The acute influence of boxing on brain health parameters in university amateur boxers

Submitted by William Edward George Wallis, to the University of Exeter as a dissertation for the degree of Masters by Research in Sport & Health Sciences, September 2021.

I certify that all material in this dissertation which is not my own work has been identified and that any material that has previously been submitted and approved for the award of a degree by this or any other University has been acknowledged.

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Abstract

The present thesis investigated the acute influence of amateur boxing on brain health parameters across two separate studies. The first study examined 20 university boxers visuomotor performance and neurocognitive function before and after 3 rounds of competitive amateur boxing. Visuomotor performance was assessed via a computer guided precision grip task, both with and without visual feedback, whilst neurocognitive function was assessed via a revised SCAT5 questionnaire and balance task. Boxers presented with no difference in average force or reaction time in both precision grip tasks, although performance error was greater after boxing when compared to pre boxing measures in the visual feedback precision grip task ($P<0.05$). Neurocognitive function was not influenced by the boxing intervention, but verbal fluency (task included in SCAT5) increased ($P<0.05$). Balance errors were greater after boxing when compared to pre-boxing measures ($P<0.05$). Boxers averaged 58 punches to the head, though these data did not correlate to any of the brain health outcomes measured ($P>0.05$). These findings provide insight into the acute effects of sub-concussive head impacts on amateur boxers. The second study investigated 18 university amateur boxers brain health parameters after sparring when compared to a seated control and boxing training with no head impacts. Cerebrovascular health was assessed via cerebral autoregulation (orthostatic stress via squat-stand manoeuvres and transfer function analysis) and cerebrovascular reactivity (breath-holding and hyperventilation procedures), using transcranial Doppler sonography. Visuomotor control (precision grip task with and without visual feedback) and neurocognitive function (SCAT5 and balance tasks) were also measured as in study 1. Head impacts were recorded by video footage and acceleration of the punches was measured via wrist-worn accelerometers. Boxers demonstrated a delayed cerebral autoregulatory response to orthostatic stress without concomitant changes in cerebrovascular reactivity after amateur boxing. We observed no change to balance or any other measures of neurocognitive function or visuomotor performance after three rounds of boxing. Boxers received an average of 40 punches to the head, resulting in an average accumulative acceleration of 290 g. This is the first study of its kind and highlights that alterations in indices of cerebrovascular integrity may be present in the immediate aftermath of boxing. Further work is now needed to understand the time

course of recovery, and whether these acute changes lead to chronic differences between those who do and do not box.

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Abbreviations

TCD	Transcranial Doppler Ultrasound
CBF	Cerebral Blood Flow
MCAv	Middle Cerebral Artery Velocity
CVR	Cerebrovascular Reactivity
CA	Cerebral Autoregulation
ND	Neurodegenerative Diseases
TBI	Traumatic Brain Injury
mTBI	Mild Traumatic Brain Injury
cTBI	Chronic Traumatic Brain Injury
CTE	Chronic Traumatic Encephalopathy
HIA	Head Impact Assessment
PGVP	Precision Grip Visuomotor Performance
$P_{ET}CO_2$	Partial Pressure of End-Tidal CO_2
MAP	Mean Arterial Pressure
SRC	Sport Related concussion
KO	Knock-Out
ASD	Autism Spectrum Disorder
ADHD	Attention Deficit Hyperactivity Disorder
MVC	Maximum Voluntary Contraction
PD	Parkinson's Disease
$PaCO_2$	Arterial partial pressure of CO_2
RMSE	Root Mean Square Error
NF	Neurocognitive Function

Chapter 1: Introduction

1.0 Introduction

Boxing, as opposed to other forms of sporting endeavour, offers a challenge which is unique. Injuries are coincidental in other sports, whereas in boxing the primary aim is to inflict damage or injury to the opponent. Specifically, the primary aim of boxing is to deliver repetitive blows to the head of an opponent in order to score points, or win by knock-out, by having the referee intervene or by rendering the opponent unconscious, which by definition is brain damage. For this reason alone, it has long been a contentious subject among the public opinion and free media. The British Medical Association, gained support from main-stream media outlets, after calling for a complete ban on boxing (amateur and professional) due to the implied risk of cumulative traumatic brain injury (TBI) (BMA., 2008; BBC, 1998; McCabe, 2009). In 2019, the dangers of boxing were never far from the media spotlight, with reports of Patrick Day, a 27 year old, world champion tragically losing his life due to a serious brain injury he sustained during his 10-round world title defence.

In addition to the acute dangers of boxing, there are thought to be long term consequences of boxing-related head impacts. Indeed Dementia Pugilistica or “punch drunk syndrome” was described nearly 100 years ago (Martland, 1928). Martland’s landmark paper outlined a “peculiar condition” that affected “prize-fighters” associated with the development of head tilting and dragging of one’s limb. Many other symptoms were noted; tremors, vertigo, deafness or Parkinsonian symptoms, as well as the need for committing to an asylum. A collection of studies followed over the following decades exclusively studying boxers and the associated TBI, however they failed to alter the rules, because delivering blows to the head is the established objective of the sport (Thomassen *et al.*, 1979; Lampert & Hardman, 1984; Butler, 1994). Despite this concern, the popularity of boxing continues to rise, with the most lucrative contract that sport has ever known recently being signed by Saul Canelo Alvarez for an 11-fight deal with streaming service DAZN worth $356 million dollars.

In addition to understanding risks to the welfare of boxers, scrutinising the influence of boxing on parameters of brain health may provide valuable insight for those who

engage in other contact sports. Boxing-related concussion have been sporadically reported, with figures of as little as 6.1% and upwards of 75% likelihood of sustaining a concussion (Loosemore *et al.*, 2015; Zazryn, McCrory & Cameron, 2009; Timm *et al.*, 1993), however the wider incidence of sport-related concussion (SRC) outside of boxing is profound. The Centre of Disease Control estimates that there are 3.8 million cases of sport related concussion per year (Harmon *et al.*, 2013a). Importantly, these estimations are based upon individuals receiving or seeking emergency care, which is a concern as perhaps only 50% of individuals will seek diagnosis (Harmon *et al.*, 2013b). SRC has been associated with a myriad of long-term complications such as poor memory, cognitive deficiency, depression and increased risk of ND (Manley *et al.*, 2017; McKee *et al.*, 2009; Mez *et al.*, 2017; Roberts *et al.*, 1990; Esopenko & Levine, 2015).

The potential link between sport related *subconcussive* head impacts and future risk of neurodegenerative diseases is also a contemporary research concern, even outside of boxing. For example, research has pointed to alterations in brain structure post mortem in American Football players (Mez *et al.*, 2017), whilst professional footballers in Scotland demonstrated a 3.5 times greater risk of neurodegenerative disease (Mackay *et al.*, 2019). A follow up paper indicated that this risk might be at least partly attributable to the repeated exposure to heading a football (Russell *et al.*, 2021). Boxing provides an excellent platform from which to understand these links.

A paper by Bailey *et al.* (2013), discovered that chronic exposure to sub-concussive head impacts throughout a professional boxing career is associated with reduced cerebrovascular function, namely impaired cerebrovascular reactivity (CVR) and cerebral autoregulation (CA)(Bailey *et al.*, 2013). CVR and CA reflect critical, integrated responses to regulate the delivery of blood flow to the brain in accordance with changes in the partial pressure of carbon dioxide (CO_2) and arterial pressure, respectively. Both, CVR and CA likely play a role in the development of ND following brain injury (Mortimer *et al.*, 1991; Allsop *et al.*, 1990; Ono *et al.*, 2012) and are highly sensitive to the effects of concussion and TBI (Wright *et al.*, 2018b, Ainslie & Duffin, 2009, Strebel *et al.*, 1997). Interestingly, the impairment in CVR observed by Bailey *et al.* was only associated with the volume and intensity of sparring during training (“sparring index”), rather than frequency of total knockouts experienced.

Thus, these data provide further evidence that repeated exposure to sub-concussive blows may be a concern regarding ND risk, and potentially more important than overt concussion.

The study by Bailey et al (2013) is insightful and warrants further exploration. One approach to do so might be to use an acute intervention model, which allows for greater experimental control. Although some rodent data has investigated the effect of repetitive head impacts, revealing acute neuroinflammation and lack of behavioural dysfunction (Shultz *et al.*, 2012). Shultz and colleagues found that rats presented no behavioural and axonal injury differences after a single mild lateral fluid percussion injury. However, the rodents presented with an acute neuroinflammatory response, involving increased macrophage and reactive astrogliosis, at 4 days post-injury. The authors suggested the neuroinflammation caused by the mild lateral fluid percussion injury may contribute to the cumulative and neurodegenerative effects of repeated sub-concussive head injuries. In comparison there is very little experimental data in humans.

Precision grip visuomotor performance (PGVP) deficits might be apparent following sub-concussive head impacts, as the broadly distributed neural network responsible for visuomotor function is known to be sensitive to concussion (Caeyenberghs *et al.*, 2010). The PGVP is quick and simple in its application and future development may see it become automated to provide quick pitch-side results with regards to checking whether an athlete should return to play. This is important, as current “pitch side monitoring” may vary across sports and the level at which they are played. For example, a clinician who can diagnose concussion is unlikely to attend amateur sporting events where head contacts are plausible (such as football, rugby). Thus, there is virtue in understanding how such visuomotor performance is influenced following head impacts, so that it may be applicable in the field.

1.1 Thesis Aims

This thesis explores the acute effect of sub-concussive blows to the head through the sport of amateur boxing on parameters of brain health. Specifically, this thesis aims to address three research questions:

1) **What is the acute effect of boxing on brain health parameters?** It was hypothesised that critical aspects of cerebrovascular function, namely cerebral autoregulation, cerebrovascular reactivity (Chapter 4) and cognitive function (Chapters 4 and 5) would be impaired in university amateur boxers.

2) **Is PGVP acutely influenced by amateur boxing?** It was hypothesised that boxing would impair precision grip visuomotor performance and cognitive function in university amateur boxers (Chapter 4 – competitive boxing, Chapter 5 – sparring).

3) **Is the magnitude of impairment in these outcomes related to the amount of force sustained/number of punches during the boxing trial**? It was hypothesised that any change in cerebrovascular (Chapter 5), cognitive and visuomotor performance (Chapters 4 and 5) would be related to the extent of head impacts sustained during boxing.

To answer these research aims, the thesis includes two experimental studies (Chapters 4 and 5). Chapter 4 examines the effect of a single bout of competitive amateur boxing on measures of visuomotor performance, neurocognitive function, and balance. Chapter 5 extends Chapter 4 by including cerebrovascular assessments, and utilises a more complex study design which allows for a control trial, a boxing trial, and a boxing trial which did not include any head impacts. The latter helps to distinguish whether the exposure to head impacts explain any changes in outcomes, rather than the physical exertion and emotional arousal of boxing.

Chapter 2: Literature review

2.0 Pathology of boxing

A knock-out (KO) is the most obvious cause of neuropathological injury. The KO is usually the product of direct blows to the head resulting in lineal or rotational acceleration (Bartsch *et al.*, 2012), resulting in the loss of consciousness. Blows to the chin result in the highest values of rotational acceleration, hence it is the primary target for most boxers (Walilko, Viano & Bir, 2005). It is worth noting, the frequency of a KO where the boxer is unable to stand in 10 s is uncommon, a recent study found that a KO occurs 0.55 times per 1000 min of boxing (Loosemore, 2015). Using the KO as a measure of neurologic injury would grossly underestimate the traumatic brain injury rate.

It has been proposed that these types of gross injuries (loss of consciousness) may not be the primary cause of neuropathology seen in many boxers (Di Virgilio *et al.*, 2019; Saigal & Berger, 2014). The cumulative effects of multiple sub-concussive head impacts appear to significantly contribute to neuropathological manifestations in boxers (Johnson *et al.*, 2014; Broglio *et al.*, 2011; Dashnaw, Petraglia & Bailes, 2012). Professional boxers with extensive fight histories have been shown to have neuropathological manifestations including functional impairment (cerebral autoregulation) and structural abnormalities (brain atrophy) (Bailey *et al.*, 2013; Jordan *et al.*, 1992). Numerous studies have identified associations between the number of recorded fights and the development of psychiatric, neurologic or histopathological manifestations of encephalopathy (Wilde *et al.*, 2016; Ross, 1983; Jordan *et al.*, 1997; Critchley, 1957). The neuropathological damage sustained by boxers includes cerebellar malformations, cerebral lesions and atrophy, degeneration of cerebral nuclear structures (e.g. substantia nigra), neurofibrillary tangles, and swelling damage to the cavum septum pellucidum (Mawdsley & Ferguson, 1963; Corsellis, Bruton & Freeman-Browne, 1973).

When an individual sustains a blow to the head, a combination of shear, compressive and stretching stresses occur on the brain (Povlishock & Katz, 2005). Shearing stresses are considered to be the most dangerous, as they cause neuronal

injury with direct relation to the magnitude of head acceleration (Beckwith, Chu & Greenwald, 2007; Viano *et al.*, 2005; Stojsih *et al.*, 2010).

Approximately 13 boxers die in the ring each year ring each year (Svinth, 2011). This is often the result of a cerebrovascular haemorrhage (Cruikshank, Higgens & Gray, 1980), which is the leading cause of death for athletes who sustain serious head injuries (Jayarao, Chin & Cantu, 2010). This fatal risk in boxing does not seem to exceed that of any other sports, although the accuracy of mortality figures have been questioned (Baird *et al.*, 2010). Indeed, other publications have found that the threat of fatality was less than in other contact sports such as American football and rugby (McCunney & Russo, 1984; Heilbronner *et al.*, 2009; Jones, 1983).

2.1 Chronic traumatic encephalopathy

Repeated blows to the head, regardless of their ability to render the athlete unconscious, may cause the development of dementia pugilistica (Unterharnscheidt, 1995; Martland, 1928). As research revealed greater insight into the subsequent clinical manifestations, the condition was successively termed chronic TBI (cTBI) and ultimately chronic traumatic encephalopathy (CTE). CTE is a common manifestation among boxers; it has been estimated that 15-40% display clinical symptoms of CTE (Clausen, McCrory & Anderson, 2005). Martland first described “mental confusion, speech hesitancy and hand tremors” which are commonly attributed to the damage of the pyramidal, extrapyramidal, and cerebellar systems respectively (Martland, 1928)(McCunney & Russo, 1984). Throughout the course of the next half-century, diagnosis progressed from gross neurological symptoms, such as memory impairment and personality changes to more specialised neuropathological assessments such as neurostructural abnormalities and geographic tauopathies (Matser *et al.*, 2000; Tagge *et al.*, 2018; McKee *et al.*, 2013). Several retrospective reports surfaced in support of Martland’s landmark paper. Corsellis and colleagues (1973) marked the first paper to explore the neuropathological evidence of CTE. Post-mortem assessment of Boxer’s brains revealed common changes among damaged brain structures including, fenestrated cavum septum pellucidum, “scarring” to the cerebellum, increased white matter in the substantia nigra and finally neurofibrillary degeneration. Each were postulated to relate to a clinical symptom observed by Martland; emotional volatility and aggression dysfunction,

impaired motor movements, Parksonian symptoms and memory deficits respectively (Corsellis, Bruton & Freeman-Browne, 1973). Neurofibrillary tangles were common to ~90% of boxers and signified extensive neurotoxic tau deposition but senile plaques (characteristic of AD) were almost absent.

CTE remains a topic of debate amongst researchers. Randolph argued that the published CTE pathology data represents a case study rather than a verifiable disease (Randolph, 2018). He concluded that the data suggests that TBI does not cause neurodegeneration and protein deposits in the brain area are a poor predictor of behavioural symptoms. In addition to this, Iverson and Gardner highlighted an increased risk of misdiagnosing CTE in men with depression, suggesting that more research needs to explore whether depression is a clinical subtype of CTE or a unique clinical disorder (Iverson & Gardner, 2019). Furthermore, Goldfinger *et al (*2018) re-evaluated Corsellis' case studies using modern immunohistochemistry and identified that only 50% of cases met the neuropathological criteria of CTE (Goldfinger *et al.*, 2018).

