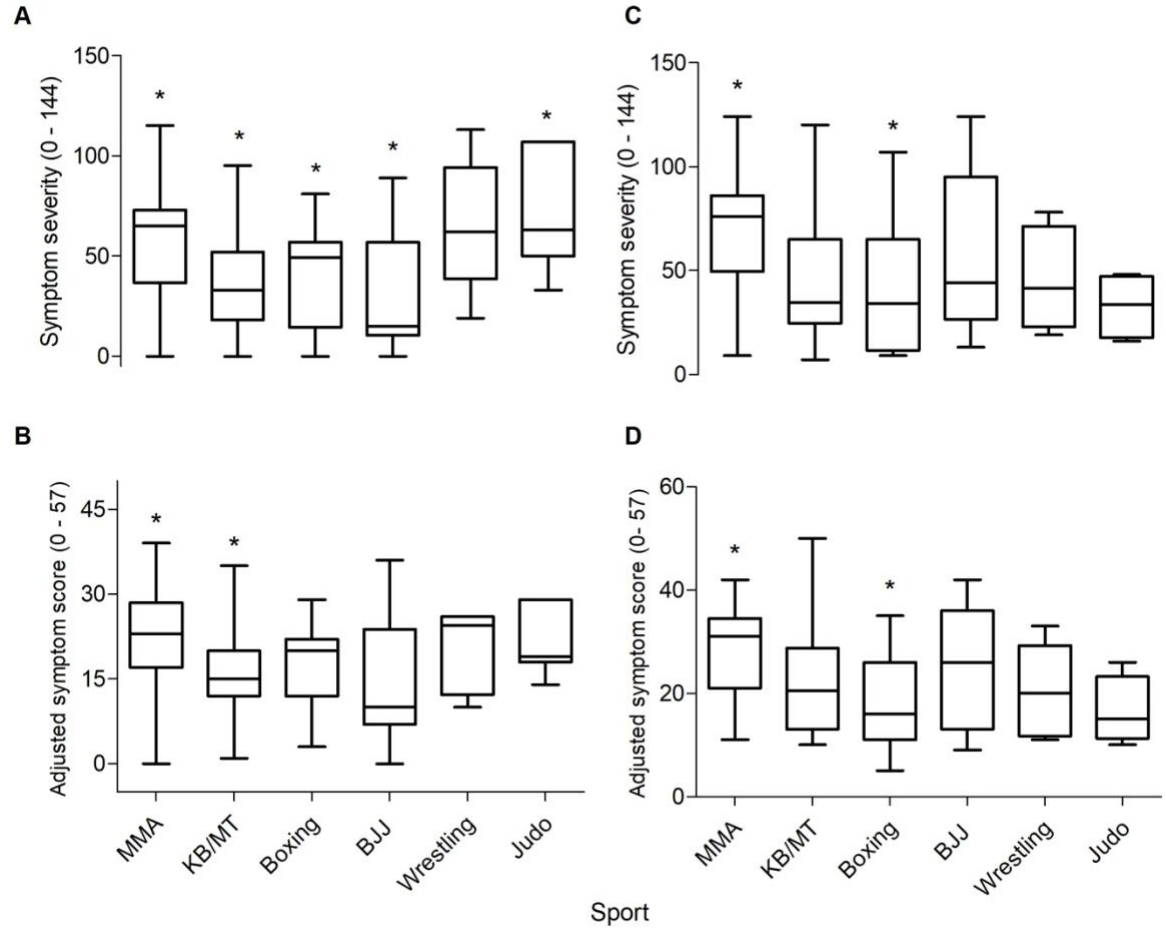

Figure 3.2: Average weight-cutting symptom severity (A), adjusted symptom score (B), concussion symptom severity (C) and adjusted symptom score (D) for each combat sport.

Of the 132 combat athletes who completed the survey, approximately two-thirds (64%) reported sustaining at least one concussion during their professional career, but only 45% were hospitalized or underwent imaging and 34% were medically diagnosed. The median (IQR) reported time before returning to training after a concussion, was 7 (12) days.

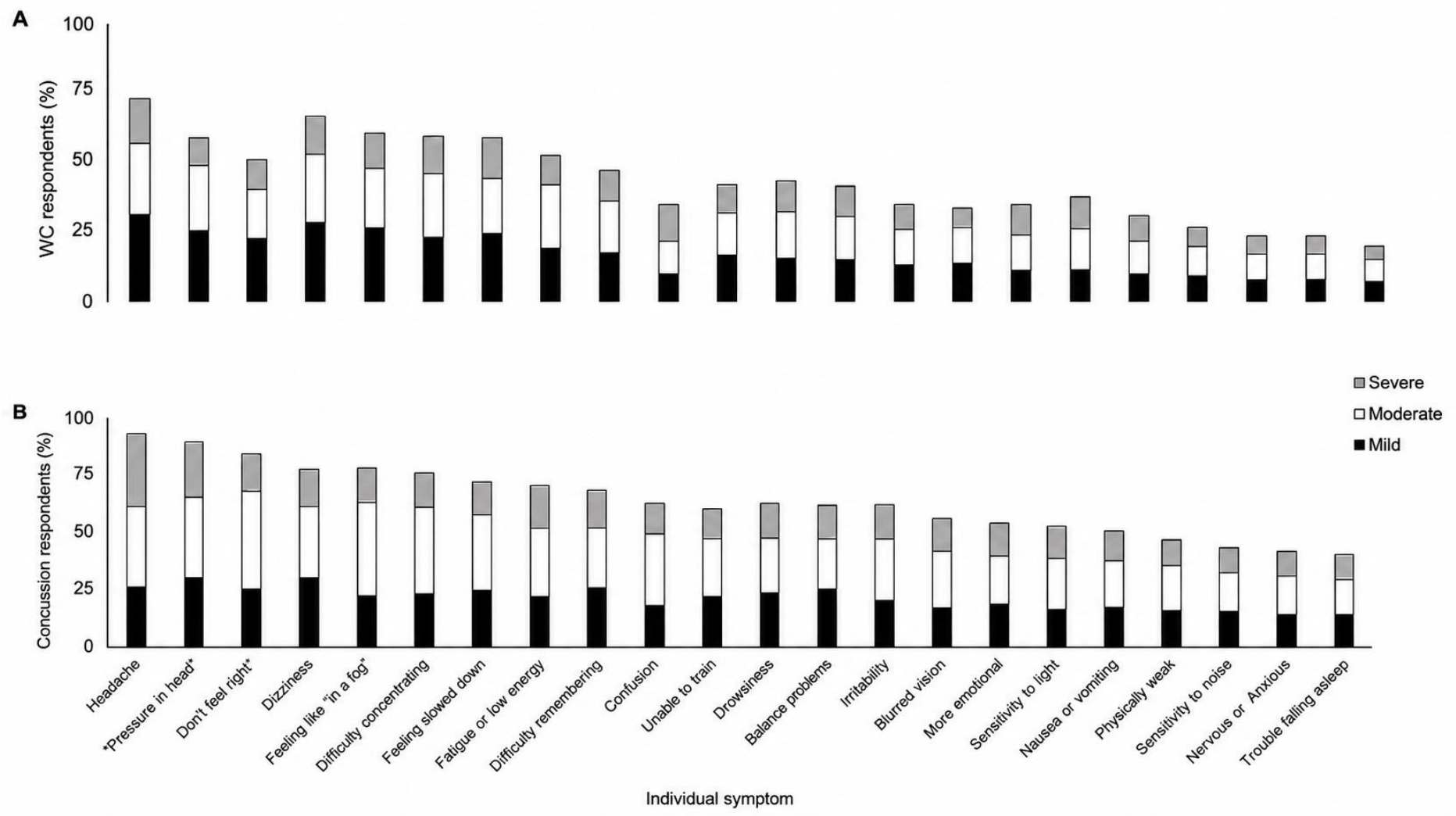

Figure 3.3: Percentage of athletes who selected each symptom severity for weight-cutting (A) and concussions (B).

The majority of athletes (70%) reported deterioration of concussion symptoms during and after a fight or sparring session, while 37% reported deterioration of WC symptoms. A summary of symptom recovery responses can be found in Table 3.2.

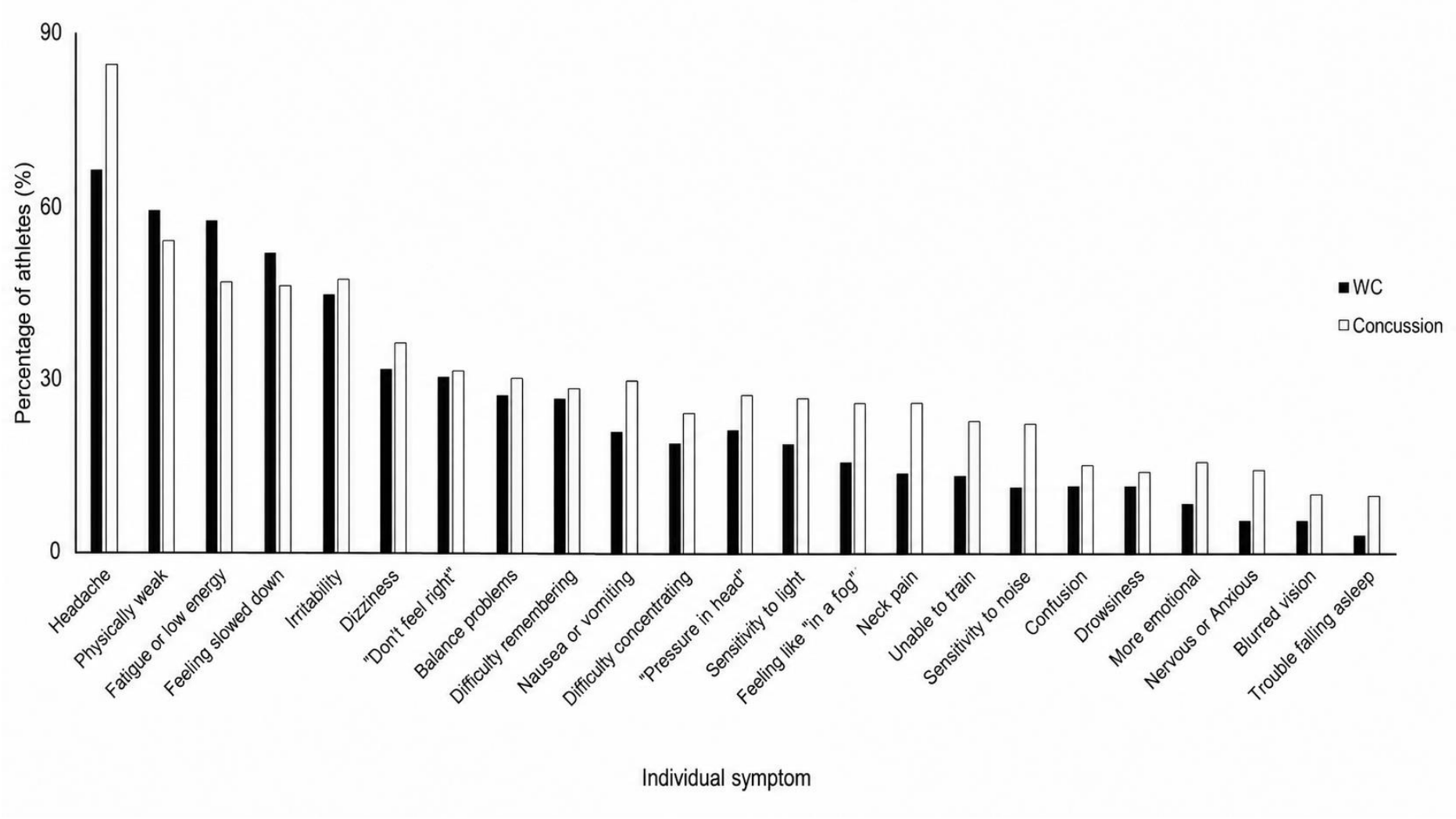

Figure 3.4: Percentage of athletes who reported symptoms to deteriorate after hypo-hydration or head impact, WC (n = 49), concussion (n = 56).

Table 3.2: Symptom recovery profile. *Y* yes *WC* weight-cut.

Symptom recovery related questions	WC		Concussion	
	% (n)	Total n	% (n)	Total n
Do you feel that these symptoms became worse during/after the fight (or sparring session) within which you were dehydrated? (Y)			70 (56)	80
Do you feel that these symptoms improved when you were able to consume fluids again? (Y)	94 (124)	132	30 (17)	56
Do you feel that these symptoms lasted longer in comparison to a camp/session when you were not cutting weight? (Y)	61 (81)	132	60 (48)	80
Do you feel that you experienced less of these symptoms in a camp/session when you were not cutting weight? (Y)	89 (117)	132	70 (56)	80
Do you feel that these symptoms became worse during/after the fight (or sparring session)? (Y)	37 (49)	132		
How long did these symptoms tend to last for?		49		56
Less than 24 h	22 (11)		15 (12)	
24 – 48 h	59 (29)		28 (23)	
3 – 5 days	10 (5)		43 (34)	
6 – 13 days	2 (1)		8 (6)	
14 – 28 days	6 (3)		4 (3)	
> 28 days	0		3 (2)	

3.4 Discussion

This study surveyed combat sports athletes to investigate the differences in symptom-onset and recovery between combat sports, and evaluated the relationships between concussion and RWL symptoms. There were strong associations between concussion and RWL symptom profiles, particularly for feelings of dizziness, headaches, physical weakness, and fatigue. The majority of athletes (60 – 70%) reported a deterioration and lengthening of concussion symptoms when undergoing a WC, suggesting that prior RWL might affect concussion symptoms.

This is the first study to assess the relationship between reported symptoms experienced as a result of previous RWL events and concussions among combat athletes. The present findings

indicate a positive relationship between symptoms associated with RWL (via dehydration and leading to hypo-hydration) and concussions, based on both the reported total number and severity of symptoms (Figure 3.1). Analysis of individual symptoms revealed that the most common WC symptoms were physical weakness, fatigue, dizziness, not feeling 'right', difficulties in concentration and irritability. The most frequently selected concussion symptoms comprised the same responses as WC, in addition to headaches, head pressure, and neck pain (Figure 3.3). These findings are in accordance with previous literature (307,325), indicating that RWL via dehydration increases concussion-related symptoms. The possible reasons for feelings of weakness and fatigue could relate to perturbations in ionic balance to restore osmotic equilibrium (326), and subsequent alterations to cell excitability and excitation-contraction coupling capabilities (105,327). Hypo-hydration also leads to a reduction in total blood volume (hypovolemia), leading to light-headedness and dizziness, while dizziness post-concussion may also be related to vestibular system damage, further increasing headaches, feelings of confusion and concentration difficulty (328). This may explain why the most frequently reported symptom (for both concussed and RWL athletes) after a WC or sparring session was headaches (314; Figures 3.3 & 3.4). Therefore, the implications of less discernible concussion-related symptoms (e.g., dizziness, headaches) in athletes undergoing RWL, might mask or exacerbate the effects of cumulative non-concussive impacts received throughout the bout. This challenges the accuracy of concussion identification tools, particularly if baseline protocols do not account for hydration status. The extreme practices of WC are likely to introduce a source of external noise to the assessment of concussion symptoms, meaning that knowledge of hydration status is necessary during all protocols to avoid erroneous results. Given that the risks associated with extreme WC extend beyond this current issue, further consideration of methods to avoid RWL is warranted.

To the best of knowledge, this study is the first to report the experiences of a WC going 'wrong', with 70 – 80% of athletes reporting a lack of energy, strength, and power, as well as 40 – 50% reporting a reduction in coordination and feeling more susceptible to concussion (Table 3.1). It was also found that 60 – 70% of athletes reported their concussion symptoms to deteriorate and lengthen during a camp where they were weight-cutting, and a fight in which they were dehydrated (Table 3.2). Furthermore, ~ 50% of athletes reported increased feelings of being 'slowed down', experiencing fatigue, irritability, and physical weakness after fighting in a hypo-hydrated state or cutting weight in camp (Figure 3.4). These reports may indicate that combat athletes perceive a worsening or prolongation of concussion-like symptoms when weight-cutting occurs close to training or competition; however, these symptoms should not be interpreted as arising solely from hypo-hydration or head trauma. An additional consideration is that combat sport competition and sparring involve substantial whole-body mechanical and metabolic stress, including repeated striking and grappling exchanges, body blows, soft-tissue trauma, high-intensity intermittent efforts and pain responses (44,329,330). These factors may independently provoke systemic inflammatory and muscle-damage responses, which could contribute to the symptom profiles reported by athletes in the present survey. For example, acute boxing competition has been shown to increase circulating inflammatory markers, including IL-1β, IL-6 and TNF-α, alongside markers of muscle damage (330), while systematic evidence from combat sport and high-intensity exercise literature indicates that strenuous exercise can acutely alter cytokine concentrations and inflammatory status (331,332). This is relevant because several symptoms commonly attributed to concussion or hypo-hydration, including fatigue, reduced energy, perceived slowing, headache, soreness and impaired concentration, may also be influenced by systemic inflammation, pain and exercise-induced muscle damage. Indeed, concussions are known to lead to hyper-glycolysis, coupled with relative depletion of energy reserves, in order to restore

ionic imbalances (4). The combination of hyper-glycolysis, alongside additional physiological stressors (in a bout) and hypo-hydration, may induce a competing substrate demand, which could be further exacerbated by reduced overall blood volume. In addition, it is possible that damage to neurons (e.g., diffuse axonal or shear injuries) is enhanced secondary to hypo-hydration due to altered membrane stability (105), tissue elasticity (298) and cerebrospinal fluid production (20), leaving the brain more vulnerable to cortical damage from trauma.

The individual reports of mishaps during WC practices, while anecdotal and possibly related to factors unknown in this study, expose a plethora of additional negative outcomes associated with RWL. In addition, the vast majority of athletes (86%) stated at least one incident of RWL to "*not go according to plan"*, indicating the perceived competitive advantage may often not be achieved by combat athletes (227). It is crucial these reports do not go ignored and athletes seek medical/professional advice with regards to RWL strategies, as many poor outcomes in individual athletes have been documented (14). In addition, MMA athletes presented higher symptom severity and adjusted scores for both concussion and RWL (Figure 3.2), indicating RWL and concussion symptoms to be more severe in MMA. Symptom severity of RWL in MMA was similar to wrestling, which is also associated with larger and more aggressive RWL magnitudes and methods (10). Interestingly, concussion symptom severity and adjusted scores were not different between sports, except between MMA and boxing. This may be explained by the higher range of weight-classes and lack of indirect head impacts (e.g., takedowns) experienced by boxing athletes in comparison to MMA. Given the differences in symptom severity across various combat sports, it is recommended that combat sport organizations aim to incorporate baseline concussion and continual hydration testing protocols to verify the effects of hypo-hydration and concussions independently.

To date, this is the first study to report on the resolution of combined RWL and concussion symptoms. Almost all athletes (94%) reported their WC symptoms to improve with fluid replenishment; however, the most frequently reported recovery time (for those with increased symptom scores following a fight) was 24 – 48 h (Table 3.2). This indicates that, despite fluid replenishment, over a day was required for said symptoms to resolve. An explanation for this could, in part, be due to a) the method of hydration i.e., bolus *vs.* tapered drinking strategies (333), b) electrolyte and carbohydrate deficient fluids insufficiently restoring blood osmolality, volume, and glucose (334), or c) concussive/non-concussive impacts further adding to alterations in cell integrity and ionic imbalances (335,336). Coaches, athletes and clinicians, should consider the implications of RWL and all aspects of training load (particularly different modes, such as sparring), as this could impact recovery and preparation for a bout.

One of the main limitations of the present study was that additional weight-loss methods used during fight preparation (e.g., food restriction) were not accounted for. Furthermore, it is possible that symptoms were misreported due to surpassed time, which could mean that the effects of RWL on concussion-related symptoms have been over- or under-estimated in the current study.

3.5 Conclusion

In conclusion, this survey demonstrates that combat sport athletes report substantial overlap between symptoms experienced during rapid weight loss and symptoms recalled after concussion. Athletes most commonly reported fatigue, headache, dizziness, physical weakness and impaired concentration across both contexts, and many perceived that concussion-related symptoms worsened or lasted longer when weight-cutting occurred during fight preparation or close to competition. These findings suggest that hydration and weight-cutting history should be considered when interpreting symptom-based concussion assessments in combat sport

athletes, and, where practicable, recommend that clinicians should monitor hydration status when performing neurocognitive tests, such as those used to screen for concussion symptoms. More generally, the findings of the current survey suggest that RWL via WC methods should be avoided near to combat training or competition, as it is possible that this could interfere with the decision making of medical professionals' diagnosis of concussion.

3.6 Linking to Chapter 4

To investigate the physiological and mechanistic basis for these self-reported findings, Chapter 4 establishes the design and reliability of laboratory procedures employed throughout the remaining chapters, in order to investigate the neurophysiological responses to both head impacts and fluid imbalances.

Chapter 4:
General methods

Part 1:
Experimental procedures

4.1 Introduction

This chapter outlines the methods used throughout the remainder of this thesis. Specific methods used in individual experimental chapters are discussed within the corresponding chapters.

4.2 Testing procedures

4.2.1 Anthropometry

Before any experimental procedure, participants height, mass and age were recorded. Height was recorded to the nearest cm using a calibrated stadiometer (Holtain, Crymych, Wales), in accordance with the International Society for Advancement of Kinanthropometry guidelines. On calibrated scales (Marsden MPCS-250, Oxfordshire, UK), mass was recorded to the nearest 0.1 kg and age was recorded to the nearest year. In line with previous work using TMS measures (337) and to avoid any change in muscle function (338) through diurnal variation, participants were tested at the same time of day in each experiment (where possible).

4.2.2 Neuromuscular function

4.2.2.1 Electromyography

EMG activity was recorded from the rectus femoris, alongside a visual inspection of biceps femoris activity, to ensure agonist activation. Placement was performed in accordance with SENIAM guidelines. Thorough skin preparation was ensured through shaving hair, removal of dead epithelial cells and wiping with isopropyl alcohol swabs. Surface electrodes (Philips, UK [Chapter 5]/Ambu, Denmark [Chapter 6 & 7]) were then placed 2 cm apart over the muscle bellies, and a reference electrode was placed over the patella. Electrode placement was marked with indelible ink to ensure a consistent placement post-trial and between trials.

4.2.2.2 Maximal voluntary contractions

A calibrated load cell was used to measure knee extensor force (N) during voluntary and evoked contractions. The load cell was fixed to a custom-built chair and connected to a noncompliant cuff attached around the participant's right leg, superior to the malleoli. Participants sat upright and strapped into the chair with hips and knees at 90° of flexion and were instructed to cross their arms or hold on to the strap for stabilisation during contractions (Figure 4.1). Following a satisfactory number of submaximal warm-up contractions, each participant was given 3 maximal attempts with verbal encouragement, each separated by 1 min; the highest of which was recorded as the MVC.

Figure 18: Example of experimental set up for all neuromuscular function assessments.

4.2.2.3 Motor nerve stimulation

Single, electrical stimuli (200 μs pulse width) was delivered to the right femoral nerve through surface electrodes (CF3200, Nidd Valley Medical Ltd, North Yorkshire, UK [Chapter 5]/Valutrode, Axelgaard Manufacturing Co, US [Chapters 6 & 7]) using a constant-current stimulator (DS7AH, Digitimer Ltd, Welwyn Garden City, Hertfordshire, UK). In line with previous literature (339,340), the cathode was positioned over the nerve, high in the femoral triangle, while the anode was placed midway between the greater trochanter and the iliac crest. Single stimuli were delivered to the relaxed muscle beginning at 40 mA and increased by 20 mA until a plateau occurred in twitch amplitude and compound muscle action potential (M_{MAX}; 309). Supramaximal stimulation was delivered by increasing the final stimulator output intensity by a further 30% (341). The positions of the stimulating electrodes were also marked with indelible ink to ensure consistent placement. For motor point stimulation, voluntary activation (VA) was assessed using the twitch interpolation method (273) and quantified by comparing the amplitude of the superimposed twitch force (SIT) to the potentiated twitch (Qtw,pot) that was delivered 3 s after each MVIC. The following equation was used offline:

$$\text{VA (\%)} = [1 - (\text{SIT} / Qtw,\text{pot})] \times 100$$

Peripheral contractility was assessed through half relaxation time (HRT) measured from peak force of the Qtw,pot until a 50% decline (342).

4.2.2.4 Transcranial magnetic stimulation

Single and paired pulse TMS were delivered using a magnetic stimulator (Magstim BiStim^2, The Magstim Company Ltd, Whitland, UK) with a double cone coil (D110, The Magstim Company Ltd, Whitland, UK). Initially, the junction of the double-cone coil was placed 1–

2 cm left of the vertex and oriented to induce posterior-to-anterior cortical current (343). Following this, the coil remained on the contralateral hemisphere to the participant's right leg, while locating the optimal stimulation site eliciting motor evoked potentials (MEPs) in the targeted muscle (rectus femoris). The coil position was marked with a semi-permanent marker ensuring consistent placement through experimental visits. To establish the TMS intensity, an isometric contraction of the rectus femoris muscle (10% MVC) was used to stabilise the contraction, while AMT was determined as the lowest stimulation intensity required to evoke peak-to-peak MEP greater than 200 μV in 3 out of 5 consecutive pulses (344). Subsequently, the AMT was recorded as a percentage of maximal stimulator output. All remaining pulses were delivered at 130% of AMT.

4.2.2.4.1 Short Interval Intracortical Inhibition

To quantify SICI, 10 single-pulse stimuli (130% AMT) and 10 short-interval paired-pulse stimuli were delivered to the primary motor cortex. The conditioning and test stimuli for paired-pulse stimulation were set at 80% and 130% AMT respectively, with an interstimulus interval of 3 ms, and subsequent posterior to anterior current flow. SICI was consequently quantified as the ratio of the paired-pulse test stimulus MEP to a monophasic single pulse MEP at 130% AMT (286,287).

4.2.2.5 Data processing

Peak-to-peak amplitudes of the evoked MEPs (MEP_{RAW}) and M_{MAX} were measured offline observing amplified EMG responses (D440 2, Digitimer North America LLC, USA). MEP amplitudes from single pulse stimuli and were averaged from 10 pulses. Duration of the cSP was calculated offline from the onset of the MEP waveform to the return of uninterrupted background EMG activity (345,346). All cSP traces were visually inspected and determined

by a single trained rater to ensure consistency in identifying the onset and offset of EMG suppression across participants and testing sessions. During AMT, MEP and cSP assessments, participants maintained a low-level voluntary contraction of approximately 10% MVC. This contraction intensity was selected to provide a stable background EMG signal while minimising fatigue, discomfort and between-trial variability that may occur during repeated higher-intensity contractions. This was particularly important because the present testing battery required multiple contractions and repeated stimulation for AMT, MEP, SICI and cSP outcomes. The use of a light voluntary contraction is consistent with common TMS protocols, where AMT and cSP measures are typically assessed during low-level contractions of approximately 10 – 20% MVC (252,254,346). Signals were amplified: gain × 1000 for EMG and × 300 for force (CED 1902, Cambridge Electronic Design, Cambridge, UK), band-pass filtered (EMG only: 20–2000 Hz), digitized (4 kHz; CED 1401, Cambridge Electronic Design), acquired and analysed off-line (Signal v8.0, Cambridge Electronic Design).

Part 2:
A between-day test-retest reliability study of TMS and MNS measures.

4.3 Introduction

Non-invasive brain stimulation methods such as TMS are widely used to evaluate corticospinal excitability and inhibition, providing valuable insights into the integrity of the motor system (246). Single-pulse TMS enables the assessment of neurophysiological markers including MEP amplitude and cSP duration, while paired-pulse protocols such as SICI offer insight into GABAergic inhibitory circuits (347,348). However, multiple methodological factors influence the reliability of TMS-based metrics, including coil positioning, target muscle selection, stimulation parameters, contraction intensity and the number of stimuli delivered over the primary motor cortex (349-352). As such, it is unsurprising that studies assessing corticospinal excitability and inhibition commonly report moderate to high test-retest reliability, with intraclass correlation coefficients (ICCs) ranging from 0.52 to 0.92 depending on the protocol and outcome (353-355).

The selection of target muscle for TMS is particularly important when considering lower-limb protocols. Unlike upper-limb muscles, the cortical representations of proximal leg muscles, such as the quadriceps, are located deeper within the interhemispheric fissure (356). These representations are smaller and more medial, often requiring the use of a double-cone coil and voluntary contraction to reliably elicit MEPs (357). The quadriceps, and in particular the rectus femoris, play a crucial role in locomotor tasks such as walking, running, and jumping, and are therefore highly relevant to both athletic performance and clinical populations (357). Their central role in force production and movement control also makes them an ideal muscle group for assessing corticomotor output. However, challenges in targeting the motor representation and increased susceptibility to coil movement artefacts may contribute to variability in responses.

Compared to upper-limb TMS, relatively few studies have explored the test–retest reliability of TMS measures in lower-limb muscles. Recent work has reported a wide range of outcomes, with reliability influenced by contraction intensity, stimulation protocol, and the experience of the experimenter (354,355). For example, rectus femoris MEP amplitudes have shown excellent reliability at moderate contraction levels (ICC = 0.8 – 0.9 at 20 – 60% MVC), but reliability deteriorates with maximal efforts (ICC ≈ 0.59; 56). In the knee extensors, O'Leary et al. (351) reported good between-day reliability for single-pulse MEP amplitude and cSP duration (ICC > 0.82), but only moderate reliability for SICI (ICC = 0.67), likely reflecting the greater sensitivity of paired-pulse protocols to methodological inconsistencies.