Despite this, a decade later Roberts *et al* (1990) furthered Corsellis' findings, linking boxing, AD, neuropathological change, and progressive dementia. Using a more modern immunohistochemistry technique, extensive amyloid-B plaques were observed among the post-mortem Boxer's brains representative of AD. Multiple papers followed, stating that CTE positively correlated with the clinical grading and frequency of boxing-related injuries as measured by number of sparring events, recorded fights, and irregular manifestations on neuroimaging (Casson *et al.*, 1984; Jordan, B. D., Matser, E. J., Zimmerman, R. D. and Zazula, 1996; Ross *et al.*, 1987). Studies that include imaging techniques such as positron electron tomography (PET) have produced mixed results but are close to be able to identify the CTE in living persons with the identification of localised p-Tau in regions potentially affected by CTE (Willer *et al.*, 2021).

The balance of evidence indicates that chronic exposure to TBI through boxing results in damage, which would increase ND risk, namely, AD (Mortimer *et al.*, 1991) PD (Davie *et al.*, 1995) and ischaemic stroke (Lee *et al.*, 2014). Despite this,

evidence that explains the mechanistic processes that elicit these neuropathological states remains limited and inconclusive.

2.2.0 Current sport-related concussion testing

Researchers have conventionally used neuropsychological testing as a non-invasive and affordable alternative, which has been argued to confer better sensitivity to detect brain structural differences than its neuroimaging counterpart (Bernick *et al.*, 2015)(Bernick C., Banks S., 2015). Wilde and colleagues (2016) showed that parameters of morphological brain abnormalities, namely, white matter diffusion TDI metrics, correlate with neuropsychological function and boxing experience. Neuropsychological evaluation is sensitive to cerebral deterioration, and generally considers a host of emotional, neurological, and psychosocial factors that may present after head impacts. To this end, neuropsychological assessments offer a means for objectively measuring both baseline cognitive function and the subsequent post-injury deficits.

Pitch-side neuropsychological assessment for a suspected concussion in most sports typically include a version of the sport concussion assessment tool (SCAT) (Echemendia *et al.*, 2017; England Rugby, 2019; Feddermann-Demont, Straumann & Dvořák, 2014). These pitch-side assessments are designed to be conducted within 3-minute window or as quickly as possible following a suspected concussive incident (Echemendia *et al.*, 2017). However, the side-line assessment and recognition of intra-game SRC incidents offers a difficult challenge for team physicians. Athletes are hyper-stimulated and motivated to return to play, leaving a small time window for physicians to evaluate and formulate a conclusion.

An expert committee of scientists in the field of concussion known as the Concussion in Sport Group, published the 5th edition of the sport concussion assessment tool (SCAT5) protocol in 2017 for the use of team physicians and medical professionals. The SCAT5 was developed to be a research-led, standardised evaluation tool to be used pitch-side and off pitch to assess symptoms, neurological, physical, coordination, cognition and balance performance. Recently a two-week test-retest study revealed the SCAT5 testing procedure held moderate to high reliability, for

symptom score (r = 0.85) and severity (r = 0.84) (Hänninen *et al.*, 2021). Although no systematic review of current literature regarding the validity of the SCAT5 in order to detect concussion has been published, its predecessor (the SCAT3) was shown to demonstrate content validity with a sensitivity of 47-72% and specificity of 78-91% when using change indices to compare against baseline and normative date (Chin *et al.*, 2016; Alla *et al.*, 2009; McLeod & Leach, 2012; Downey, Hutchison & Comper, 2018).

An important component of the SCAT5 is the modified balance error scoring system (mBESS). Previous studies have found significantly higher modified balance error scores in people who have suffered a diagnosed concussion (Buckley, Munkasy & Clouse, 2018) with a testing sensitivity of 60-71% (Riemann & Guskiewicz, 2000; Buckley, Munkasy & Clouse, 2018; Hunt *et al.*, 2009).

It should be noted, that the SCAT5 should not be used singularly to exclude or diagnose a concussion, as athletes may have concussion but score “normally” in the SCAT5 (Echemendia *et al.*, 2017). The subjective nature of the Maddox questions, SCAT5 and mBESS protocols has resulted in high-profile incidents where the decision of the medical staff, to allow players to continue playing, has been criticised for compromising player welfare. It should be noted that traditionally, loss of consciousness was a requirement of concussion diagnosis, though it is now believed to be present in only 5-10% of cases. A high-profile example of this is provided by the professional football player Lorius Karius, who sustained a head collision but did not lose consciousness in the 2018 UEFA Champions league final. This head collision was later identified by Massachusetts General Hospital after the game as a concussive incident. Professional medical opinion remarked that this incident would have “likely affected performance”. His two personal mistakes led his team to defeat in the most prestigious game in world club football (Zafonte & Herget, 2018).

2.2.1 Head injury assessment and guidelines in Boxing

Most professional sporting organisations have developed specific intra-game head injury protocols. For example, the Rugby Football Union have developed the Head Impact Assessment protocol (RFU, 2017) and the National Football League have

developed the Head, Neck and Spine protocol (NFL, 2016). Despite this, current intra-bout concussion and return to play policies in boxing are decided on a country-to-country basis and vary between boxing commissions. Some boxing commissions follow stringent written guidelines whereas others do not, and in those instances the procedures are at the discretion of the ringside physician. It should be noted that, ringside physicians differ greatly in their experience and medical background, and this naturally leads to variation in SRC evaluation and post-bout recommendations. To this end, Moriarity and colleagues (2012) found that amateur boxers with absence of diagnosed concussion by ringside physicians, showed acute decline of cognitive performance in post-bout computerised cognitive testing. Although many participants in the study safely competed without evidence of sustaining a concussion, it may be considered that a greater number of unrecognised concussive injuries may occur at events with less optimal organisation or supervision.

Commonly, boxers who have received a head injury are considered to be fit to return to play, based on a clinician's opinion. The clinician most frequently bases this opinion on non-standardised cognitive function testing or the athlete-reported symptoms (Dicker & Maddocks, 1993). This lack of standardisation is a concern, for example if a boxer returned to the ring prematurely and sustained another concussion, resulting in second impact syndrome (Cantu, 1998). Ultimately, the ability to quickly and objectively determine whether an individual has sustained a concussion remains elusive.

2.3 Boxing & Neuropsychological testing

Typically, active and retired professional boxers, who have sustained an extensive history of head impacts, present with decreased performance in fronto-temporal outcomes. Primarily, boxers are most sensitive to impairments in memory, information processing, concentration and attention (Roberts, 1969, Johnson, 1969, Neubuerger *et al.*, 1959, Thomassen *et al.*, 1979, Drew *et al.*, 1986, McLatchie *et al.*, 1987).

In 1986, researchers found cognitive deficits of professional boxers were correlated to both the number of bouts and also total number of losses plus draws (Drew *et al.*,

1986). A decade later, Jorden *et al.* (1996) reported that impaired neuropsychological performance is also strongly associated with an increased sparring history. Subsequent studies by Stiller *et al.* (2014) and Montenigro *et al.* (2017) have found that the risk of developing neurological sequelae is associated with a history of sub-concussive blows via a sparring index or "cumulative head impact index" respectively. This evidence suggests that history of sparring frequency over one's boxing career, rather than number of bouts or boxing career length, is associated with poor cognitive function, and is further evidence that repeated head impacts in professional boxing may promote unfavourable long-term changes.

In contrast, papers examining neuropsychological performance of amateur boxers show inconclusive results, with many studies finding mild, if any impairment (Hart *et al.*, 2017; Moriarity *et al.*, 2004; Porter, 2003). Porter (2003) reported that 20 amateur boxers showed no evidence of decreased neurocognitive test performance over a 9-year period. In contrast, the boxers performed significantly better when compared to age-matched controls. Similarly, Hart and colleagues (2017) found that after a season of boxing, neuroimaging and neuropsychological assessment failed to detect any evidence of brain injury. In fact, amateur boxers performed better in areas of attention and concentration. The authors suggested this could be a result of boxing successfully improving concentration in order to focus on the opponent and to avoid being hit. A learning effect through repeating the tasks could also have confounded the results and without a control cohort the interpretation of the data must be viewed with a wider scope, that both neuroimaging and psychological testing were insensitive to any significant deterioration after a season of amateur boxing. This line of evidence has led researchers to determine that cautiously measured durations of amateur boxing may permit boxers to evade neuropsychological damage, provided that both frequency and duration are restricted (Butler, 1994).

2.4 Cerebrophysiological testing

2.4.1 Cerebral autoregulation

Typically, boxing data has focused on morphological (Lampert & Hardman, 1984) and neuropsychological (Drew *et al.*, 1986) consequences of repetitive sub-concussive blows, leaving the potential functional deficits of the cerebral circulation

to be omitted from consideration. Evidence suggests alterations in cerebrovascular function are implicated in the progression of ND (Raz, Knoefel & Bhaskar, 2016). To this end, many papers acknowledge the need for research to be directed towards the examining the cerebrovascular response in hope to underpin the mechanisms behind sport-induced TBI (Wilberger *et al.,* 2006; McCrory *et al.*, 2009a)(McCrory *et al.*, 2009b).

Alterations in cerebral blood flow (CBF) are believed to be important in the pathophysiology underlying TBI. Reduced CBF has been reported following SRC and has been related to initial symptom severity (Meier *et al.*, 2015; Churchill *et al.*, 2017). SRC-dependant CBF disturbances may arise from an impaired ability of the cerebrovasculature to maintain cerebral perfusion despite fluctuations in blood pressure (BP); a process referred to as cerebral autoregulation (CA) (Lassen, 1959; Jünger *et al.*, 1997; Strebel *et al.*, 1997; Golding *et al.*, 1999; Czosnyka *et al.*, 2001). CA is governed by the complex interaction of myogenic, neurogenic and metabolic mechanisms, in order to maintain cerebrovascular integrity during hypertensive surges and prevent ischaemia during hypotensive periods (Paulson, Strandgaard & Edvinsson, 1990; Chillon & Baumbach, 2002). CA dysfunction has been persistently reported following both mild and severe TBI (Kirkness *et al.*, 2001; Riberholt *et al.*, 2016), with impaired CA being a significant predictor of long term (6-month) compromised neurocognition (Chaiwat *et al.*, 2009).

Transcranial Doppler ultrasound can be used to non-invasively assess CA dynamics during periods of orthostatic stress (Claassen *et al.*, 2016). By using this method, CA dysfunction has been observed in a large cohort of non-concussed contact sport athletes (Ice hockey and American football), and CA impairment was associated with the total number and severity of head impacts (Wright *et al.*, 2018a). The authors concluded that repetitive sub-concussive blows sustained during a season of contact sports are associated with CA dysfunction in an exposure-dependant manner. Previous data had found that professional boxers with extensive international experience exhibit preserved CA when compared to “actively matched” controls (Bailey *et al.*, 2011b). However in recent years the body of evidence suggests that CA is impaired following traumatic head injury (Riberholt *et al.*, 2016; Wright *et al.*, 2018b; Calviello *et al.*, 2017; Heilbronner & Ravdin, 2020).

Many reproducible methods to drive blood pressure oscillation for quantification of CA have been documented; Lower-body negative pressure oscillation (Tzeng *et al.*, 2011; Tan, Hamner & Taylor, 2013; Brothers *et al.*, 2009; Hammer *et al.*, 2004), sit-stand procedure (Oudegeest-Sander *et al.*, 2014; van Beek *et al.*, 2010, 2012) deep breathing (Joshi, Young & Diehl, 1996; Reinhard *et al.*, 2011, 2003; Gommer *et al.*, 2010), passive leg raises (Elting *et al.*, 2014) and squat-stand manoeuvres (SSM) (Brassard *et al.*, 2017; Smirl *et al.*, 2015a; Claassen *et al.*, 2009; Wright *et al.*, 2018b; Bishop *et al.*, 2017; Birch *et al.*, 1995). In a 2015 landmark paper by Smirl and colleagues, the SSM was coined the "gold standard" for assessing dynamic cerebral autoregulation, after comparison with other technique revealed it to hold considerably greater reproducibility, whilst also presenting the only method to produce coherence values of ~0.9 (Smirl *et al.*, 2015b). Which far surpasses the current "one-size fits" all threshold for coherence values, which is widely reported as 0.5, which indicates only half of the alteration in CBF may be related to blood pressure changes (Claassen *et al.*, 2015).

During the last 30 years, various methods have been developed for non-invasive assessment of CA data based on transient oscillations in BP and CBF i.e. multimodal pressure-flow analysis (Mitsis *et al.*, 2002), transfer function analysis (TFA) (Czosnyka *et al.*, 1996), non-linear analyses using Laguerre expansions of Volterra kernels (Tiecks *et al.*, 1995), the autoregulatory index (Piechnik *et al.*, 1999), autoregression (Mitsis *et al.*, 2002), as well as correlation coefficient analysis (Panerai *et al.*, 1995). There is no universally accepted method to analyse CA metrics among scientific studies. Nonetheless, given that cerebral autoregulation is commonly explained as the concept of the dynamic relationship between oscillating BP (input) and CBF (output), transfer function analysis (TFA) has emerged as a recommended and commonly used method to assess the autoregulatory response transient oscillations of BP (Panerai, 1998; Smirl *et al.*, 2015b). TFA gives an estimate on parameters which explain the dynamic nature of the human CA system, with the assumption that CA is a linear control system. Due to the changes in CBF resulting from BP changes and other variables that affect CBF, such as CO_2, the CA system is, in fact, non-linear. Nevertheless, it is possible to gain insight into the effect of stimuli or clinical populations on the cerebrovasculature by considering the CA as

a ‘simplified’ linear system and keeping the caveats in mind when interpreting the results of TFA. To this end, the SSM is attractive, as it routinely elicits coherence values of >0.9, with 1.0 demonstrating a perfectly linear relationship between mean arterial pressure (MAP) and middle cerebral artery velocity (MCAv), as described above (Smirl *et al.*, 2015b).

2.4.2 Cerebrovascular reactivity

Another physiological parameter which is believed to be an important indicator of TBI pathology and holds relevance regarding ND is the impaired responsiveness of cerebrovasculature to fluctuating levels of arterial carbon dioxide (CO_2; impaired cerebrovascular reactivity). CBF is strongly influenced by oscillations in arterial partial pressure of CO_2 ($PaCO_2$), and the ability to regulate this (cerebrovascular reactivity - CVR) is fundamental to neurophysiological health (Ide, Eliasziw & Poulin, 2003; Rangel-Castilla *et al.*, 2008).

Unlike other steady-state parameters, CVR reflects the dynamic vascular ability to dilate and constrict cerebral blood vessels in response to challenges. Previous studies have found individuals who have suffered mTBI and SRC to present with impaired CVR metrics (Enevoldsen & Jensen, 1978; Dodd *et al.*, 2020; Lee *et al.*, 2001; Stephens *et al.*, 2018; Len *et al.*, 2011; Bailey *et al.*, 2013; Dewitt & Prough, 2003; Golding *et al.*, 1999). Increased arterial CO_2 concentrations (hypercapnia) cause cerebral vessels to dilate and increase in CBF to flush out CO_2 from the brain tissue. Whereas reduced arterial CO_2 concentrations (during hypocapnia) cause cerebral vessels to constrict and CBF to decrease. CVR is a vital homeostatic physiological response and reacts rapidly (within 6s) to regulate central pH values (Ainslie & Duffin, 2009).

In order to deduce CBF velocity from TCD ultrasonography, estimates rely upon the assumption that the insonated vessel (usually the middle cerebral artery; MCA) diameter remains relatively constant. Although $PaCO_2$ is a known potent vaso-modulator, many papers have reported that MCA diameter, in humans, does not significantly change as a result of fluctuating arterial CO_2 concentrations (Serrador *et al.*, 2000; Valdueza *et al.*, 1997; Giller *et al.*, 1993). A recent study investigated the

effect of an array of end-tidal partial pressure of CO_2 ($P_{ET}CO_2$) concentrations, which can been used to deduce $PaCO_2$, via the use of magnetic resonance imaging (McSwain *et al.*, 2010; Verbree *et al.*, 2014). Small variations in $P_{ET}CO_2$ (±7.5mmHg) led to no significant changes in MCA vessel diameter, however a larger increase (+15mmHg) exhibited a 6.8% increase in diameter (Verbree *et al.*, 2014). It is worth noting that Coverdale and colleagues (2014) showed that CBF_V may underestimate CBF during modest hyper/hypocapnia (Coverdale *et al.*, 2014).
It can be summarised that findings remain inconclusive, and more research is required in order to determine the boundaries of significance of $P_{ET}CO_2$ on MCA diameter and how that effects CVR metrics. More relevant to the current study, specifically the assessment of CA, data has revealed no change in MCA diameter during lower-body negative pressure, which is an alternative method for inducing rapid BP oscillations and is widely used in literature to explore CA (Serrador *et al.*, 2000). As such, authors concluded that the changes in MCAv are representative of relative changes in CBF, provided that they remain within 7.5% of baseline $P_{ET}CO_2$ values (Verbree *et al.*, 2014).