These findings highlight that corticomotor excitability and inhibition in the lower limbs are inherently more variable than upper-limb outcomes. Consequently, there is a strong need for robust baseline characterisation when using lower-limb TMS metrics. The experience and consistency of the experimenter play a critical role in minimising inter-session and inter-operator variability, particularly with lower-limb muscles where optimal coil placement is more challenging to maintain (357). Familiarisation sessions, along with procedures to determine smallest detectable change (SDC) or standard error of measurements (SEM), have been strongly recommended to improve data quality (355).

The purpose of the present study was therefore to evaluate the reliability of key TMS-derived variables when applied to the rectus femoris muscle. By conducting repeated baseline testing under standardised conditions, this chapter aims to determine whether consistent measurement of corticospinal excitability (MEP_{RAW}, MEP/M_{MAX}), inhibition (SICI, cSP), and VA can be achieved in a lower-limb context. Although the reliability of several TMS-derived measures has previously been reported, reliability estimates are highly protocol-specific and depend on methodological factors such as stimulation parameters, target musculature, contraction

intensity, coil placement and analysis procedures (351,352,356,357). Consequently, reliability estimates derived from previous studies cannot be assumed to apply directly to the methodological framework used within the present thesis. Establishing the test–retest reliability of the specific assessment battery used was therefore necessary to quantify typical error and allow meaningful interpretation of subsequent experimental findings. For these reasons, a preliminary reliability assessment was conducted to characterise the stability of the neurophysiological measures employed in the later chapters.

4.4 Methods

4.4.1 Participants

Twelve healthy males (age: 32 ± 6 yrs, stature: 179 ± 5 cm, BM: 83 ± 15 kg) without any self-reported neurologic conditions or lower limb disabilities, or disease were recruited at the University of Essex. While the specific participant population differed from that used in the later experimental chapters, the reliability assessment aimed to characterise the stability of the measurement techniques rather than population-specific physiological responses. In addition, the decision to include male participants only, was to reduce potential variability associated with sex-related physiological differences that may influence neurophysiological measures, including hormonal fluctuations across the menstrual cycle that have been shown to affect corticospinal excitability and intracortical inhibition (373 Restricting the sample to males therefore allowed a more controlled evaluation of the reliability of the measurement techniques prior to their application in the upcoming experimental studies.

Individuals who suffer from a chronic disease, take medication for an underlying condition (358) or sustained an injury in the last 3 months were not eligible to take part of the present study. This was determined using an institutional screening form for neurostimulation

(Appendix 5). Participants were excluded where the following is applicable: History/family history of seizures or syncope, heart diseases, neurological or psychiatric diseases, epilepsy, migraine, sleep deprivation, alcoholism, pregnancy. In addition, participants with implanted medical devices (e.g., pacemakers, cochlear implants) or ferromagnetic implants, those currently taking tricyclic antidepressants, neuroleptic agents or any drug that might lower seizure threshold were excluded. The previous statements are supported by the safety and recommendations for TMS use in healthy subjects and patient populations (358) and as per the standard St Mary's University and University of Essex neuromodulation screening committee guidelines. Finally, informed consent was obtained in alignment with institutional ethical approval.

4.4.2 Sample size justification

Sample size was determined using a web app developed by Arifin (2018) and available at https://wnarifin.github.io/ssc/ssicc.html. Using recent TMS reliability studies in healthy participants (359,360) and a minimum acceptable reliability ICC of 0.6 (standard for "good" reliability [361]), an alpha level of 0.05, 80% power and two repetitions per participant the recommended sample size was twelve.

4.4.3 Study design

Prior to familiarisation (> 24 h), participants were provided with an information sheet and then screened for any self-reported neurological disorders and contraindications arising from the use of TMS (323) and a physical activity questionnaire. Participants admitted through the screening process proceeded to a familiarisation session, to assess baseline measurements, and participant comfort with the observational protocol.

Participants were asked to refrain from vigorous physical activity, consuming alcohol, and caffeine or smoking for 24 h prior to each study session. Prior to commencing data collection, participants arrived at the laboratory for a familiarisation session, during which they completed all outcome measures to acquaint them with the assessment procedures and minimise later learning effects. Following familiarisation, participants reported to the laboratory for 2 further experimental days, spaced by a minimum of 48 h and a maximum of 2 weeks apart.

4.4.4 Neuromuscular function

4.4.4.1 Electromyography

For electromyography, please see ‘General Methods’ section 4.2.2.1.

4.4.4.2 Maximal voluntary contractions

For full procedures of the MVC, please see ‘General Methods’ section 4.2.2.2.

4.4.4.3 Motor nerve stimulation

For motor nerve stimulation, please see ‘General Methods’ section 4.2.2.3.

4.4.4.4 Transcranial magnetic stimulation

For transcranial magnetic stimulation, please see ‘General Methods’ section 4.2.2.4.

4.4.4.5 Data processing

For data processing methods, please see ‘General Methods’ section 4.2.2.5.

4.4.5 Statistical analysis

All data analyses were performed using SPSS for Windows (v 23; SPSS Inc., USA). The inter-day reliability of all variables was assessed using ICC, 95% confidence intervals (CI), and coefficients of variation (CV). All ICC values were interpreted as follows: ≤ 0.39 = poor; 0.40–

0.59 = fair; 0.60–0.74 = good; 0.75–1.00 = excellent (325). To quantify absolute reliability and determine the threshold for meaningful change, the SEM and typical error (TE) were calculated. The SEM was derived from the SD and ICC using the following equation:

$$SEM = SD \times \sqrt{1 - ICC}$$

The TE was calculated from the SD of the test–retest difference scores using the following equation:

$$TE = \frac{SDdiff}{\sqrt{2}}$$

Where *SDdiff* represents the standard deviation of the difference scores between repeated measurements. Session changes exceeding TE × 1.5 were then used to indicate potentially meaningful change beyond measurement noise.

4.5 Results

The reliability analysis revealed that most neurophysiological and performance measures demonstrated excellent test-retest reliability. Findings are summarised below in Table 4.1.

Table 4.1: Test–retest reliability of neurophysiological and neuromuscular function measures.

Variable	Trial 1	Trial 2	Difference	Between test *p* value	Classification	ICC (95% CI)	CV (%)	TE (x 1.5)	SEM
MEP_{RAW} (mV)	1.92 ± 1.04	1.80 ± 0.83	-0.12	0.75	Excellent	0.77 (0.21 – 0.94)	24.61	0.86	0.55
MEP/M_{MAX}	0.34 ± 0.19	0.36 ± 0.15	0.02	0.74	Excellent	0.87 (0.55 – 0.96)	17.94	0.12	0.12
SICI	0.18 ± 0.10	0.17 ± 0.10	-0.01	0.90	Fair	0.53 (–0.64 – 0.86)	33.11	0.12	0.03
cSP (s)	0.13 ± 0.02	0.14 ± 0.02	0.01	0.38	Excellent	0.75 (0.14 – 0.93)	6.32	0.02	0.01
AMT (%)	33 ± 6	33 ± 6	0.08	0.97	Excellent	0.97 (0.90 – 0.99)	3.07	2.41	5.22
M_{MAX} (mV)	6.19 ± 2.14	5.23 ± 1.07	-0.96	0.28	Excellent	0.89 (0.60 – 0.97)	14.19	1.43	1.42
MVC (N)	650.11 ± 110.91	631.74 ± 116.51	-18.36	0.70	Excellent	0.94 (0.79 – 0.98)	5.00	54.10	82.63
VA (%)	94.42 ± 4.03	95.75 ± 4.14	1.33	0.43	Excellent	0.84 (0.45 – 0.95)	1.61	0.02	2.43
HRT (ms)	0.08 ± 0.03	0.07 ± 0.03	-0.004	0.71	Excellent	0.92 (0.72 – 0.98)	13.00	0.36	0.02

4.6 Discussion

Most neurophysiological and neuromuscular measures demonstrated excellent between-day test–retest reliability, with ICC values exceeding 0.75 for MEP_{RAW}, MEP/M_{MAX}, cSP, MVC, VA, and HRT. Notably, AMT and M_{MAX} displayed the highest reliability (ICC = 0.97 and 0.89, respectively), while SICI showed fair reliability (ICC = 0.53), consistent with previous reports of greater variability in intracortical inhibition.

The TMS-derived variables in the present study generally demonstrated good inter-day reliability, aligning with prior literature on knee extensors. In particular, rectus femoris MEP_{RAW} showed excellent reliability (ICC ~ 0.77), which is comparable to reports in vastus lateralis where between-

day MEP ICC values around 0.82 have been observed (362). Notably, normalising MEP_{RAW} to the M_{MAX} improved its consistency (ICC ~ 0.87, CV ~ 18%), an approach supported by other studies showing better reliability for MEPs expressed relative to M_{MAX} (359). While raw MEP amplitudes may be sufficiently stable for within-session comparisons (360,363), accounting for peripheral excitability via M_{MAX} becomes critical for reducing variability across days, especially in longitudinal designs. This suggests that accounting for peripheral excitability can reduce inter-session variability in corticospinal excitability measures. In contrast, SICI exhibited fair reliability across days in the present data (ICC ~ 0.53). This finding is in line with the wider literature: for example, O'Leary et al. (351) found SICI in the active quadriceps had lower between-day repeatability (ICC ~ 0.67) than other TMS measures, and more recent work has reported SICI ICC values often below 0.6 in lower-limb muscles (360). Such variability in SICI likely reflects its sensitivity to slight changes in primary motor cortex inhibition state and TMS targeting, highlighting that paired-pulse intracortical metrics are inherently less stable than single-pulse measures (364). By contrast, the cSP duration proved highly reliable between days (CV ~ 6%, ICC ~0.75) in this study, consistent with previous reports of excellent reliability for CSP in knee extensors (ICC ≥ 0.82; 362). The AMT was the most reproducible cortical measure (ICC ~ 0.97, CV ~ 3%), indicating minimal day-to-day fluctuation. This reinforces that AMT can be measured very consistently in young healthy adults (365), especially with standardised criteria for EMG response. Overall, these results demonstrate that TMS metrics like MEP_{RAW}, MEP/M_{MAX}, CSP, and AMT can be obtained excellent inter-session reliability in the rectus femoris, whereas SICI is more variable; a pattern echoed by multiple studies on lower-limb musculature (351,366).

The peripheral nerve stimulation and muscle performance measures in this study showed consistently high inter-day reliability, which agrees with published findings in similar populations. Maximal force of the quadriceps was very reproducible (ICC ~ 0.9+), as was VA

assessed via the interpolated twitch technique (ICC ~ 0.84, CV ~ 1 – 2%). These values closely match those reported by Park and Hopkins (367), who found between-session ICCs of ~ 0.92 for quadriceps MVC torque and ~ 0.86 for the central activation ratio (a VA index) in healthy young males. Such excellent reliability confirms that with familiarisation and consistent effort, maximal force production and voluntary neural drive can be reliably reproduced on separate days. Likewise, the electrically evoked muscle responses were very stable. The M_{MAX} amplitude showed excellent reliability (ICC ~ 0.89, CV ~ 14%), in line with prior studies reporting ICCs on the order of 0.8 – 0.9+ for quadriceps M_{MAX} (364,3380). This consistency indicates a reliable stimulation technique and stable peripheral excitability of the muscle and nerve across sessions. A high reliability for twitch contractile properties such as HRT (ICC ~ 0.92) was also observed. This is expected because intrinsic muscle twitch kinetics tend to remain unchanged in the absence of interventions. Indeed, recent work has identified HRT and related twitch times as among the most reliable contractile parameters in the quadriceps (368).

4.7 Conclusion

Taken together, the results demonstrate strong between-day reliability for both corticospinal and peripheral neuromuscular measures in the rectus femoris within the present sample. Cortical excitability and inhibition metrics such as MEP_{RAW}, MEP/M_{MAX}, cSP duration, VA and AMT all showed excellent reproducibility. While SICI displayed greater variability, this is consistent with the wider literature and reflects the known sensitivity of paired-pulse protocols to subtle physiological and methodological fluctuations. On the peripheral side, MVC, VA, M_{MAX}, and HRT also demonstrated excellent reliability, reaffirming that neuromuscular function can be consistently assessed between days when testing procedures are standardised and participants are familiarised. These findings demonstrate acceptable test–

retest reliability of the neurophysiological measures within the present sample and provide an estimate of the typical measurement error associated with the experimental techniques used in subsequent chapters.

Chapter 5:

Modulations of corticospinal and peripheral excitability, and serum UCH-L1, following hypo-hydration and rehydration.

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5.1 Introduction

RWL strategies in combat sports typically involve acute fluid restriction or active dehydration, leading to a reduction in total body water (316). The process of intracellular fluid loss, and the resulting hypertonic extracellular environment, is termed intracellular dehydration or hypertonic hypovolemia (91-93) and may influence both central and peripheral nervous system function. As noted in Chapter 3, athletes engaging in RWL often report symptoms that overlap with concussion, raising the possibility that hypo-hydration may affect neurocognitive and neuromuscular function. However, the physiological basis for these overlaps remains poorly understood, particularly under *in-vivo* conditions. This chapter also builds on the biomarker literature outlined in Chapter 2. UCH-L1 is a neuron-enriched protein that has gained attention as a blood-based biomarker of neuronal injury and has been incorporated, alongside GFAP, into clinical decision-support tools for the evaluation of suspected mild traumatic brain injury (369-372). In combat sport settings, the interpretation of such biomarkers may be complicated by the fact that athletes are often exposed to exercise, heat stress, dehydration, RWL and RHIs within a similar time frame. Therefore, determining whether heat-induced hypo-hydration and rehydration alter circulating UCH-L1 in the absence of a diagnosed head injury is important for understanding whether non-traumatic physiological stressors may act as potential confounders when interpreting blood-based brain injury biomarkers in sport.

While hypo-hydration is known to impair endurance performance through mechanisms such as cardiovascular strain (95), reduced aerobic metabolism (96), and impaired thermoregulation (97), its specific effects on neuromuscular function and brain-to-muscle communication are less clear. Evidence for a loss of maximal strength remains mixed (326), likely due to methodological inconsistencies across dehydration studies, including variation in fluid restoration protocols (87,188), dehydration type (active vs. passive, or fluid restriction vs.

sweat loss) (317,373), and the outcome measures used to assess neuromuscular function (15,16,87,113).

The present chapter therefore builds on the survey findings in Chapter 3 by experimentally manipulating hydration status to evaluate its impact on corticospinal excitability, VA, and MVC under controlled conditions. By doing so, it seeks to clarify the extent to which hypo-hydration alone may contribute to central fatigue or impaired neuromuscular performance, and whether fluid restoration can mitigate these effects.

Relatively few studies have used TMS and MNS to provide mechanistic reasoning for the reduction (or lack of change) in maximal strength after hypo-hydration (for review, see 78). While it has been reported that VA is unaffected (16,93,94), Bowtell et al. (87) speculated that hypo-hydration (3% BMR) resulted in different neural strategies (relative to the euhydrated group) to preserve motor output, as observed through unchanged corticospinal inhibition and increased sarcolemma excitability (during an MVC). It has been proposed that hypo-hydration could lead to a reduction in neuromuscular function, but compensatory mechanisms (i.e., reduced cSP) might occur to minimise force loss during an MVC (374), but this hypothesis is yet to be tested. In addition, Barley et al. (16) reported a reduction in high-intensity efforts 3 h and 24 h after 5% BMR despite *ad libitum* fluid and food intake, indicating the need for understanding a time-course of recovery, and associated mechanisms, after dehydration and subsequent rehydration. Indeed, weight-classification sports vary in recovery times (e.g., judo *vs.* professional boxing), requiring athletes to compete as quickly as 2 h or as far as 36 h after a weigh-in. Therefore, it is crucial to investigate if a) the proposed compensatory mechanisms of reduced neuromuscular function are observed after dehydration of greater severities e.g., 5% BMR and b) what the recovery time-course of such changes/compensations are after fluid restoration.

While TMS and MNS provide an indirect assessment of neural transmission efficacy between the brain and skeletal muscle, blood-based biomarkers of the CNS may provide a mechanistic reasoning for the observed changes in neural output. The BBB plays a vital role in the tight regulation of exchange of ions, molecules and cells between the CNS and peripheral circulation (375,376). Watson et al. (376) reported that plain-water ingestion equal to the volume lost during exercise, attenuated the increase of Calcium Protein Binding b (S100b) in the peripheral bloodstream, indicating that rehydration might limit the increase in exercise-induced BBB permeability, and the associated dehydration process. It is possible that the osmotic pressure caused by hyperosmolality of the extracellular fluid causes a shift in fluids from the brain to the peripheral bloodstream (377-379) and the shrinkage of the endothelial cells of the BBB, leading to transient opening of the tight junctions (380). Unlike S100b, Ubiquitin C-terminal hydrolase -L1 (UCH-L1) is highly and specifically expressed in neurons and is pivotal in the removal of misfolded or oxidised proteins (381). To date, it is unknown if severe hypo-hydration, or variation in the dehydration and rehydration time-course, affects circulating peripheral UCHL-1, which would provide evidence of increased BBB permeability, as well as reduced neuronal integrity. Such a reduction may be synonymous with decreased NM function, or the proposed compensatory changes observed with TMS and MNS after hypo-hydration (374). Furthermore, if blood biomarkers related to BBB integrity are affected by hypohydration, this is likely to have important clinical implications, since their contribution to the identification of traumatic brain injury would be more questionable if fluid imbalance is likely to have occurred in close proximity to the measurement (382).

The primary aims of the study were to investigate; (a) the corticospinal and peripheral responses to hypo-hydration (intracellular dehydration) and (b) the time-course of NM function resulting from gradual and rapid rehydration. A secondary aim of the study was to investigate

if UCH-L1 (a systemic blood marker linked to BBB and neuronal integrity) concentration is affected by intracellular dehydration and subsequent rehydration strategies. It was hypothesised that after dehydration, there would be a reduction in maximal strength, muscle contractility and corticospinal inhibition, increase in sarcolemma excitability and UCH-L1 concentrations. Therefore, it was hypothesised there would be a reduction in MVC, muscle twitch parameters and an increase in the TMS-evoked silent period and M_{MAX}, after intracellular dehydration.

5.2 Methods

5.2.1 Participants

A total of 12 healthy male participants (age: 29 ± 7 yr; BM: 86.4 ± 9.5 kg; stature: 185 ± 12 cm) were assigned (via a randomised crossover design) to undergo a control or experimental protocol, intended to induce a BMR of ~ 5%. Male participants were recruited to reduce biological variability within this initial experimental study. Sex-related differences in thermoregulation, total body water, sweat rate, substrate metabolism and hormonal profile may influence physiological responses to exercise-heat stress and dehydration (77,383,384). In addition, ovarian hormone fluctuations across the menstrual cycle may influence corticospinal excitability, intracortical inhibition and motor cortical plasticity, which may increase between-day variability in TMS-derived outcomes if menstrual cycle phase or hormonal contraceptive use are not controlled (385,386). Therefore, the inclusion of males only was intended to improve internal validity and reduce variability in the neurophysiological and thermoregulatory responses to the protocol. Ethical approval was provided by the institutional ethics committee of St Mary's University College (SMEC_2018-19_055) which was conducted in accordance with the 2013 Helsinki declaration, besides pre-registration on an

open database. All participants provided written informed consent before data collection. Participants were required to be physically active, free from injury (6 months), not taken part in outdoor hot-weather training (3 months) and have no pre-existing medical conditions. All exercise trials were separated by a minimum of 7 days to minimise tiredness from the previous trial. Participants were requested to keep a personal food diary from the night before the trial, then replicate this for the subsequent trials. It was also requested that they avoid strenuous exercise, and the consumption of nutritional supplementation (e.g., caffeine) and alcohol.

5.2.2 Experimental design

Participants took part in a randomised crossover study, with a control condition (CON), heat-induced dehydration with rapid rehydration (RHY2) and heat-induced dehydration with gradual rehydration (RHY24) condition. The control trial was the completion of a prolonged submaximal cycling (Monark, Varburg, Sweden) protocol (3 h at 60 W) in temperate conditions (25 ± 5 °C, 30 ± 3% relative humidity), whereby electrolyte fluid intake was permitted throughout, to account for body mass reduction every 30 min. Participants undergoing both dehydration trials completed the same exercise protocol twice, but in an environmental chamber (Sporting Edge, UK) under a hotter dry-bulb temperature of 45 ± 3 °C and relative humidity of 35 ± 3%. During this time, participants were restricted from fluid intake, to induce a 5% reduction of body mass through dehydration (387).

Pre-testing dietary intake was controlled in the form of a standardised breakfast meal, consisting of rice cereal, milk, bread, butter, jam, and orange juice (Tesco, Welwyn Garden City, Hertfordshire, UK) and water (10 mL/kg), providing 20% of their estimated energy requirements (EER) and 1 g of carbohydrate/kg body mass (388,389). Daily energy expenditure was estimated using the equations of Mifflin-St Jeor (390) with a physical activity

factor of 1.8 (403,404). Post-testing standardised meals consisted of a pasta or sandwich with a protein source, crisps and biscuits (Tesco, Welwyn Garden City, Hertfordshire, UK). All post-testing meals were homogenous, providing energy from 35 to 40% of their EER, and 14, 33 and 53% from proteins, fats and carbohydrates, respectively. All meal selections were provided to the participants and replicated for each trial. A text message was sent to each participant to reiterate the eating and drinking protocol after the trial. In addition, communication was maintained with each participant to ensure that there was no confusion, and the required fluid volume and meal selections were consumed at the correct times. No deviations for the meals consumed outside of the laboratory (i.e., breakfast and dinner) were reported.

Before each protocol, a post-breakfast urine sample, nude body mass, NM function, capillary and venous blood samples were obtained. Participants who were identified to be in a mildly dehydrated state (> 600 mOsml/kgH_2O) prior to the protocol, were rehydrated with 250 mL of water followed by 30 min of seated rest before being re-tested and allowed to begin the protocol. Prior to commencement of the protocol, participants inserted a rectal thermometer (Edale Instruments Ltd., Cambridge, UK) 10 cm beyond their anal sphincter for assessment of core temperature. In addition, baseline resting blood pressure, heart rate, perceived thirst (391) and thermal comfort (392) were assessed.

A cycling power output of 60 W was chosen to increase core temperature and promote sweating but minimise muscle glycogen depletion (387,393). An absolute workload was used rather than exercise intensity relative to lactate threshold because the primary aim was to reproduce a standardised dehydration stimulus and body mass reduction protocol (387), rather than to match relative metabolic strain across participants. Upon completion, participants rested for 60 min in a thermo-neutral environment (19 ± 3 °C and ~ 30% relative humidity), thereby

facilitating core temperature to return to baseline. Throughout each protocol (every 30 min), participants were assessed for nude body mass (after being towelled down), whole body sweat rate (WBSR), rectal core temperature, blood pressure, heart rate and ratings of thermal comfort, perceived exertion, and thirst. In the case of (1) heart rate exceeding 180 beats/min for five consecutive minutes, (2) rectal temperature exceeding 39.5 °C, (3) displaying signs or symptoms of an exercise-induced heat illness (394), or (4) a request to stop exercising, the protocol ceased, and participants were placed in a thermo-neutral environment to allow cooling and recovery (117). Rectal temperature was used as the primary index of core temperature throughout the protocol. Intramuscular temperature was not measured because this would have required invasive needle thermometry, increasing participant burden, infection risk and ethical complexity in a study that already included repeated neuromuscular testing, venous and capillary blood sampling, rectal thermometry and exercise-heat exposure.

In the case of participants dehydrating too rapidly (i.e., reaching near to 5% dehydration target ahead of schedule; > 1.75 L/h), participants consumed a small volume of water (250 ml) during exercise, to ensure only 5% dehydration. Participants who were not predicted to reach a target loss of 5% body mass within the 3 h time frame (whole body sweat rate of < 1.25 L/h) were instructed to wear an additional layer of clothing (Track suit, Everlast, UK; Sweat suit, Everlast, UK). Participants were permitted to urinate post-protocol, following a BM measurement, to offset fluid losses unrelated to heat-stress.

5.2.2.1 Rehydration protocol

During the control trial, participants were permitted to consume an electrolyte beverage throughout the protocol, which was calculated as: 1.5 × BMR (as observed in the 30 min interval body-mass recordings). Participants undergoing rapid rehydration (RHY2) aimed to

achieve complete rehydration in 120 min through the consumption of an electrolyte beverage (25 mL/kg/h) in tapered stages. Thereafter, they consumed 5.5 mL/kg/h for the remaining 8 h. The second experimental trial rehydrated over a 24 h period (RHY24) and were instructed to consume 15 mL/kg/h of the electrolyte beverage in tapered stages in the first 120 min, followed by 7.5 mL/kg/h for the remaining hours. Consumption of fluids began 60 min after completion of the dehydration protocol to allow for cooling and assessment of neuromuscular function, except the control group, who were able to drink ad libitum. Rehydration electrolyte beverages were consumed in the form of water (obtained from a cool-water dispenser) and a commercial rehydration tablet, consisting of 2% CHO, 30 mmol/L sodium, 12.34 mmol/L potassium and 24.68 mmol/L chloride (Oral Rehydration Solutions, Clinova Ltd., Southampton, UK). Assessment of fluid, electrolyte and dietary intake throughout the post-testing period was achieved through measurement of urine volume, plasma volume, electrolytes and the provided dietary choices. A summary of the protocol can be found in Figure 5.1.

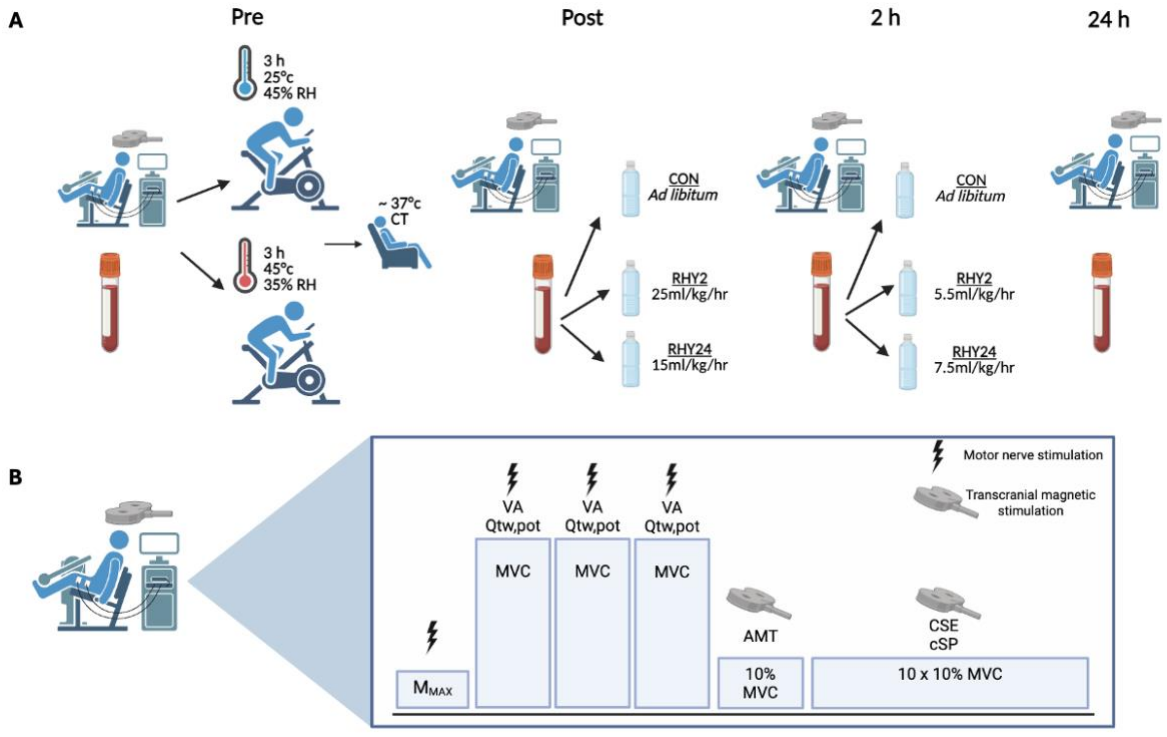

Figure 5.1: A) Experimental design for all three conditions and, B) breakdown of transcranial and peripheral motor nerve stimulation protocol. *AMT* active motor threshold, *cSP* cortical silent period, *CT* core temperature, *MEP* motor evoked potential, M_{MAX} maximum compound muscle action potential, *MVC* maximum voluntary contraction, *Qtw,pot* potentiated twitch, *RH* relative humidity, *VA* voluntary activation

5.2.3 Neuromuscular function

For details of the study protocol related to TMS, MNS and neuromuscular function, please see Section 4.2.4 in 'General Methods – Part 1'.

5.2.4 Blood measures

Vacutainers (6 mg) containing lithium heparin and serum separating gel were used to collect venous blood samples, which were centrifuged either immediately (plasma) or after 30 min (serum), for 15 min at 1000 g at 4 °C (Mikro 220 R Hettich, Tuttlingen, Germany). Plasma and serum supernatant were aliquoted to microcentrifuge tubes and stored at − 80 °C until analysis. Serum UCH-L1 concentration was determined using a commercially available enzyme immunoassay kit (abx251226, Abbexa Ltd, Cambridge, UK) per the manufacturer's instructions. The detection range was 100–2300 pg/ml with a sensitivity of 47 pg/mL, with inter- and intra-assay coefficients of variation below < 10%. All assays were run in duplicate. To calculate PV change and correct concentrations for any changes in PV with dehydration, a capillary blood sample was taken pre- and post-exercise and haematocrit was measured using a micro-haematocrit centrifuge and reader (Hawksley, Sussex, UK). All blood samples were obtained after 30 min rest in a semi-reclined position, with arm height constant. Percent change in plasma volume (PV) was calculated using the following formula:

$$\%\ \text{change in PV} = \left(\frac{100}{100\ Hct\ pre}\right) \times 100\left(\frac{[\text{Hct pre} - \text{Hct post}]}{\text{Hct post}}\right)$$

(395,396).

5.2.5 Urine measures

For the assessment of baseline hydration status (urine osmolality) and fluid retention, participants were instructed to collect midstream samples (50 ml) of urine 2 h post-breakfast and all urine (24 h urine volume) post-dehydration. A digital refractometer (Osmocheck, Vitech Scientific Ltd, West Sussex, UK) was used to provide an indicative reading of urine osmolality (U_{osm}) with a manufacturer's reported testing accuracy of ± 20 mOsml/kgH^2O and between-run coefficient (CV) of 0.3%. Participants were also requested to report fluid intake pre- and post-trial completion.

5.2.6 Statistical analysis

All data were assessed for normality and sphericity using a visual inspection of the Q-Q plots and Mauchly's test of sphericity, respectively. Where data were parametric, the dependent variables were assessed using a two-way (Condition [CON, RHY2, RHY24] x Time [PRE, POST, POST2, POST24] repeated measures ANOVA. Mid-protocol variables were similarly analysed, but with a difference in time points (Condition [CON, RHY2, RHY24] x Time [Baseline, 30, 60, 90, 120, 150, 180, Rest]). Follow-up Bonferroni post hoc analyses were conducted if a significant main and interaction effect was observed. Data are presented as mean ± SD, and all statistical analyses were carried out using SPSS for Macintosh v28 (SPSS Inc., USA), with a significance level of $p \leq 0.05$ applied. Effect sizes are reported as partial eta squared (η_p^2) with a threshold of 0.01, 0.06 and 0.14 as a small, medium and large effect, respectively (397).

5.3 Results

5.3.1 Neuromuscular function

MVC reduced significantly across time ($F_{(3,33)} = 34.9$, $p < 0.001$, $\eta_p^2 = 0.76$) and a condition*time interaction ($F_{(6,66)} = 2.3$, $p = 0.044$, $\eta_p^2 = 0.17$) was observed for MVC. Post-hoc analyses determined no significant changes in the control group across time points; however, MVC significantly reduced between PRE and POST in the RHY2 condition (−19.3%, $p < 0.001$) and RHY24 condition (−19.1%, $p = 0.001$). There was also a significant improvement of MVC between POST and POST2 (8.7%, $p = 0.004$) in the RHY2 condition only (Figure 5.2).

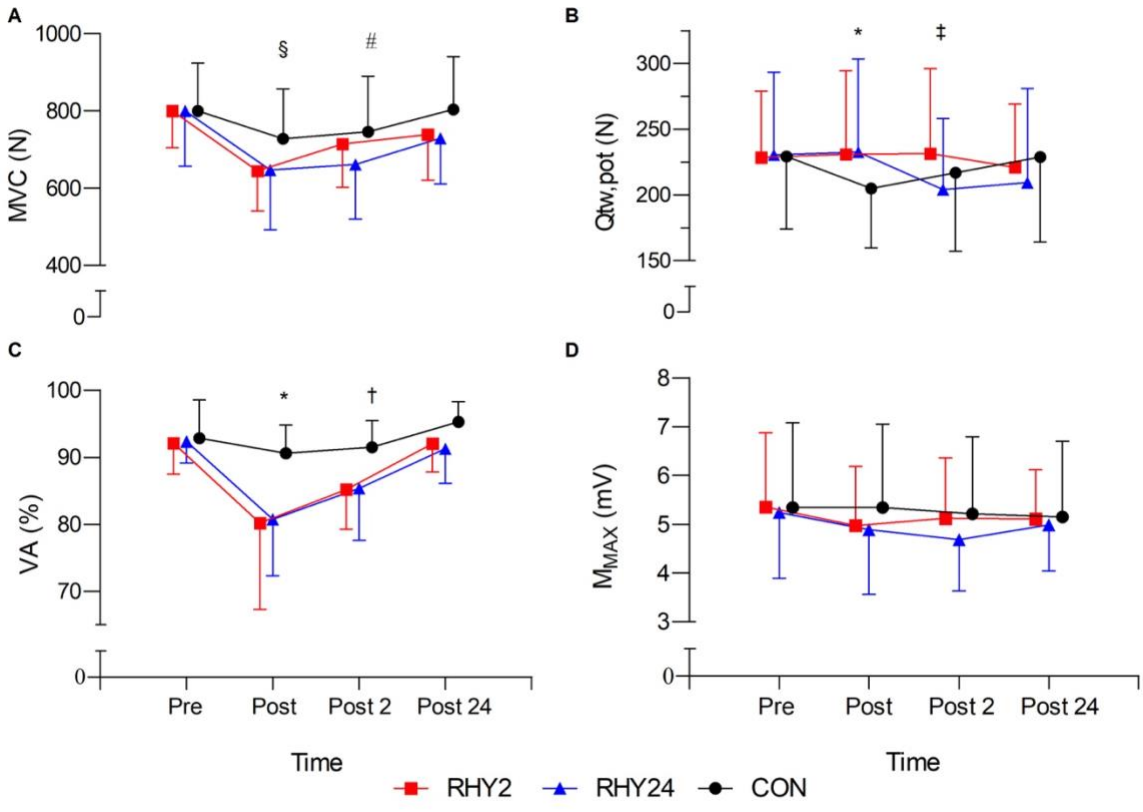

Figure 5.2: Maximal voluntary contraction (MVC; A), Potentiated twitch (Qtw,pot; B), Voluntary activation; C), and Maximum compound muscle action potential (M_{MAX}; D) across

time-points for each condition. Main interaction effects: Between condition differences for: * CON *vs.* RHY2, † CON *vs.* RHY24, ‡ RHY2 *vs.* RHY24. Within condition difference for: § RHY2 and RHY24 only, # RHY2 only. Time points are jittered for visual clarity.

A condition*time interaction effect was observed for Qtw,pot ($F_{(6,66)} = 4.1$, $p = 0.002$, $\eta_p^2 = 0.27$), with post-hoc analyses demonstrating a significant reduction in Qtw,pot at POST during CON relative to the RHY2 condition (−11.5%, $p = 0.045$), and at POST2 during RHY24 relative to the RHY2 condition (−10.5%, $p = 0.015$).

A main effect of condition ($F_{(2,22)} = 6.6$, $p = 0.006$, $\eta_p^2 = 0.37$), time ($F_{(3,33)} = 26.183$, $p < 0.001$, $\eta_p^2 = 0.70$) and condition*time interaction effect ($F_{(2.548, 28.029)} = 3.3$, $p = 0.043$, $\eta_p^2 = 0.23$) were observed for VA. Post-hoc analyses showed VA at POST was lower during RHY24 *vs.* CON (−10.5%, $p = 0.037$), and at POST2, lower during RHY2 *vs.* CON (−6.3%, $p = 0.017$). No main effects ($p = 0.407$) or interactions ($p = 0.593$) were observed for $M_{MAX.}$

5.3.2 Corticospinal measures

There was a main effect of time ($F_{(3,33)} = 7.3$, $p < 0.001$, $\eta_p^2 = 0.40$) and interaction effect ($F_{(6,66)} = 2.8$, $p = 0.018$, $\eta_p^2 = 0.20$) for MEP_{RAW}. Post-hoc analyses demonstrated a higher MEP_{RAW} at POST2 during RHY2, relative to the RHY24 condition (8.9%, $p = 0.02$), and a lower MEP_{RAW} at POST24 during RHY24, relative to the CON condition (−10.3%, $p = 0.018$) (Figure 5.3A). In addition, there were within-condition differences at PRE *vs.* POST for RHY2 (−20.9%, $p = 0.025$) and RHY24 (−20.2%, $p = 0.045$) (Figure 5.3A). No condition effect was observed ($p = 0.166$).

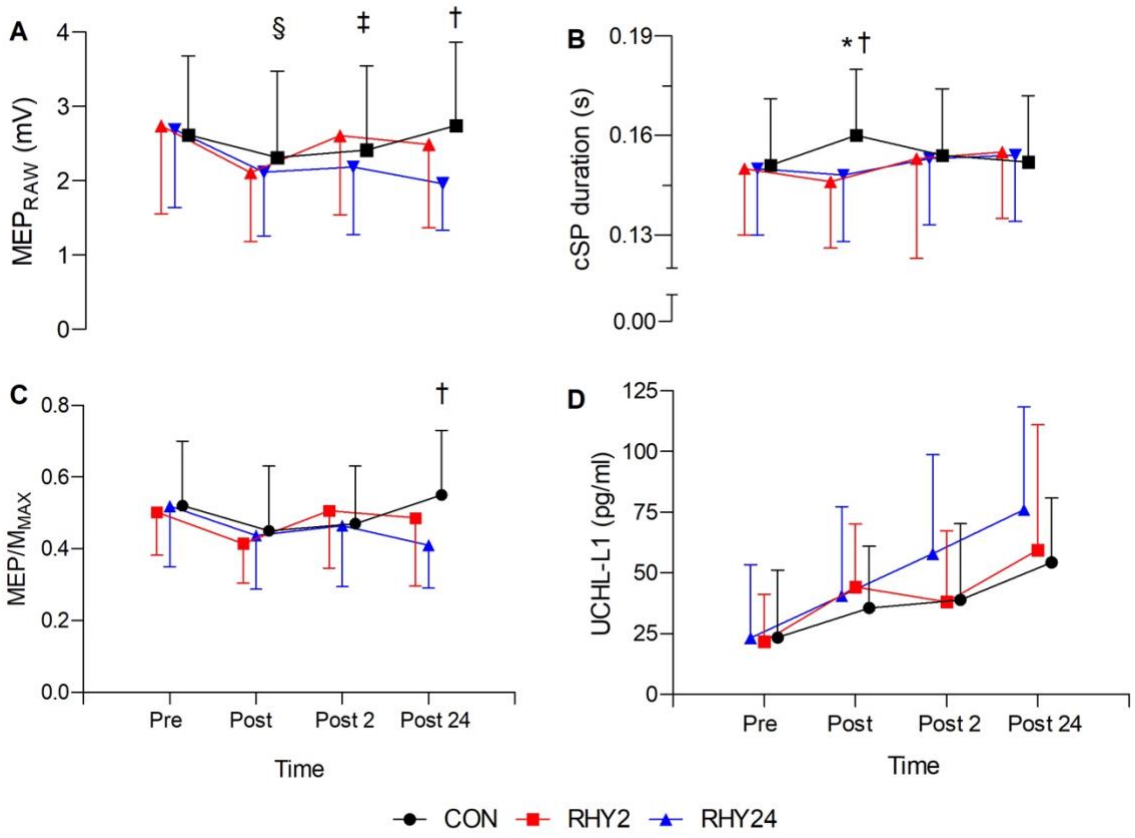

Figure 5.3: Motor evoked potential amplitude (MEP_{RAW}; A), Cortical silent period (cSP; B), MEP/M_{MAX} ratio (C), and UCH-L1 (D; $n = 10$) across time-points for each condition. Main interaction effects: Between condition differences for: * CON *vs.* RHY2, † CON *vs.* RHY24, ‡ RHY2 *vs.* RHY24. Within condition difference for: § RHY2 and RHY24 only. Time points are jittered for visual clarity

An interaction effect was observed for cSP ($F_{(6,66)} = 5.4$, $p < 0.001$, $\eta_p^2 = 0.33$). Post-hoc analyses showed a significant lengthening in POST exercise cSP duration during the CON condition (0.16 ± 0.006 ms [6.3%]) relative to the RHY2 condition (0.146 ± 0.005 ms, $p = 0.011$) and RHY24 condition (0.148 ± 0.005 ms, $p = 0.002$) (Figure 5.3B).

A main effect of time ($F_{(3,33)} = 4.6$, $p = 0.09$, $\eta_p^2 = 0.29$) and interaction effect ($F_{(6,66)} = 2.8$, $p = 0.016$, $\eta_p^2 = 0.21$) was observed for MEP/M_{MAX} ratio. Post-hoc analyses showed a significant decrease from PRE (0.51 ± 0.04) to POST (0.43 ± 0.03) MEP/M_{MAX} ratio

($p = 0.009$) across all conditions. Additionally, MEP/M_{MAX} ratio at POST24, was significantly lower during RHY24, relative to the CON condition (−25.6%, $p = 0.026$). No main ($p = 0.196$) or interaction effects ($p = 0.946$) were observed for AMT.

5.3.3 Serum UCH-L1

A time effect was observed for UCH-L1 ($F_{(3,27)} = 9.3$, $p < 0.001$, $\eta_p^2 = 0.51$). Post-hoc analyses showed a significant increase in peripheral UCH-L1 concentration at all time points (POST [$p = 0.015$]; POST2 [$p = 0.022$]; POST24 [$p = 0.009$]) relative to baseline ($n = 10$; Fig. 5.3D). No condition effect was observed ($p = 0.816$).

5.3.4 Body mass, plasma volume and fluid intake

Body mass was significantly higher in the CON condition ($F_{(2,22)} = 20.1$, $p < 0.001$, $\eta_p^2 = 0.65$), and at baseline relative to all time points ($F_{(3,33)} = 261.6$, $p < 0.001$, $\eta_p^2 = 0.96$). An interaction effect between the dehydration conditions and CON was also observed ($F_{(2.5,27.9)} = 140.9$, $p < 0.001$, $\eta_p^2 = 0.93$) for body mass (Figure 5.4). A main effect of time ($F_{(2,22)} = 243.6$, $p < 0.001$, $\eta_p^2 = 0.96$) and an interaction effect were also observed in delta PV change ($F_{(4,44)} = 18.3$, $p > 0.001$, $\eta_p^2 = 0.63$). Pairwise differences are depicted in Figure 5.4.

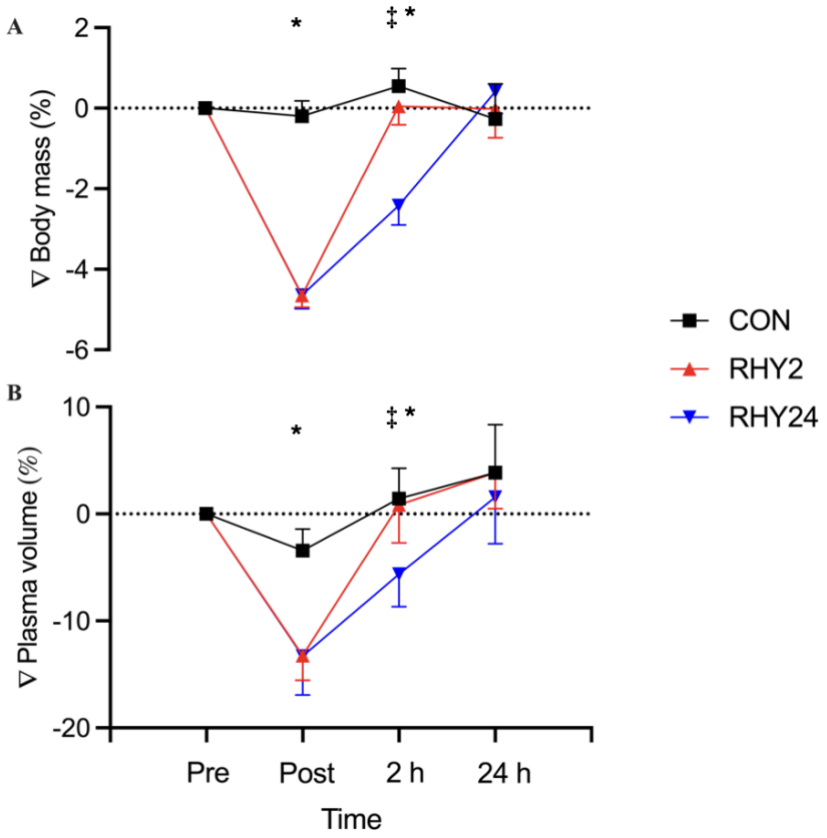

Figure 5.4: Changes in body mass (A) and plasma volume (B). Δ = % change from baseline. * Significant pairwise difference from CON. ‡ Significant pairwise difference between RHY2 and RHY24.

5.3.5 Mid-protocol variables

There were main and condition*time interaction effects for rectal temperature, heart rate, thermal comfort and thirst scale scores, with ($p < 0.001$). Post-hoc analyses showed significant differences between CON and both dehydration conditions ($p < 0.005$), but not between RHY2 and RHY24 ($p > 0.005$) (Figure 5.5). The RHY2 and RHY24 trials produced a transient state of hyperthermia during the 3 h cycling protocol, with rectal temperature rising progressively to 38.9 ± 0.3°C and 39.0 ± 0.2°C at 180 min, respectively. However, following the 60 min recovery period in thermo-neutral conditions, rectal temperature returned to baseline in both

experimental conditions. Average fluid intake during RHY2 and RHY24 were 375.4 ± 68.0 mL/h and 330.8 ± 47.0 mL/h respectively. Urine volume during RHY2 and RHY24 was 168.2 ± 50.0 mL/h and 113.2 ± 26.2 mL/h, respectively. Consequently, fluid retention was 55.2 and 65.8% during RHY2 and RHY24, respectively.

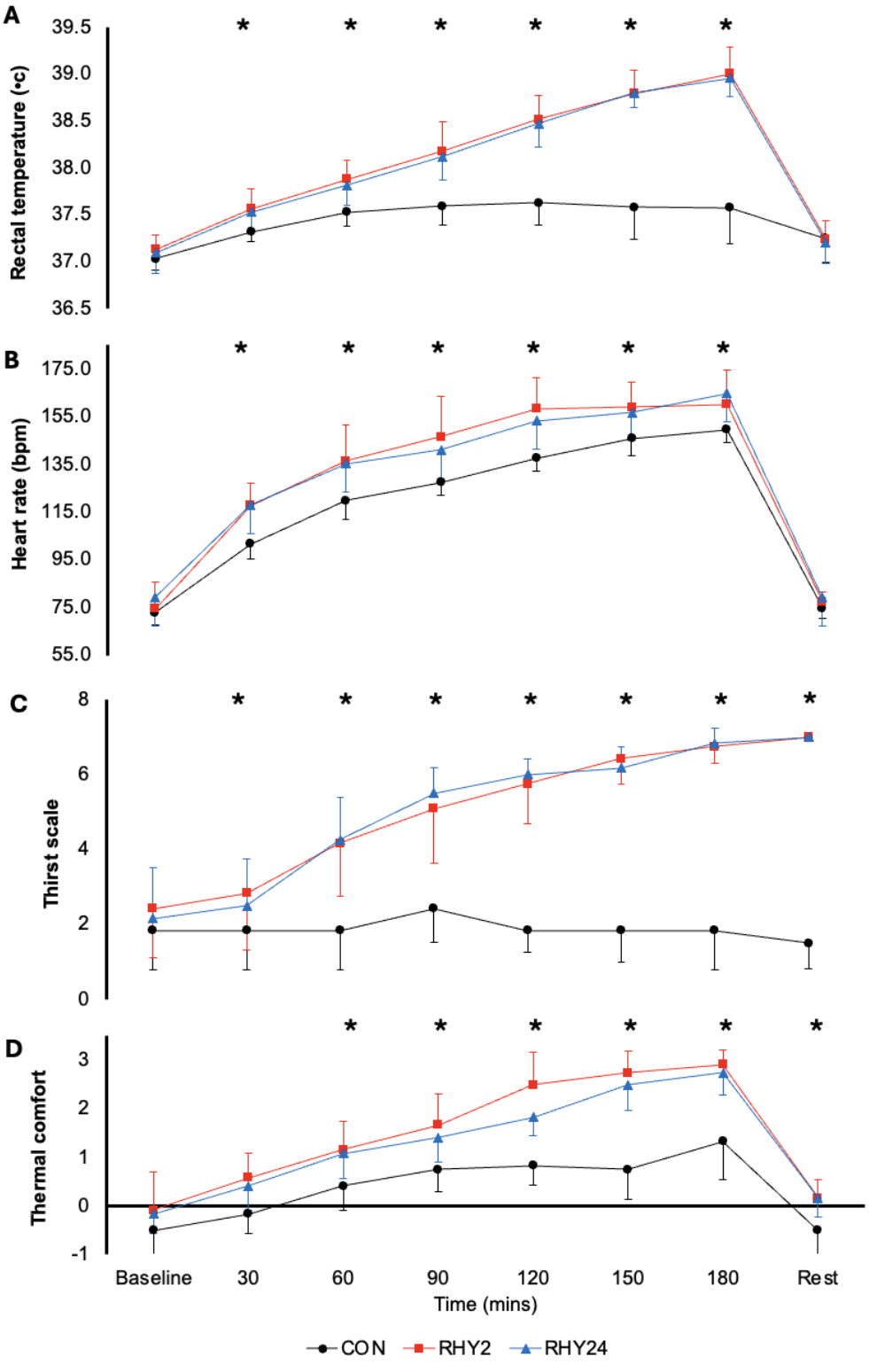

Figure 5.5: Average change for mid-protocol variables. * Significant pairwise difference from RHY2 and RHY24.

5.4 Discussion

The aims of the study were to (a) investigate the corticospinal and peripheral responses to heat-induced hypo-hydration (intracellular dehydration) and rehydration and (b) the time-course of neuromuscular function resulting from gradual and rapid rehydration. Findings indicate that neuromuscular function is impaired after heat-induced hypo-hydration, as characterised by a reduction in MVC of the lower-limb, but the mechanisms of impairment appear to differ from that of prolonged low-intensity exercise. Heat-induced hypo-hydration led to a reduction in VA and corticospinal excitability in both experimental conditions, while exercise in a euhydrated state increased corticospinal inhibition and reduced muscle contractility. In addition, rehydration after 2 h partially restored maximal strength and CSE but this remained depressed at 24 h despite neuromuscular function returning to baseline.

The present findings add to a large body of evidence of MVC reduction due to hypo-hydration (15,87,114-119,398). However, it is of interest that MVC increased from POST to POST2 during RHY2, but not during RHY24, indicating a partial improvement in strength with rapid restoration of fluid balance, but not with gradual fluid restoration. This agrees with the findings of Schofstall et al. (119), where a restoration of maximal strength after two hours of rest and rehydration (1.5% BMR) was reported, whilst also speculating that more time might be required to restore strength losses after dehydration of larger magnitude. While it is generally recommended that rehydration is more efficient when fluid consumption is progressively tapered (333), this pattern may not be optimal to the restoration of maximal strength, and in part, this can be explained by changes in the CNS and PNS. VA did not differ as a function of the rehydration strategy, indicating this restoration of maximal strength was not related to a reduction in central fatigue (399). On the other hand, the reduction in the Qtw,pot occurring

immediately after exercise in the euhydrated state, and at POST2 during RHY24 indicates a reduced myofibrillar Ca^{2+} sensitivity and/or force produced at a cross-bridge level. Indeed, Periard et al. (134) reported an increased muscle relaxation rate and decreased relaxation time after hyperthermia, yet a centrally mediated rate of activation to overcome the faster relaxation rates. It is plausible that the increase of muscle temperature had a residual effect on muscle-contractile properties, initially protecting the muscle from the effects of reduced contractility. Indeed, increased muscle temperature is reported to accelerate the opening and closing of voltage-gated Na^{+} channels, leading to a more rapid onset depolarisation and faster MFCV (141). It is possible that as core temperature returned to baseline, but plasma volume remained ~ 7% below baseline, muscle volume remained below baseline, and Ca^{2+} sensitivity and myosin phosphorylation decreased as dehydration ensued (105). Overall, hypo-hydration leads to a loss of maximal strength capacity, and acute losses (2 h) were partially attenuated by rapid restoration of fluids.

In the current study, a 12% reduction of VA was observed after both dehydration trials, which differs to the findings of others (16,87,94), where VA (from TMS and MNS) and corticospinal excitability were reported to be unaffected by a 2 – 5% BMR via heat-induced hypo-hydration and fluid restriction. However, the present findings agree with Del Coso et al. (93), where a 5% reduction in VA was observed after 2 h of moderate-intensity exercise in the heat (3.8% BMR), demonstrating that higher levels of hypo-hydration might be sufficient to impair VA (87). In addition, Del Coso et al. (93) observed similar changes in VA when exercising in the heat with plain water ingestion, but minimal changes to VA when ingesting a 6% carbohydrate electrolyte solution, implying carbohydrate availability influences VA. This might explain why rehydration type did not influence VA recovery after exercise, as both groups consumed a standardised meal during the recovery period, but it is worth noting that 2 h of gradual or rapid

rehydration and recovery (in addition to a cooling period) is not sufficient to restore VA after heat-induced hypo-hydration. It is plausible that the sustained central fatigue could be attributed to mechanisms of supraspinal origin and the result of increased hypothalamic temperature. Goodall et al. (127) and Ross et al. (400) suggested that an increase in central fatigue was related to cerebrovascular responses to heat. Hypo-hydration also notably increases perceptions of fatigue and pain (200-203). It is possible that conscious signals originating from both central and peripheral afferent pathways could mediate behaviour, such that motivation to perform simple motor tasks is reduced to minimise discomfort (198). While the exercise modality in this study was less intense than that of Del Coso et al. (93), participants did not undergo preliminary heat acclimation training and exercised for 60 min longer, possibly leading to increased pain and discomfort, and the observed larger reductions in VA (~ 10%). Overall, VA is reduced after hypo-hydration and recovery is not influenced by gradual or rapid rehydration strategies.

A novel finding of this study was the differential corticospinal responses to prolonged light-intensity exercise and hydration status, which may explain the reduction in neuromuscular function. The M_{MAX}, is evoked when all the motor axons of a target muscle are activated simultaneously. By normalising the MEP amplitude to the M_{MAX} (expressed as MEP/M_{MAX}), a methodological advantage is introduced. This normalisation allows one to consider the relative contribution of the corticospinal volley within the pool of activated motor neurons. However, as Bowtell et al. (87) have previously demonstrated that the M_{MAX} changes during maximal contractions after hypo-hydration, corticospinal excitability was examined as both MEP_{RAW} and MEP/M_{MAX} in this study. No changes in sarcolemma excitability were observed in the present study; however, MEP/M_{MAX} significantly decreased after light-intensity exercise and further decreased at 24 h for the RHY24 condition (relative to CON; Figure 5.3C). This was equally

observed with $MEP_{RAW,}$ with the addition of MEP_{RAW} at POST2 being significantly higher after rapid rehydration (RHY2), relative to RHY24. This indicates a reduced excitability of several depolarised pyramidal tract neurones (401,402) when in a hypo-hydrated state, which has been previously hypothesised (374). It is of interest that MVC and MEP_{RAW} did not improve at POST2 during RHY24, and this was accompanied by a marked reduction in Qtw,pot (Figure 5.2D). The present findings demonstrate that reduced maximal strength after hypo-hydration is accompanied with a reduction in MEP_{RAW} and VA, and despite a restoration of maximal strength and VA, this reduction in corticospinal excitability may persist after gradual rehydration.

While intracortical changes were not measured, the present findings are supported by Bowtell et al. (87), where cSP was unchanged among hypo-hydrated subjects (despite a reduction in force), but cSP was lengthened in euhydrated subjects (Figure 5.3B). The cSP is attributed to a combination of spinal inhibitory mechanisms (i.e., after-hyperpolarisation and recurrent inhibition of the motor neuron pool), and intracortical mechanisms mediated by slower metabotropic $GABA_B$ receptors (via the opening of K^+ channels) in the primary motor cortex, leading to the reduction of corticospinal drive. Overall, this would imply that following euhydration, there is a preferential increase in synaptic efficacy of $GABA_B$ neurones which act to increase synaptic efficiency of pyramidal cells, thus inhibiting them. The mechanisms for these differential responses in corticospinal excitability and inhibition warrant further investigation.

The present findings also show an effect of time in the change of a blood marker linked to BBB permeability after low-intensity exercise. Pre-clinical studies have suggested that physical exertion may upregulate genetic expression of UCH-L1 e.g., increased striatal and hippocampal mRNA expression of UCH-L1 in rats undergoing aerobic exercise (403-405).

These findings were speculated to be the result of exercise-induced signalling from mTOR or calcium/calmodulin dependent protein kinases (406). Bazarian et al. (406) reported an increase in UCH-L1 concentrations post-physical exertion in humans and reported exercise duration (but not brain white matter integrity) to have a linear correlation to increases in UCH-L1. In addition, no between-group differences in UCH-L1 concentrations were observed, despite the numerically faster rate of rise in the RHY24 condition. These results are contrary to the findings of Watson et al. (376), which showed a restoration of s100B concentrations after rehydration. Further research is required to understand if hypo-hydration has an isolated effect on blood markers of BBB integrity. Overall, the present data might have important implications for the use of blood-based biomarkers, such as UCH-L1, as on-field point-of-care tests, as physical exertion (and possibly hydration status) may confound the results and do not appear to be associated with neuromuscular function. In addition, as discussed in Chapter 2, RWL may also involve metabolic changes beyond fluid loss, including reduced carbohydrate availability, energy restriction and possible increases in circulating ketone bodies. Such changes may theoretically influence neural outcomes through altered cerebral substrate availability or BDNF-related signalling (229-231). However, ketone bodies and BDNF were not measured in the present study.