CVR is determined by a variety of protocols which measure some index of blood flow, for example blood oxygen level dependant (BOLD) signal change measured by functional magnetic resonance imaging or more frequently, blood flow velocity measured by ultrasound in response to a vasoactive stimulus (Vicenzini *et al.*, 2007; Churchill *et al.*, 2020; Glodzik *et al.*, 2013; Bailey *et al.*, 2013). Hypercapnia (increased arterial CO_2 concentration) is the most commonly used to bring about an increase in blood flow via vasodilation. Hypercapnia can be achieved through various methods including inhalation of CO_2-enriched air, rebreathing and breath-holds. Similarly, hypocapnia (decreased blood carbon dioxide (CO_2) concentration) can be used to bring about a reduction in blood flow via vasoconstriction. Commonly, hypocapnia is induced by hyperventilation protocols. CVR has been shown to be impaired in athletes who have suffered mTBI, when presented with a physiologically challenging protocol, such as breath-holds (BH) and hyperventilation (HV)(Len *et al.*, 2011).

Breath holds (BH) and controlled hyperventilation (HV), have proven to be reliable and reproducible methods of inducing CO_2 fluctuations and measuring CVR (Totaro R, Marini C, Baldassarre M, 1999; Settakis *et al.*, 2002; Stoll *et al.*, 1996). Using BH and HV, Len and Neary (2010) demonstrated that CVR is disrupted in the days immediately following an mTBI. In a second study, Len and colleagues described a marked impairment to CVR during induced hypo- and hypercapnic stress (BH & HV) in concussed athletes, when compared to controls, despite no differences in MCAv at rest (Len *et al.*, 2011).

Our understanding of the relationship between sub-concussive blows and the regulation of vessel diameter in response to a CO_2 challenge to cerebrovasculature remains poorly understood. Female collegiate football players demonstrated disrupted CVR measures throughout the season, which persisted 4-5 months after the season had ended (Svaldi *et al.*, 2017). Similarly, Bailey *et al* (2013) found that professional active non-concussed boxers exhibited impaired CVR which was significantly negatively associated with their total exposure to head impacts, estimated from the number of rounds they had sparred, but not concussion history. This also corresponded with poor neurocognitive performance primarily in areas concerning attention, memory, and executive function. More recently, rugby players exhibited a decline in cerebrovascular reactivity across all positions on the field (Owens *et al.*, 2021). Further analysis uncovered players in the forward positions exhibited a greater decline in the ability to adapt to periods of hypercapnia when compared to backs. The authors concluded that it may be associated with the data that forwards were found to sustain a greater number of concussions across the season when compared to backs. These studies suggest that head impacts both concussive and sub-concussive may be associated with a decline in cerebrovascular reactivity. To our knowledge there is no paper that has investigated the effects of amateur boxing on CVR.

It is worth noting, that despite both being disrupted by head impacts, CA and CVR operate independently, meaning both need to be measured, as one cannot be inferred from the other. Previous studies have reported that CVR does not correlate with CA, in both healthy, TBI and diseased subjects (Singhal & Markus, 2005; Gommer *et al.*, 2008; Carrera *et al.*, 2009). Authors suggested that, the factors that

are responsible for CA under orthostatic stress differ from mechanisms that regulate CBF under hypercapnia (Atkinson, Anderson & Sundt, 1990; Iadecola, 1992). Given the high coherence of SSM 0.9, which we can infer the myogenic and sympathetic effect of CBF regulation (Smirl *et al.*, 2015b), the CVR protocol is needed to better infer metabolic pathway of cerebrovascular regulation.

2.4.3 Visuomotor function

Common indices for identifying mTBI are loss of consciousness, loss of coordination (ataxia), a loss of awareness and cognitive dysfunction. Initial head injury assessments subjectively test for physical and cognitive impairment in isolation (Echemendia *et al.*, 2017), however mTBI can present with a wider range of symptoms across a number of neurophysiological systems, such as visuomotor control.

Precision gripping has been used as a way to explore the functioning of the visuomotor system (Gölge *et al.*, 2004; Tazoe & Perez, 2017) and is an important task for activities of daily living. The visuomotor control of precision gripping is associated with activation in a distributed neural system consisting of the parietal cortex, dorsolateral prefrontal cortex, premotor cortex, supplementary motor area, primary motor cortex, the nuclei of the basal ganglia, motor regions of the cerebellum, and visual motion areas namely, V3 and V5 (Coombes, Corcos & Vaillancourt, 2011; Neely *et al.*, 2013a; Vaillancourt, Mayka & Corcos, 2006). More specifically, the parietal and motor cortices are vital for processing sensory feedback, generating motor commands and remaining on task (Vaillancourt, Mayka & Corcos, 2006) and the basal ganglia are critical for the organisation of grip force (Prodoehl, Corcos & Vaillancourt, 2009).

The underlying skull anatomy and the specific point of impact often dictates the severity and the subsequent symptoms of TBI. As visuomotor control of grip force is dependent on such a widely distributed system, it is susceptible to dysfunction in any one of these regions. This is important because recent neuroimaging, neurochemical, genetic, and neuropsychological literature are in agreement of a neural systems-based anatomy of SRC, wherein impairment is thought to affect

areas such as the basal ganglia, thalamus, cortex and cerebellum– all known factors in visuomotor control processes (Coombes, Corcos & Vaillancourt, 2011; Neely *et al.*, 2013a; Vaillancourt, Mayka & Corcos, 2006).

Isometric precision-grip test performance deficits have been identified in clinical populations with ND such as PD (Neely *et al.*, 2013b). The ability to perform this test has is also sensitive to alterations in other neurological conditions such as attention deficit hyperactive disorder and autism spectrum disorder (Neely *et al.*, 2016, 2019). Pertinently, individuals who have a history of concussion have displayed visuomotor performance impairment (Raikes, Schaefer & Studenka, 2018; Studenka & Raikes, 2019). Visuomotor deficits have been associated with TBI, previous research postulates a history of TBI results in an incapacity for visual processing or integrating sensory feedback (Gera *et al.*, 2018; Raikes, Schaefer & Studenka, 2018; Putukian, 2017; Padula *et al.*, 2017). Recent studies demonstrate deficits in sensorimotor timing and accuracy and deficits in visuomotor tracking in young athletes with TBI (Caeyenberghs *et al.*, 2010).

Subsequently, it is feasible that fine motor impairments through head impacts in boxing are associated with inability to integrate visual feedback for control of an isometric contraction. Such a discovery would provide important insights to the neuropathology of boxing-related mTBI. This test may offer further insight because it represents the integration of many neurological processes, when compared to widely used psychometric tests such as SAC or standardised Maddocks questions. This is also exciting to explore as precision-grip performance testing can occur quickly and potentially ring-side.

There has been various methodological inconsistencies in the scientific literature about how to test for visuomotor function, such as altered degrees of visual feedback (from continuous to intermittent (every 50 ms) to the complete absence of visual feedback) (Vaillancourt, Slifkin & Newell, 2001; Vaillancourt, Mayka & Corcos, 2006; Slifkin & Newell, 2000; Neely *et al.*, 2016) and different target force lines (ranging from 5 to 95% MVC) (Studenka & Newell, 2013; Slifkin & Newell, 2000; Sosnoff & Newell, 2006a, 2006b; Neely *et al.*, 2016). Evidence suggests that this task appears

to be sensitive to different conditions despite a variety of methodological approaches being used.

Although concussion and repetitive head impacts being found to result in a loss of dexterity, sensory abnormalities and inhibition of motor control (Stewart *et al.*, 1994; Brooks *et al.*, 1987; Jordan, 1987; Ross *et al.*, 1987), no study to our knowledge has tested the complex and highly integrated process of precision grip performance in amateur boxers who have sustained multiple sub-concussive head impacts.

Chapter 3: Methods

3.0 Introduction

This chapter provides an overview of the research techniques used in order to answer the research aims (see section 1.1). Both studies were approved by the institutional Ethics Committee (Appendix 1). Chapter 4 investigates the acute effect of amateur boxing on visuomotor performance (using a pinch grip computer guided task) and neurocognitive function (via a SCAT5 questionnaire and balance assessment). Chapter 5, includes these approaches, as well as cerebrovascular measures (cerebral autoregulation via squat-stand manoeuvres and cerebrovascular reactivity via breath-holds and hyperventilation procedures). In order to address the third thesis aim, and to investigate whether there is a dose-dependent impairment to cerebral health, punches to the head were recorded by video footage in chapter 4 and also by using wrist-worn accelerometers in chapter 5. Table 3.1 indicates which of the measures explained in this chapter were used in each experimental paper in order to address the research aims.

	CA	CVR	NF	PGVP	Balance	Head Impacts
Chapter 4			X	X	X	Observation
Chapter 5	X	X	X	X	X	Observation + punch trackers

Table 3.1 – Table indicating which measures are featured in which chapter of this thesis. An " X " indicates the presence of the measure in the chapter. (CA = Cerebral autoregulation, CVR = Cerebrovascular reactivity, NF = Neurocognitive function, PGVP = Precision grip visuomotor performance)

3.1 Participants

For both Chapters 4 and 5, all participants were recruited via purposive sampling from the University of Exeter amateur boxing club performance squad, who typically complete at least 3 rounds of sparring per week. All participants were competitive squad members and were given an opportunity to ask further questions about the study, prior to providing written informed consent to take part. Participants were instructed that they were free to drop out of the study at any time, without providing a reason and without penalty. Participant demographics are displayed in the method section of each experimental paper (section 4.2.1 and 5.3 respectively). All participants were members of the Amateur Boxing Association of England and as such had passed a medical examination which cleared them to participate in competitive amateur boxing.

3.2.0 Assessment of cerebral autoregulation

CA is the ability of the cerebrovasculature to maintain cerebral blood flow despite changes to perfusion pressures. In chapter 5, CBF was assessed by MCAv, and this was captured via transcranial Doppler ultrasound. In addition, MAP was assessed through beat-to-beat finger plethysmography. Dynamic CA was assessed using the repeated SSM, which is recommended above other methods as it provides the greatest coherence between blood pressure and CBF, and superior between day reliability than other accepted protocols (Smirl *et al.*, 2015b). Data captured throughout the SSM procedures would be later analysed via transfer function analysis in order to generate parameters of CA function, namely coherence, phase, gain and normalised gain. In addition, SSM at 0.05 Hz and 0.10 Hz have shown that all Transfer Function Analysis metrics (coherence, gain, absolute gain and phase) have biologically acceptable reproducibility as reflected in a between-day, within-subject coefficient of variation of <20% (Smirl *et al.*, 2015b; Barnes *et al.*, 2017).

3.2.1 Transcranial Doppler Ultrasound

MCAv was insonated using a 2 MHz pulsed transcranial Doppler (TCD) ultrasound probe (Doppler-Box X, DWL, Compumedics, Germany). With the participant seated, the TCD probe was positioned over the right trans-temporal window, following the searching procedures described by Willie *et al.* (2011)(see Figure 3.1). The MCAv

signal was identified via assessment of velocity and waveform and optimised by adjusting the signal depth (Aaslid *et al.,* 1982) and finally secured in position by a headset (DWL®, DiaMon®, Compumedics, Germany, GmbH) (Figure 3.1). The headset facilitated an optimal signal insonation throughout the data collection and negated signal drop or probe movement. Researchers attempted to ensure replication of the insonation angle for within-day measurements and signal depth across visits in Chapter 5.

Beat-by-beat mean arterial pressure (MAP) was continuously measured by finger photoplethysmography (Finometer PRO, Netherlands). Throughout the SSM participants were asked to maintain the Finapres at heart-height, in order to abate any movement artifact and minimise hydrostatic errors in the blood-pressure data (Imholz *et al.*, 1998). After measuring height and body mass, the Finapres height correction unit was calibrated for each participant before data collection.

MCAv and MAP were sampled continuously at 200 Hz using an analogue-to-digital converter (Powerlab; model - 8/30, ADInstruments, Colorado Springs, CO, USA) linked with a laptop computer. All data were stored for later analysis using commercially accessible software (LabChart v8, AD Instruments, Oxford, UK). Cerebrovascular data collection was completed within 1 hour pre and post each trial condition.

The majority of previous literature exploring CA has primarily expressed data as mean values, however recent work suggests that each phase of the cardiac cycle may be differentially regulated (Smirl *et al.*, 2018; Wright *et al.*, 2018b; Burma *et al.*, 2020). Most notably, Smirl et al (2018) showed that systolic BP fluctuations are more effectively dampened by the CA than diastolic fluctuations, which is thought to reflect a protective mechanism against cerebral haemorrhage due to over perfusion. Studying CA data across the whole cardiac cycle allows researchers to detect changes in the buffering capacity of BP fluctuations at both extremes together with the mean. Indeed, Burma et al (2020) identified the differential response in CA metrics following a bout of acute exercise, depending on whether the diastolic or systolic components were measured. This highlights the insight that may be lost if

nuanced data analysis is not explored. Accordingly, CA metrics were explored during systole and diastole, as well as the more traditional mean of the cardiac cycle.

Figure 3.1 **Transcranial Doppler ultrasound technique.**
Diagram showing the trans-temporal placement of the Doppler ultrasound transducer and the headset used during the squat-stand protocol.

3.2.2 Squat-stand manoeuvre (SSM)

Participants completed a 1-minute standing resting period to attain baseline MCAv and MAP data. In order to drive oscillations in blood pressure, participants completed SSM tests at both 0.10 Hz and 0.05 Hz. This corresponds to the subject alternating between squatting and standing every 5 seconds (0.10 Hz) or every 10 seconds (0.05 Hz) for 5 minutes (Smirl *et al.*, 2015b). These frequencies of oscillations in MAP are within the experimental range that CA is believed to have the most impact on pressure-flow dynamics (Zhang *et al.*, 1998), as oscillations >0.20 Hz are not dampened by the cerebrovasculature. It has been suggested that driven oscillations at 0.05 Hz frequency are thought to reflect myogenic properties of

cerebrovasculature, whilst oscillations at 0.10 Hz are thought to reflect sympathetic activation (Smirl *et al.*, 2015b; Tan, Hamner & Taylor, 2013; Andersen *et al.*, 2018).

Starting in a standing position; the participant squatted down to achieve a 90° knee bend. Subjects held this position in line with the 0.05 Hz or 0.10 Hz frequency protocol, before returning to a standing position for the same length of time. Participants were prompted by the experimenter and a metronome to ensure the correct cadence of postural transition was completed.

3.2.3 Transfer Function Analysis

In order to quantify parameters of CA, the outcomes of coherence, gain, normalised gain (normalised to percentage change in CBF per unit of BP; %.mmHg^{-1}) and phase of the actively driven blood-pressure oscillations were generated by extracting at the point estimate of the selected driven frequency (0.05 or 0.10 Hz).

Coherence expresses "the fraction of output variance that can be explained by the corresponding input power at each frequency"(Claassen *et al.*, 2016). High coherence values offer greater reliability in the interpretation of the results of the *phase* and *gain* metrics and results in greater within-participant stability of phase and gain (Smirl *et al.*, 2015b). *Gain* explains an amplitude ratio between output (MCAv) and input (BP), where a high *gain* indicates large magnitude of BP being transferred to the cerebrovasculature, presented as cm/s^{-1}/mmHg^{-1} (Smirl *et al.*, 2015b). *Normalised gain* is similar to gain but presents the data as a percentage of mean gain values (%.mmHg^{-1})*. Phase* reflects the temporal characteristics or "timing offset" between input and output oscillations, measured in radians. When considering CA, a low *phase* value suggests a slower adjustment of cerebrovasculature to changes in BP. Finally, data were checked for negative phase values, or 'phase wrap-around', which may impede correct interpretation of phase estimates (Claassen et al., 2016). However, no such data were present.

3.2.4 Electrocardiogram & $P_{ET}CO_2$

Subjects wore a three-lead electrocardiogram (ECG) for measurement of heart rate during the SSM. The ECG data was stored for R-R interval calibration during TFA.

Partial pressure of end-tidal CO_2 ($P_{ET}CO_2$) was sampled via a mouthpiece and recorded using an online gas analyser (ML206; ADInstruments, Colorado Springs, CO). A nose clip was applied throughout to ensure inspiration and expiration was performed through the mouthpiece.