There are some potential limitations of the current study, which include a lack of strict standardisation of the rehydration beverage temperature. Whilst fluids were dispensed from the same water cooler in the laboratory, the beverages will have inevitably changed temperature across the longer 24 h periods. It is currently uncertain whether the assumed increases in fluid temperature would affect the variables measured, and improved methods to control fluid temperature would help to remove this uncertainty in future research. Further, based on the current sample size, it was not feasible to statistically account for the variation in the methods

used to induce ~ 5% BMR among a small number of the participants (e.g., participants requiring additional layers of clothing or additional rest during the protocol). In addition, the inability to investigate intracortical activity via the use of paired-pulse stimulation measures e.g., SICI, as well as the addition of a no-exercise trial, was a limitation of the current study.

Despite the low exercise intensity, the prolonged duration of the dehydration protocol (~3 h) may have resulted in glycogen depletion, which could independently influence neuromuscular function. In addition, dietary intake outside of laboratory control was not recorded, and the use of a standardised (rather than individualised) electrolyte solution may have introduced variability in substrate availability and electrolyte balance (and therefore, neuromuscular function) between participants and conditions. Finally, the inclusion of local muscle temperature measurements may clarify the role of temperature on contractile function and peripheral neuromuscular responses. Future studies should consider adopting these measures to provide mechanistic insights into the cSP (as observed) and other intracortical changes of the CNS. This would further clarify the exclusive role of intracellular dehydration after exercise and heat-induced hypo-hydration.

5.5 Conclusion

In summary, the present findings provide novel evidence of the different neurophysiological responses to heat-induced hypo-hydration, demonstrating an increase in corticospinal inhibition after low-intensity exercise with fluid ingestion, but a decrease in corticospinal excitability after intracellular dehydration and low-intensity exercise in heat. In addition, maximal strength is impaired after hypo-hydration, which is likely a result of reduced CSE and VA, but this can be partially attenuated by rapid restoration of fluids. Collectively, these results

underscore the sensitivity of the brain-to-muscle pathway to fluid balance and suggest that hydration status may modulate neural drive and motor performance.

5.6 Linking to Chapter 6

Chapter 6 builds on these findings by exploring how sparring-induced head impacts affect corticospinal function, and whether these changes are influenced by hydration and head impact characteristics.

Chapter 6

This chapter explores the acute neurophysiological consequences of RHIs during sparring, a key training modality in combat sports. Building on the findings of Chapter 5, which demonstrated the neurophysiological effects of hypo-hydration and its potential to modulate central drive, the present chapter shifts focus to the interaction between hydration status head impact kinematics and corticospinal function. The chapter is divided into two parts. Part 1 investigates the time course of changes in corticospinal excitability and inhibition following a single sparring session. Part 2 expands this analysis by incorporating instrumented mouthguard (IMG) data to quantify head impact exposure across multiple sessions, enabling a model of the relationship between cumulative impact load and neurophysiological change. Together, these investigations aim to advance the understanding of how RHIs in sparring may alter neuromotor function in combat athletes, and if hydration status influences this.

Part 1:

The neurophysiological responses to repetitive head impacts in a single sparring session.

6.1 Introduction

Combat sports athletes often undergo RWL via dehydration to meet weight class requirements, exposing them to fluctuating hydration states across a typical training week (407). The findings of Chapter 5 demonstrated that hypo-hydration significantly altered corticospinal excitability and neuromuscular function, with an increase in cortical inhibition after exercise and fluid ingestion, but not after exercise and dehydration. These changes suggest a differential neurophysiological response to exercise under varying hydration states and a possible neural compensatory mechanism to protect further losses in muscle force production after intracellular water loss. The importance of examining repetitive head impacts in combat sport extends beyond acute concussion diagnosis. Although the true incidence and prevalence of CTE remain unknown, boxing has historically provided some of the earliest descriptions of chronic neurological decline after repetitive head trauma, and older epidemiological estimates suggested clinical evidence of chronic traumatic brain injury in approximately 17% of professional boxers from earlier eras (408,409). More recent reviews indicate that a substantial minority of professional boxers may develop chronic TBI during their careers, with symptoms of chronic brain injury reported in up to 40% of retired professional boxers in some cohorts (410,411). However, these estimates should be interpreted cautiously because diagnostic criteria, exposure histories and ascertainment methods vary substantially, and CTE can currently only be definitively diagnosed post-mortem (40,412). Nevertheless, the intentional and repetitive nature of head contact in boxing, kickboxing and mixed martial arts makes combat sports a particularly important context for investigating acute markers of neural perturbation that may precede, or contribute to, longer-term neurological consequences.

RWL may further complicate this issue because combat athletes frequently undergo acute dehydration close to training or competition. Hypo-hydration has been associated with

reductions in brain and cerebrospinal fluid volume, ventricular expansion, altered cerebral perfusion and impaired cognitive or perceptual function (17,19-21). These changes do not demonstrate that RWL directly increases neurodegenerative disease risk, but they provide plausible reasons why hydration status may influence the interpretation of symptoms or neurophysiological responses in athletes exposed to head impacts. In addition, symptoms associated with hypo-hydration and RWL, including fatigue, dizziness, headache and impaired concentration, overlap with symptoms used in concussion assessment, potentially complicating recognition and removal decisions in combat sport settings (323,324). Combat sports athletes are exposed to RHIs, both in training and competition (318), and as demonstrated in the findings of Chapter 3, there is an overlap between concussion and hypo-hydration symptoms. This could mean that concurrent hypo-hydration and concussion lead to exacerbation of symptoms, which would also obfuscate mTBI diagnosis. It may also indicate shared underlying neurophysiological mechanisms that are yet to be determined. However, to date, the interaction between hydration status and brain trauma remains unexplored in athletes who routinely experience both.

In contrast to other contact sports such as rugby, where full-contact training is restricted to 15 min per week and head impacts are now routinely monitored at the elite level using IMGs (413,414), combat sports remain far less regulated in this regard. Combat sports also present a unique context in which head impacts are frequent, deliberate, and often unmonitored in training. While competition-based concussion incidence rates are high, with estimates ranging from 16 to 25 per 100 exposures in boxing and MMA (46,47), they likely underestimate the total head trauma sustained during sparring. Sparring is a key component of preparation for competitive bouts and may occur multiple times per week yet remains largely unregulated and may be devoid of medical oversight, particularly in sub-elite settings. Consequently, the

cumulative burden of RHIs in training is not well quantified and its relationship to neuromuscular function is not well understood.

While RHIs during sparring may be non-concussive, producing no observable clinical symptoms, they are reported to provoke alterations in brain function (9). Neurophysiological assessments, such as TMS, offer a means of capturing subtle, subclinical changes in corticospinal function that often go undetected through standard assessments. Previous studies have reported increased inhibition and reduced excitability following concussion (after 72 h; 7,415) and non-concussive exposures (between 24 – 48 h; 8,9), but this research has focused on acute, isolated events or artificial models such as controlled sparring or heading drills. Importantly, the current literature has not examined the functional changes and time-course of recovery, that occur during naturalistic training scenarios like sparring, where athletes often accumulate a high burden of non-concussive RHIs across time (60). This limits the ecological validity of existing findings and presents a gap in understanding the relationship between RHIs and neuromuscular function.

Recent evidence demonstrates that combat sport athletes may train in a hypo-hydrated state and fail to fully rehydrate during the session (363). In a study of university-level taekwondo athletes, 72 – 77% of participants started training with a urine specific gravity (USG) above 1.020, indicating hypo-hydration (416). Despite *ad libitum* access to water during training, fluid intake was insufficient to offset sweat loss, and athletes remained hypo-hydrated post-training. The study also found significant increases in thirst perception and urine colour following each session, supporting the physiological signs of fluid imbalance following training (77). Exposure to RHIs may interact with underlying states of hypo-hydration, particularly during fight camp, when athletes train intensely while undergoing weight manipulation strategies (407). Given that both RHI and hydration status independently affect

corticospinal and neuromuscular pathways, it is critical to investigate whether RHIs in typical sparring sessions further exacerbate these neurophysiological changes. Therefore, the aim of the present study was to observe the acute neurophysiological changes following a typical uninterrupted sparring session, with the hypothesis that corticospinal inhibition will increase after sparring and recover within 48 hours.

6.2 Methods

6.2.1 Participants

Thirteen healthy male combat sports athletes (age: 23 ± 5 yrs, stature: 175 ± 8 cm, BM: 74 ± 17 kg) without neurologic conditions, lower limb disabilities, or disease were recruited from a local combat sports gymnasium. Individuals who suffered from a chronic disease, took medication for an underlying condition or sustained an injury in the last 3 months were not eligible to take part. Participants completed a neurostimulation safety screening form to identify those at risk so that they can be excluded from participation (358). The participants signed an informed consent before participation, previously approved by the institutional review board (ETH-2425-0793).

6.2.2 Sample size justification

Using a one way repeated-measures ANOVA model (Pre vs. Post vs. Post48), and data from soccer headers in amateur athletes (8) showing an increase in corticospinal inhibition (ES: 0.3), a total of 12 combat sports athletes was required for the study. The sample size was determined using G*Power (v 3.1). The significance level was set at 0.05 and power at 0.9.

6.2.3 Study design

Baseline measurements of hydration status, body mass, neuromuscular function and corticospinal excitability (PRE) were followed by two post-sparring assessments: immediately after (within 60 min; POST) and 48 hours later (POST 48). Participants attended as usual to a typical sparring session (3 × 5 min rounds) and drank fluids *ad libitum* in between rounds. Sparring involved participants mimicking a fighting bout/scenario (punches, kicks, takedowns, clinches etc.) with no protective gear besides a mouthguard and shin pads and rotating with other sparring partners in each round (if available). In line with the naturalistic design of the study, no attempt was made to alter the training environment, schedule or recovery practices. While it was attempted to capture a sample of euhydrated or exercising control group data, this was not included in the present study due to both practical and ecological considerations i.e., euhydration or an exercise-only session with no impacts could not be controlled for all participants. This approach was necessary to preserve ecological validity and reflects real-world training conditions in combat sports. As such, participants rested as they normally would following sparring, providing an exertion rating (RPE), then undergoing repeated baseline measurements at POST and POST 48.

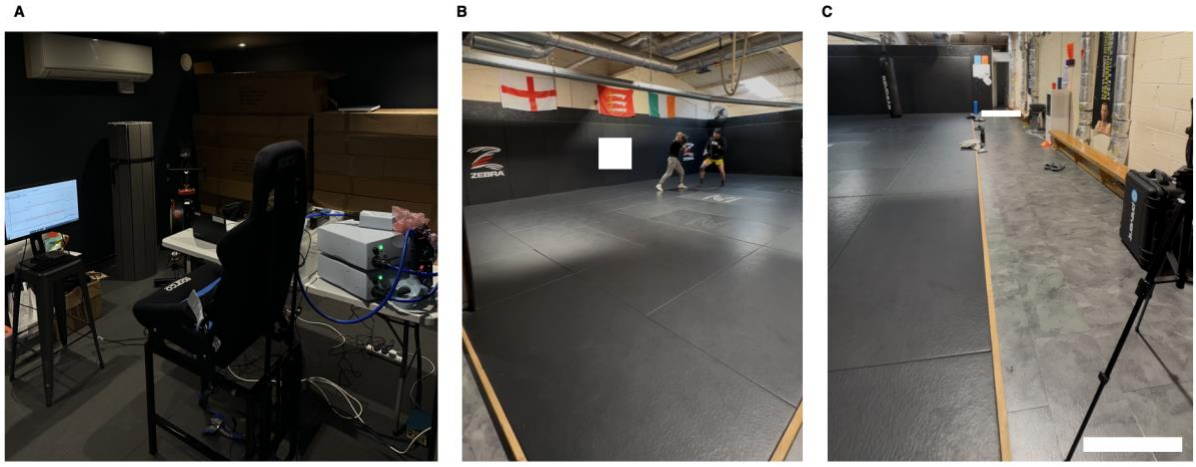

Figure 6.1: Study set-up: A) Assessment of neuromuscular function in separate treatment room within gymnasium. B) Sparring location and C) camera set-up on either side of the matted area (white bars).

6.2.4 Neuromuscular function

6.2.4.1 Electromyography

For electromyography, please see ‘General Methods’ section 4.2.2.1.

6.2.4.2 Maximal voluntary contractions

For full procedures of the MVC, please see ‘General Methods’ section 4.2.2.2.

6.2.4.3 Motor nerve stimulation

For motor nerve stimulation, please see ‘General Methods’ section 4.2.2.3.

6.2.4.4 Transcranial magnetic stimulation

For transcranial magnetic stimulation, please see ‘General Methods’ section 4.2.2.4.

6.2.4.5 Data processing

For data processing methods, please see ‘General Methods’ section 4.2.2.5.

6.2.5 Rating of perceived exertion

Session intensity was assessed using an RPE scale ranging from 1 (very light) to 10 (maximum effort). Participants were asked to report their overall RPE immediately after the sparring session to provide a subjective measure of internal load. Training load was calculated for each sparring session using session RPE (sRPE) using the following equation (417):

$$sRPE = Session\ length \times RPE$$

6.2.6 Urine specific gravity

USG was measured using the Atago PAL-10S digital refractometer pen (Atago Co., Ltd., Tokyo, Japan), a handheld optical device validated for field and laboratory hydration monitoring. Participants provided midstream urine samples in sterile containers upon arrival, and measurements were taken immediately using the refractometer, following manufacturer guidelines. The device reports USG to three decimal places with a typical range of 1.000–1.050, demonstrates high reliability with strong agreement to manual refractometry ($r = 0.998$) and minimal measurement bias (mean difference 0.0002 ± 0.0004; 418), and has also shown high correlation ($r = 0.91$), sensitivity (93.9%), and specificity (85.8%) in field validation studies (419). Values ≥ 1.020 were classified as indicative of hypo-hydration, consistent with thresholds used in previous athletic hydration studies (78).

6.2.7 Data capture (video analysis)

Sessions were recorded at 30 frames per second by two video cameras with a 720p resolution (iPad 8th Gen, Apple, USA). Frame by frame analyses were used to confirm each head impact using a video analysis software (In-Play Sports, South Yorkshire, UK). All video footage was independently reviewed by two trained researchers and discrepancies were resolved by consensus. A head impact was defined as visible contact to the head and face region that resulted in observable head motion. Events not meeting these criteria or deemed inconclusive were excluded. Head impacts were quantified as described above to support interpretation of neurophysiological responses; however, accelerometery data were not included in this section, as not all participants in this single-session study were fitted with a custom instrumented mouthguard (see Section 6.8.6).

6.2.8 Statistical analysis

All data analyses were performed using SPSS for Windows (v 23; SPSS Inc., USA), and values are reported as mean ± SD or median (IQR). Assumptions of normality and equality of variance were assessed through visual inspection of Q–Q plots and residual vs fitted value plots. All dependent variables were assessed using a one-way repeated measures ANOVA. Where a significant main effect was identified, Bonferroni-adjusted post hoc comparisons were conducted. Partial eta squared (η^2) was calculated as a measure of effect size, with thresholds of 0.01, 0.06, and 0.14 interpreted as small, medium, and large effects respectively (397,420). For pairwise comparisons, 95% confidence intervals were reported to quantify the precision of observed effects. Statistical significance was set at $p \leq 0.05$.

6.3 Results

6.3.1 Corticospinal excitability and neuromuscular function

There was a significant main effect of time on AMT [$F_{(2, 24)} = 9.94$, $p < .001$, $\eta^2 = .453$], with *post hoc* tests demonstrating an increase from PRE to POST ($p = .024$ [95% CI, 0.27 – 4.04]) and a reduction from POST to POST 48 ($p = 0.004$ [95% CI, -2.73 – 1.04]). MEP_{RAW} also demonstrated a significant effect of time [$F_{(2, 24)} = 6.65$, $p = .005$, $\eta^2 = .357$], with amplitudes reduced from PRE to POST ($p < .001$ [95% CI, -0.84 – -0.24]) and from PRE to POST 48 ($p = .034$ [95% CI, 0.03 – 0.86]). The cSP duration was significantly affected across time [$F_{(2, 24)} = 3.43$, $p = .049$, $\eta^2 = .222$], with a significant increase from PRE to POST ($p = .044$ [95% CI, 0.00 – 0.02]), while other comparisons were not significant. In contrast, MEP/M_{MAX} ratio did not significantly change across time [$F_{(2, 24)} = 2.33$, $p = .119$, $\eta^2 = .162$], nor SICI [$F_{(2, 24)} = 2.33$, $p = .119$, $\eta^2 = .162$]. A summary of individual and group changes is summarised below in Figure 6.2.

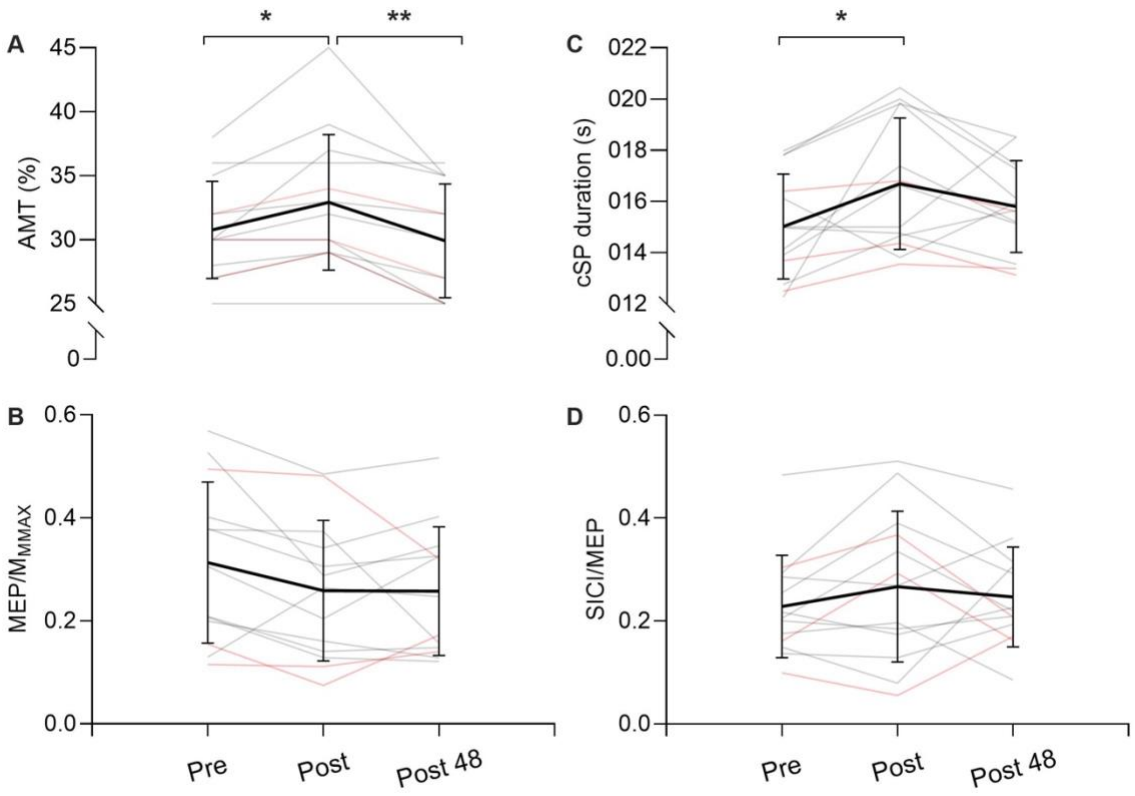

Figure 6.2: Average and individual traces for changes in Active Motor Threshold (AMT; A), Motor Evoked Potential normalised to the Maximum compound muscle action potential (MEP/M_{MAX}; B), Cortical Silent Period (cSP; C) and Short-Interval Intracortical Inhibition (SICI; D). * $p < 0.05$, ** $p < 0.01$. *Red traces denote participants with USG > 1.02 (possible hypo-hydration).*

A significant main effect of time was found for MVC [$F_{(2, 24)} = 5.86$, $p = .008$, $\eta^2 = .328$], with a reduction in maximal strength from PRE to POST ($p = .031$ [95% CI, - 0.52 – - 0.02]). VA exhibited a significant effect of time [$F_{(2, 24)} = 24.86$, $p < .001$, $\eta^2 = .674$], with VA declining from PRE to POST ($p < .001$ [95% CI, -3.71 – -1.22]), but increasing significantly from POST to POST48 ($p < .001$ [95% CI, - 0.96 – 3.04]), although not fully returning to baseline. M_{MAX} did not significantly change across time points [$F_{(2, 24)} = 2.251$, $p = 0.127$, $\eta^2 = .158$], while HRT showed a significant main effect [$F_{(2, 24)} = 3.44$, $p = .048$, $\eta^2 = .223$], but no significant pairwise comparisons were identified (Figure 6.3). No significant changes were observed in Qtw,pot.

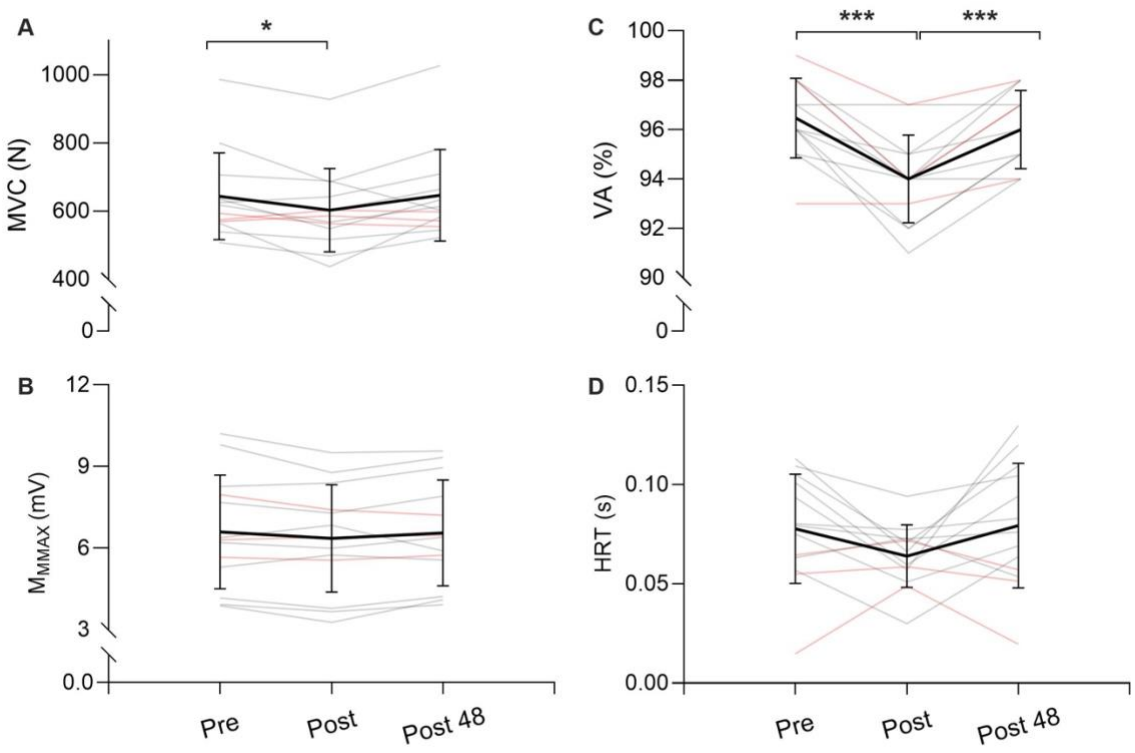

Figure 6.3: Average and individual traces for changes in Maximal Voluntary Contraction (MVC; A), Maximum compound muscle action potential (M_{MAX}; B), Voluntary Activation (VA; C) and muscle half relaxation time (HRT; D). * $p < 0.05$, *** $p < 0.001$. *Red traces denote participants with USG > 1.02 (possible hypo-hydration).*

6.3.2 Body mass and hydration status

BM demonstrated a significant main effect of time [$F_{(2, 24)} = 3.68$, $p = .040$, $\eta^2 = .235$]. Pairwise comparisons showed a statistically significant reduction in BM from PRE to POST ($p = .002$ [95% CI, -0.76 – -0.18]). USG was also significantly affected by time [$F_{(2, 24)} = 6.48$, $p = .006$, $\eta^2 = .351$], with a significant increase in USG from PRE to POST ($p = .001$ [95% CI, 0.001 – 0.003]). Figure 6.4 summarises changes in BM (% change) and USG.

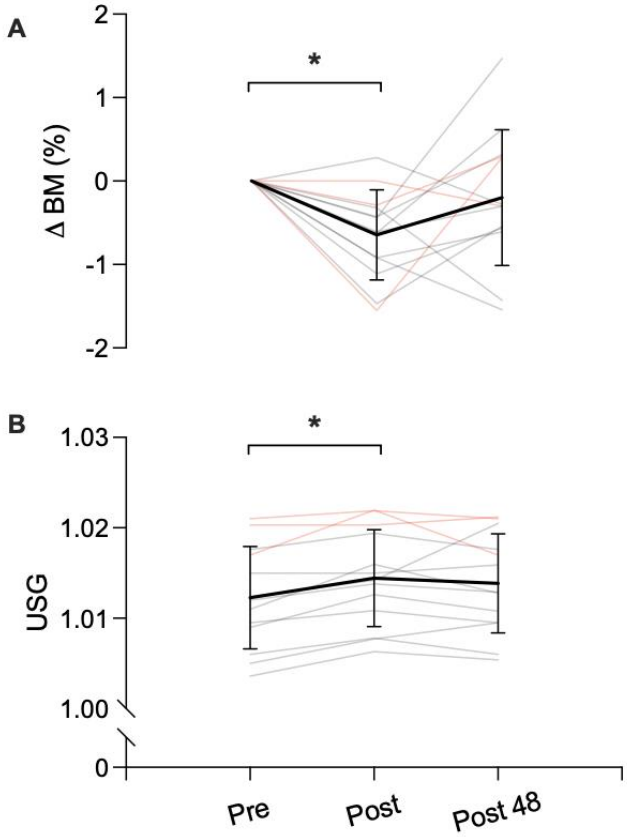

Figure 6.4: Average and individual traces for changes in BM (A) and USG (B). * $p < 0.05$, *Red traces denote participants with USG > 1.02 (possible hypo-hydration). BM* Body mass *USG* Urine specific gravity.

6.3.3 Head impacts and session metrics.

Participants sustained a median of 13 (IQR: 11 – 22) head impacts during the sparring session. The number of impacts ranged from a minimum of 7 to a maximum of 40 per participant. Post-sparring RPE scores indicated a median of 7 (IQR: 6 – 8), median training load was 90 (IQR: 72 – 120) and session length was 15 min (IQR: 12 – 15).

6.4 Discussion

A single sparring session induced clear acute neurophysiological changes in the primary motor cortex, marked by increased corticospinal inhibition and reduced excitability. Immediately post-sparring, participants showed a significant prolongation of the cSP, alongside an elevated AMT and suppressed MEP_{RAW}, indicating enhanced GABAergic inhibition and reduced

cortical excitability, which recovered within 48 h. These changes occurred in the context of repeated head contact, with participants sustaining a median of 13 head impacts during the sparring session, ranging from 7 to 40 impacts. In addition, although significant reductions in BM were observed, the magnitude of hypo-hydration remained physiologically minor during a typical sparring session.

The present findings align with studies of repetitive non-concussive impacts, where amateur boxers' soccer ball heading have been shown to acutely increase cSP duration, reflecting a transient rise in GABA-mediated inhibitory activity in the primary motor cortex (8,9). While MEP_{RAW} was significantly reduced, this was not found to be significant when normalised to the M_{MAX}. Despite this, an increase in the AMT implies that excitatory inter-neuronal circuits within the primary motor cortex were less effective at recruiting the corticospinal pathway, possibly due to increased GABAergic tone after a sparring session. The potential contribution of head impact load to these neurophysiological changes is an important consideration and is examined in greater detail in Chapter 6, Part 2, however, the magnitude of cSP increase observed in the present study (11%) exceeded that reported by Di Virgilio et al. (9), who noted a 6% increase. Both studies assessed cSP duration from the rectus femoris, but methodological differences may partially account for the discrepancy, including contraction intensity (10% MVC *vs.* MVC), bout duration (3 × 5 min rounds with 1 min rest *vs.* 3 × 3 min rounds with 2 min rest) and the quantification of head impacts, which was not reported by Di Virgilio et al. (9).

Notably, the recovery of TMS measures in the present cohort (typically normalising by 48 hours post-exposure) suggests that the neurophysiological disturbances from a brief sparring session are short-lived. This contrasts with concussive impacts, where TMS abnormalities can persist beyond 72 h (280-287) or even in the post-acute period months after injury (6). The

transient nature of the changes in our study implies an effect of RHIs on recovery time, but it is yet to be determined if this is in relation to the number of impacts sustained or the magnitude and severity of impacts sustained within the session. In essence, a single session of subconcussive RHIs appear to trigger reversible functional changes that resolve within two days, whereas a single or several concussive impacts produce longer-lasting cortical alterations (7). Nonetheless, it is noteworthy that the pattern of acute changes we observed (increased inhibition and reduced excitability) is qualitatively similar to that reported in mTBI (60). This supports the notion that even non-concussive sparring impacts can momentarily disrupt the excitation–inhibition balance in the brain. Such a GABAergic cortical response may represent an initial, subclinical manifestation of brain trauma, albeit one from which the brain can swiftly recover given the lower severity of insult.

Measures of hydration status revealed a consistent increase in USG and a corresponding reduction in BM (~ 0.6%) following a single sparring session. While these changes were not sufficient to constitute moderate hypo-hydration (typically > 2% BMR; 78), they nonetheless reflect acute fluid shifts associated with sparring. Despite this, it is unlikely that any significant neurophysiological alterations were observed that could be directly attributed to these fluid shifts (as seen in Chapter 5), possibly due to the relatively mild magnitude of fluid losses and the fact that participants drank fluids *ad-libitum* throughout the session. Notably, these findings contrast with those reported by Belfort et al. (416), where the majority of athletes began training in a hypo-hydrated state and failed to restore fluid balance. Combat athletes frequently restrict both calories and fluids in the final stages of fight camp, often achieving > 5% BMR (317,362). If sparring sessions were to occur during this period, it is plausible that the cumulative burden of hypo-hydration and head impact exposure could amplify central and peripheral neuromuscular dysfunction, compounding the risk of impaired motor control or delayed recovery following RHIs. However, it is also notable that in most structured fight camps,

sparring typically ceases in the final week before competition (44), coinciding with the most aggressive phase of RWL (318). As such, the timing of hydration manipulation may act as a natural buffer that reduces the likelihood of concurrent exposure to both sparring-induced RHIs and hypo-hydration. While this practice may mitigate acute risk, it does not eliminate the broader concern regarding RHIs during a competitive bout where an athlete is already dehydrated.

The decline in MVC force immediately after sparring appears to be performance fatigue driven by central rather than peripheral mechanisms, as this was accompanied by a reduction in VA, with no change in M_{MAX} amplitude. Our findings mirror those of Di Virgilio et al. (9) who also noted a ~ 6% MVC decrement after sparring. Crucially, the authors observed a similar MVC reduction in a control group that performed exercise without head impacts (9). In their work, both the sparring and non-sparring exercise led to a comparable reduction in MVC, indicating that a short bout of exercise alone can induce performance fatigue, but only the group sustaining head impacts showed a 6% increase in the cSP. This dissociation suggests that the heightened corticospinal inhibition reported herein and elsewhere after sparring is not simply a byproduct of general physical fatigue (9). Additionally, the absence of a robust prolongation in HRT or any drop in M_{MAX} amplitude indicates that peripheral fatigue was minimal, consistent with prior work showing that these variables reflect peripheral excitation–contraction impairments (421,422). In the present study, athletes' inability to fully activate their quadriceps immediately post-sparring (lower VA) may underscore a central mechanism of impairment, and similarly in Di Virgilio et al. (9), an earlier recruitment of high threshold motor units was observed. The central fatigue could be attributed to the elevated corticospinal inhibition (e.g., longer cSP) or increased perceived effort, gating the output of primary motor cortex, thereby limiting motor unit recruitment (272). It is worth noting that this interpretation is based on non-invasive assessments of a single muscle i.e., rectus femoris, yet

the consistency in the observed pattern across the present study and previous work (9) is encouraging and reinforces the validity of the central fatigue hypothesis following non-concussive head impacts.

The current observations of relatively faster recovery in neurophysiological metrics have important practical implications, but they also raise critical questions about cumulative effects. The present study captured the neurophysiological response to a single sparring session; however, combat sport athletes typically undergo repeated sparring sessions and head impacts over a training camp or competitive season. While each isolated exposure may cause only transient changes, the concern is whether incomplete recovery or subtle deficits could accumulate with frequent repetition. It is possible that more frequent or heavier sparring sessions would extend the recovery timeline of cortical metrics, blurring the line between non-concussive and concussive effects. To better understand these dynamics, longitudinal studies are needed to monitor fighters across an entire training period (e.g., a fight camp) and a robust method of monitoring head impact severity. Establishing the trajectory of neurophysiological changes (or lack thereof) across repeated non-concussive impacts is crucial for assessing long-term risk, potentially explaining how routine sparring, over years, could contribute to cumulative neurological impairment and conditions like chronic traumatic encephalopathy in some athletes (423).

6.5 Conclusion

This chapter investigated the time course of changes in corticospinal excitability and inhibition following a single sparring session. The findings demonstrate that a single bout of sparring (~ 15 min) can transiently reduce corticospinal excitability and increase cortical inhibition,

mimicking neurophysiological features of an mTBI, though with an accelerated recovery time (48 h). In addition, a reduction in maximal strength is observed after sparring, accompanied by a reduction in central drive, but no markers of increased peripheral fatigue. These central alterations occurred alongside minimal fluid losses, suggesting that central fatigue and inhibitory changes were not confounded by peripheral or hydration-related factors.

6.6 Linking to Chapter 6, Part 2

The implications of the present findings remain unclear as to whether these translate to multiple sparring sessions. The following section build on these findings by investigating how repeated sparring exposures across time may affect neurophysiological function, incorporating objective head impact metrics via IMG technology to explore potential relationships.

Part 2:

A kinematic and neurophysiological analysis of repetitive head impacts in combat sports.

6.7 Introduction

Chapter 6, Part 1 established that a single sparring session can transiently reduce corticospinal excitability and increase cortical inhibition, with minimal fluid losses and peripheral fatigue. While recovery was observed within 48 h, the extent to which these changes accumulate over time remains unclear. This second part extends these findings by investigating the neurophysiological responses across repeated sparring sessions and examining whether head impact exposure, as measured using IMG technology, predicts the magnitude or persistence of central changes.

At the onset of a head impact, the cerebral cortex undergoes impulse force waves that radiate from the point of contact across cortical tissue layers (424). In combat sports, the brain is frequently subjected to both linear and angular accelerations through RHIs (425). Both forms of acceleration contribute to brain injury mechanisms, with linear acceleration associated with translational forces and coup–contrecoup effects, and rotational acceleration linked to shear strain and diffuse axonal injury (426). Rotational acceleration is often highlighted as a key predictor of mTBI (59), with striking sports often exceeding the rotational thresholds typically observed in contact sports, such as rugby or American football (427). While the literature reports wide-ranging thresholds for concussive injuries (30 – 96 *g* for linear acceleration and 2911 – 5582 rad/s² for angular acceleration; 427), emerging evidence suggests that non-concussive impacts below 20 *g* may still induce cumulative neural trauma at the cellular level, especially when occurring repetitively (428).

A critical and often overlooked factor in this context is hydration status. Neural tissue is highly sensitive to fluid balance, and even modest levels of hypo-hydration can alter cerebrovascular function, reduce cerebral blood flow, and increase neuronal vulnerability to mechanical stress (22,429). As reported in Part 1 of this chapter, a typical sparring session in combat athletes

leads to an increase in USG and BMR. Although these changes do not meet thresholds for hypo-hydration, they nonetheless reflect a statistically significant perturbation in fluid homeostasis, with unknown consequences for brain-to-muscle communication. It is plausible that even a transient fluid imbalance may interact with the biomechanical forces of sparring to amplify neurometabolic strain, particularly when fluid restriction or aggressive WCs precedes training. More specifically, the reduction in total body water may impair the buffering capacity of CSF and interstitial fluid, potentially increasing the effective strain transmitted to neural tissue during impact.

This becomes particularly relevant when considering the neurophysiological consequences of RHIs, which may go undetected by clinical symptom assessments. As shown in Part 1 of the current thesis chapter, a single session of sparring results in a marked increase in cortical inhibition. While, prior work has primarily explored these outcomes in response to controlled laboratory exposures or isolated concussive events, no studies have investigated a naturalistic observation of real-world sparring loads, particularly multiple sessions, and how they relate to head impact characteristics and the neurophysiological responses. To address this gap, the current study employed IMG technology to quantify head impact frequency (HIF) and magnitude across multiple sparring sessions. To date, only one study has implemented instrumented mouthguards in mixed martial arts, with Tiernan et al. (60) reporting high-magnitude (> 50 *g*) head acceleration events during sparring and competition. However, the effect of these head impacts on neurophysiological function has not been explored, nor has the potential interacting effects with whole-body hydration status. By integrating objective head impact metrics with TMS-based assessments of cortical excitability and inhibition, this study aimed to explore potential relationships between cumulative head impact kinematics and corticospinal activity. We hypothesised that higher cumulative impact load would be associated with greater cortical inhibition and reductions in corticospinal excitability.

6.8 Methods

6.8.1 Participants

For participant details, please see Chapter 6 section 6.2.1. In addition, five participants from this cohort (age: 25 ± 6 yrs, stature: 180 ± 11 cm, BM: 83 ± 26 kg) wore IMGs (detailed in 6.8.6).

6.8.2 Study design

This chapter presents a naturalistic observational study designed to explore how cumulative head impact exposure across multiple sparring sessions relates to neurophysiological function. Unlike the previous chapter, which focused on the time-course of acute changes following a single session, the present study extends the investigation over several sessions to examine whether the accumulation and magnitude of head impacts alter cortical inhibition and excitability. Participants were monitored longitudinally across their typical fight camp training, with TMS and neuromuscular assessments repeated after each sparring session. This repeated Pre–Post design allowed for the calculation of cumulative head impact exposure (e.g., total number of impacts, cumulative linear and angular acceleration) and its association with session-to-session changes in corticospinal measures. The decision to re-analyse the same neurophysiological outcomes was driven by the aim of this chapter to determine whether session-wise accumulation of RHIs correlate with and predict the observed neurophysiological responses in Part 1. Measures such as session RPE and whole-body hydration status were also included to examine potential interacting factors. For the remaining details related to study design, please see Chapter 6 section 6.2.3.

6.8.3 Neuromuscular function

6.8.3.1 Electromyography

For electromyography, please see ‘General Methods’ section 4.2.2.1.

6.8.3.2 Maximal voluntary contractions

For full procedures of the MVC, please see ‘General Methods’ section 4.2.2.2.

6.8.3.3 Motor nerve stimulation

For motor nerve stimulation, please see ‘General Methods’ section 4.2.2.3.

6.8.3.4 Transcranial magnetic stimulation

For transcranial magnetic stimulation, please see ‘General Methods’ section 4.2.2.4.

6.8.3.5 Data processing

For data processing methods, please see ‘General Methods’ section 4.2.2.5.

6.8.4 Rating of perceived exertion

Session intensity was assessed as described in Chapter 6, Part 1, section 6.2.5.

6.8.5 Urine specific gravity

USG was assessed as described in Chapter 6, Part 1, section 6.2.6.

6.8.6 Instrumented mouthguard

Upon completion of a three-dimensional oral scan performed by a dentist, subjects were fitted with a custom instrumented mouthguard device (Prevent Biometrics, Edina, MN). This device has been independently tested and verified in previously published studies across multiple athletic activities, including boxing (430-434). Accelerometer and gyroscope ranges were set at ± 200 *g* and ± 2,000 deg/s, respectively. The sensor accuracy is cited as ± 1% across the full-scale range. The accuracy of the system (after transform to the head centre of gravity) has been shown to have a concordance correlation coefficient of 0.98 (450). In addition, the Prevent Biometrics IMG is reported to demonstrate a mean linear bias of – 1.0% with ratio Limits of

Agreement (LoA) ranging from – 16.9% to 14.8%, and a mean angular bias of – 6.8% with LoA from – 21.7% to 8.0%, and a sensitivity of 75% (95% CI: 67–83%) for detecting true-positive head acceleration events during guided analysis (435).

A sensor acceleration event, which is the IMG data that approximates a head acceleration event at the head's centre of gravity (436) was recorded by the IMG when one of the axes on the medial–lateral, anterior–posterior, and vertical planes exceeded 8 *g*, capturing 50 ms of head kinematics (10 ms pre-trigger; 40 ms post-trigger). The peak linear acceleration (PLA; *g*) and peak angular acceleration (PAA; rad/s^2) of each event was calculated from resultant values. Thereafter, a trigger threshold of 5 *g* was used to initiate data collection exports. This threshold was selected in order to include indirect head acceleration events, as suggested by Tooby et al. (437), and to allow for the capture of low-magnitude, potentially non-concussive impacts that may otherwise be excluded. To account for tangential and acceleration effects (34), linear head kinematics were transformed to the 50th percentile male head centre of gravity from the IMG location. Prevent Biometrics' in-house algorithm categorised signal noise for each head acceleration event recorded as minimal (0; $n = 890$), moderate (1; $n = 66$), or severe (2; $n = 15$). A 4-pole, zero-phase, low-pass Butterworth filter was employed, with class 0, 1, and 2 signals using 200, 100, and 50 Hz cut-off frequencies, respectively.

6.8.7 Data capture (video analysis)

Sessions were recorded at 30 frames per second by two video cameras with a 720p resolution (iPad 8th Gen, Apple). Frame by frame analyses were used to confirm each head impact using a video analysis software (In-Play Sports, South Yorkshire, UK). All video footage was independently reviewed by two trained researchers, who were blinded to the mouthguard data. Inter-rater reliability was assessed on 20% of randomly selected clips and showed substantial agreement (Cohen's $\kappa = 0.80$, $p < 0.001$), based on the interpretative benchmarks proposed by Landis and Koch (438). Discrepancies were resolved by consensus. A head impact was defined

as visible contact to the head and face region that resulted in observable head motion. Events not meeting these criteria or deemed inconclusive were excluded.

6.8.8 Statistical analysis

All statistical analyses were conducted using R (version 4.2.1, R Core Team). Data were visually inspected for normality using Q–Q plots and presented as mean ± SD or median (IQR). For each neurophysiological outcome, a linear mixed effects model (LMM) was used to examine changes from pre to post sparring, with TimePoint (PRE, POST) included as a fixed effect and participant modelled as a random intercept to account for repeated measures. Degrees of freedom and p-values were estimated using the Satterthwaite approximation via the 'lmerTest' package. Where appropriate, pairwise comparisons were conducted using Tukey's HSD to adjust for multiple testing. To explore relationships between head impact exposure and neurophysiological outcomes, repeated measures correlation was performed using the 'rmcorr' package. This method estimates within-subject associations while controlling for inter-individual variability, making it suitable for repeated observations across time. Head impact metrics and other confounding factors included HIF, cumulative linear acceleration (CLA), cumulative angular acceleration (CAA), hydration status (USG), and session RPE. These were correlated against neurophysiological measures including cSP, MEP_{RAW}, MEP/M_{MAX}, AMT, SICI, MVC and VA. LMM were also used to examine whether head impact characteristics predicted post-sparring changes in neurophysiological variables. For example, cSP duration was modelled as a function of CAA, with CAA included as a fixed effect and Participant as a random effect to account for within-subject clustering. Models containing multiple predictors were specified with additive fixed effects, denoted using '+'. Statistical significance was set at $p < 0.05$ and correlation coefficients were interpreted according to the following classification: weak for values between 0.10 and 0.39, moderate for values between 0.40 and 0.69, strong for

values between 0.70 and 0.89, and very strong for values equal to or above 0.90, as recommended by Schober et al. (439). No correction for multiple comparisons was applied to p-values in the repeated measures correlation analyses, as these were considered exploratory in nature. The primary aim was to identify potential patterns of association between head impact exposure and neurophysiological change, to guide future hypothesis-driven research. This approach is consistent with best-practice recommendations for early-phase exploratory work (440).

6.9 Results

6.9.1 Session and head impact metrics

On average, all participants took part in 15 ± 2 min of sparring (min: 8 min, max: 20 min), with the majority of sessions consisting of 3 x 5 min rounds. Average RPE in each session was 6 ± 1 (min: 4, max: 9) and training load was 94 ± 19.

In total, 942 head impacts (> 5 *g*) were recorded across all sparring sessions, 5 sparring sessions were removed due to incomplete IMG data, and 715 were analysed following data processing and video verification. The highest PLA and PAA recorded were 50 *g* and 5775 rad/s², respectively (Figure 6.5A – B). A summary of head impact locations can also be found in Figure 6.5D. Cumulative load (sum of all impact load) per session totalled 240 ± 136 *g* for PLA and 20,421 ± 11,910 rad/s² for PAA. Across a single sparring session (3 x 5 min), participants sustained a median (IQR) of 19 (10) head impacts. Mean PLA per impact was 11 ± 4 *g*, while mean PAA was 924 ± 379 rad/s².

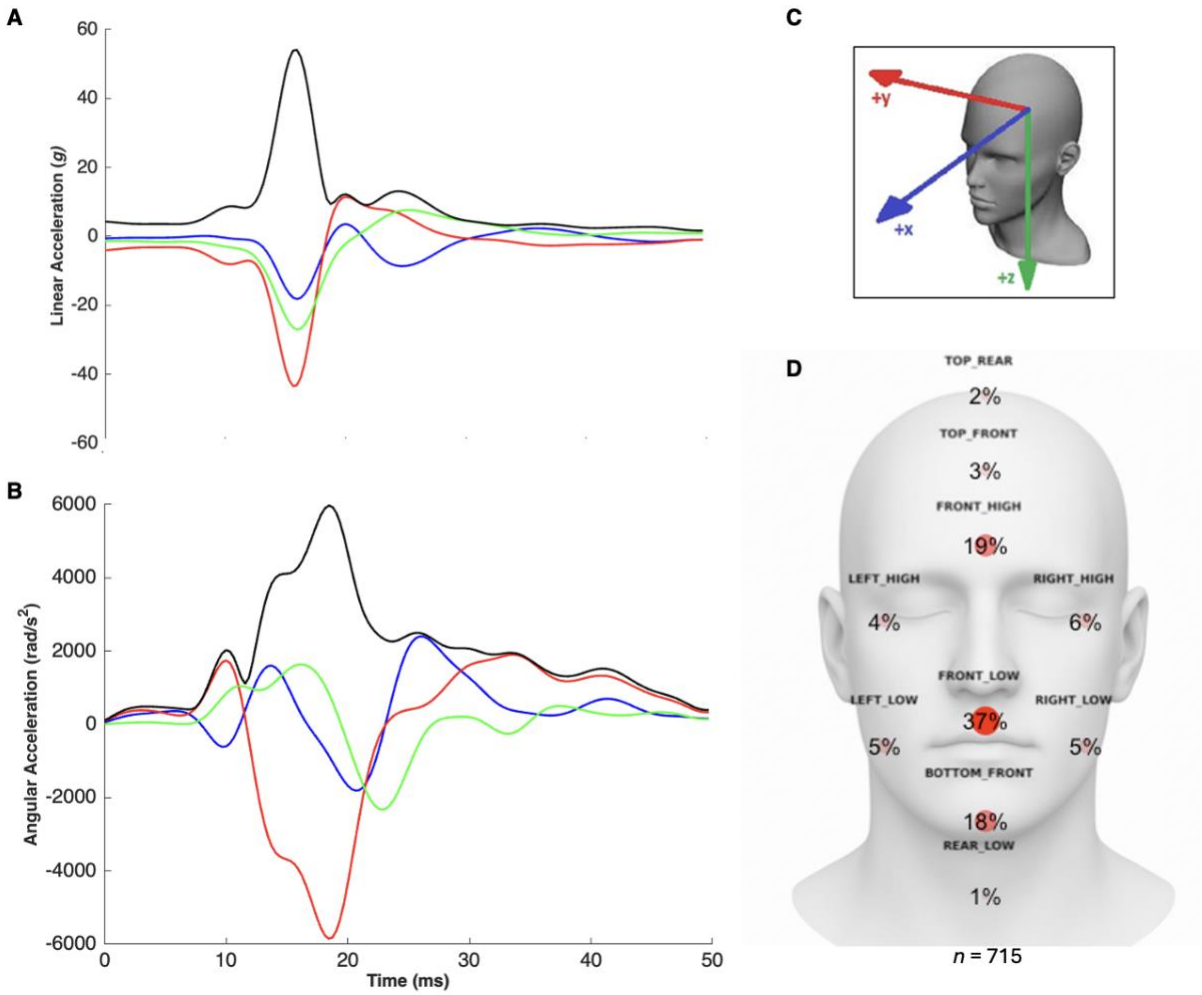

Figure 6.5: Representative traces for PLA (A), PAA (B), triaxial movement key (C) and summary of impact region frequency (D). *CLA* Cumulative linear acceleration *CAA* Cumulative angular acceleration.

To balance false positives and false negatives in the context of combat sports, two threshold combinations were evaluated for impact detection: 5 *g* + 400 rad/s² (a commonly used standard in rugby; 441) and a reduced threshold of 5 *g* + 300 rad/s². The latter demonstrated reduced mean bias (– 0.76 *vs*. – 1.97) and narrower limits of agreement (– 7.99 to 6.48 *vs*. – 10.49 to 6.55) when compared to tallied impacts (Figure 6.6). This suggested that the 300 rad/s² threshold demonstrated a more accurate threshold to validate head acceleration events in striking-based sports, where meaningful impacts may occur with high linear acceleration but

relatively modest angular acceleration, and the remaining analyses were performed according to this.

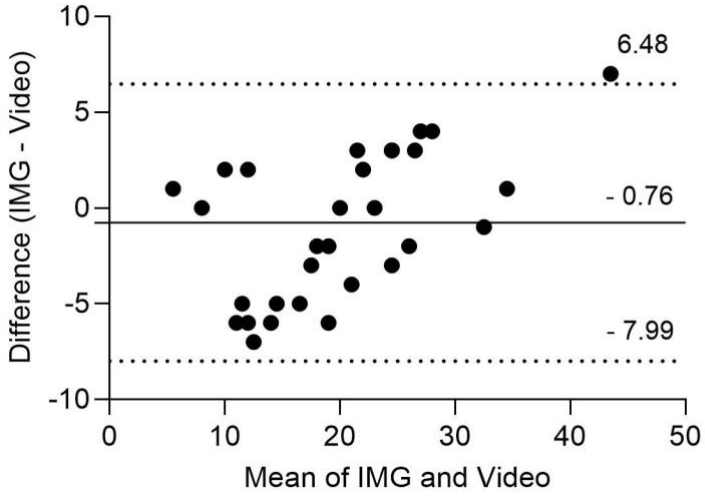

Figure 6.6: Bland-Altman plot for mean difference in IMG-recorded (video verified) and tallied impacts.

6.9.2 Corticospinal excitability and neuromuscular function.

Significant neurophysiological changes were observed following sparring: AMT (β = -1.18, SE = 0.35, t(44.00) = -3.38, 95% CI [-1.86, -0.49], p = .002) and MEP/M_{MAX} ratio (β = -0.051, SE = 0.01, t(44.00) = -3.54, 95% CI [-0.08, -0.02], p < .001). In addition, cSP duration increased post-sparring (β = 0.009, SE = 0.003, t(44.00) = 2.99, 95% CI [0.003, 0.016], p = .005). Maximal voluntary contraction decreased (β = -25.92, SE = 7.34, t(44.00) = 3.53, 95% CI [-0.268, -0.076], p < .001) after sparring; however, no changes were observed in M_{MAX} amplitude (β = -0.14, SE = 0.20, t(44.00) = -0.70, p = .491). There was also a reduction of VA (β = -1.69, p < .001) and HRT from after sparring (β = -0.0113, p = .00085) (Figure 6.7). No change was observed in SICI between PRE and POST (β = 0.22 ± 1.23, t(44) = 0.18, p = .861).

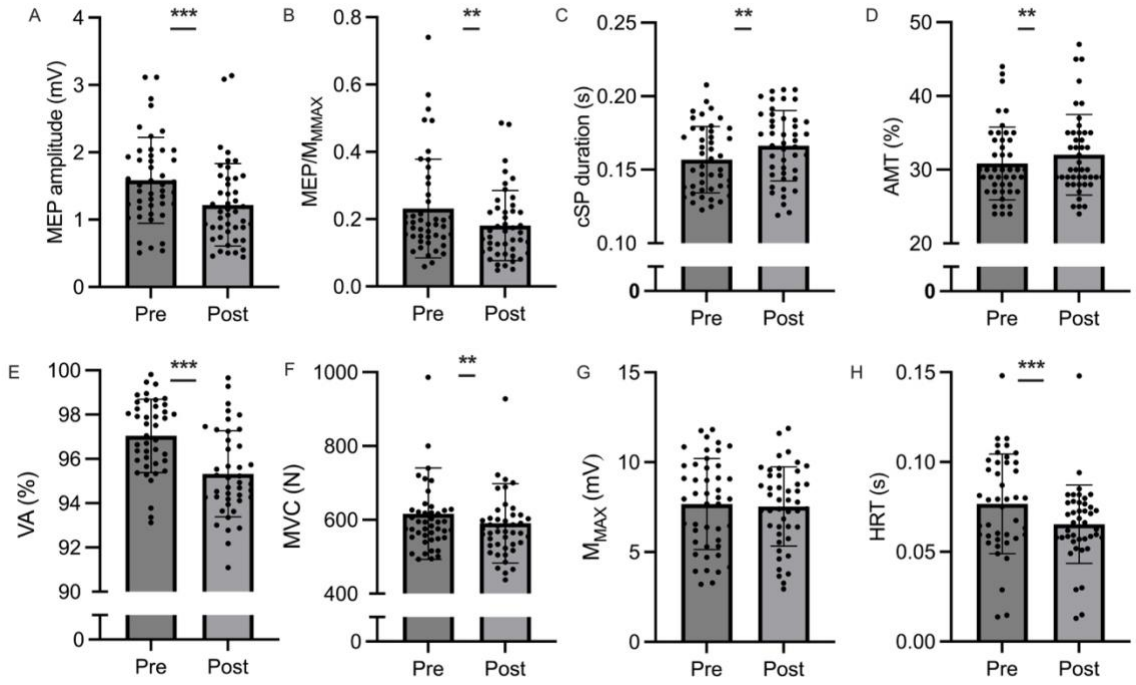

Figure 6.7: Summary of corticospinal and neuromuscular changes following sparring. MEP_{RAW} (A), MEP/M_{MAX} ratio (B), cSP (C), AMT (D, VA (E), MVC (F), M_{MAX} (G) and HRT (H). *cSP* Cortical silent period *MEP* Motor evoked potential M_{MAX} Maximum compound muscle action potential *AMT* Active motor threshold *VA* Voluntary activation *MVC* Maximum voluntary contraction *HRT* Muscle half relaxation time. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

6.9.3 Hydration status

There was no significant change in USG from PRE to POST time points (1.012 ± .004 *vs*. 1.02 ± .031; β = 0.008, SE = 0.004, $t(44.00) = 1.83$, $p = 0.074$).

6.9.4 Associations between head impacts and neurophysiological variables

Significant positive repeated measures correlations were observed between cSP duration and several head impact variables. cSP change was strongly correlated with HIF ($r = 0.69$, 95% CI [0.41, 0.85], $p < .001$; Figure 29A). Additionally, CSP change showed very strong associations with both CLA ($r = 0.89$, 95% CI [0.77, 0.95], $p < .001$) and CAA ($r = 0.91$, 95% CI [0.81, 0.96], $p < .001$; Figure 29B & C). No other repeated measures correlations reached statistical significance (Figure 6.8D – O).

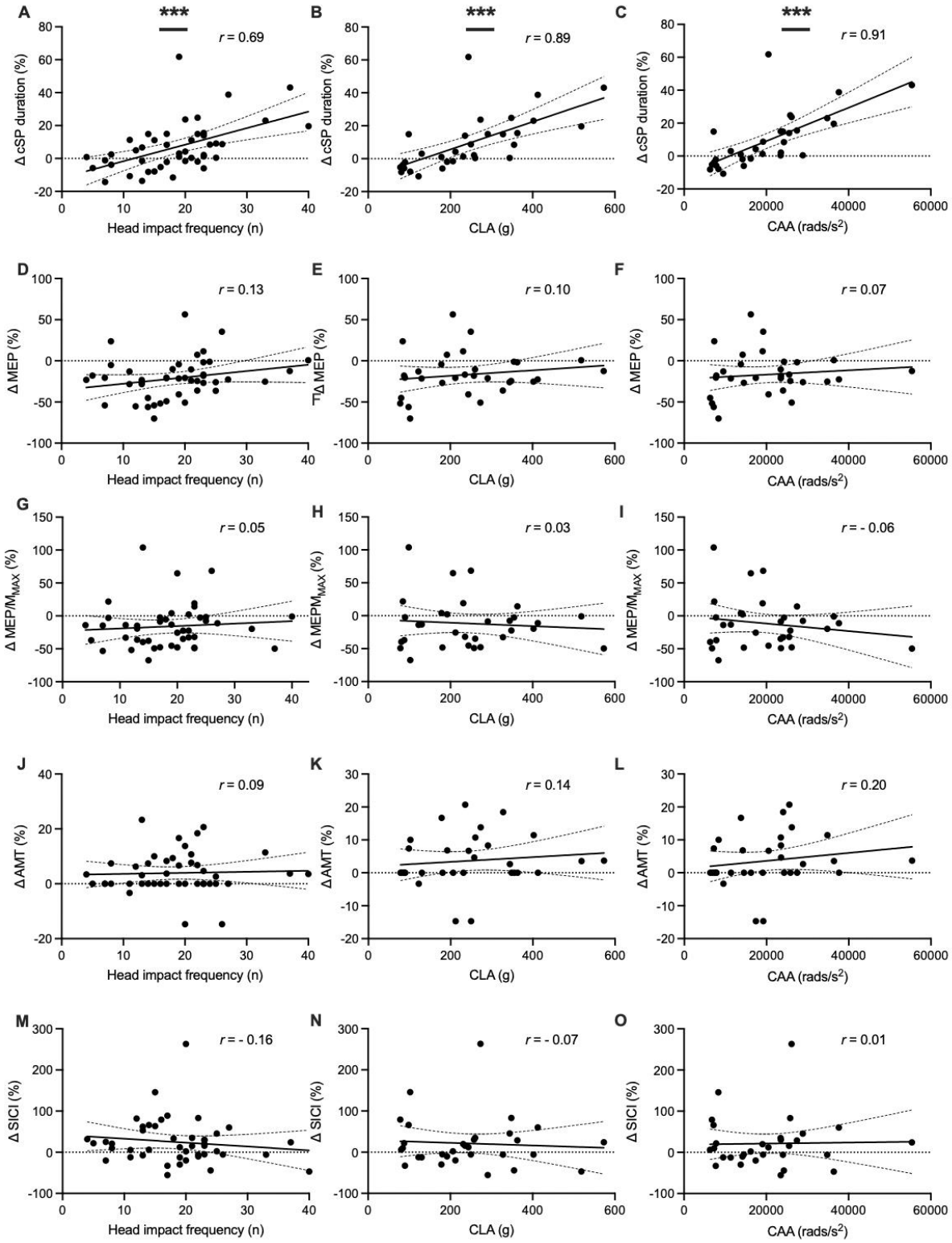

Figure 6.8: Repeated measures correlations for all neurophysiological variables against head impact frequency (HIF), cumulative linear acceleration (CLA) and cumulative angular acceleration (CAA). *cSP* Cortical silent period *MEP* Motor evoked potential M_{MAX} Maximum compound muscle action potential *AMT* Active motor threshold *SICI* Short-interval intracortical inhibition. *** $p < 0.001$.