Research has confirmed the use of end-tidal partial pressure of CO_2 ($P_{ET}CO_2$) concentrations, in order to infer $PaCO_2$ (McSwain *et al.*, 2010; Verbree *et al.*, 2014). $P_{ET}CO_2$ has been shown not to change during SSM to an extent which would cause changes in MCA diameter, and thus invalidate our use of TCD to infer flow (Smirl *et al.*, 2015b). However, $P_{ET}CO_2$ was measured in our protocol in order to check this assumption.

3.3.0 Cerebrovascular reactivity (CVR) protocol

In chapter 5, CVR was assessed by measuring MCAv (via TCD) and MAP (via finger plethysmography) data as outlined in section 3.2. In contrast to the CA protocol in section 3.2, participants completed both hyper and hypocapnic challenges assess the ability to maintain CBF despite changes to $PaCO_2$. There are many methods to elicit a hyper- and hypocapnic challenge. In the current study, CVR was determined by two separate protocols; breath-holding as a hypercapnic stimulus (CVR^{BH}) and hyperventilation to induce hypocapnia (CVR^{HV}). These approaches have been shown to be sensitive to changes in CVR post-concussion (Len *et al.*, 2011).

3.3.1 Breath-holding protocol - hypercapnia

Following CA assessment, described in section 3.2, each subject remained seated whilst baseline data was collected for 1 min. Then, following a normal inspiration, subjects completed a 20 s breath hold (BH) (Figure 3.2). This was practiced and coached in a prior familiarisation laboratory visit, where the participants were instructed to try to remain relaxed and avoid performing the Valsalva manoeuvre. The researcher provided a countdown for each subject for the BH. Each BH was held for 20 s and was followed by 40 s of recovery (normal breathing). This protocol of BH-recovery was repeated five times in order to replicate the approach by Len et al. (Len *et al.*, 2011).

3.3.2 Hyperventilation protocol - hypocapnia

Following the CVR^{BH} protocol and after 1 min of normal breathing in a seated position, the subjects performed 20 s of HV at 36 breaths per minute. After 40 s of recovery, HV was repeated a further four times (Figure 3.4). Recovery data was then collected for an additional 1 min following the final HV. Cadence during the HV protocol was provided by an electronic metronome and supervised by a researcher to ensure the participant was executing the protocol correctly.

3.3.3 CVR^{BH} data analysis

CVR during hypercapnia was determined as the greatest increase in mean MCAv in the 12 seconds immediately after the breath-holds ($MCAv^{max}$), always expressed as the percentage increase from the very first "1 minute baseline" MCAv mean, for each of the five breath-holds (Equation 3.1). CVR^{BH} was then defined as the average of these five attempts (Figure 3.3). To identify the presence of a Valsalva manoeuvre, and minimise any increases in MAP (which may occur during BH and thus better isolate any changes in $P_{ET}CO_2$) beat-to-beat MAP was recorded via finger plethysmography (Portapress, Finapres, Netherlands), and the change from baseline during the final 5 s of the breath-hold was calculated. The increase was analysed by two separate researchers, and if MAP was greatly elevated (>15 mmHg) post BH, the breath-hold would be omitted from data analysis. This occurred in 9 breath-hold attempts across all 540 participants, trials, and attempts in Chapter 5.

$$\mathrm{CVR^{BH}} = \left(\frac{MCAv^{\,max}}{Baseline\ MCAv\ mean}\right) x\ 100$$

Equation 3.1: Cerebrovascular reactivity to hypercapnic stimulus as induced by a breath-hold protocol (CVR^{BH}). $MCAv^{max}$ explains the greatest increase in mean MCAv across the 12 seconds immediately following each BH attempt. Baseline data was computed by averaging the collected data during the first minute of resting baseline measures.

3.3.4 CVR^{HV} data analysis

CVR during hypocapnia was determined as the lowest mean MCAv value ($MCAv^{min}$) in the 10 seconds immediately after the hyperventilation protocol, always expressed as the percentage decrease from the initial baseline MCAv mean (Equation 3.2). The

CVR percentage decrease from MCAv mean baseline (CVR%) was then averaged across all five HV attempts to give CVR^{HV} (Figure 3.5)

$$\mathrm{CVR}^{\mathrm{HV}} = \left(\frac{MCAv^{min}}{Baseline\ MCAv\ mean}\right)\ x\ 100$$

Equation 3.2: Cerebrovascular reactivity to hypocapnic stimulus as induced by a hyperventilation protocol (CVR^{HV}). $MCAv^{min}$ explains the lowest mean MCAv value across the 12 seconds immediately following the HV attempt. Baseline data was computed by averaging the collected data during the first minute.

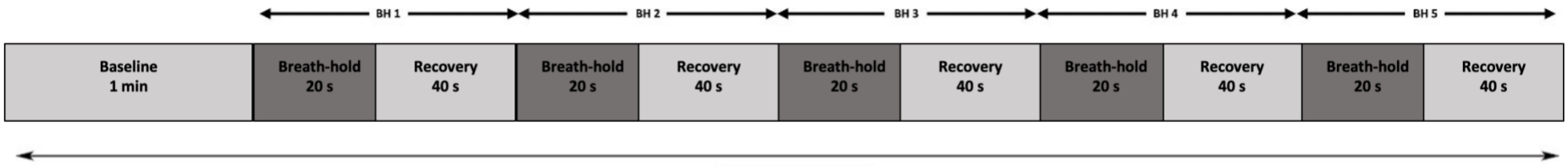


Figure 3.2 Breath hold (BH) protocol to induce a hypercapnic stimulus. Temporal representation of the 5 breath holds.

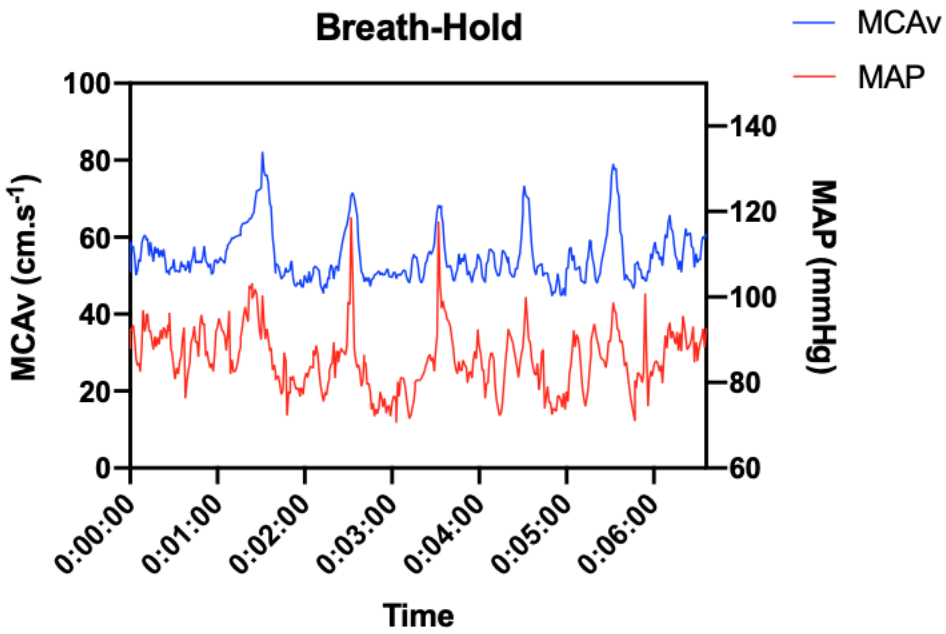


Figure 3.3 An example visual representation of the regulation of both middle cerebral artery velocity and mean arterial pressure during the 5 attempts of the breath-hold procedure.

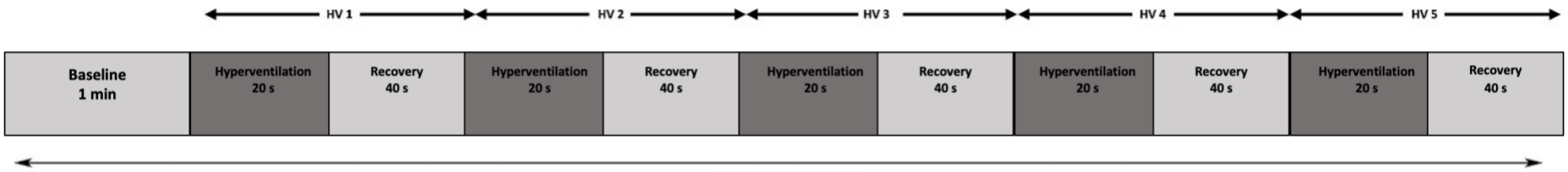


Figure 3.4 Hyperventilation protocol (HV) to induce a hypocapnic stimulus. Temporal representation of the 5 hyperventilation sequences.

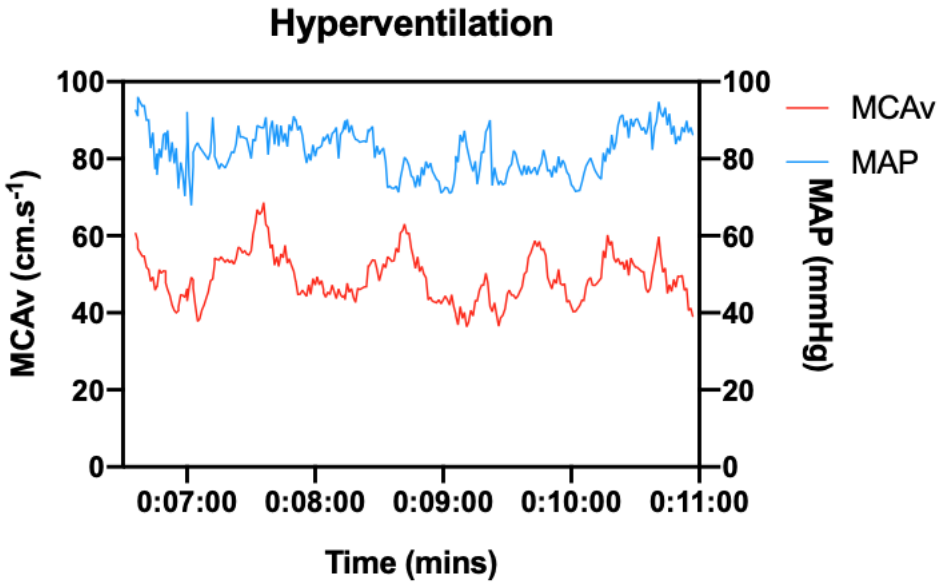


Figure 3.5 An example visual representation of the regulation of both middle cerebral artery velocity and mean arterial pressure during the 5 attempts of the hyperventilation procedure

3.4.0 Neurocognitive function

Neurocognitive function was assessed in both experimental chapters (4 & 5) via a revised version of the SCAT5 questionnaire, whereby participants had to undergo tasks and answer questions.

3.4.1 Sports concussion assessment tool 5th edition (SCAT5)

Components were taken from the most recent form of the internationally recognised SCAT5 (Echemendia *et al.*, 2017). SCAT5 is a holistic evaluation tool that includes manual methods shown to be useful in detecting sports-related concussion and NF. Following the work of Echemendia *et al* (2017) this study used the modified Balance Error Scoring System (mBESS) (McCrea *et al.*, 1998) and standardised assessment of concussion (SAC) (Lovell *et al.*, 2006). Both these components of the SCAT5 were found to be the most sensitive to differentiation between concussed from non-concussed athletes immediately post-injury (Echemendia *et al.*, 2017). The SAC questions were adopted from the SCAT5, including Maddocks questions considering orientation (Maddocks, Dicker & Saling, 1995); episodic memory (see below) consisting of immediate and delayed word recall; and concentration via repeating a string of numbers in reverse order and the months in reverse order.

3.4.2 Balance

Balance deficits, as a measure of neurocognitive dysfunction, have been considered a sensitive measure of cumulative, sport-related, sub-concussive head impacts (Miyashita, Diakogeorgiou & Marrie, 2017). The mBESS test measured barefoot balance on a firm surface in three postural variations for 20 seconds: double-leg (ankles together), single-leg (the dominant leg raised), and tandem (heel-to-toe with the dominant foot in front). Each 20 second trial is scored by counting the number of errors. The maximum number of errors for each condition is 10. Example errors are hands off iliac crest, opening eyes, step or stumble, lifting forefoot or heel, remaining out of position for more than 5 s and moving hips. According to the 5th International Consensus Statement on Concussion in Sport (Echemendia *et al.*, 2017) the m-BESS is the recommended postural control assessment, with a higher score showing greater imbalance.

3.4.3 Executive function

In order to assess executive function, participants completed the DSST test (Wechsler, 1981) and the verbal fluency task (Canning *et al*., 2004). The DSST is a manual test containing a 9 digit-symbol key and an accompanying sequence of random digits. Below each digit, an answer space is provided for the participant to write the corresponding symbol. The participant completed as many correct symbols as possible over 60 seconds.

Following the Consortium to Establish a Registry for Alzheimer's Disease (CERAD) participants completed a verbal fluency task (Canning, S. J *et al*., 2004; Morris JC *et al.,* 1988). Participants had 60 s to name as many words as possible starting with a certain letter (informed by the researcher). The letters used were standardised per trial (visit 1: R + P, visit 2: S + M, visit 3: T + B). Participants had to avoid pluralization and repetition.

3.4.4 Episodic memory

The CERAD (Morris *et al.*, 1988) also includes subtest of episodic memory which involves oral introduction to 10 words by the experimenter at a rate of one word every second. Immediately after, the participants attempted to recall the words. This task was repeated 3 times. Episodic memory was determined as the average number of words recalled. In Chapter 5 only, a delayed CERAD recall test was taken the morning after each trial whereby the participants were asked to recall the 20 words they had been presented the day before.

3.4.5 Visual search tasks

Smirl and colleagues (2016) identified the use of Where's Wally© as a visual search challenge used in conjunction with TCD to assess neurovascular coupling. In the absence of the cerebrovascular laboratory set up in Chapter 4, the first experimental study used the Where's Wally search task in isolation as a surrogate measure post sub-concussive head blows to test cognitive function (specifically the ability to search and identify). Participants completed a visual search task by searching on a page for an object character of specific shape and colour pattern ("Wally") that is hidden

amongst a landscape of characters designed to distract the participant. Participants had 20 s for each trial, a “success” was scored each time the character was identified and physically pointed out.

3.5.0 Visuomotor function

Visuomotor function was assessed in both experimental chapters (4 & 5) via computer guided precision grip test. Participants followed on screen instructions and completed the task both with and without visual feedback.

3.5.1 Precision grip visuomotor performance test protocol

Participants sat at a table, in front of a 13-inch HP LCD monitor (Hewlett-Packard, California, USA; resolution: 1920 × 1080; refresh rate: 120 Hz) located 0.5 m away. All participants affirmed that they could see the display clearly. Participants were instructed to hold a small force-transducer between their pollex (thumb) and index finger (Figure 3.6A) in a precision pinch grip in their dominant hand. Multiple precision grip sensorimotor deficits have been identified in the dominant hand relative to the non-dominant hand in certain neurodegenerative diseases (McKinney *et al.*, 2019). Using their dominant index finger interphalangeal joint, subjects registered force through a pinching motion, using index finger abduction. Data were sampled at 1652 Hz. The task protocol was administered in, and all data collected through, LabView. Participants were familiarised with the equipment and the test protocol before registered data were collected. Participants received both a verbal and visual explanation of how to complete the test. The test protocol consisted of two conditions (Full Vision (with visual feedback) and No vision (in the absence of visual feedback)).

In the first experimental precision grip performance task labelled “full vision”, a target force was represented by a line along the centre of the screen. After 10 seconds of baseline sampling, the screen prompted participants to begin to squeeze the force transducer, by presenting a green light, to reach the target force line (Figure 6C). In this trial participants performance was led by the visual sensory feedback of the magnitude of pressure they were exerting.

In the second precision grip performance task labelled “no vision” visual feedback was removed after 10 seconds of pressure; participants could no longer see the live

performance line but were still encouraged to maintain a constant pressure prompted by a green light. After 10 seconds a red light prompted participants to release the precision grip force transducer (Figure 6B).