An LMM was conducted on the predictors that demonstrated a strong, statistically significant positive association with cSP in the repeated measures correlation analysis. The LMM demonstrated that HIF was a significant predictor of cSP change after accounting for participant-level variability (β = 1.27, SE = 0.24, t(36.75) = 5.29, p < .001). In addition, both CLA (β = 0.91, SE = 0.10, t(23.87) = 9.58, p < .001) and CAA (β = 1.08, SE = 0.10, t(23.90) = 10.97, p < .001) significantly predicted cSP change (Table 6.1). Intercepts for all three models were not statistically significant (p > .05).

Table 6.1: LMM estimates for the association between head impact metrics (HIF, CLA, CAA) and cSP duration *HIF* Head impact frequency *CLA* Cumulative linear acceleration *CAA* Cumulative angular acceleration. CLA was scaled x 10 and CAA was scaled x 1000 for clarity.

Model	Estimate (β)	Std. Error	*t* value	*p* value
HIF	1.27	0.24	5.29	< .001 ***
CLA	0.91	0.10	9.58	< .001 ***
CAA	1.08	0.10	10.97	< .001 ***

6.9.5 Associations between hydration status and neurophysiological variables

No significant repeated measures correlations were observed between USG at baseline or delta change and all neurophysiological variables. In addition, inclusion of hydration status (USGPre or USGDelta) as a covariate in the LMM did not alter the significance or strength of the main predictors (HIF, CLA, or CAA) of cSP, nor was independently associated with cSP across any model (p > 0.05; Table 6.2).

Table 6.2: Full parameter estimates from LMMs testing head impact metrics (HIF, CLA, CAA) with and without hydration covariates (USGPre, USGDelta) as predictors of cSP duration. *HIF* Head impact frequency *CLA* Cumulative linear acceleration *CAA* Cumulative angular acceleration *USG* Urine specific gravity.

Model	Predictor	Estimate (β)	Std. Error	t value	p value
HIF + USGPre	HIF	1.24	0.25	5.01	< .001 ***
	USGPre	–156.16	380.55	–0.41	0.68
HIF + USGPost	HIF	1.24	0.24	5.08	< .001 ***
	USGPost	–38.29	54.38	–0.70	0.49
HIF + Δ USG	HIF	1.25	0.24	5.12	< .001 ***
	USGDelta	–0.39	0.58	–0.67	0.51
CLA + USGPre	CLA	0.91	0.15	9.01	< .001 ***
	USGPre	28.86	291.77	0.10	0.92
CLA + USGPost	CLA	0.91	0.15	9.12	< .001 ***
	USGPost	4.16	35.71	0.12	0.91
CLA + ΔUSG	CLA	0.91	0.15	9.17	< .001 ***
	USGDelta	0.04	0.38	0.11	0.92
CAA + USGPre	CAA	1.08	0.11	10.58	< .001 ***
	USGPre	209.70	261.30	0.81	0.43
CAA + USGPost	CAA	1.08	0.11	10.46	< .001 ***
	USGPost	3.84	31.94	0.12	0.91
CAA + ΔUSG	CAA	1.08	0.11	10.51	< .001 ***
	USGDelta	0.01	0.34	0.03	0.98

6.9.6 Associations between session length, RPE and neurophysiological variables

No significant repeated measures correlations were observed between session duration and any of the neurophysiological variables. Similarly, session RPE was not significantly associated with cSP, AMT, SICI and VA. However, higher session RPE was negatively correlated with MEP_{RAW} (r = -0.56, p = 0.002, 95% CI [-0.77, -0.24]) and MEP/M_{MAX} (r = -0.48, p = 0.008, 95% CI [-0.72, -0.14]).

An LMM was conducted to examine the relationship between corticospinal excitability and subjective exertion ratings (RPE). MEP_{RAW} significantly predicted RPE, with a negative association observed (β = –0.027, SE = 0.005, $t(43)$ = –5.00, $p < .001$), indicating that lower excitability was associated with higher perceived effort. Similarly, association with MEP/M_{MAX} was found (β = –0.010, SE = 0.005, $t(43)$ = –2.09, p = .043). Intercepts for both models were significant ($p < 0.001$), although both models demonstrated singular fits, likely due to minimal variance in random intercepts.

6.10 Discussion

This study aimed to determine whether neurophysiological changes following sparring could be explained by RHI exposure, quantified using IMG technology, and hypothesised that higher RHI load would be associated with greater cortical inhibition and reductions in corticospinal excitability. The main finding was that changes in cSP duration were significantly correlated with cumulative acceleration forces and RHI frequency, indicating a relationship between head impact load and corticospinal inhibition. Participants sustained an average of 19 head impacts per session, with a mean PLA of 34.4 *g* and PAA 3499 rad/s². In a larger sample than Part 1, and across repeated sessions, significant post-sparring increases in cSP and AMT were observed, alongside reductions in MEP/M_{MAX}, VA and MVC. Finally, session intensity (as indexed by RPE) was negatively associated with changes in corticospinal excitability (MEP/M_{MAX}).

The present study observed an average of 19 head impacts per sparring session, with a mean PLA of 11 *g* and PAA of 924 rad/s². While these are representative of a cohort of athletes from one gymnasium, these values are broadly comparable to the only other findings in MMA by Tiernan et al. (60) and in boxing (using the same IMG system; 430) but notably exceed the head kinematic loads reported in rugby training. For example, an IMG study by Tooby and

colleagues (441) in professional rugby league players found a median PLA of 7 *g* and PAA of 600 rad/s², but approximately 52 head acceleration events per match. Collectively, these comparisons suggest that the neurophysiological changes observed in combat sport athletes may be driven by a higher concentration of head acceleration events (which appear to be non-concussive) within shorter exposure windows compared to team sport counterparts. While the present study focused on immediate post-sparring effects, it was found in Part 1 that the magnitude of these changes appeared to diminish by 48 h. This may indicate a short-lived, subclinical disturbance in cortical function, with a relatively rapid recovery trajectory, likely reflecting the moderate impact burden (34 *g*) compared to concussive impacts (87 *g*; 60).

A key strength of the present study was the objective quantification of head impact exposure using instrumented mouthguards, which revealed strong linear associations between cumulative head impact load and head impact frequency with changes in cSP duration (Figure 6.8A–C). While cumulative head acceleration exposure over the course of an athletic career has been implicated in elevated risk for early neurodegenerative diseases, including chronic traumatic encephalopathy (442-445), the cumulative burden sustained across single or multiple sparring sessions remains poorly studied, particularly with respect to its acute effects on central nervous system function. Luke et al. (446) reported median cumulative PLA and PAA values of 661 *g* and 64,990 rad/s², respectively, across a rugby athlete's season (from captured events only). In comparison, the present study observed cumulative PLA and PAA values of 573 *g* and 55,408 rad/s² within a single sparring session, highlighting the substantial head impact load experienced in combat sport training and its relative intensity compared to collision-based team sports.

The present findings reflect acute alterations in corticospinal excitability and inhibition consistent with previous reports following non-concussive impacts (8,9). The observed

increase in cSP duration also aligns with our findings suggesting transient $GABA_B$-mediated inhibitory upregulation post-impact (Figure 6.7C). However, the current findings also support a relationship between cumulative head impact burden and cortical inhibition for the first time. The observed increase in cSP with higher volume-related head impact metrics may represent a compensatory inhibitory response to preserve cortical integrity, although the underlying mechanisms remain unclear. The absence of significant associations between SICI or MEP/M_{MAX} (Figure 6.8D – O) and impact metrics may reflect differential cortical circuitry sensitivity or temporal dynamics, as previously observed changes (415,447) follow a single concussive event. In contrast, our data point to the possibility that the accumulation of lower-magnitude accelerations might selectively influence $GABA_B$-mediated pathways (reflected in cSP). Interestingly, the strongest predictor of cSP prolongation was CAA (Table 6.1), which aligns with concussion and chronic traumatic encephalopathy literature, identifying rotational acceleration to be the most damaging in concussion symptomology (59) and risk of chronic traumatic encephalopathy diagnosis (448).

Central drive (VA), corticospinal excitability and maximal strength significantly declined following sparring, with no changes in M_{MAX} amplitude, supporting a central rather than peripheral origin of fatigue. These reductions in supraspinal drive mirror TMS findings following intense exercise (341). Notably, reductions in MEP_{RAW} were significantly correlated with session RPE, indicating that subjective perceptions of exertion might influence corticospinal suppression. Indeed Brasil-Neto et al. (449) attributed the post-exercise reduction in MEP_{RAW} to a decreased efficiency in the generation of descending volleys in the primary motor cortex. While the observed decreases in VA and MEP_{RAW} may reflect disrupted cortical output, the underlying cause of force reduction is likely multifactorial, involving both inhibitory adaptations and psychological arousal shifts inherent to exercise (450). Despite reductions in MVC, peripheral excitability (M_{MAX}) remained unchanged, indicating intact

neuromuscular propagation. The isolated reduction in HRT is, therefore, unlikely to reflect fatigue-induced slowing of Ca^{2+} reuptake into the SR, which typically occurs following high-volume exercise. Instead, it may reflect the effects of muscle warming, which is known to enhance enzymatic activity and accelerate Ca^{2+} reuptake, thereby shortening muscle relaxation time (451). These data reinforce the predominantly central origin of neuromuscular changes in response to sparring.

In contrast to the modest yet statistically significant hydration changes observed in Part 1, the current data revealed a non-significant increase in USG post-sparring, with the LMM indicating negligible between-subject variability (Table 6.2). Additionally, no significant associations were found between hydration status and any neurophysiological variables. As with Part 1, these findings contrast with those reported by Belfort et al. (416), where most athletes began training in a hypo-hydrated state and failed to restore fluid balance. This suggests that, within the context of these sparring sessions, fluid shifts were likely too small or inconsistent to elicit measurable cortical and neuromuscular alterations. This reinforces the earlier interpretation that a more substantial or prolonged state of hypo-hydration, such as that seen during RWL phases, may be necessary to assess if there are any interactions with RHI-induced neurophysiological changes.

While these findings extend prior TMS-based investigations in combat sports and repetitive head impacts, it is worth noting that individual variability in sparring intensity, protective gear, and technical skill likely influence head impact exposure. Moreover, the study did not evaluate longer-term recovery beyond the immediate post-sparring period. Several methodological limitations should also be acknowledged: the head impact data were limited to peak linear and angular acceleration values, which provide only a partial representation of the full acceleration-time profile. This approach may overlook potentially severe or sustained impacts that fall short

of peak-based thresholds but carry biomechanical significance. In addition, while session RPE was obtained, no objective physiological monitoring e.g., heart rate, or pre-session screening tools (such as SCAT5 symptom checklists) were employed. It is therefore possible that some athletes entered the sparring session with existing symptoms or elevated fatigue, which could influence neurophysiological responses. Future research should consider repeated TMS assessments across a training camp, with concurrent physiological monitoring and baseline screening, to better capture the cumulative effects of repetitive non-concussive trauma under real-world conditions.

A further consideration when interpreting these data is the relationship between head acceleration and impact force. While accelerometery-derived metrics such as PLA and PAA are commonly used as proxies for head impact severity, they reflect the kinematic response of the head rather than the direct force applied. The force experienced by the head is dependent on both acceleration and the mass involved in the collision, meaning that similar acceleration values may arise from impacts with differing force magnitudes depending on the nature of the impact. Moreover, angular acceleration is more closely related to shear strain within brain tissue, whereas linear acceleration is associated with translational forces and pressure gradients (424,451,452). As such, PLA and PAA should be interpreted as complementary indicators of impact severity rather than direct measures of force. This distinction is important when considering injury risk, as brain deformation (thus neurophysiological disruption) is likely influenced by both the magnitude and direction of acceleration, as well as the temporal characteristics of the impact.

6.11 Conclusion

This study demonstrates that RHIs from combat sports sparring can elicit measurable cortical changes, particularly increased cortical inhibition, which is predicted by both the frequency and magnitude of RHIs. This is a novel finding that provides mechanistic insight into how cumulative non-concussive load translates into neurophysiological disruption and suggests that head impact metrics could serve as objective predictors of brain function change. Such insights have important implications for the development of athlete monitoring strategies and preventative frameworks, enabling early identification of individuals at risk of impaired neuromuscular function. In addition, these changes occur independently of peripheral fatigue, pointing to a central mechanism of neuromuscular impairment. The integration of IMG technology with neurophysiological assessments such as TMS, offers a valuable approach for monitoring acute brain responses and recovery following RHIs.

6.12 Linking to Chapter 7

Group-level averages may mask important inter-individual variability in susceptibility, recovery and neurophysiological response patterns. Therefore, the next chapter adopts a case study approach, examining longitudinal changes in corticospinal function across a full fight camp in three individual athletes. This allows for a more detailed characterisation of cumulative head impact exposure and athlete-specific neuromuscular responses over time, in correspondence with the details of their fight preparation camp.

Chapter 7:

Individual-level analysis of neurophysiological responses to repetitive head impacts – a case study approach.

7.1 Introduction

Combat sports athletes are frequently exposed to repeated non-concussive impacts during sparring and competition (52). This is a concern, given mounting evidence that such exposure can lead to measurable neurophysiological changes (7). In Part 1 of Chapter 6 of the current thesis, acute changes in corticomotor and neuromuscular function of thirteen athletes were monitored following a single sparring session. Here, increases in cortical inhibition and reduced corticospinal excitability were identified. Part 2 extended these findings by assessing longitudinal patterns across multiple sparring sessions during a fight camp, alongside quantified head impact exposure using IMGs. These analyses revealed associations between head impact metrics and cortical inhibition across time.

To date, no previous study has tracked combat athletes longitudinally across a full fight preparation camp using either TMS and IMG technology, nor the combination. The integration of these two tools allows for a novel mapping of how brain function and head impact exposure interact during one of the most physiologically and cognitively demanding periods in an athlete's calendar. While previous research has examined the physiological responses to a fight preparation camp (318) and cumulative RHIs have been monitored across competitive seasons in Rugby (453), the cyclical and individualised nature of fight camp preparation remains underexplored.

While group-level analyses provide valuable insights, they can obscure meaningful inter-individual variability (454). This variability is increasingly recognised in studies using neuromodulatory and neurophysiological techniques. For instance, responses to single- and paired-pulse TMS show high individual heterogeneity, with only around 60% of participants demonstrating the expected pattern of plasticity following stimulation protocols (455,456).

Several factors likely contribute to this variability, including skull thickness, cortical folding, baseline excitability, and individual neurochemical profiles (457,458).

To address individual-level change, this case study adopted the TE as a benchmark for evaluating measurement variability. TE provides an estimate of within-subject variation expected under stable conditions and is widely used in athlete monitoring and sports performance research to assess whether observed changes exceed the bounds of measurement noise (454,459). A threshold of 1.5 multiplied by the TE was applied to define meaningful change, aligning with conventions in applied sport science where smallest worthwhile change (SWC) or SDC is not directly calculated (460). This threshold was preferred over alternatives such as the SWC or SDC, which require additional distributional or confidence assumptions that may not align with the exploratory and applied nature of neuromuscular monitoring in small samples.

This approach is especially relevant during a fight preparation camp, where combat athletes undergo substantial variation in training intensity, sparring volume, and weight management strategies. In the current chapter of the thesis, a case study was conducted to monitor neurophysiological and head impact data from three combat sport athletes across 9 – 13 sparring sessions during a fight preparation camp, incorporating descriptive statistics, and with the aim of examining group-level patterns while also using TE-based thresholds to explore individual-level responses over time.

7.2 Methods

7.2.1 Participants

Three participants took part in the present study as part of a fight preparation camp. A summary of participant and fight preparation camp details can be found below in Table 7.1.

Table 7.1: Participant and fight preparation camp details (self-reported).

Participant	Age (y)	Stature (cm)	Fighting level	Fighting at end of camp?	Body mass at start of camp (kg)	Body mass at weigh-in (kg)	Weight-cut in final week (kg)	Weight-cut in final day (kg)	Weight-cutting method	Range of training sessions per week	No. of full-contact sparring per week
1	18	170	Amateur	No. Regular sparring partner	65	-	-	-	-	9-10	2
2	29	185	Amateur	Yes	76	66	5.8	4.2	"Water loading, reduce caloric intake focusing on fats and proteins. Hot water bath in final day"	10-15	2-3
3	28	173	Professional (Debut fight)	Yes	70	56.68	7.0	4.5	"Cutting salts, carbs and water cutting in the final evening"	12-14	3

7.2.2 Study design

This chapter presents a naturalistic observational study designed to explore the cumulative head impact exposure and session-to-session neurophysiological change across the entirety of a fight-preparation camp. Three participants were monitored across a 10-week fight preparation camp for an amateur and professional MMA bout (Cage Warriors Academy). For the remaining details related to study design, please see Chapter 6 section 6.2.3 and 6.7.2.

7.2.3 Neuromuscular function

7.2.3.1 Electromyography

For electromyography, please see 'General Methods' section 4.2.2.1.

7.2.3.2 Maximal voluntary contractions

For full procedures of the MVC, please see 'General Methods' section 4.2.2.2.

7.2.3.3 Motor nerve stimulation

For motor nerve stimulation, please see 'General Methods' section 4.2.2.3.

7.2.3.4 Transcranial magnetic stimulation

For transcranial magnetic stimulation, please see 'General Methods' section 4.2.2.4.

7.2.3.5 Data processing

For data processing methods, please see 'General Methods' section 4.2.2.5.

7.2.4 Rating of perceived exertion

Session intensity was assessed as described in Chapter 6, Part 1, section 6.2.5.

7.2.5 Instrumented mouthguard

For details of instrumented mouthguards, please see Chapter 6 section 6.6.4.

7.2.6 Data capture (video analysis)

For details of data capture methodology, please see Chapter 6 section 6.6.5.

7.2.7 Statistical analysis

Descriptive statistics were reported for head impact exposure, and for each neurophysiological variable the mean pre-to-post change, standard deviation (SD), standard error (SE), and 95% confidence intervals (CIs) were calculated separately for each participant across sparring sessions. In addition, the typical error (TE) was derived from the SD of the Pre-Post difference scores using the following equation:

$$TE = \frac{SDdiff}{\sqrt{2}}$$

Where *SDdiff* represents the standard deviation of the difference scores between Pre and Post measurements. Session changes exceeding TE × 1.5 were then used to indicate potentially meaningful change beyond measurement noise. This approach enabled sensitivity to true physiological changes at the session level while accounting for intra-individual reliability.

7.3 Results

Across the monitored sparring sessions, session duration ranged from 720 to 1,200 s (typically 3 × 5 min rounds), with reported RPE values between 4 – 9 and total RHIs per session ranging from 8 to 40, reflecting moderate variability in session intensity and impact exposure both within and between participants (Table 7.2).

Table 7.2: Individual session details for each participant. RPE rating of perceived exertion. RHIs repetitive head impacts (video verified).

Participant	Measure	Session									
		1	2	3	4	5	6	7	8	9	10
P1	Session length (s)	900	900	900	900	920	900	1080	900	900	
	No. of rounds x round length (min)	3 x 5	3 x 5	3 x 5	3 x 5	3 x 5	3 x 5	5 x 3	3 x 5	3 x 5	
	RPE	7	4	5	7	4	7	5	6	7	
	Training load	105	60	75	105	60	105	75	90	105	
	RHIs	23	22	23	22	20	22	23	23	18	
P2	Session length (s)	900	900	900	720	1080	900	1200	780	900	
	No. of rounds x round length (min)	3 x 5	3 x 5	3 x 5	4 x 3	5 x 3	3 x 5	4 x 5	4 x 3.25	3 x 5	
	RPE	7	9	6	6	6	8	6	6	6	
	Training load	105	135	90	72	90	120	120	52	90	
	RHIs	11	20	15	33	20	27	23	26	13	
P3	Session length (s)	900	900	920	900	720	900	900	1200	900	900
	No. of rounds x round length (min)	3 x 5	3 x 5	3 x 5	3 x 5	4 x 3	3 x 5	3 x 5	4 x 5	3 x 5	3 x 5
	RPE	6	4	5	7	7	7	6	7	8	6
	Training load	90	60	75	105	84	105	90	140	120	90
	RHIs	40	8	17	14	37	17	19	16	25	19

Tables 7.3, 7.4 & 7.5 present the mean change from Pre-Post session, and the associated SD, SE and 95% CIs. shows the number of trials exceeding the TE threshold across neurophysiological variables. Notably, some metrics (e.g., MEP_{RAW} and VA) showed consistent directional changes and relatively narrow CIs across sessions, while others (e.g., SICI/MEP) demonstrated greater within-participant variability. A summary of all variables can be found in Table 7.6.

Table 7.3: Participant 1 – Change scores and 95% confidence intervals across all trials *(N = 9 trials; Pre–Post differences per session)*

Measure	Mean Change	SD	SE	95% CI Lower	95% CI Upper
MEP_{RAW} (mV)	-0.07	0.33	0.11	-0.33	0.18
SICI/MEP	0.84	7.97	2.66	-5.29	6.96
cSP (s)	0.01	0.02	0.01	-0.01	0.02
AMT (%)	1.67	2.40	0.80	-0.18	3.51
MEP/M_{MAX}	-0.02	0.07	0.02	-0.07	0.03
VA (%)	-1.75	1.22	0.41	-2.69	-0.81
M_{MAX} (mV)	0.32	1.31	0.44	-0.68	1.32
MVC (Nm)	-0.14	0.32	0.11	-0.39	0.11
HRT (s)	-0.008	0.019	0.006	-0.022	0.007

Table 7.4: Participant 2 – Change scores and 95% confidence intervals across all trials *(N = 9 trials; Pre–Post differences per session)*

Measure	Mean Change	SD	SE	95% CI Lower	95% CI Upper
MEP_{RAW} (mV)	-0.42	0.57	0.19	-0.86	0.02
SICI/MEP	3.44	5.70	1.90	-0.94	7.82
cSP (s)	0.01	0.03	0.01	-0.01	0.03
AMT (%)	0.67	3.94	1.31	-2.36	3.69
MEP/M_{MAX}	-0.09	0.17	0.06	-0.21	0.04
VA (%)	-1.43	1.82	0.61	-2.82	-0.03
M_{MAX} (mV)	-0.22	1.02	0.34	-1.00	0.57
MVC (Nm)	-0.23	0.50	0.17	-0.62	0.16
HRT (s)	-0.02	0.02	0.01	-0.03	-0.004

Table 7.5: Participant 3 – Change scores and 95% confidence intervals across all trials *(N = 9 trials; Pre–Post differences per session)*

Measure	Mean Change	SD	SE	95% CI Lower	95% CI Upper
MEP_{RAW} (mV)	-0.29	0.35	0.11	-0.53	-0.04
SICI/MEP	-1.89	9.96	3.15	-9.01	5.23
cSP (s)	0.01	0.02	0.01	-0.01	0.02
AMT (%)	0.80	1.32	0.42	-0.14	1.74
MEP/M_{MAX}	-0.03	0.06	0.02	-0.07	0.01
VA (%)	-1.68	2.80	0.89	-3.68	0.32
M_{MAX} (mV)	-0.53	2.01	0.63	-1.97	0.90
MVC (Nm)	-0.05	0.23	0.07	-0.21	0.12
HRT (s)	-0.01	0.01	0.004	-0.01	0.002

Table 7.6. Number and percentage of individual sessions exceeding the TE × 1.5 threshold across all measured variables for each participant.

Participant		MEP_{RAW} (mV)	SICI/ MEP	cSP (s)	AMT (%)	MEP/ M_{MAX}	VA (%)	M_{MAX} (mV)	MVC (Nm)	HRT (s)
1	TE x 1.5	0.35	8.45	0.02	2.54	0.07	1.30	1.38	0.34	0.02
	n > TE x1.5	2	3	4	3	3	6	2	3	3
	%	22	33	44	33	33	67	22	33	33
2	TE x 1.5	0.61	6.05	0.03	4.18	0.18	1.93	1.08	0.54	0.02
	n > TE x 1.5	2	3	3	3	1	3	3	4	3
	%	22	33	33	33	11	33	33	44	33
3	TE x 1.5	0.37	10.56	0.02	1.40	0.06	2.97	2.13	0.24	0.12
	n > TE x 1.5	4	2	2	2	1	6	2	5	3
	%	40	20	20	20	10	60	20	50	30

n = sessions

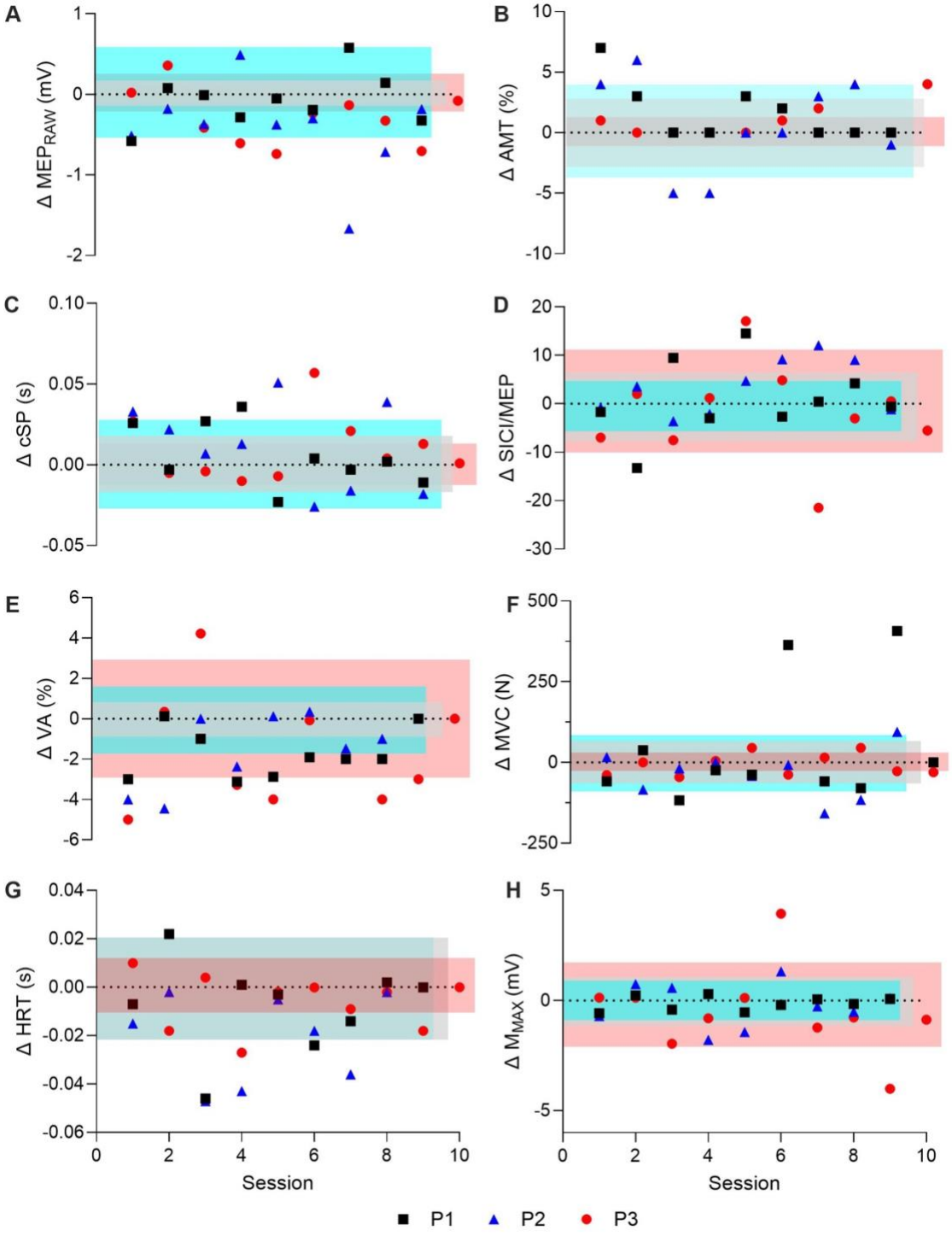

Figure 7.1: Summary of Δ Pre-Post change of MEP (A), AMT (B), cSP (C), SICI/MEP (D), VA (E), MVC (F), HRT (G) and M_{MAX} (H) per sparring session and participant. Shaded bands indicate participant-specific TE × 1.5 thresholds (Grey: P1, Blue: P2, Red: P3). *cSP* Cortical silent period *MEP* Motor evoked potential *M_{MAX}* Maximum compound muscle action potential *AMT* Active motor threshold *SICI* Short interval intracortical inhibition.

Table 7.7 outlines the head impact characteristics for each participant across the fight camp. The number of head impacts per participant was relatively consistent, although the CLA and CAA varied between individuals.

Table 7.7: Head impact frequency, cumulative load and peak load across the fight preparation camp. *HIF* Head impact frequency *CLA* Cumulative linear acceleration *CAA* Cumulative angular acceleration *PLA* Peak linear acceleration *PAA* Peak angular acceleration.

Variable		Participant 1 (n = 10)	Participant 2 (n = 12)	Participant 3 (n = 13)
HIF (n)	Mean ± SD	21.8 ± 1.7	20.9 ± 7.2	21.2 ± 10.1
	Range	18 – 23	11 – 33	8 – 40
	Sum	238	246	249
CLA (*g*)	Mean ± SD	263.2 ± 73.2	251.2 ± 113.3	224.8 ± 194.8
	Range	180.6 – 362.7	102.3 – 412.5	78.3 – 573.0
	Sum	2279	2364	2368
CAA (rad/s²)	Mean ± SD	20,609 ± 5,264	22,332 ± 10,751	18,737 ± 16,988
	Range	14,225 – 27,434	8,343 – 37,641	6,333 – 55,408
	Sum	178,256	209,301	200,405
PLA (*g*)	Mean ± SD	12.5 ± 6.1	11.8 ± 5.1	11.9 ± 5.4
	Peak	35	30	37
PAA (rad/s²)	Mean ± SD	958 ± 541	1,030 ± 521	1,000 ± 629
	Peak	3001	2908	5207

n: sessions

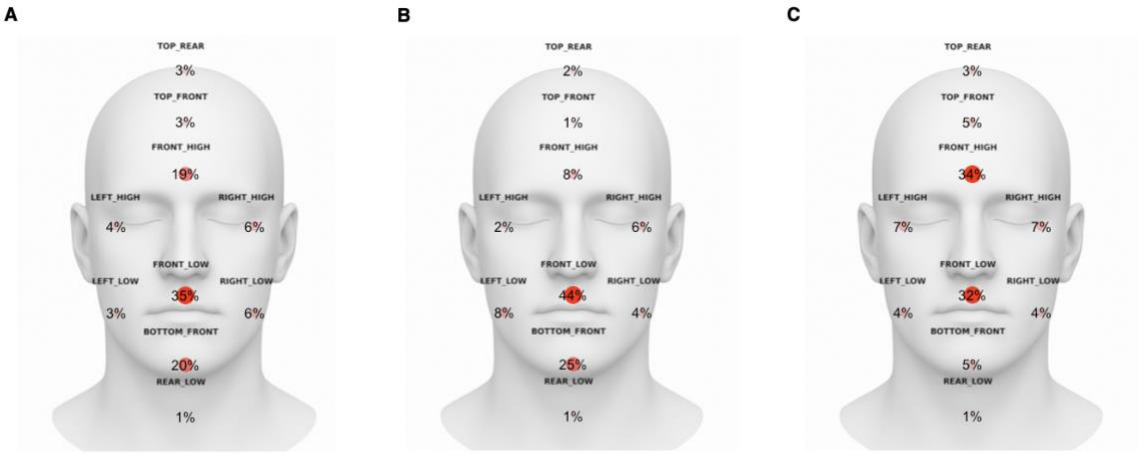

Figure 7.2: Head impact region frequency (%) across participants slightly different in the front regions 1 (A), 2 (B) and 3 (C).

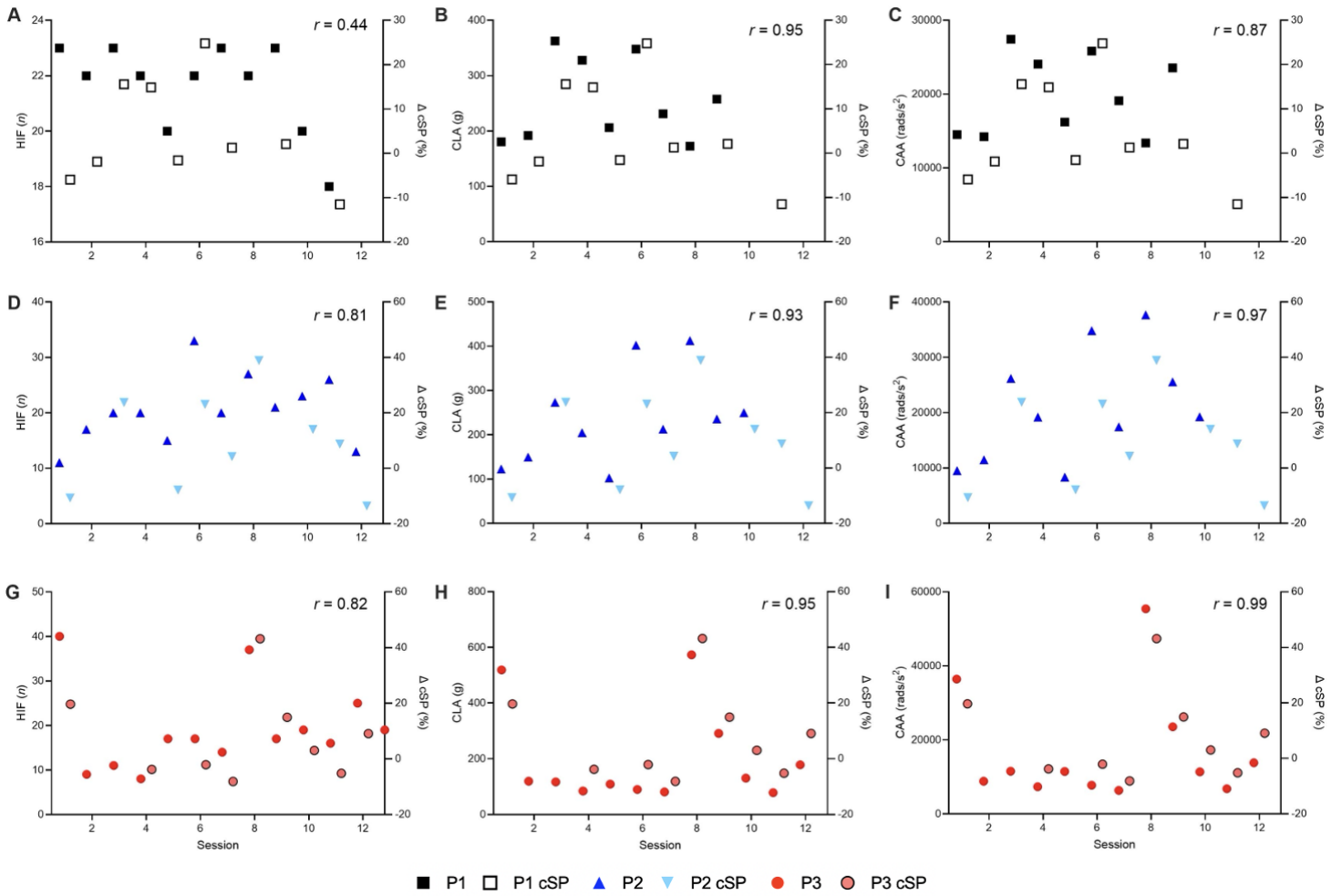

Figure 7.3: Visual depiction of head impact metrics alongside changes in cSP duration per session and participant. *HIF* Head impact frequency *CLA* Cumulative linear acceleration *CAA* Cumulative angular acceleration *cSP* Cortical silent period duration. Pearson's *r* values are displayed in the top-right of each plot as a descriptive indicator of potential associations. These correlations are presented as exploratory and non-inferential**,** given the limited sample size and case study design.

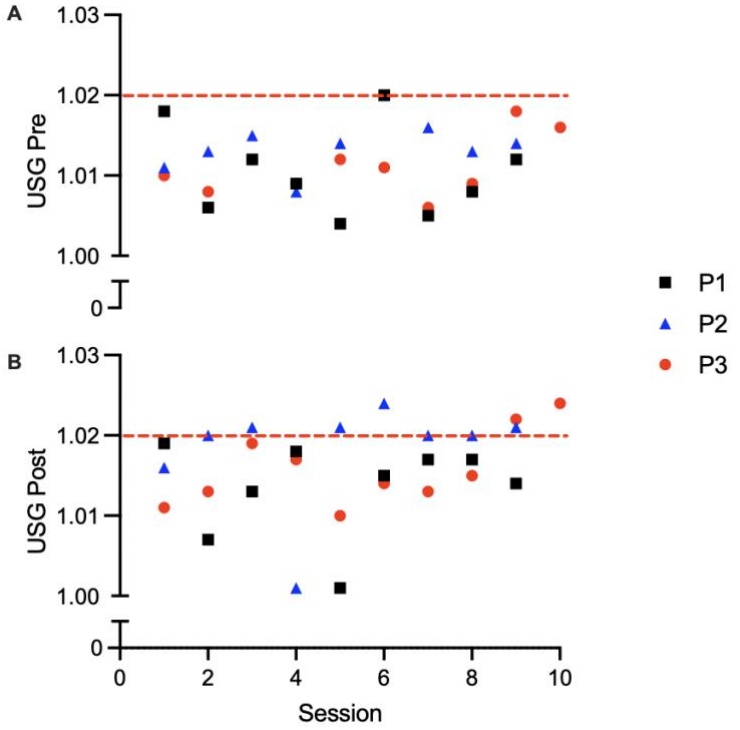

Figure 7.4: Breakdown of USG Pre (A) and Post (B) value per sparring session and participant. A 1.02 threshold has been applied to depict sessions in which participants were potentially dehydrated after sparring. *USG* Urine specific gravity.

7.4 Discussion

This case series provides novel insights into the neurophysiological responses and head impact burden experienced across a fight preparation camp in combat athletes. Across ~ 10 sparring sessions per athlete, the cumulative number of recorded RHIs ranged from 238 to 249, with PLA values between 11.8 – 12.5 *g* and peak values reaching 37 *g*. Similarly, PAA was ~ 1000 rad/s² for each athlete, with peak values reaching 5207 rad/s². Despite broadly similar session counts and impact volumes, there was notable between-athlete variability in the magnitude and distribution of impact location. From a neurophysiological standpoint, changes in VA and MVC most frequently exceeded the TE × 1.5 threshold (12 – 15 of 29 sessions) across the camp, followed by cSP duration and HRT (9 of 29), while MEP/M_{MAX} demonstrated relatively

few threshold-exceeding changes (5 of 29), highlighting differential sensitivity across outcome measures.

Across the three participants, there were clear discrepancies in training intensity and weight-cutting practices that may have shaped the physiological responses observed throughout the fight camp (Table 7.1). While all athletes completed between 9 to 15 training sessions per week, the total weekly sparring exposure was greatest in P3, who completed up to 3 full-contact sessions weekly. P3 was preparing for their professional debut, which may be the reason for the overall higher training volume. Despite this, session durations remained similar between all three athletes (Table 7.2). Importantly, both P2 and P3 underwent weight cuts, reducing body mass by 10 kg and 13.3 kg, respectively, with over 4 kg lost in the final 24 h before weigh-in. These cuts were achieved using common but physiologically stressful methods, including water loading, carbohydrate restriction, and heat exposure (362). RWL of this magnitude, particularly involving dehydration, is known to impair neuromuscular performance and central drive (77) but unlikely to contribute to the individual differences in neurophysiological outcomes reported here as hydration status (measured via USG; Figure 7.4) was measured prior to these shifts in fluid balance (final week of camp). Participants generally maintained euhydration at baseline across sessions, but post-sparring USG values regularly exceeded the hypo-hydration threshold in P2, possibly reflecting greater sweat losses in line with their higher body mass reductions. In contrast, P3 showed a gradual drift toward hypo-hydration in later sessions, potentially linked to increasing fluid restriction or dietary manipulation closer to fight day. These patterns suggest that hydration status may not have been a primary driver of neurophysiological alterations during the study period, particularly in a training context where fluid balance was otherwise well maintained.

Threshold-based analysis revealed consistent changes exceeding the TE × 1.5 criterion in key neurophysiological variables, particularly in VA and MVC, followed by cSP duration (Table 7.6). A plausible explanation is that these neuromuscular measures are less susceptible to measurement error and intersession variability than TMS-derived parameters. MVC and VA reflect mechanical, whole-system outputs that integrate both central and peripheral contributions to voluntary force, and when measured under standardised conditions (e.g., consistent posture, instructions, and joint angles), they tend to exhibit higher reliability across repeated sessions (360,461). In contrast, MEP_{RAW}, MEP/M_{MAX} and AMT, are influenced by a range of fluctuating physiological and technical factors including cortical state, coil positioning, background muscle activation, and synaptic responsiveness**,** which can elevate their within-subject variability (462,463). Among TMS measures, the cSP may offer greater utility for repeated assessments than excitability metrics like MEP_{RAW}. Di Virgilio et al. (355) reported that corticomotor inhibition showed better reproducibility and lower CV than corticospinal excitability in athletes exposed to non-concussive impacts. In soccer players, the CV for cSP was 15.2%, compared to 41.6% for excitability measures, suggesting a higher signal-to-noise ratio when using inhibitory outcomes (355). Taken together, these findings support the idea that inhibitory measures (i.e., cSP) and peripheral neuromuscular outputs such as VA and MVC may provide more robust indicators in monitoring head impact-related effects.

Figure 7.3 further reinforces this interpretation by plotting HIF, CLA, and CAA against session-wise changes in cSP duration for each participant. In addition, to further illustrate the potential interaction between RHI exposure and cortical inhibition, exploratory Pearson correlations were conducted between head impact metrics and the percentage change in cSP duration. As shown in Figure 7.3, moderate to strong positive correlations were observed in all three participants. These trends support the findings from Chapter 6, where group-level increases in cortical inhibition were significantly associated with head impact metrics. The

convergence of these results strengthens the interpretation that repetitive non-concussive exposures may contribute to cumulative changes in cortical inhibition across fight preparation.

The present study presents novel findings on the cumulative head impact load sustained across a full fight preparation camp (Table 7.7). Athletes completed between 20 and 30 full-contact sparring sessions over the course of the camp (Table 7.1), with IMG and video data captured from approximately 35% of these sessions. Based on this sampling rate, the observed total of ~ 240 RHIs per athlete can be conservatively estimated to reflect only one-third of total exposure, suggesting a potential true camp-wide total exceeding 700 RHIs per athlete. The average impact measured was approximately 12 *g* (PLA) and 1000 rad/s² (PAA), although this likely reflects the lower end of exposure. Head impact load may vary considerably depending on gym culture, sparring intensity, opponent style, or level of competition, and could be significantly higher in professional athletes preparing for longer bouts or title fights. In comparison, a season-long study in amateur rugby union (38 male players) reported 20,687 head impacts (> 10 *g*), averaging 95 ± 133 impacts per player per match across a typical competitive season (453). While rugby players experience a higher total number of RHIs per season, it is important to contextualise this against the structure of combat sport competition cycles. Amateur and professional combat athletes may engage in 2 – 4 fight camps per year, depending on promotion schedules, injury status, and WCs (312). Therefore, the cumulative load across multiple camps within a single year could approach or exceed the total head-impact exposure seen in other contact sports, particularly if fighters engage in heavy sparring blocks for each bout. Moreover, combat sport impacts often involve direct, rotationally dominant strikes to the head, which may carry a higher per-impact neurological burden despite their lower frequency (59). In a recent analysis of 631 former American football players, each additional 1,000 estimated head impacts sustained over a career was associated with a ~21% increase in the odds of receiving a post-mortem diagnosis of chronic traumatic encephalopathy

(448). Similarly, for every extra 10,000 *g* of cumulative linear acceleration, the odds of CTE increased by ~ 20%, and for every additional 1,000,000 rad/s² of cumulative rotational acceleration, the odds rose by ~ 22%. These findings support the growing consensus that long-term chronic traumatic encephalopathy risk is primarily driven by cumulative exposure to RHIs, rather than by isolated concussive events. This reinforces the potential value of tracking cumulative impact burden in combat athletes as a tool for monitoring long-term brain health across training and competitive seasons.

This study was observational in nature, and several limitations should be acknowledged. First, no physiological monitoring data such as heart rate or internal training load metrics were collected. Although an attempt was made during pilot sessions to incorporate heart rate, tympanic temperature, oculomotor testing, and SCAT symptom checklists via Qualtrics, this approach was ultimately abandoned due to discomfort with HR monitors and a need to minimise disruption to the testing process and athlete routines. Second, no symptom-based data were recorded following sparring sessions, which limits the ability to contextualise neurophysiological changes relative to perceived head trauma. Third, while athletes completed 20 to 30 sparring sessions, only a subset (~ 35%) were monitored using IMGs and video, potentially underestimating total head impact exposure. Finally, all participants were amateur MMA athletes, and findings may not generalise to professional cohorts who may differ in training intensity, experience, and bout preparation. Despite these limitations, the study aimed to capture meaningful longitudinal data within a real-world training environment while maintaining ecological validity.

7.5 Conclusion

This case series described longitudinal neurophysiological and head impact responses across a fight preparation camp, revealing key individual-level patterns that both align with and extend

previous group-level findings. Notably, changes in VA and MVC most frequently exceeded the TE × 1.5 threshold, followed by cSP and HRT highlighting their relative sensitivity to training and RHI exposure. In contrast, MEP/M_{MAX} among other excitability measures displayed far fewer threshold-exceeding changes, consistent with their higher intra-session variability and lower reliability in repeated assessments. These findings support the utility of TE-based thresholds as a practical approach for identifying potentially meaningful intra-individual changes across sessions, complementing descriptive statistics of cumulative head impact burden. Moreover, the combined application of TMS-derived measures and IMG technology allowed for a detailed mapping of neurophysiological responses relative to head impact exposure, reinforcing their potential value for monitoring athlete brain health across fight preparation camps where RHIs are routine.

Chapter 8:
General discussion

The overarching aim of this thesis was to examine how deficits in body water associated with RWL and exposure to RHIs during combat sparring influence neuromuscular function, and to consider whether these stressors may share common mechanisms affecting the corticospinal tract. The research questions spanned from subjective reports to reliability testing, followed by experimental and longitudinal monitoring, to comprehensively address: (a) how combat sport athletes subjectively perceive the interaction between WC and concussion-related symptoms, (b) the specific neurophysiological changes induced by hypo-hydration and subsequent rehydration, (c) the acute neurophysiological responses to sparring-induced head impacts and (d) the relationships between hydration status, head impact kinematics and changes in brain excitability. By integrating findings across five experimental studies and a survey, this thesis provides a multifaceted discussion of how hypo-hydration and head impacts influence the combat sport athlete's brain and neuromuscular system.

8.1 Summary of key findings

Chapter 2 provided the conceptual foundation for this thesis by reviewing the neurophysiological consequences of both hypo-hydration and brain trauma. The literature demonstrated that hypo-hydration could impair neuromuscular performance, typically without major loss of strength unless hypo-hydration exceeds 2 – 3 % BM. However, the central mechanisms remain less clear, with studies suggesting altered cortical excitability (87), potentially due to changes in ionic balance or reduced CSF volume (24,25). In parallel, studies on concussive and non-concussive impacts consistently showed increased cortical inhibition, reduced corticospinal excitability, and prolonged cSP durations (280-288). These effects are attributed to a heightened GABAergic response following mechanical loading of the brain. Together, these two stressors share a neurophysiological signature characterised by impaired cortical output and disrupted voluntary drive. Moreover, both hypo-hydration and brain trauma

appear to influence motor and cognitive function in ways that could converge during combat sport training, especially when RWL and RHIs co-occur. This overlap formed the rationale for examining their combined effects and for investigating whether hypo-hydration might exacerbate or mimic brain trauma pathophysiology in athletes.

Chapter 3 explored athlete perceptions through a large-scale survey of over 100 combat sport athletes. The findings revealed a strong overlap (~ 70%) in symptomology between hypo-hydration and mTBI, with dizziness, confusion, and fatigue frequently reported during WC practices. Notably, 60 to 70 percent of athletes believed hypo-hydration worsened their concussion symptoms, and nearly half reported feeling more vulnerable to brain trauma while hypo-hydrated. These insights, drawn from athletes from combat sports, established the foundation for the subsequent experimental chapters. The findings also highlighted a culture of acceptance around extreme weight cutting, despite perceived negative impacts on brain function and performance.

Chapter 4 established the methodological framework for the remaining studies using TMS in conjunction with neuromuscular function assessments of the rectus femoris muscle. It validated the reliability of all neurophysiological measurements via a test-retest protocol in healthy controls. These data enabled later studies to compare meaningful change between trials using the TE for individual athletes. This chapter confirmed that TMS and neuromuscular assessments could be used with sufficient precision to monitor athletes in both lab and field environments.

Chapter 5 investigated the effects of hypo-hydration on neuromuscular and corticospinal function in a controlled experimental setting. Athletes underwent a 4 – 5 % BMR through heat exposure and low-intensity exercise, then were rehydrated either rapidly or gradually. Compared to hydrated controls, the hypo-hydrated group exhibited a reduction in VA,

corticospinal inhibition, and maximal strength. Interestingly, while cSP duration increased after exercise under hydrated conditions, this was absent in the hypo-hydrated state, which was also observed by Bowtell et al. (87). Rapid rehydration appeared to support strength recovery more effectively than slow rehydration, despite limited recovery of central drive. These findings provided mechanistic support for the subjective experiences reported in Chapter 3 and suggested that hypo-hydration might disrupt neuromuscular function in ways not seen with regular exercise-induced fatigue.

Chapter 6 examined the neurophysiological responses to RHIs in sparring through a naturalistic observational study of 13 combat sport athletes across a varied number of sparring sessions: Part 1, observed the acute neurophysiological responses to a single sparring session in combat athletes. After sparring, athletes displayed increased cortical inhibition (longer cSP), elevated AMT, reduced MEP_{RAW}, and a decline in VA and MVC, while peripheral muscle excitability remained unchanged. These changes were evident despite minimal signs of hypo-hydration (< 1% BM reduction) and resolved within 48 h. These findings suggest that RHIs during a single sparring session produce a transient suppression of CNS function (as observed and discussed by Di Virgillio et al. [8,9,355]), distinguishable from exercise-induced-fatigue or hydration-related effects.

Chapter 6, Part 2, extended this work longitudinally, tracking athletes across multiple sparring sessions, but with the additional implementation of IMGs in six participants. Head impact metrics revealed an average of 13 impacts per session with linear and rotational accelerations exceeding 30 g and 3000 rad/s² respectively. Here, several positive relationships emerged: higher cumulative head impact burden, particularly PAA (followed by HIF and PLA), predicted greater increases in cortical inhibition. These findings strengthen the case that repetitive, non-concussive RHIs can induce measurable changes to corticospinal tract activity

(8,9), and that rotational forces are particularly disruptive. Again, hydration metrics (USG and BM changes) showed minimal influence on neurophysiological outcomes, supporting the idea that fluid balance (particularly during sparring) is relatively well maintained.

Chapter 7 presented a case-series investigation tracking neurophysiological responses and head impact burden across a 10-week fight preparation camp in three amateur MMA athletes, IMG data with repeated TMS and neuromuscular assessments. Across 9 – 13 monitored sparring sessions per athlete, cumulative RHI counts exceeded 230 impacts. Notably, VA and MVC exhibited the most frequent changes exceeding the TE × 1.5 threshold, followed by cSP duration and muscle HRT, supporting their utility as sensitive, session-to-session markers. Excitability-based measures, including MEP_{RAW} and MEP/M_{MAX}, showed fewer threshold-exceeding changes, likely reflecting higher inter-session variability and lower reliability. Although hydration status remained stable for most participants, two athletes underwent aggressive weight cuts involving RWL in the final 24 hours before weigh-in, with USG data revealing late-camp drift toward hypo-hydration in one case. While not all sparring sessions were captured using IMGs, the estimated cumulative head impact exposure was conservatively over 700 RHIs per athlete. The findings align with emerging evidence (464) that cumulative head impact load, rather than isolated concussions, may be a key driver of long-term brain health risk.

Considered collectively, these findings suggest that hypo-hydration and RHIs can both alter the brain-to-muscle pathway, but with distinct neurophysiological profiles. Heat-induced hypo-hydration was associated with reduced central drive and maximal strength, without changes in peripheral excitability, suggesting a predominantly central limitation. In contrast, RHIs produced a distinct pattern characterised by increased cortical inhibition, and reduced corticospinal excitability, reflected by prolonged cSP and reduced MEP_{RAW}, as well as a

reduction in central drive. Both stressors ultimately constrained voluntary force production through altered corticospinal output, though via different pathways: hypo-hydration primarily reducing excitatory drive, and RHIs increasing inhibitory tone within the motor cortex. While the additive effect of hypo-hydration and potential risk of exacerbated brain trauma remains unknown, the addition of IMG data demonstrated that such an increase in inhibitory tone after sparring, can be predicted by head impact magnitudes. Therefore, the value of the thesis lies not in demonstrating a direct interaction between dehydration and head impacts, but in identifying measurable neurophysiological outcomes that could be used in future studies designed to examine this interaction prospectively. Together, these findings highlight the vulnerability of the combat athlete's neuromuscular system to both fluid imbalance and repetitive brain trauma, underscoring the importance of managing hydration and head impact exposure concurrently.

The thesis adds novel evidence to the WC literature**,** corroborating anecdotal claims and case reports e.g., extreme dehydration episodes leading to collapse or poor performance (11-14), by systematically showing that symptoms of fluid imbalance can resemble and potentially exacerbate post-concussion symptoms. This is a new contribution: whereas numerous studies have catalogued the physiological effects of hypo-hydration on endurance, strength, and cognition, few (299,307) have discussed how hypo-hydration might modulate mTBI risk or symptomology. Furthermore, Chapter 5 challenges some earlier conclusions (e.g., 16,96) suggesting that hypo-hydration does not impair central drive or corticospinal excitability*,* attributing performance loss mostly to peripheral factors. The discrepancy could arise from differences in methodology or from the inclusion of a rehydration manipulation highlighting how prolonged hypo-hydration can sustain central fatigue. Nevertheless, the current findings build on prior research by integrating non-invasive brain stimulation methods and tracking neuromuscular function more precisely. The observed absence of increased cSP duration in the

hypo-hydrated group suggests that the expected inhibitory modulation post-exercise is blunted under thermal stress and fluid deficits. This not only challenges previous assumptions that post-exercise inhibition is a universal protective mechanism but suggests that hypo-hydration may interfere with the homeostatic regulation of cortical output. Therefore, our findings align more closely with Bigard et al. (15) and Bowtell et al. (87) who noted increased central fatigue after hypo-hydration.

Our observations of increased cSP after sparring mirror those reported after repetitive soccer heading (9) boxing sparring (9) and are congruent with findings of acute primary motor cortex inhibition following mTBI (e.g., 463). This reinforces the reliability of cSP as a marker of brain perturbations and extend its utility to sub-clinical monitoring in high-impact sports. Importantly, the dissociation in inhibitory response between hypo-hydration and RHIs clarifies that although both conditions impact motor output, they may do so via different pathways; hypo-hydration reducing excitability without altering inhibition, and RHIs enhancing inhibition alongside suppressed excitability. The repeated-sparring study revealed a relationship between cumulative angular acceleration and increased cortical inhibition. This aligns with biomechanical theories that rotational forces are more detrimental to neural tissue than linear forces due to shearing effects at the grey-white matter interface. The association between rotational kinematics and prolonged cSP is supported by previous findings from rugby and American football, where angular acceleration has been linked to more severe neurophysiological and cognitive outcomes (38). This thesis contributes further by showing that even within non-concussive thresholds, the burden of repeated impacts can produce measurable central changes.

The lack of interaction between hydration status and RHIs in this thesis may be due to these stressors not occurring simultaneously. Athletes rarely trained in a hypo-hydrated state,

reserving RWL for the final 48 – 72 h before weigh-in, by which point sparring had ceased. This behavioural pattern, confirmed in the case-study data, limits the co-occurrence of fluid deficits and head impacts during typical training blocks. However, this does not eliminate the risk posed by their combination, particularly if hypo-hydrated during a competitive bout. While this was not directly observed in the current studies, athlete reports in Chapter 3 suggest that these scenarios do occur and are associated with worsened symptoms, which is most likely a retrospective reflection of the bout as opposed to the fighting camp itself. This supports the idea of a synergistic effect that merits further investigation, by investigating fluid balance and head impact metrics in combat sports bouts.

8.2 Implications for training and athlete safety

The collective findings of this thesis carry several important implications. Firstly, the evidence strongly suggests that hypo-hydration (particularly induced by RWL) and brain trauma should not be viewed in isolation when considering athlete safety, as they can interact and potentially exacerbate each other's harm. Practically, this means coaches and sports medicine professionals should monitor hydration status as part of concussion protocols. In particular, conducting concussion baseline tests (neurocognitive or balance tests) in a dehydrated state could yield misleadingly poor results; thus, establishing procedures to test athletes when euhydrated (or noting hydration status) is important for accuracy.

For both the general population and athletes who are likely to face periods of thermal strain and subsequent fluid losses, the findings of Chapter 5 demonstrate the likely time-course of changes in neuromuscular function and how rapidly rehydrating across 2 h may partially improve muscle force production, which would not be the case with more progressive strategies. This suggests that athletes who undergo RWL in close proximity to their sporting event (e.g., boxers, judokas and MMA athletes), are likely to experience a decrement in

performance, particularly when fluids are not rapidly restored. In addition, the clinical utility of UCH-L1 as a blood-based biomarker of traumatic brain injuries is well established (371,465). Situations in which a rapid and accurate point-of-care diagnosis is required, are critical in combat and contact sporting environments. However, with the observed increases in the concentration of UCH-L1 after exercise, and the concurrent occurrences of intracellular dehydration and risk of brain injury in contact sports (466), further research is warranted to establish if physical activity may present as a confounder in the clinical utility of point-of-care blood tests, particularly in sporting events.