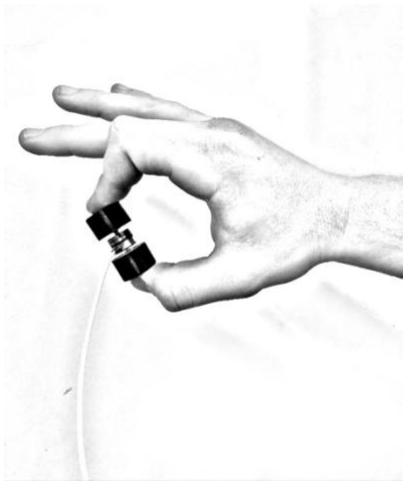
The subjects were instructed to react as quickly as possible to the starting visual cue (green screen) and pinch the device to match the force trace to the target line and minimise the distance between the two lines throughout. Each trial consisted of 20 seconds of force followed by 10 seconds of rest. Participants completed the No force-force-rest procedure three times for each condition. In the full vision condition participants used visual feedback of past performance to influence their ongoing performance. The no vision condition removes visual feedback and requires the participant to use proprioception and short-term memory to maintain the target force (Vaillancourt, Mayka & Corcos, 2006).

3.5.2 Precision grip visuomotor performance data analysis

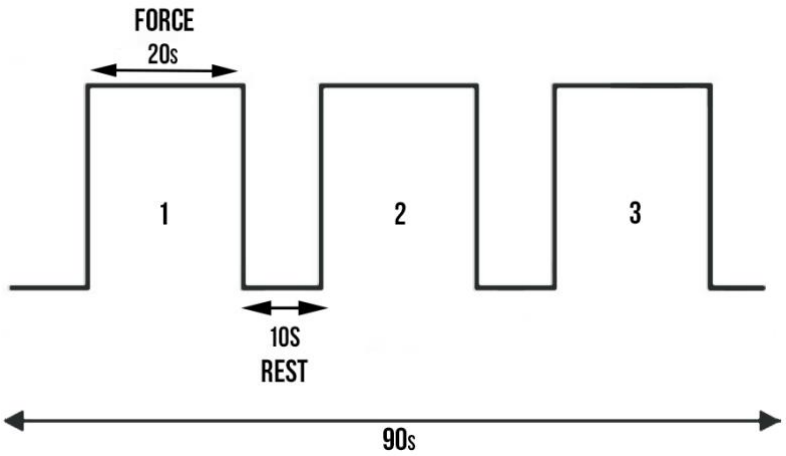
PGVP data from the three repeats were analysed. The raw output from the force transducer was time stamped in Microsoft Excel (Version 16) at 1652 Hz, before selecting the raw data for the pinch sequences only.

During the “initial phase” of the trial, in which individuals squeezed the device’s load cells to reach the target level, we examined reaction time (RT), rate of rise (RoR) (i.e., the maximum value of the first derivative of the force trace) and percentage overshoot (OS) (the accuracy of the initial 1.5s of force output compared to the target force). Reaction time was calculated as the point after the start cue at which the force increased beyond resting levels (~0.42 V) for more the 100ms (Grafton & Tunik, 2011). For the initial phase, the data was averaged into 0.25 s time bins, producing four data points per second. The rate of rise was defined as the change in force (resting to max force) divided by the duration of initial max force output. The force overshoot for the initial phase was defined as the maximum force in the first 1.5s of onset of force as a percentage of the target force. Perfect accuracy was explained by values of 0. Values above 0 indicate overshooting the target force and values below 0 reflect an undershooting of the target force. If participants pinched the force transducer device before the onset cue the trial was excluded and the attempt was repeated.

6A



6B



6C

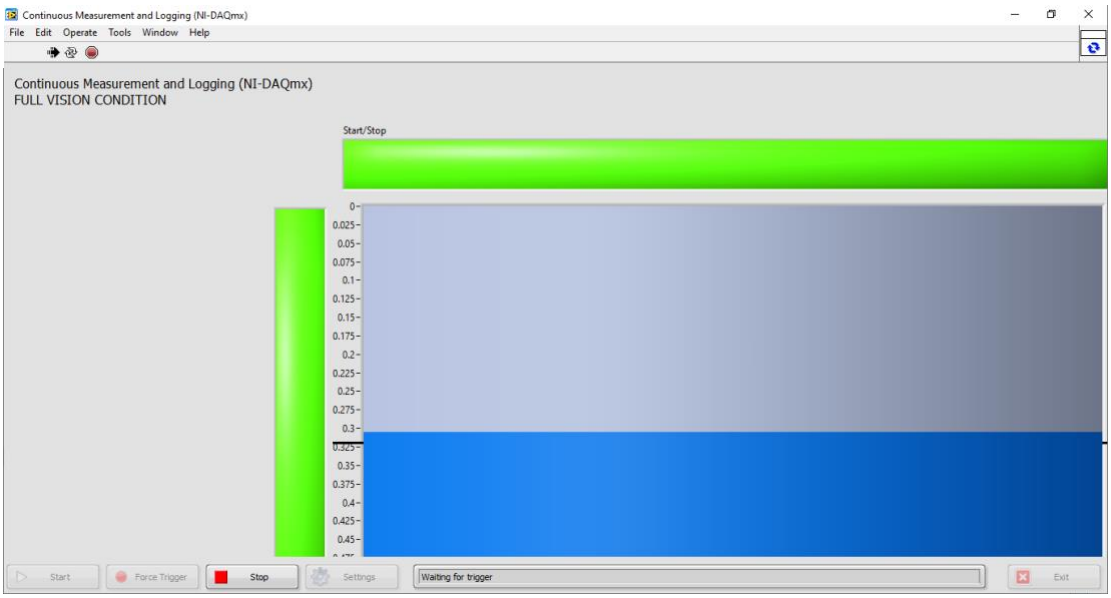


Figure 3.6 **Precision grip testing.**

Figure 3.6 (3.6A) Custom-made precision grip tracking task apparatus. Participant holds device between thumb and forefinger. (3.6B) An infographic of the experimental protocol of each precision grip task. Participants pinched the load cells whilst looking at the visual cue on the screen. Figure 3.6C Visual-motor performance task display. The horizontal black line on the scale is the target line. The blue line is the participants produced force.

To establish whether participants could sustain a constant level of force with and without visual feedback, the steady-state portion of the trial was analysed. The steady-state portion of the trial was defined as the final 10 seconds before the offset cue, in the ‘no vision’ trial this was the point at which the visual feedback was removed. The participants visuomotor ability to complete the task was determined by

mean force output. Visuomotor performance error was determined as the mean root-mean-square-error (RMSE) during the trial the final 10 seconds of the trial (Raikes, Schaefer & Studenka, 2018; Studenka & Raikes, 2019). RMSE explained the amount of force on average, a participants force trace deviated from the target force line Secondly, the standard deviation (SD) of the force output was calculated. Steady-state phase data was excluded if participants force dipped to baseline for >1s (Raikes, Schaefer & Studenka, 2018).

$$RMSE = \sqrt{\frac{\Sigma(s - f_{\iota})^2}{\eta - 1}}$$

Equation 3.3: Visuomotor performance error was determined as root-mean square-error over the last 10 seconds of each trial, in the "No vision" trial this is where the visual cue is removed where S is the target value (3.2 x 10^{-5} N), f_i is the force sample and *n* is the number of sample points (Sosnoff & Newell, 2006b)

3.6.0 Punch demographics

In order to address the third thesis aim, and to investigate whether punches to the head leads to dose-dependent impairment of cerebral health, punches to the head were recorded by GoPro video cameras in chapter 4 and also wrist-worn accelerometers in chapter 5. In order to discriminate between data that was a result of punches to the head and discard data that corresponded to body punches and blocked punches, the boxing bouts were video recorded (GoPro Hero 5, GoPro, California, USA) and later analysed. Only head impacts were counted in Chapter 4 via the video footage from the GoPro cameras.

To measure the accumulated acceleration delivered to the head of the opponents in Chapter 5, participants were equipped with wrist-mounted wearable punch trackers (Corner Boxing Trackers, Corner Wearables Ltd, Manchester, UK) under their hand-wraps. The compact thumb-sized tri-axial accelerometers were synced to the smartphone app via Bluetooth and data was collected during real-time and stored for later analysis. Both punch power data from the punch trackers and the video footage were time stamped and synchronised. Total punches to the head and total power taken to the head were then analysed by two separate researchers and a mean

value was generated to eliminate human error or bias. Intraclass correlation coefficient estimates, and their 95% confident intervals were calculated using SPSS v.26 (SPSS, Chicago, USA), absolute-agreement, 2-way mixed-effects model.

Chapter 4: The acute influence of competitive amateur boxing on precision visuomotor performance, balance, and neurocognitive function.

4.1 Introduction

Research has begun to acknowledge both short-term, and chronic neuropathological effects of sub-concussive head impacts (Svaldi *et al.*, 2017; Ling *et al.*, 2017; Bailey *et al.*, 2013; Di Virgilio *et al.*, 2019). For example, emerging data suggesting head impacts from heading the ball in soccer are associated with an increased risk mortality from ND, specifically an increased risk of ND in positions associated with increased head impacts such as defending, and lowest in goalkeepers (Mackay *et al.*, 2019; Russell *et al.*, 2021). The dangers associated with multiple sub-concussive blows to the head in soccer highlight wider issues in other sports where athletes are routinely subjected to sub-concussive head impacts, such as combat sports.

Boxing has long been regarded as a high-risk sport, due to the participants deliberately targeting punches to an opponent's head to secure points or win by virtue of knock out. A career in boxing is linked to increased risk of ND (Pearce, Gallo & McElvenny, 2015). Di Virgilio and colleagues reported delayed motor unit recruitment and decreased force thresholds with late motor unit activation, after a single sparring session in amateur boxers, (Di Virgilio *et al.*, 2019).

The visuomotor control of precision gripping involves the activation of many neural systems for example parietal cortex, dorsolateral prefrontal cortex, premotor cortex (Coombes, Corcos & Vaillancourt, 2011; Neely *et al.*, 2013a; Vaillancourt, Mayka & Corcos, 2006). Dynamic visual motor processing impairments have been identified following concussive head impacts (Parker *et al.*, 2007; Raikes, Schaefer & Studenka, 2018). Since visuomotor control of grip force is reliant on such a broadly distributed system, it is vulnerable to deficits in any one of these regions. This is important because recent neuropsychological, neuroimaging, neurochemical, and genetic articles are converging on a neural systems-based anatomy of SRC wherein dysfunction is thought to affect areas such as the basal ganglia, thalamus, cortex and

cerebellum – all known factors in visuomotor control processes (Coombes, Corcos & Vaillancourt, 2011; Neely *et al.*, 2013a; Vaillancourt, Mayka & Corcos, 2006).

To our knowledge, no study has assessed traditional concussion testing alongside precision grip visuomotor performance in the acute phase post-amateur boxing. Such a discovery would provide important insights regarding any neuropathological changes post-boxing as it represents the integration of many neurological processes, when compared to widely used psychometric tests such as SAC or standardised Mattocks questions (McCrea *et al.*, 1998).

Furthermore, precision grip visuomotor performance (PGVP) is a relatively rapid and simple testing method and could be used in conjunction with standardised pitch-side sport concussion assessment tools for example the SCAT5 (Echemendia *et al.*, 2017).

The purpose of this study was to examine if a single bout of competitive amateur boxing influenced precision visuomotor control, neurocognitive function and balance.

4.2 Methods

4.2.1 Participants

Twenty, amateur boxers (see *Table 4.1* for descriptive characteristics) from The University of Exeter amateur boxing club gave written informed consent to take part prior to an amateur boxing competition. The study was approved by the institutional Ethics Committee (Appendix 1). The participants had 3 – 24 months of boxing experience. Exclusion criteria included any history of medically diagnosed concussion diagnosis and contraindications to participation in competitive boxing. Participants were excluded if they reported any neurological or musculoskeletal disorder that could cause atypical sensorimotor functioning, or a history of medications known to affect sensorimotor functioning.

Data is displayed for 20 participants; a participant did not return for testing post-boxing match due to a prior engagement with the media reporters.

Age (years)	Sex	Height (cm)	Body Mass (kg)	BMI (kg/m^2)
20 ± 1	15♂ 5♀	172 ± 7	70 ± 9	23.2 ± 2.0

Table 4.1 Participant characteristics. Data presented as mean ± standard deviation. *n* = 20

4.2.2 Experimental procedure

Participants completed a battery of tests before and after (within 20 minutes) a standard format (3 x 3 mins with a 1 min break between rounds) amateur boxing match. Individuals completed a revised SCAT5 test, a balance test (mBESS) and an assessment of visuomotor function via a precision-grip performance task. Boxing bouts were recorded using a video camera and subsequently two researchers independently assessed the total number of punches to the head that were received by each boxer. Punches that landed to the body and blocked were omitted. The total number of head impacts sustained was determined as the mean of the two tallies. The total punch values never differed by more than 5 punches between the two researchers (Intraclass correlation coefficient = 0.988 with 95% confident interval = 0.972-0.995, p = <0.01).

4.2.3 Visuomotor performance (PGVP)

Participants sat at desk, facing a laptop screen and completed two precision pinch grip performance tasks in order to measure visuomotor function as described in section 3.5.2. Participants were instructed to squeeze the force transducer between pollex and the right index finger of their dominant hand (Figure 3.6A) in line with on screen cues regarding target force (Figure 3.6C).

PGVP was analysed in the initial stage and the sustained stage of the task as explained in section 3.5.2. The primary PGVP outcome was performance error as determined by Root mean square error as described in section 3.5.2. In addition, PGVP was determined by percentage overshoot (OS), rate of rise (RoR) and reaction time (RT) which are described in 3.5.2.

4.2.4 Neurocognitive function & balance

Participants completed a revised Sport assessment concussion tool 5th edition (SCAT5) test, consisting of tests to assess episodic memory (see section 3.4.5), balance (see section 3.4.3), orientation (see section 3.4.2) and concentration (see section 3.4.2) as well as completing separate tests of executive function (see section 3.4.4), visual search and recognition (see section 3.4.6). Results from each individual test were analysed separately before being summed to generate total SCAT5 metric to assess NF.

4.2.5 Statistical Analysis

All data were analysed using IBM SPSS statistics (v26, IBM Corporation, USA). Normality of distribution was assessed using the Shapiro-Wilk test for all outcomes. Pre- and post-boxing PGVP (RT, RoR and OS) and cognitive function were assessed via paired samples t-tests. The balance scores violated the assumption of normality, and were subsequently assessed using a Wilcoxon Signed Rank test. Additionally, changes in visuomotor performance (RMSE, mean force) were explored using a 2 (pre- and post-boxing) by 10 (each second of data collection) repeated measures ANOVA. A Pearson's correlation was used to assess the relationship between number of head impacts and change in average RMSE values after boxing. Statistical significance was set at the $p<0.05$ level. Effect sizes from the repeated measures ANOVA main effect and interaction effects were provided. Effect size thresholds were defined as small ($\eta^2= 0.01$), medium ($\eta^2 = 0.06$) and large ($\eta^2= 0.14$) (Cohen, 1988). The magnitude of the pairwise differences were described using Cohen's *d*, as small (0.2), medium (0.5) and large (0.8) (Cohen, 1988).

4.3 Results

4.3.1 Initial phase of PGVP

There were no significant differences between pre- and post-boxing values in the 'Full vision or "No vision" trials for RT, percentage OS or RoR from target force (see table 4.2).

	Reaction Time	Rate of Rise	Percentage Overshoot
'Full vision'	$P = 0.591$, $\eta^2 = 0.122$	$P = 0.872$, $\eta^2 = 0.037$	$P = 0.704$, $\eta^2 = 0.086$
'No vision'	$P = 0.132$, $\eta^2 = 0.353$	$P = 0.449$, $\eta^2 = 0.173$	$P = 0.142$, $\eta^2 = 0.343$

Table 4.2 Statistical output from paired-samples t-test of pre- and post-boxing initial phase metrics.

4.3.2 Sustained phase of PGVP

4.3.2.1 Full vision average force

Average force remained at target force level (3.20×10^{-5}) when visual feedback was present (Pre-boxing 3.19×10^{-5} N ± 1.30×10^{-6} ; Post-boxing 3.05×10^{-5} N ± 6.99×10^{-6}). There was no effect of test duration ($P = 0.565$, $\eta^2 = 0.041$), boxing ($P = 0.345$, $\eta^2 = 0.045$) or interaction effect ($P = 0.350$, $\eta^2 = 0.056$) for average force.

4.3.2.2 No vision average force

Average force was not altered by boxing when visual feedback was removed ($P = 0.266$, $\eta^2 = 0.061$). (Pre-boxing 3.29×10^{-5} N ± 1.74×10^{-6} ; Post-boxing 3.11×10^{-5} N ± 7.40×10^{-6}). Average force decreased over the duration of the test ($P = 0.002$, $\eta^2 = 0.308$), but this was not influenced by the boxing intervention (no interaction effect was found, $P = 0.748$, $\eta^2 = 0.016$).

4.3.2.3 Full vision root mean square error (RMSE)

Participants exhibited a main effect of trial with greater RMSE after boxing ($P = 0.002$, $\eta^2 = 0.397$); Figure 4.1) compared to pre-boxing values (4.39×10^{-5} N and 2.41×10^{-5} N respectively). There was no main effect of time ($P = 0.812$, $\eta^2 = 0.057$), or interaction effect ($P = 0.458$, $\eta^2 = 0.453$).

4.3.2.4 No Vision RMSE

RMSE increased during the precision grip task without visual feedback eyes ($P = <0.001$, $\eta^2 = 0.518$) (see Figure 4.2). However, this increase over time was not

altered by the boxing intervention (P = 0.751, η^2 = 0.05), nor was there an interaction effect (P =0.426, η^2 = 0.042).

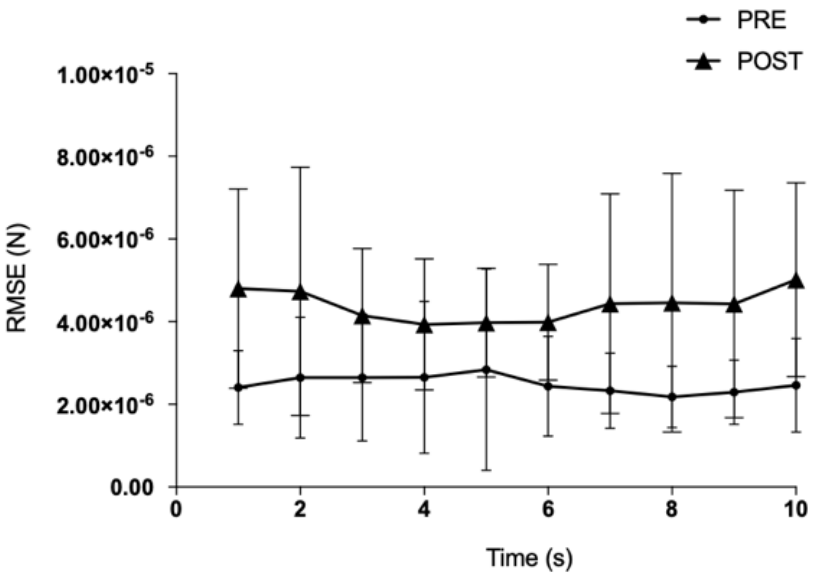


Figure 4.1 Mean performance error data with real-time visual feedback pre- and post-boxing. Data represents the last 10 s average across all participants. *Error bars* represent standard deviation. RMSE was significantly greater post boxing (P = 0.002, η^2 = 0.397),

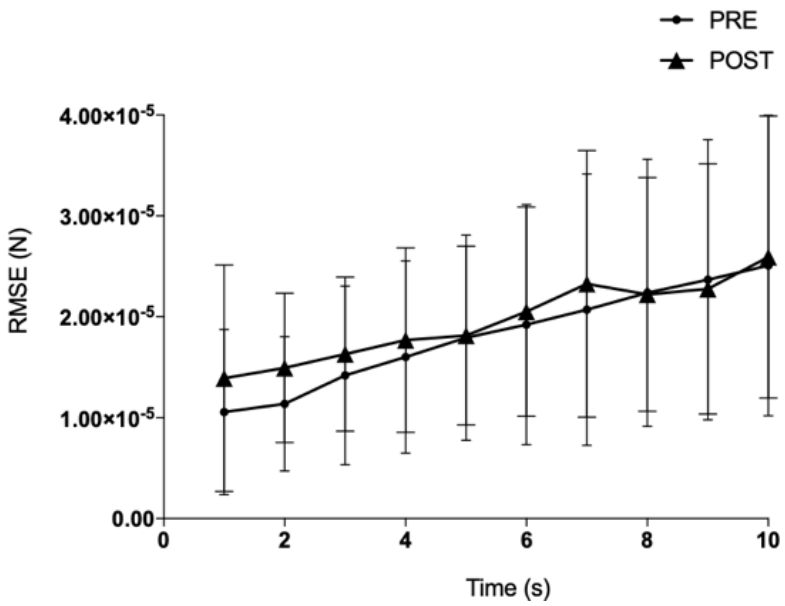


Figure 4.2 Mean performance error data without real-time visual feedback pre- and post-boxing. Data represents the last 10 s average across all participants. *Error bars* represent standard deviation. RMSE was significantly greater as a function of time (throughout the trial) in both pre- and post-boxing, but there was no interaction effect of the boxing intervention

4.3.3 Neurocognitive Function

There was no observed difference between pre (32 ± 5) and post-boxing (33 ± 4) measures in SCAT5 score (P = 0.137) or visual search task (Where's Wally?) (1 ± 1, 1 ± 1 successes, P = 0.724). Post-boxing verbal fluency scores were greater (18 ± 5) when compared to pre-boxing measurements (13 ± 4; P = <0.001, d = 1.331). There was greater error in executing balance poses after boxing (4 ± 3), compared to pre boxing measures (2 ± 2; P = 0.001, d = 0.887).

4.3.4 Head impacts

Participants received an average of 58 ± 18 punches to the head. The number of head impacts a participant sustained was not related to the difference in average RMSE in the presence of visual feedback pre- and post-boxing; r = 0.250, P = 0.259. (Figure 4.3). The number of head impacts was not related to changes in: verbal fluency (r = 0.107, P = 0.645), balance (r = -0.094, P = 0.686), visual search tasks (r = 0.301, P = 0.184), full-vision RT (r = 0.012, P = 0.959), no vision RT (r = -0.069, P = 0.765), full-vision OS (r = -0.277, P = 0.225), no-vision OS (r = 0.178, P = 0.441), full-vision RoR (r = -0.102, P = 0.660) no-vision RoR (r = -0.273, P = 0.231)

4.4 Discussion

The purpose of this study was to examine if a single bout of competitive amateur boxing influenced PGVP, NF and balance. We hypothesised PGVP, NF measures and balance would be impaired post-boxing. We observed greater PGVP performance error in amateur boxers after boxing when compared to pre-boxing measures, when visual feedback was available, and impaired balance. However verbal fluency performance improved after boxing.

4.4.1 Precision grip visuomotor performance

When participants were presented with visual feedback, performance error was greater in the precision pinch grip task after boxing, when compared to pre-boxing measures. This performance error was evaluated during the steady-state component of the trial, whereby participants had to analyse real-time visual feedback to perform a continuous isometric pinch on the force transducer. Continuous spacio-motor control is associated with increased localised brain activity in the cerebellum and

posterior parietal cortex (Desmurget *et al.*, 1999; Desmurget & Grafton, 2000). Neely and colleagues (2013) added that similar visual-guided isometric hand-grip tasks were associated with increased brain activity in the same brain regions. Similar to results seen in the present study, impaired visuomotor function, specifically deficiency in force regulation, is a common behavioural characteristic across neurodegenerative populations such as PD and more specifically individuals who have suffered a sports-related-concussion (Stelmach & Worrincham, 1988; Raikes, Schaefer & Studenka, 2018). Development of parkinsonian symptoms are well documented in ex-boxers, though not an exclusive manifestation, it is thought to be a result of neuronal atrophy as determined by reduced N-acetylaspartate (neuronal marker) in extrapyramidal and putamen structures (Davie *et al.*, 1995). The findings here are particularly of interest, given that they indicate disturbances to visuomotor ability are apparent even in young healthy adults immediately post competition. Although, amateur boxers had greater performance error after boxing than pre-boxing measures, they were able to reach the target force both, before and after, the boxing intervention. That is, the time taken to achieve the target force ("initial phase") was not different. So, this increase in RMSE post-boxing reflects error in ability to continuously regulate force, rather than react. The sub-concussive head impacts through amateur boxing resulted in impaired visuomotor performance when visual feedback was available, suggesting that there might be a compromised neural network governing processes, such as feed-forward processing, predictive control, and visual feedback processing.

It is interesting that the post-boxing alterations in RMSE were not apparent when visual feedback was removed. Previous work with individuals with neuropsychiatric disorders (ADHD) and ND (PD), have found a greater rate of visuomotor performance error and force decay. This force decay is known to present following 1.5s after a motor-memory source is removed, which marks the limit of the temporal capabilities of the short-term memory to store precise visuomotor information (Vaillancourt & Russell, 2002). Vaillancourt and Russel proposed that the limits of short-term visuomotor memory explain force decay, rather than the shortfalls of sensory reflex pathways or dysfunction of motor output signals from the cortical regions of the brain. This was determined because force decay was evident between 1.5-2.5s after the removal of visual feedback (Vaillancourt, Slifkin & Newell, 2001; Neely *et al.*, 2016, 2019; Vaillancourt & Newell, 2002). It is proposed that this motor

memory decay is coupled with the decline in motor neuron excitation and consequently decreased force output (Vaillancourt & Russell, 2002). With regards to the current study population, amateur boxers are reported to present no evidence of visuospatial, attentional or information processing impairment when compared to age matched controls (Lesiakowski, Zwierko & Krzepota, 2013; Butler, 1994) and we did not observe any acute changes in the rate of force decay post-boxing. Therefore, head impacts sustained during the boxing intervention may not have been enough to afflict the visuomotor memory or neural systems governing the visuomotor control performance. Further research is required to explore the change in full vision RMSE despite no change SCAT5, which is a tool typically used to detect concussions.

4.4.2 Neurocognitive Function

In the current study, amateur boxers exhibited an increase in verbal fluency after boxing when compared to pre-boxing measures. Similar to the current study, Moriarty and colleagues found an improvement of certain cognitive performance, specifically learning tasks, in 82 amateur boxers after sparring, when compared to non-boxing controls (Moriarity *et al.*, 2004). The finding in the current study may be explained by the increase in arousal post-bout. Acute bouts of high intensity cardiovascular exercise provides post-exercise enhancements to several cognitive functions including, mental processing, attention and executive function (Hacker *et al.*, 2020; Chang *et al.*, 2012; Marchant *et al.*, 2020), which are associated with increases in arousal (Hillman, Snook & Jerome, 2003). Anecdotally, during this study, researchers commented on the difficulty in retaining the attention of the participants between tasks and participants reported of hyperawareness of oneself. The SCAT5 assessment was not sensitive to the increase in verbal fluency, as it was not altered post-boxing. No differences between pre- and post-boxing measures were found in any other cognitive function measures of recognition, memory, attention, orientation.

4.4.3 Balance

Greater indices of sparring exposure (frequency/intensity) have been shown to be associated with poorer performance balance in professional boxers (Stiller *et al.*, 2014). The present data are the first to demonstrate that balance is acutely altered post-amateur boxing. Compromised balance has long been an associated symptom

and consequence of concussion (Guskiewicz, Ross & Marshall, 2001; Riemann & Guskiewicz, 2000; McCrea *et al.*, 2003), and is used in the internationally recognised SCAT5 (Echemendia *et al.*, 2017). However, it should be noted that its inclusion as a concussion assessment point has not gone without criticism; Buckley *et al* (2018) identified that the mBESS test sensitivity and specificity to detect SRC in collegiate athletes was 71.4% and 65.7%, respectively. It is therefore interesting that we were able to detect changes in balance performance, despite no differences in SCAT5, which indicates that an impairment in balance may not be reserved to post concussion states, but rather present following sub-concussive impacts.

Alternatively, it could mean that concussions were sustained in the present study, but the SCAT5 was not sensitive to these incidences. However, this alteration in balance performance was not correlated with the number of head impacts sustained, therefore this alteration in balance may also be driven by increased arousal. Indeed, increased physiological arousal has previously been associated with impaired postural sway performance (Maki & McIlroy, 1996). Further work is needed to establish whether alterations in balance are truly indicative of concussion, rather than being reflective of exposure to sub-concussive events. Further work may explore whether alterations to balance following sub-concussive head impacts are associated with impaired visuomotor performance present in this study.

4.4 Limitations

Our current findings should be considered in light of our methodological limitations. Our main goal was to observe how head impacts received during amateur boxing influences precision visuomotor control performance. Previous investigations typically use a target force based on a percentage of the participants registered maximal voluntary contraction (Neely *et al.*, 2015, 2016, 2019; Sosnoff, Vaillancourt & Newell, 2004; Vaillancourt & Russell, 2002; Prodoehl, Corcos & Vaillancourt, 2009; Poon *et al.*, 2011; Mosconi *et al.*, 2015). In this study the target force was set at 3.20 x 10^{-5} N for all participants, meaning the force output required was not standardised against participants grip strength. The extent to which this normalisation of target force would be beneficial to our method is not clear, however it is known that greater force variability and force decay is associated with larger target forces (%MVC)

during isometric visuomotor tasks (Vaillancourt & Russell, 2002). Thus, those with weaker pinch forces might be at greater risk of error.

In addition, the cohort of amateur boxers within this study were young healthy university educated athletes. Force variability of isometric contractions are known to have age-related differences; therefore, it is difficult to translate our findings across the whole sport of amateur boxing, due to widely accessible participation. Further research should examine participants across broader age spans to observe whether boxing-related differences in PGVP measures are influenced by head impacts. Evidence has shown that middle-aged individuals present with precision grip visuomotor control deficits at low force outputs, when compared to other age groups, suggesting a gradual decline in grip force control form adulthood to middle-aged (Lindberg *et al.*, 2009). It should be explored whether this hypothesis of gradual decline is evident after testing precision visuomotor control following boxing related head impacts.

The cohort of this study included both females and males. There is evidence that females may experience greater microtrauma than men from sub-concussive head impacts (Rubin *et al.*, 2018), possibly due to differences in neck strength (Tierney *et al.*, 2005), which could lead to greater acceleration of the skull (Bretzin *et al.*, 2017) Only 5 females volunteered to take part, so it was beyond the scope of this study to consider if sex (or neck strength) influenced these acute responses to boxing. Similarly, the study included a wide range of prior experience (3-24 months), and it is plausible that a more junior boxer might have less developed neck strength. Further study regarding whether prior experience and or neck strength can protect against the changes observed here is warranted.

To further our understanding on the influence of head impacts on neuropathological manifestations, future studies should measure the magnitude of the blows to the head. It may be that the individual who took more blows to the head did not sustain as much force by nature of the power of each punch. This information may be recorded by commercially available wrist-worn accelerometers and may give a greater insight into the visuomotor impairments evident in the present study. It should also be considered that in order to put our data in context, further research is needed

to examine the effect of clinically diagnosed concussion on PGVP, and how this related to the data collected in the present study.

4.6 Conclusion

The current study demonstrates acute deficits visuomotor performance during visual feedback task and a decrease in balance performance in amateur boxers after competitive boxing, whilst verbal fluency improved. These observations occurred in the absence of any changes in the SCAT5 score. To distinguish the effect of repetitive head impacts as a stimulus, further research should investigate whether the impairments to visuomotor performance differs between boxers with a longer history of boxing, competitive bouts, sparring index, post KO and whether this affect is accumulative following multiple bouts at an amateur boxing tournament. The addition of physiological measures to provide potential mechanistic insight would also be beneficial, as well as addressing whether the response is the same in males and females, once controlling for differences in neck strength and weight categories. Such research may reveal a novel test to explore the effect of repetitive sub-concussive head impacts, which challenges the functionality and integration of signals governed by multiple brain regions to coordinate a dynamic response.

Chapter 5: The acute influence of boxing on brain health parameters of university amateur boxers.

5.1 Introduction

Recent evidence has implicated that exposure to sub-concussive head impacts (heading in soccer) may increase the risk of ND (Mackay *et al.*, 2019; Russell *et al.*, 2021). Evidence from both animal and human studies suggest sub-concussive blows can lead to acute alterations to functional connectivity of the brain and damage to the central nervous system (Dashnaw, Petraglia & Bailes, 2012; Johnson *et al.*, 2014; Bauer *et al.*, 2001). In addition, normal CVR responses are acutely disrupted following diagnosed mTBI (Len *et al.*, 2011). Whilst Bailey *et al. (*2013) reported that chronic exposure to sub-concussive head impacts through professional boxing results in reduced parameters of cerebrovascular health (Bailey *et al.*, 2013). Specifically, professional boxers, when compared to age and fitness-matched controls, displayed impaired CVR, CA and NF (Bailey *et al.*, 2013). Rugby players have been found to exhibit a decline in cerebrovascular reactivity following a season of professional rugby. More specifically the magnitude of the decline is associated with the position on the field and the amount of concussions sustained throughout the season (Owens *et al.*, 2021).

Tagge *et al.* (2018) recently found that, sport-related head traumas, independent of concussive symptoms, may result in neuropathological sequelae and associated surrogate characteristics of CTE in teenage athletes who have suffered from a mild head injury (Tagge *et al.*, 2018). Similarly, Di Virgilio *et al.*, (2019) found that amateur boxers acutely after repetitive sub-concussive head impacts through boxing sparring, exhibited acute and transient brain changes reminiscent of those seen in TBI. With national participation numbers in boxing rising (ABAE 2012; Sport England, 2019), early detection of cerebrovascular impairments is important, considering the potential to prevent cTBI and protect against an increase in ND risk (Kutcher & Giza, 2014).

Many papers acknowledge the need for the research focus to be directed towards the cerebrovascular response in hope to underpin the mechanisms behind sport-induced TBI (Wilberger, Ortega & Slobounov, 2006; McCrory *et al.*, 2009b). To that end, available studies have found that boxing is associated with reduced

haemodynamic function, characterised by altered regional cerebral perfusion and hypometabolism (Rodriguez *et al.*, 1983; Rodriguez, Vitali & Nobili, 1998; Jordan BD, Dane SD, Rowen AJ, 1999; Provenzano FA, Jordan BD, Tikofsky RS, 2010). The associated periods of oscillating hypo- and hyperperfusion, as a consequence of dysfunctional cerebral perfusion-pressure regulation, is associated with damage to the cerebrovascular endothelium and blood-brain-barrier leakage, with further damage leading to cerebral oedema (Markus, 2004; Bailey *et al.*, 2011a).

CVR is known to be acutely compromised in athletes who have sustained concussion (Churchill *et al.*, 2020; Len *et al.*, 2011), but to our knowledge there are no studies investigating the effect of sub-concussive head impacts on CVR, nor any studies investigating the effects of amateur boxing.

Wright *et al.* (2018) found altered CA metrics in contact-sports athletes who sustained a concussion, specifically, reduced phase (ability to adjust cerebrovascular resistance to changing MAP) and increased gain (magnitude of MAP oscillation transferred to cerebrovasculature), when compared to pre-season controls. The altered CA parameters suggests deficits to both the magnitude and temporal control of the cerebrovascular pressure-buffering system capacity, after a single concussive incident, in athletes. Wright *et al.* (2018) suggested that repetitive sub-concussive head impacts following a season of contact-sports are associated with impairments to cerebrovascular pressure-buffering capacity. In addition, the magnitude of CA and neurocognitive impairment has been correlated with sparring index in professional boxers (Bailey *et al.*, 2013; Jordan, B. D., Matser, E. J., Zimmerman, R. D. and Zazula, 1996). While a decline in CA, CVR and NF have previously been implicated in experienced professional boxers (Bailey *et al.*, 2013; Jordan, B. D., Matser, E. J., Zimmerman, R. D. and Zazula, 1996), the acute effects of amateur boxing on parameters of brain health, remains to be investigated.

The aim for the present study was to investigate whether compromised brain health parameters, previously discussed by Bailey *et al.*(2013) (impaired dynamic CA, CVR and NF), among other measures, are accrued in amateur boxers with >1 year boxing experience, after three rounds of boxing. The study was designed to test the following hypotheses.

1.) Cerebral health parameters, namely CA, CVR, visuomotor function and NF would be altered in amateur boxers with >1 year of experience post-boxing.
2.) The magnitude of cerebral health impairment would be related to the amount of head impacts received during the boxing trial.

5.2 Methods

5.2.1 Ethics

This study was approved by the University of Exeter Sport and Health Sciences Ethics committee, prior to data collection. All procedures were carried out in accordance with the Declaration of Helsinki (World Medical Association, 1964). Participants were provided with participation information sheets, explaining the study aims, experimental protocols, potential benefits, and risks of participation. Following this, subjects were given the opportunity to ask any questions, confirm their understanding and decide if they wanted to begin the study.

We recruited eighteen healthy competitive amateur boxers with at least 1 year of boxing experience. The sample size for the study was estimated using G* Power (3.1.9.3) calculation, based upon a power of 80%, alpha level of 0.05, and our own unpublished pilot data, which assessed cerebral autoregulation pre- and post-boxing and a time-matched pad-boxing trial. Specifically, the study was powered to detect an expected change in phase (partial eta squared (η^2) = 0.464 for a trial by time interaction in a repeated measures design). This power calculation determined that 17 participants would be required. To err on the side of caution and because the study must be completed in pairs due to the nature of boxing, the final output for sample size was 18.

All boxers provided written informed consent before participation. Participants were active members of the competitive squad at the University of Exeter amateur boxing club and took part in at least 3 rounds of sparring per week**.** All participants had passed a medical examination and were members of the Amateur Boxing association of England. All participants were free of contraindications to exercise and had no history of drug or cigarette abuse. Participants were free of medication that may influence blood pressure, heart rate, vascular function or visuomotor control.

5.2.2 Experimental design

Participants attended the laboratory on 4 separate occasions and took part in a randomised, cross-over, repeated-measures experimental design. On their first visit, participants were measured for baseline physical characteristics and familiarised with the study procedure and laboratory testing environment. Following this preliminary visit, 3 subsequent experimental visits were completed. Each trial was separated by approximately one week. On the day of all laboratory visits, participants were instructed to arrive at the laboratory, having not completed any vigorous exercise in the 24 hours prior to each experimental visit. The three experimental visits consisted of 1) boxing (BOX), 2) pad-boxing (PAD), and 3) a seated control trial (CON). The order of experimental trials was randomised to minimise potentially confounding carry-over learning effects. It was not possible to blind the investigators to the condition during data collection.

The BOX trial consisted of three separate rounds, each three minutes in duration, separated by one minute of rest, as specified by the English amateur boxing rulebook (ABAE, 2018). This was a routine sparring session between two sex and weight-matched athletes overseen by medically trained professional boxing coaches. The PAD trial was designed to replicate the exertion of a boxing session but without the exposure to head impacts. This trial consisted of punching hand pads held by a sparring partner for the same three, 3 minutes rounds. The CON trial consisted of time-matched seated rest.

5.2.3 Cerebrovascular assessments

With participants seated the researchers followed the CA, CVR (BH & HV) protocol described in section 3.2.1 and 3.3.1 respectively.

5.2.4 Cerebral autoregulation (CA)

CA was quantified by transfer function analysis of spontaneously occurring oscillations in MAP and MCAv (Zhang *et al.*, 1998). To deduce robust gain and phase estimates, coherence values of less than 0.6 were deemed poor quality and were subsequently omitted from use (average coherence was 0.95 ± 0.07, 0 files were omitted from the analysis). After an initial 1 minute standing baseline, participants completed the squat-stand manoeuvres described in section 3.2.2. CA

data was analysed across the whole cardiac cycle allowing researchers to detect changes in the buffering capacity of BP fluctuations at both extremes (systole and diastole) together with the mean.

5.2.5 Cerebrovascular resistance (CVR)

In order to measure CVR participants completed a breath hold and hyperventilation protocol as outlined in section 3.3.2 & 3.3.3.

5.2.6 Visuomotor performance (PGVP)

Participants sat at desk, facing a laptop screen and completed two precision pinch grip performance tasks in order to measure visuomotor function as described in both section 3.5.1 and replicated in chapter 4.

5.2.7 Neurocognitive function

Participants completed a battery of psychometric tests in a fixed order, designed to assess NF as described in both, section 3.4.

5.2.8 Punch demographics

Boxing bouts were video recorded to measure number of punches landed to the head in the same manner as Chapter 4. The total punch values never differed by more than 5 punches between the two researchers (ICC = 0.998 with 95% confident interval = 0.997-0.999, $p < 0.01$). Additionally, participants wore wrist-mounted, tri-axial punch trackers (Corner Boxing Trackers, Corner Wearables Ltd, Manchester, UK) under their hand-wraps, as outlined in section 3.6.1. The acceleration for each punch which connected to the head (but not body) of the opponent was noted, and summed to provide an estimate of accumulated total acceleration experienced by that opponent.

5.2.9 Statistical analysis

Normality of distribution was confirmed using the Shapiro-Wilk test. The acute effect of amateur boxing on measures of cerebral health was determined using a 3 x 2 (trial by time) (for CA and NF) and 3 x 2 x 5 (trial by time by the 5 BH or HV attempts within

each test – please refer to Figure 3.2 and Figure 3.4) (for CVR outcomes) repeated measures ANOVA. LSD post-hoc analysis was used to identify any significant differences. Statistical significance was established when $p<0.05$. ANOVA main and interaction effects are presented as p values and partial eta squared (η_p^2), interpreted as small (<0.06), moderate ($0.06 < \eta_p^2 < 0.14$) and large (>0.14) effects. The magnitude of post-hoc pairwise comparisons were explored using standardised effect sizes (Cohen's *d*) and interpreted as small (<0.20), moderate (0.20-0.50) and large (>0.50). Pearson's correlation was used to investigate the relationship between the change in cerebral autoregulation indices and punches to the head as well as accumulated acceleration. All statistical analyses were completed using SPSS (IBM Corp, USA, v26.0).

5.3 Results

Baseline participant characteristics are presented in Table 5.1. All participants completed all trials. On average participants had 3 ± 2 years of boxing experience. The number of concussions previously sustained were as follows: 0 concussions = 14, 1 concussion = 3, 2 concussions = 1 and 3 concussions = 1. Participants received an average of 40 ± 16 punches to the head during the boxing trial resulting in an average accumulative acceleration of 290 ± 135 *g*.

Sex	13 ♂ 5 ♀
Age (y)	21 ± 1
Body mass (kg)	76.5 ± 14.6
Stature (m)	1.74 ± 0.09
BMI (kg/m^2)	25.0 ± 2.3
Brachial systolic blood pressure (mmHg)	123 ± 9
Brachial diastolic blood pressure (mmHg)	74 ± 8

Table 5.1: Participant characteristics. Data presented as mean ± SD. BMI = Body mass index.

5.3.1 Resting cerebrovascular values

Resting values are presented in table 5.2. There was no interaction effect for resting MCAv (P = 0.11, η^2 = 0.12), MAP (P = 0.73, η^2 = 0.02) or $P_{ET}CO_2$ (P = 0.18, η^2 = 0.11).

	CON	PAD	BOX
Pre			
MCAv (cm/s)	67.4 ± 14.9	65.2 ± 13.2	65.5 ± 14.7
MAP (mmHg)	79.4 ± 16.8	77.1 ± 13.2	79.8 ± 14.9
$P_{ET}CO_2$ (mmHg)	42.2 ± 3.2	41.9 ± 3.4	41.5 ± 4.6
Post			
MCAv (cm/s)	65.2 ± 13.2	67.7 ± 12.0	68.2 ± 13.3
MAP (mmHg)	76.8 ± 11.2	77.8 ± 15.4	80.5 ± 15.1
$P_{ET}CO_2$ (mmHg)	40.6 ± 6.6	37.8 ± 10.9	42.0 ± 3.6

Table 5.2 Resting physiological values from 1 minute seated baseline. Data presented as mean ± standard deviation. MCAv – middle cerebral artery velocity, MAP – mean arterial pressure, $P_{ET}CO_2$ – partial pressure end-tidal Carbon dioxide.

5.3.2 Cerebral autoregulation

There was no interaction effect for the power spectrum densities for MAP (P = 0.13, η^2 = 0.11) or MCAv (P = 0.07, η^2 = 0.16) during SSM at 0.05 Hz (see table 5.3). No trial by time interaction effects were observed for coherence (P = 0.98, η^2 = 0.01), gain (P = 0.78, η^2 = 0.01), and normalised gain (P = 0.78, η^2 = 0.02) (Figure 5.1). However, there was a significant trial by time interaction (P = 0.05, η^2 = 0.16) for phase. Post-hoc analysis of the interaction revealed a lower phase after boxing (mean difference = -0.13 radians, d = 0.74, P = 0.02), when compared to pre-boxing measures (PRE = 0.75 ± 0.18 vs POST = 0.62 ± 0.17 radians). Further scrutiny identified that phase was significantly lower after boxing when compared to pre-boxing measures in both systolic (Pre 1.33 ± 0.12 vs Post 1.00 ± 0.12 radians, d = 2.75, P = 0.01) and diastolic (Pre 0.648 ± 0.037 vs Post 0.520 ± 0.051 radians, d = 2.87, P = 0.01) portions of the cardiac cycle.

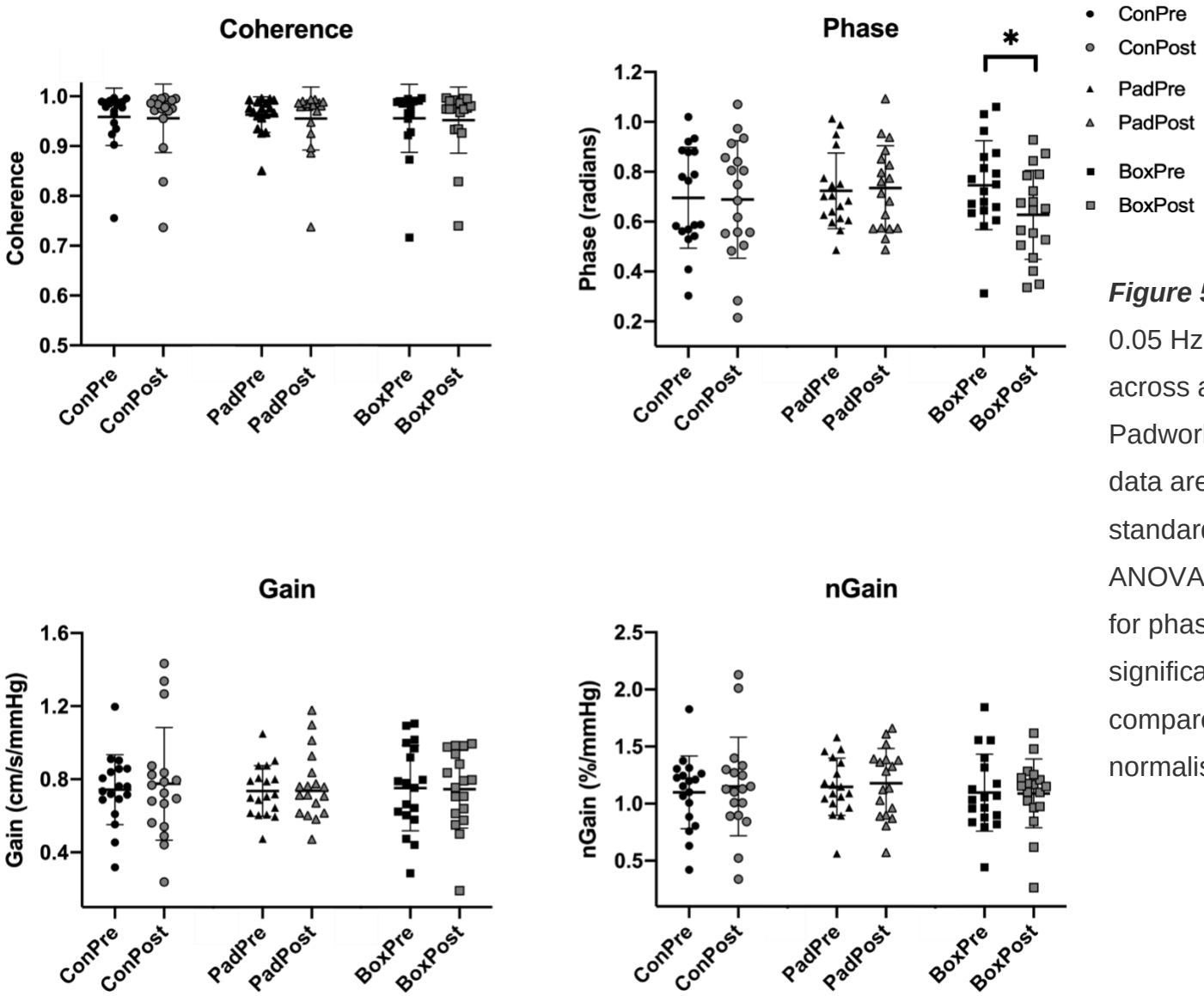


Figure 5.1 Transfer function analysis output from the 0.05 Hz frequency of the squat-stand manoeuvres across all three conditions (Con = Control, Pad = Padwork and Box = Boxing). Individual participant data are plotted with error bars representing the standard deviation and mean. A repeated measures ANOVA revealed a significant time by trial interaction for phase only ($P = 0.05$, $\eta^2 = 0.16$). * indicates a significantly reduced phase after the boxing trial when compared to pre-boxing measures ($P = 0.02$). nGain = normalised gain

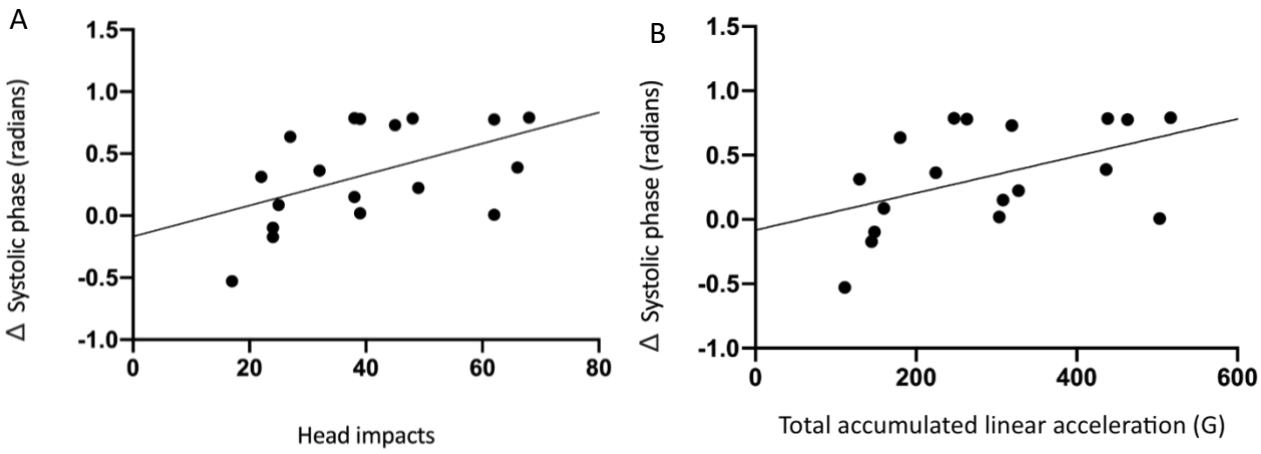


Figure 5.2 (5.2A) Participants Δ systolic phase (pre-systolic phase – post-systolic phase) as a function of total head impacts sustained in the boxing trial (r = 0.50, *P* = 0.03). (5.2B) Participant Δ systolic phase (pre-systolic phase – post-systolic phase) as a function of total accumulated acceleration sustained in the boxing trial (r = 0.48, *P* = 0.04). (Δ= change)

The difference between the change in systolic phase post-boxing (delta phase) was related to the amount (r = 0.50, *P* = 0.03) (Figure 5.2A) and accumulated total linear acceleration (r = 0.48, *P* = 0.04) (Figure 5.2B) of head impacts sustained during the boxing trial. But not for diastolic (r = 0.07, *P = 0.78,* r = 0.15, *P = 0.55)* or mean delta phase (r = 0.16, *P = 0.53,* r = 0.18, *P = 0.47)* when related to head impacts or accumulated total acceleration of head impacts, respectively.

At the 0.10Hz frequency during SSM the MAP (*P* = 0.89, η^2 = 0.01) and MCAv (*P* = 0.16, η^2 = 0.11) power spectrum density revealed no trial by time interaction effect (see table 5.3). No trial by time interaction effects were observed for coherence (*P* = 0.56 η^2 = 0.03), phase (*P* = 0.65, η^2 = 0.02), gain (*P* = 0.29, η^2 = 0.07), or normalised gain (*P* = 0.85, η^2 = 0.01) (Figure 5.3).

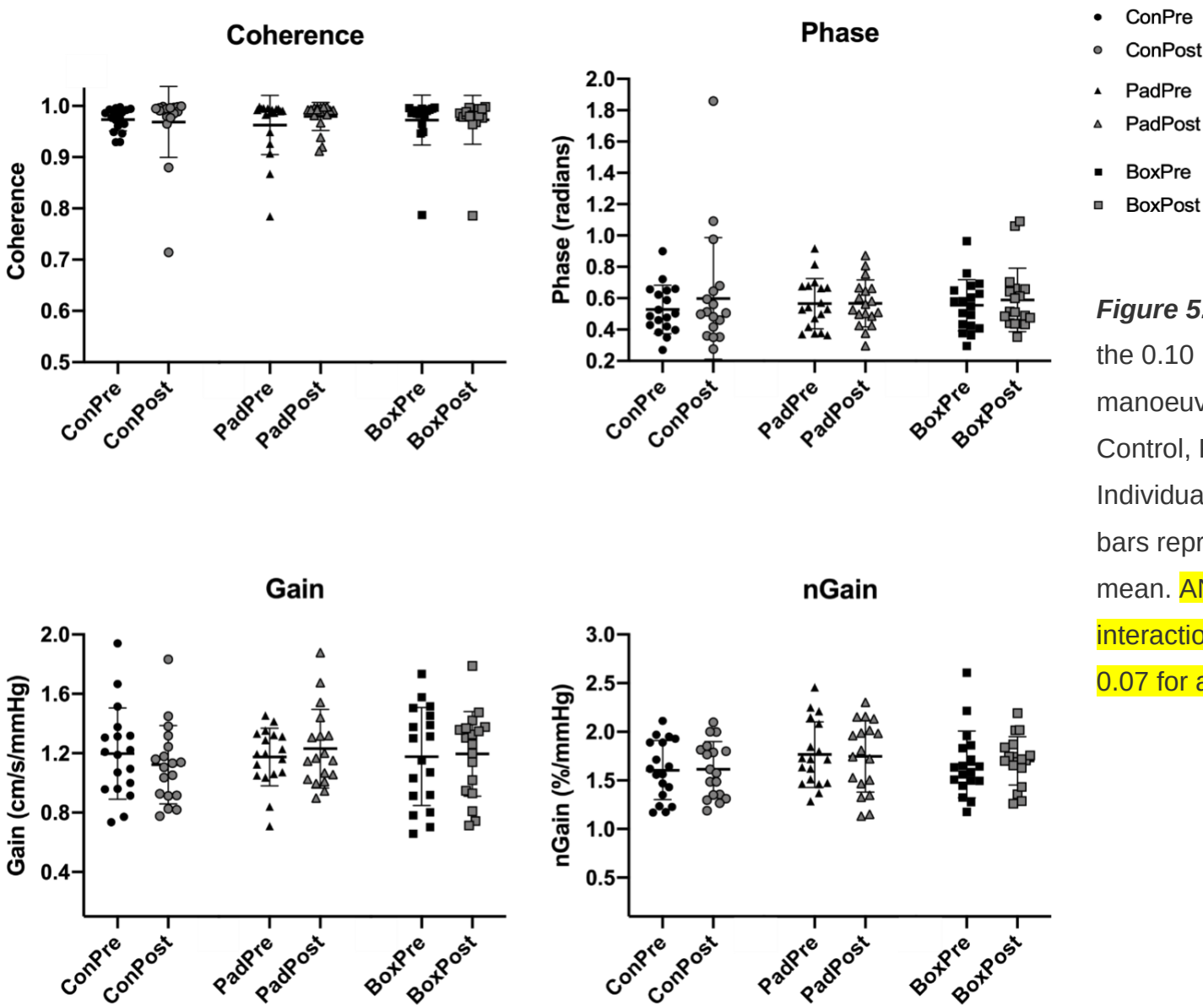


Figure 5.3 Transfer function analysis output from the 0.10 Hz frequency of the squat-stand manoeuvres across all three conditions (Con = Control, Pad = Padwork and Box = Boxing). Individual participant data are plotted with error bars representing the standard deviation and mean. ANOVA analysis revealed no time by trial interaction effect for any CA metric ($P > 0.29$, $\eta^2 < 0.07$ for all). nGain = normalised gain.

	CON		PAD		BOX		*P* value
	Pre	Post	Pre	Post	Pre	Post	
0.05 Hz squat-stand							
MAP PSD $(mmHg)^2/Hz$	38048 ± 21410	31897 ± 20222	39419 ± 23944	35970 ± 23993	34261 ±16861	39020 ± 26370	0.13
MCAv PSD $(cm/s)^2/Hz$	26568 ±11914	18307 ±11914	24075 ± 18889	25962 ± 30277	24048 ± 219991	25520 ± 19335	0.19
0.10 Hz squat-stand							
MAP PSD $(mmHg)^2/Hz$	29218 ± 15117	29428 ± 11836	22888 ± 13172	24871 ± 8547	21452 ± 6369	22263 ± 10035	0.89
MCAv PSD $(cm/s)^2/Hz$	46091 ± 30183	40168 ± 24996	32890 ± 18053	41443 ± 32219	34368 ± 22344	34594 ± 18795	0.16

Table 5.3 Power spectrum densities of oscillations in MAP and MCAV during SSM. Data are displayed as mean ± standard deviation. P = represents alpha values of trial by time interaction effect following ANOVA analysis. Greater power spectral power was observed in the control trial compared to padwork and boxing trials.

5.3.3 Breath-Hold (Hypercapnia)

MCAv data for each BH attempt are presented in Table 5.3. Within each BH assessment of CVR, there was a significant increase in MCAv across the 5 repeated BH attempts ($P < 0.05$), with the peak MCAv value apparent within 5 seconds of the end of each BH attempt. There was no interaction effect for MCAv for each of the 5 BH attempts (trial by time by attempt interaction $P = 0.85$ $\eta^2 = 0.03$).

MAP was not statistically different across the trials after each BH attempt, with no interaction effect being observed ($P = 0.14$, $\eta^2 = 0.14$). MAP after BH attempts averaged 90.6 ± 17.2 and 90.1 ± 15.0 mmHg before and after the control trial, 87.7 ± 15.7 and 90.2 ± 12.3 mmHg before and after the padwork trial and 91.6 ± 16.7 and 95.3 ± 14.2 mmHg, before and after the boxing trial.

Mean $P_{ET}CO_2$ was not statistically different across the trials after each BH attempt, with no observed interaction effect present ($P = 0.53$, $\eta^2 = 0.06$). Mean $P_{ET}CO_2$ after BH attempts averaged 45.3 ± 5.0 and 45.6 ± 4.6 mmHg before and after the control trial, 45.6 ± 5.0 and 45.0 ± 4.7 before and after the padwork trial and 43.7 ± 7.6 and 44.6 ± 4.0 mmHg, before and after the boxing trial.

	CON (%)	PAD (%)	BOX (%)
Pre			
Attempt 1	18.1 ± 12.9	21.1 ± 18.2	13.7 ± 11.4
Attempt 2	19.7 ± 16.2	14.4 ± 14.3	11.2 ± 15.5
Attempt 3	22.7 ± 12.2	21.3 ± 18.4	9.8 ± 13.3
Attempt 4	13.7 ± 14.5	13.9 ± 14.3	14.1 ± 18.8
Attempt 5	17.8 ± 12.3	13.2 ± 13.9	14.3 ± 11.1
Mean	18.4 ± 13.0	16.8 ± 15.2	12.6 ± 13.5
Post			
Attempt 1	13.5 ± 17.3	20.1 ± 8.70	14.5 ± 17.3
Attempt 2	19.5 ± 14.0	8.5 ± 18.2	14.6 ± 17.3
Attempt 3	23.0 ± 14.2	14.5 ± 16.2	13.2 ± 19.0
Attempt 4	11.7 ± 15.5	14.3 ± 13.4	15.1 ± 19.6
Attempt 5	19.9 ± 11.9	10.3 ± 11.2	16.4 ± 18.1
Mean	17.5 ± 14.7	13.5 ± 13.6	14.7 ± 17.1

Table 5.4 Cerebrovascular reactivity to each breath hold challenge, expressed as the percentage change in middle cerebral artery blood velocity above baseline following (~5 s) each breath hold attempt. Data are presented as means ± standard deviations. CON = Control trial, PAD = Padwork trial, BOX = Boxing trial. Repeated measures ANOVA revealed no main effect of trial (P = 0.35, η^2 = 0.08), time (P = 0.60, η^2 = 0.02) or interaction effect (P = 0.85, η^2 = 0.03).

5.3.4. Hyperventilation (Hypocapnia)

Minimum MCAv values during each HV attempt ensued within 10s of the termination of hyperventilation and the resuming of standard breathing. MCAv decreased following each HV attempt (P < 0.05), however there was no interaction effect of trial by time by attempt (P = 0.75, η^2 = 0.03). Data for each HV attempt are presented in Table 5.4.

There was no interaction effect for differences in MAP after each HV attempt (P = 0.30, η^2 = 0.10). MAP after BH attempts averaged 68.2 ± and 71.0 ± 15.0 mmHg

before and after the control trial, 67.9 ± 12.9 and 69.0 ± 12.7 mmHg before and after the padwork trial and 75.8 ± 16.9 and 76.4 ± 18.7 mmHg, before and after the boxing trial.

Mean $P_{ET}CO_2$ was not statistically different across the trials after each HV and there was no interaction effect of trial by timepoint by attempt (P = 0.53, η^2 = 0.08). In the control trial after each HV attempt, $P_{ET}CO_2$ averaged 29.9 ± 5.7 mmHg and 29.5 ± 5.7 mmHg before and after the intervention respectively. Compared to 30.2 ± 5.9 mmHg and 28.8 ± 5.1 before and after the padwork trial and 28.0 ± 5.4 mmHg and 28.1 ± 5.0 pre and post the boxing trial.

	CON (%)	PAD (%)	BOX (%)
Pre			
Attempt 1	-32.0 ± 14.1	-33.4 ± 11.7	-36.1 ± 11.3
Attempt 2	-25.7 ± 9.2	-20.8 ± 25.9	-30.6 ± 10.5
Attempt 3	-25.6 ± 9.1	-14.5 ± 33.3	-26.6 ± 11.9
Attempt 4	-18.0 ± 13.6	-26.8 ± 12.1	-25.3 ± 11.8
Attempt 5	-23.5 ± 7.8	- 26.3 ± 7.7	-23.6 ± 9.4
Mean	-25.0 ± 15.0	-24.4 ± 16.9	-28.4 ± 11.6
Post			
Attempt 1	-32.2 ± 14.8	-30.1 ± 10.5	-31.3 ± 12.3
Attempt 2	-29.4 ± 10.1	-33.4 ± 13.5	-30.1 ± 12.0
Attempt 3	-24.6 ± 15.6	-28.7 ± 12.0	-25.7 ± 10.8
Attempt 4	-23.6 ± 9.9	-28.4 ± 11.1	-24.0 ± 15.1
Attempt 5	-21.0 ± 16.0	-27.2 ± 15.6	-24.5 ± 13.7
Mean	-26.2 ± 12.8	-29.6 ± 12.5	-27.1 ± 11.9

Table 5.5 Cerebrovascular reactivity to each hyperventilation challenge, expressed as the percentage change in middle cerebral artery blood velocity from baseline following each 20 s hyperventilation. Data are means ± standard deviations. Repeated measures ANOVA revealed no main effect of trial (P = 0.94, η^2 <0.01), time (P = 0.52, η^2 = 0.04) or interaction effect (P = 0.75, η^2 = 0.03)

5.3.5 Precision grip visuomotor function (PGVP)

There were no main effects of time or trial or interaction effect in the ‘Full vision or “No vision” trials for RT, percentage OS or RoR from target force during the precision grip tasks. (see table 5.6). There was no effect of trial (*P* = 0.58, η^2 = 0.03), visit (*P* = 0.51, η^2 = 0.02), time (*P* = 0.79, η^2 = 0.03) or interaction effect (*P* = 0.80, η^2 = 0.03) for average force in the ‘Full vision’ trial.

There was no main effect of trial *(P* = 0.74, η^2 = 0.01, visit (*P* = 0.71, η^2 = 0.01) or interaction effect (*P* = 0.10, η^2 = 0.12) on the average force in the ‘No vision’ trial. There was a main effect of time (*P* = 0.002, η^2 = 0.40), with post-hoc pairwise comparisons revealing average force decreased over the ten seconds, all seconds were significantly different to one another (mean difference between timepoint 1 and timepoint 10 = -0.014, *P* = 0.04, η^2 = 0.77, T1 = 0.321, T10 = 0.335.

In the ‘Full vision’ trial, there was a no main effect of trial (*P* = 0.47, η^2 = 0.04), time (*P* = 0.39, η^2 = 0.06), or interaction effect (*P* = 0.83, η^2 = 0.03) observed when analysing RMSE. There was main effect of visit (*P* = 0.002, η^2 = 0.45), with post-hoc pairwise tests describing an increase in performance