Secondly, the demonstration of acute yet transient cortical inhibition from sparring indicates that rest days and spacing of sparring sessions are optimal for recovery of neuromuscular function. The present data demonstrated recovery by 48 h, suggesting that athletes sparring (in a similar fashion) should rest from further sparring for a minimum of two days to avoid the possibility of accumulating neurophysiological deficits, potentially elevating risk of poorer performance or even injury. Therefore, coaches should consider the "neural load" of sparring, in a similar fashion to how training is periodised around muscle recovery. Tools such as IMGs could eventually help quantify a "safe weekly head impact dose", somewhat akin to managing pitch counts in baseball or weekly mileage in running. Additionally, at an organisational level, the findings may lend support to policies that limit sparring intensity or frequency during fight camps, which is an approach some coaches/athletes already adopt by balancing 'hard' sparring with technical sparring (485,486). More generally, this has already been implemented in rugby, where contact time has been reduced to 15 min per week (413).

Finally, regarding weight cutting, these results add to the growing call for reforming extreme dehydration practices. The survey evidence that hypo-hydration can mask concussion and worsen symptoms means that fighters who dehydrate themselves might be competing with a

more vulnerable neuromuscular system, rendering the physiological consequences of RHIs more dangerous. Combat sport governing bodies may need to strengthen WC regulations e.g., implementing hydration testing as part of weigh-ins, as some amateur wrestling programs do, or introducing longer periods for weight descent). In the interim, fighters and coaches should be educated that on the balance between the 'benefits' and disadvantages of hypo-hydration (203), and therefore, the smartest approach is to avoid hypo-hydration prior to sparring or competing, wherever possible. It is essential to appreciate the nuances of athlete behaviour and buy-in, and thus, the call to abolish WC practices is acknowledged to not be a feasible approach, but rather a focus on organisational monitoring and athlete education.

8.3 Limitations and future recommendations

Chapter 3 set the scene through establishing the perceived link of weight-cutting to exacerbated concussion symptoms, but was retrospective and correlational, relying on self-reported memories which could introduce bias or inaccuracies. Athletes might overestimate or underestimate their symptoms, and those who cut more weight might also be inherently different (more experienced, etc.) which could confound results. Thus, while suggestive of a link, the survey cannot prove causation between hypo-hydration and exacerbation of brain trauma pathophysiology; however, it had set the stage for more controlled prospective research in the thesis and proved to be more than an anecdotal experience. A limitation of Chapter 5 was the potential confounding of hyperthermia and exercise, which was attempted to be minimised by ensuring baseline core temperature, and an exercise-only condition. Nevertheless, a hypo-hydrated group exercising in temperate conditions, and a group that dehydrated passively would have strengthened the design. Similarly, we did not include paired-pulse TMS measures e.g., SICI/ICF in that study due to equipment availability, which could have provided direct insight into intracortical inhibitory/excitatory circuit changes.

Caution is advised when generalising the present findings, as the recruited cohort were observed from one gymnasium, and may not represent the variability in combat sporting cultures and gymnasiums. Constraints on the study designs also meant it was not possible to fully isolate the combined effect of hypo-hydration and head impacts in one controlled experiment. The initial plan was to study each factor separately (hypo-hydration in Chapter 5, RHI in Chapter 6) and then observe interactions across several bouts in various hydration states. The ideal experiment was to have a) athletes perform sparring while hypo-hydrated vs. hydrated in a crossover manner or b) spar in naturally different hydration states due to the summer and heat in the gymnasium, and c) compete while noting the outcome and investigating the physiological and hydration status before and after the bout. This was refrained from, due to the following reasons: a) in order to ensure this was a truly naturalistic observation. Due to testing taking place between January and April in the early morning, b) was not possible either, and c) due to many ethical constraints in the utilisation of TMS before and after a sanctioned fight event. As a result, our conclusions about the combined effects remain somewhat inferential, and a mechanistic link might be more pronounced if further probed into. In addition, a concussion symptom survey (as used in Chapter 3) was prepared for athletes in the experimental studies of Chapter 5, 6 & 7, along with measures of heart rate, oculomotor function and tympanic temperature; however, this was not successfully executed due to the number of variables that needed to be prioritised (in the interest of time and participant retention) and participant discomfort (heart rate monitors during sparring).

This thesis opens several avenues for further inquiry. Future research should build on this thesis through a staged progression of studies. First, larger prospective observational studies should monitor combat athletes across full fight preparation camps to establish typical ranges of head-impact exposure, hydration status and neurophysiological variability. These studies should combine practical field measures with more detailed hydration indices where feasible,

including urine osmolality, plasma osmolality, sweat rate, sweat sodium concentration and individualised rehydration assessment. Second, controlled experimental studies should examine hypo-hydration and repetitive head impacts concurrently, either by manipulating hydration status before standardised sparring or by using controlled impact-simulation models where ethically appropriate. Third, future work should include female combat sport athletes, with menstrual-cycle phase, hormonal contraceptive use, training status and weight-cutting history appropriately recorded or controlled. Finally, multi-modal monitoring approaches should integrate instrumented mouthguards, symptom scales, hydration assessment, TMS/MNS, cognitive testing, blood biomarkers such as GFAP, UCH-L1 and neurofilament light, and markers of inflammation and muscle damage. This approach would help determine whether the measures used in this thesis can contribute to practical athlete-monitoring frameworks and whether modified sparring schedules, hydration monitoring, individualised rehydration strategies or reduced head-contact training can attenuate adverse neurophysiological responses in combat sports.

8.4 Conclusion

In conclusion, this thesis demonstrates that hypo-hydration and RHIs independently influence neuromuscular and corticospinal function in ways that are relevant to combat sport performance and athlete safety. Heat-induced hypo-hydration reduced maximal force and voluntary activation, while sparring-related RHIs produced transient increases in cortical inhibition and reductions in central drive. The thesis also demonstrates that head-impact magnitude, particularly cumulative angular acceleration, is associated with the degree of corticospinal inhibition after sparring, supporting the use of instrumented mouthguards and neurophysiological testing as complementary monitoring tools. Importantly, the thesis does not establish whether hypo-hydration directly worsens the neurophysiological response to head

impacts. Instead, it provides initial evidence that both stressors can alter components of the brain-to-muscle pathway and identifies a clear need for future studies that assess hydration status, head-impact exposure and neurophysiological outcomes concurrently. The practical contribution of this work is therefore to support more cautious monitoring of hydration practices and sparring exposure in combat athletes, while providing a methodological foundation for future research into their combined effects.

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Appendices

Appendix 1

St Mary's University Twickenham London

Approval Sheet

(This sheet must be signed at all relevant boxes)

Name of proposer(s)	Nasir Uddin
Name of supervisor(s)	140211@live.stmarys.ac.uk
Programme of study	Dr Jamie Tallent, Dr Mark Waldron, Dr Stephen Patterson, Dr Stacy Winter
Title of project	Perceptions of concussion and weight cutting symptomology in combat athletes.

Supervisors, please complete section 1. If approved at level 1, please forward a copy of this Approval Sheet to the Faculty Ethics Representative for their records.

SECTION 1: To be completed by supervisor.(for student research projects)			
Approved at Level 1. Refer to Faculty Ethics Representative for consideration at Level 2 or Level 3.			
Name of Supervisor:	Jamie Tallent		
Signature of Supervisor:		Date:	3/7/2020

SECTION 2: To be completed by Faculty Ethics Representative.			
Approved at Level 2. Level 3 consideration is required by Ethics Sub-Committee.			
Name of Faculty Ethics Representative:	Lubna Ahmed		
Signature of Faculty Ethics Representative:	Lubna Ahmed	Date:	25/7/20

Appendix 2

Study 1 – Concussion & Weight Cutting Questionnaire

Inclusion Criteria: 1) Active (fighting within a year prior to Covid lockdown), 2) experienced a concussion, 3) cuts weight/has cut weight for a competition.

Section 1: Please answer the following questions about you and your medical history.

Q1 What is your age? (years)

Q2 What is your gender?

- Male
- Female

Q3 What is your primary combat sport?

- MMA
- Wrestling
- Brazilian Jiu-jitsu
- Kickboxing/Muay-Thai
- Boxing
- Judo
- Other _____

Q4 What age did you start practicing your primary combat sport? (years)

Q5 How much do you usually weigh when you are not preparing for a competition/fight? (kg/lbs)

Q6 What is your competition weight and category? (kg/lbs and category)

Q7 How tall are you? (cm)

Q8 At which level do you compete?

- Professional (receive a purse for competition)
- Amateur (do not receive a purse but paid to be in training)
- Amateur (not paid for competition/training or recreational)

Q9 What is your sporting/fight record?

- Win _____
- Lose _____
- Draw _____

Q12 How many concussions do you think you have had in the past? _____
Q13 How many of these were confirmed by a medical professional? _____
Q14 How long did you take out before returning to training, from your most recent concussion?
Q15 Have you ever been hospitalized or had medical imaging for a head injury? Y N

Section 2a: Please answer the next series of questions about your experiences with preparing and cutting weight for competition. Weight *'cutting'* specifically refers to *the loss of water* via sauna, fluid restriction, diuretics, sweating gels, hot water baths, laxatives, fat burners, vomiting and/or water loading. This also may include any other method used to reduce your body water.

Q1a Do you generally have a fight preparation camp before a competition?

- Yes
- No

If No –

Q1b Please explain your reasoning for not having a fight preparation camp.

_____

If Yes –

Q1b What is the usual time frame you have for a fight camp? (weeks/days)
Q1c What is the shortest time frame you've had for a fight camp? (weeks/days)
Q1d What is the longest time frame you've been in a fight camp? (weeks/days)

Q2 How much weight do you usually cut before a competition/fight? (kg/lbs)

Q3 How many days prior to a competition/fight do you usually cut weight?

- 1-3 days
- 4-5 days
- 6-7 days
- 8-10 days
- 11-14 days
- 15 days or more

Q4a What is the shortest amount of time you have had to cut weight within? (days)

Q4b How much weight was lost? (kg/lbs)

Q5 How much weight do you usually cut in the final 24 hours before weigh-ins? (kg/lbs)

Q6a What is the usual time frame you have between weigh-ins and competition?

- 24-36 hr
- 12-23 hr
- 6-12 hr
- Less than 6 hr

Q6b How much weight do you regain within that time? (kg/lbs)

Q7 How many times have you cut weight during your career?

Q8 What is the shortest period of time you have had in between two different weight cuts? (months)

- Less than 1 month
- 1-2 months
- 3-4 months
- 5 months or above

Q9 What is the usual time period between each weight cut? (months)

- 1-3 months
- 4-7 months
- 8 months or above

Q10a Have you ever had a weight cut not go according to plan?

- Yes
- No

If Yes

Q10b Please specify why (select as many as applicable):

- o You still felt dehydrated during the fight.
- o You felt a lack of energy because you had lost too much in a short period of time.
- o You felt a lack of strength/power because you had lost too much in a short period of time.
- o You felt it was easier to get 'rocked' during the fight.

- You felt your coordination and reaction times were not optimal.
- You initially failed to make weight.
- You felt too bloated going into the fight (from the rehydration and refeed period).
- Other _____

Section 2b: The following questions are related to your symptoms from weight cutting.

Q1 During a fight camp that you were cutting weight within, did you experience any of the following? For each item listed below please indicate the severity of each symptom, on the following 0-6 scale:
0) None, 1) Mild -, 2) Mild +, 3) Moderate -, 4) Moderate +, 5) Severe -. 6) Severe +.

	None	Mild		Moderate		Severe	
Headache	0	1	2	3	4	5	6
"Pressure in head"	0	1	2	3	4	5	6
Neck pain	0	1	2	3	4	5	6
Nausea or vomiting	0	1	2	3	4	5	6
Dizziness	0	1	2	3	4	5	6
Blurred vision	0	1	2	3	4	5	6
Balance problems	0	1	2	3	4	5	6
Sensitivity to light	0	1	2	3	4	5	6
Sensitivity to noise	0	1	2	3	4	5	6
Feeling slowed down	0	1	2	3	4	5	6
Feeling like "in a fog"	0	1	2	3	4	5	6
"Don't feel right"	0	1	2	3	4	5	6
Difficulty concentrating	0	1	2	3	4	5	6
Difficulty remembering	0	1	2	3	4	5	6
Fatigue or low energy	0	1	2	3	4	5	6
Confusion	0	1	2	3	4	5	6
Drowsiness	0	1	2	3	4	5	6
More emotional	0	1	2	3	4	5	6
Irritability	0	1	2	3	4	5	6
Sadness	0	1	2	3	4	5	6
Nervous or Anxious	0	1	2	3	4	5	6
Trouble falling asleep	0	1	2	3	4	5	6

Q2 Do you feel that these symptoms were worse during/after a fight (or sparring session) in which you were dehydrated?

- Yes
- No

Q3 Do you feel that these symptoms were worse during/after a fight camp that you had to cut a large amount of weight for?

- Yes
- No

Q4 Do you feel that these symptoms lasted longer during/after a fight camp that you had to cut a large amount of weight for?

- Yes

- No

Q5 If you answered 'Yes' to any of the last 3 questions, please select which symptoms worsened:

- Headache
- "Pressure in head"
- Neck pain
- Nausea or vomiting
- Dizziness
- Blurred vision
- Balance problems
- Sensitivity to light
- Sensitivity to noise
- Feeling slowed down
- Feeling like "in a fog"
- "Don't feel right"
- Difficulty concentrating
- Difficulty remembering
- Fatigue or low energy
- Confusion
- Drowsiness
- More emotional
- Irritability
- Sadness
- Nervous or Anxious
- Trouble falling asleep

Q6 How long do these symptoms tend to last for?

- 1-5 days
- 6-13 days
- 14-28 days
- Over 28 days

Q7 Do you feel that these symptoms improved when you were able to consume fluids again?

- Yes
- No

Section 3: The following questions are now related to your understanding and knowledge regarding weight cutting methods before a fight.

Q1 Do you feel like ***your*** weight cutting method(s) is the best approach for your overall health?

- Yes
- No

If No:

Q2b Please specify why (select as many as applicable):

- You are fully aware of weight cutting effects on health.
- You are not totally aware of weight cutting effects on health.
- There may be alternative methods but unsure.
- You don't feel at your best during/after the fight after weight cutting.

Q2 Below are various weight cutting methods for a fight. Which of these methods do you think work best for you?

- Gradual dieting

- Water loading
- Sauna
- Hot water baths
- Training in hot rooms/with sweat suits
- Diuretics/laxatives
- A combination of some of the above.
    - If 'a combination of the above', then which methods do you combine?

_____

Q3 Which of these methods do you think are the best option for your health?

- Gradual dieting
- Water loading
- Sauna
- Hot water baths
- Training in hot rooms/with sweat suits
- Diuretics/laxatives

Q4 If you had to select which method you think is the worst for your health. Which method would it be?

- Gradual dieting
- Water loading
- Sauna
- Hot water baths
- Training in hot rooms/with sweat suits
- Diuretics/laxatives

Q5 Who has had the biggest influence on you, In terms of advice for weight loss prior to competition?

- Training partner/ other combat sports athlete
- Physician/Doctor
- Physical trainer/S&C Coach
- Fighting coach
- Parents
- Dietitian
- Internet
- Other

Section 4: Please read the following statements related to your concussion knowledge and circle TRUE or FALSE for each question.

1. There is a possible risk of death if a second concussion occurs before the first one has healed. **TRUE /FALSE**
2. Running everyday does little to improve cardiovascular health.
TRUE /FALSE
3. People who had one concussion are more likely to have another concussion.
TRUE /FALSE
4. Training mats provide cushioning for athletes when they hit the ground.
TRUE /FALSE
5. In order to be diagnosed with a concussion, you have to be knocked out.
TRUE /FALSE
6. A concussion can only occur if there is a direct hit to the head.
TRUE /FALSE
7. Being knocked unconscious always causes permanent damage to the brain.
TRUE /FALSE
8. Symptoms of a concussion can last several weeks.

TRUE /FALSE

9. Sometimes a second concussion can help a person remember thing that were forgotten after the first.

TRUE /FALSE

10. Weightlifting helps to tone and/or build muscle.

TRUE /FALSE

11. After a concussion occurs, brain imaging (CAT scan, MRI, X-ray etc.) typically shows visible physical damage (bruise, blood clot) to the brain.

TRUE /FALSE

12. If you receive one concussion and you have never had a concussion before, you will become less intelligent.

TRUE /FALSE

13. After 10 days, symptoms of a concussion are usually completely gone.

TRUE /FALSE

14. After a concussion, people can forget who they are and not recognize others but be perfect in every other way.

TRUE /FALSE

15. College students and secondary/high school students are the same age.

TRUE /FALSE

16. Concussions can sometimes lead to emotional disruptions.

TRUE /FALSE

17. An athlete who gets knocked out after getting a concussion is experiencing a coma.

TRUE /FALSE

18. There is rarely a risk to long-term health and well-being from multiple concussions.

TRUE /FALSE

Please read each of the following scenarios and circle TRUE or FALSE for each question that follows the scenarios

Scenario 1: During sparring, fighter Q and fighter X collide with each other and each suffers a concussion. Fighter Q has never had a concussion in the past. Fighter X has had 4 concussions in the past.

- It is likely that fighter Q's concussion will affect his long-term health and well-being.

TRUE/FALSE

- It is likely that fighter X's concussion will affect his long term-health and well-being.

TRUE/FALSE

Scenario 2: Fighter F suffered a concussion during a bout. He continued to fight despite the fact that he continued to feel the effects of the concussion.

- Even though fighter F is still experiencing the effects of the concussion, his performance will be the same as it would, had he not suffered a concussion.

TRUE /FALSE

Please select all the following that you believe are a symptom of concussion:

Abnormal sense of smell
Abnormal sense of taste
Amnesia
Blurred vision

Black eye
Chest pain
Confusion
Dizziness
Headache
Loss of consciousness
Nausea
Nosebleed
Numbness/tingling in the upper extremity
Sharp burning pain in the neck
Sleep disturbances
Weakness of neck range of motion

Section 5: Please answer the next series of questions about your symptoms/experience of concussions/head impacts.

Q1 Following a suspected concussion during a fight/sparring, please rank how severe your following symptoms were? For each item listed below please indicate the severity of each symptom, on the following 0-6 scale:
0) None, 1) Mild -, 2) Mild +, 3) Moderate -, 4) Moderate +, 5) Severe -. 6) Severe +.

	None	Mild		Moderate		Severe	
Headache	0	1	2	3	4	5	6
"Pressure in head"	0	1	2	3	4	5	6
Neck pain	0	1	2	3	4	5	6
Nausea or vomiting	0	1	2	3	4	5	6
Dizziness	0	1	2	3	4	5	6
Blurred vision	0	1	2	3	4	5	6
Balance problems	0	1	2	3	4	5	6
Sensitivity to light	0	1	2	3	4	5	6
Sensitivity to noise	0	1	2	3	4	5	6
Feeling slowed down	0	1	2	3	4	5	6
Feeling like "in a fog"	0	1	2	3	4	5	6
"Don't feel right"	0	1	2	3	4	5	6
Difficulty concentrating	0	1	2	3	4	5	6
Difficulty remembering	0	1	2	3	4	5	6
Fatigue or low energy	0	1	2	3	4	5	6
Confusion	0	1	2	3	4	5	6
Drowsiness	0	1	2	3	4	5	6
More emotional	0	1	2	3	4	5	6
Irritability	0	1	2	3	4	5	6
Sadness	0	1	2	3	4	5	6
Nervous or Anxious	0	1	2	3	4	5	6
Trouble falling asleep	0	1	2	3	4	5	6

Q2 Do you feel that these symptoms were worse after a fight (or sparring session) in which you were dehydrated?

- Yes
- No

Q3 Do you feel that these symptoms were worse during/after a fight camp that you had to cut a large amount of weight for?

- Yes
- No

Q4 Do you feel that these symptoms lasted longer during/after a fight camp that you had to cut a large amount of weight for?

- Yes
- No

Q5 If you answered 'Yes' to any of the last 3 questions, please select which symptoms worsened:

- Headache
- "Pressure in head"
- Neck pain
- Nausea or vomiting
- Dizziness
- Blurred vision
- Balance problems
- Sensitivity to light
- Sensitivity to noise
- Feeling slowed down
- Feeling like "in a fog"
- "Don't feel right"
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- Difficulty remembering
- Fatigue or low energy
- Confusion
- Drowsiness
- More emotional
- Irritability
- Sadness
- Nervous or Anxious
- Trouble falling asleep

Q6 How long do these symptoms tend to last for?

- 1-5 days
- 6-13 days
- 14-28 days
- Over 28 days

Q7 Do you feel that these symptoms improved when you were able to consume fluids again?

- Yes
- No

Would you be willing to take part in a follow-up interview/focus group?

- Yes
- No

Would you be willing to take part in/hear about any other concussion/weight cutting related studies?

- Yes
- No

Thank you for completing the questionnaire.

Grading:

Section 1 – Ungraded
Section 2a – Higher scores indicate higher severity of RWL malpractice.

- Q1a: 1 Point if NO
- Q2: 1 Point per Kg
- Q3: 5 points for 1-3 days, DEDUCT 1 point for each bullet point lower
- Q4: **4b – 4a**
  - 4a: 2 points per day,
  - 4b: 1 point per kg
- Q5: 2 points per kg
- Q6: 24-36hr = 1 point, 12-23hr = 2, 6-12hr = 3, <6hr = 4
- Q7: 1 point per time
- Q8: Less than 1 month = 3, 1-2 months = 2, 3-4 months = 1, 5 months or above = 0
- Q9: 1-3 months = 2 points, 4-7 months = 1, 8 months or above = 0
- Q10: 10a x Number of selected options for 10b
  - 10a: No = 0, Yes = 2
  - 10b: Each selected option = 1

Section 2b – 2 scores. Higher scores indicate higher risk and severity of RWL/concussion symptoms.
Symptoms - Q11 Symptom score + Q12-17 score. Max score = 51
Severity – Q11 Severity score. Max score = 132

- Q1: 2 scores = Symptom score, Severity score.
  - Symptom score: *n of 22 (higher score indicates higher risk of concussion-like symptoms from RWL)*
  - Severity score: *n of 132 (higher score indicates higher risk and severity of RWL symptoms)*
- Q2: No = 0, Yes = 1
- Q3: No = 0, Yes = 1
- Q4: No = 0, Yes = 1
- Q5: Each selected symptom = 1
- Q6: 1-5 days = 0, 6-13 days = 1, 14-28 days = 2, 29 days above = 3 points
- Q7: No = 1. Yes = 0

Section 3 – Ungraded. *Aim is to address educational gaps, influences and anecdotal experiences.*
Section 4 – Concussion Knowledge Index (CKI) = *n of 17 (higher score indicates better knowledge)*
Section 5 – Refer to **Section 2b**

Appendix 3

St Mary's University Twickenham London

6 September 2019 SMEC_2018-19_055

Nasir Uddin (SHAS): 'The effects of hypo-hydration and rehydration on peripheral and corticospinal excitability'

Dear Nasir

University Ethics Sub-Committee

Thank you for re-submitting your ethics application for consideration.

I can confirm that all required amendments have been made and that you therefore have
ethical approval to undertake your research.

Yours sincerely

Dr Jamie North
Chair, Ethics Sub-Committee

Cc Dr Mark Waldron, Dr Jamie Tallent, Dr Stephen Patterson

Appendix 4

11/02/2025

Mr Nasir Uddin, Miss Leeza Voyevoda, Mr Thomas Adams, Mr Patrick Schoenmakers, Dr Jamie Tallent, Dr Benjamin Jones

Sport, Rehabilitation, and Exercise Sciences, Sport, Rehabilitation, and Exercise Sciences, Sport, Rehabilitation, and Exercise Sciences, Sport, Rehabilitation, and Exercise Sciences, Sport, Rehabilitation, and Exercise Sciences, Sport, Rehabilitation, and Exercise Sciences, Sport, Rehabilitation, and Exercise Sciences

University of Essex

Dear Nasir,

Ethics Committee Decision

Application: ETH2425-0793

I am pleased to inform you that the research proposal entitled "An analysis of the kinematics and acute neurophysiological responses to a martial arts training session.." has been reviewed on behalf of the Ethics Sub Committee 2, and, based on the information provided, it has been awarded a favourable opinion.

The application was awarded a favourable opinion subject to the following **conditions**:

Extensions and Amendments:

If you propose to introduce an amendment to the research after approval or extend the duration of the study, an amendment should be submitted in ERAMS for further approval in advance of the expiry date listed in the ethics application form. Please note that it is not possible to make any amendments, including extending the duration of the study, once the expiry date has passed.

Covid-19:

Please note that the current Government guidelines in relation to Covid-19 must be adhered to and are subject to change and it is your responsibility to keep yourself informed and bear in mind the possibility of change when planning your research. You will be kept informed if there are any changes in the University guidelines.

Yours sincerely,

Izzie Easton

Appendix 5

PLEASE NOTE: Your response to these questions are crucial for your safety and may result in exclusion from the study, but the researcher will discuss with you in full length.

Safety Screening Questionnaire for Transcranial Magnetic Stimulation (TMS)

(Version 1.1, Jan 08 2013)

Participant Name: _____ **Date:** _____

Current Age: _____ (in years) **Handedness:** Left Right Ambi

Have you ever had an adverse reaction to TMS?	☐Yes	☐No
Do you have epilepsy or have you ever had a seizure/convulsion?	☐Yes	☐No
Have you ever had a fainting spell or syncope? If yes, describe.	☐Yes	☐No
Have you ever had a stroke?	☐Yes	☐No
Have you ever had a serious head injury (with loss of consciousness)?	☐Yes	☐No
Have you ever had neurosurgery of any type (including brain or spinal cord)?	☐Yes	☐No
Do you have hearing problems or ringing in your ears?	☐Yes	☐No
Do you have any metal in your body such as shrapnel, surgical clips, or fragments from welding or metalwork?	☐Yes	☐No
Do you have any implanted devices such as cardiac pacemakers, aneurysm clips, cochlear implants, medical pumps, deep brain stimulators, or intracardiac lines?	☐Yes	☐No
Do you have a medication infusion device	☐Yes	☐No
Do you suffer from frequent or severe headaches?	☐Yes	☐No
Have you ever had any other brain-related condition?	☐Yes	☐No
Have you ever had any illness that caused brain injury?	☐Yes	☐No
Are you taking any psychiatric or neuroactive medications? (please list)	☐Yes	☐No
Are you taking any other medications or other drugs/substances?	☐Yes	☐No
Are you pregnant or do you have any reason to believe that you may be?	☐Yes	☐No
Do you, or does any family member, have epilepsy/history of seizures?	☐Yes	☐No
Do you hold a heavy goods vehicle driving license or bus license?	☐Yes	☐No
Have you consumed alcohol in the past 24 hours?	☐Yes	☐No
Did you have adequate sleep last night?	☐Yes	☐No
Have you participated in a TMS study within the past 24 hours?	☐Yes	☐No

Potential Contraindication Drugs

Strong Potential Hazard: imipramine, amitriptyline, doxepine, nortriptyline, maprotiline, chlorpromazine, clozapine, foscarnet, ganciclovir, ritonavir, amphetamines, cocaine, (MDMA, ecstasy), phencyclidine (PCP, angel's dust), ketamine, gamma-hydroxybutyrate (GHB), alcohol, theophylline

Relative Potential Hazard: mianserin, fluoxetine, fluvoxamine, paroxetine, sertraline, citalopram, reboxetine, venlafaxine, duloxetine, bupropion, mirtazapine, fluphenazine, pimozide, haloperidol, olanzapine, quetiapine, aripiprazole, ziprasidone, risperidone, chloroquine, mefloquine, imipenem, penicillin, ampicillin, cephalosporins, metronidazole, isoniazid, levofloxacin, cyclosporin, chlorambucil, vincristine, methotrexate, cytosine arabinoside, BCNU, lithium, anticholinergics, antihistamines, sympathomimetics.

Withdrawal Hazard: alcohol, barbiturates, benzodiazepines, meprobamate, chloral hydrate.

Appendix 6

School of Sport, Rehabilitation and Exercise Science
Physical Activity Readiness Questionnaire (PAR-Q) FORM

This screening form must be used in conjunction with an agreed Consent Form.

Full Name: Date of Birth:
Height (cm): Weight (kg):

Have you ever suffered from any of the following medical conditions? If yes please give details:

	Yes	No	Details
Heart Disease or attack	□	□	_____
High or low blood pressure	□	□	_____
Stroke	□	□	_____
Cancer	□	□	_____
Diabetes	□	□	_____
Asthma	□	□	_____
High cholesterol	□	□	_____
Epilepsy	□	□	_____
Allergies	□	□	_____
Other, please give details	□	□	_____

Do you suffer from any blood borne diseases? If yes please give details;

Please give details of any **medication** you are currently taking or have taken regularly within the last year:

Please give details of any **musculoskeletal injuries** you have had in the **past 6 months** which have affected your capacity to exercise or caused you to take time off work or seek medical advice:

Other Important Information
During a typical week approximately how many hours would you spend exercising?

If you **smoke** please indicate how many per day:

If you drink **alcohol** please indicate how many units per week:

Are you currently taking any **supplements or medication?** Please give details:

Is there any reason not prompted above that would prevent you from participating within the relevant activity?

By signing this document I agree to inform the relevant individual(s) of any change(s) to my circumstances that would prevent me from participating in specific activities.
Signature (Participant): Date:

Signature (Test Coordinator*): Date: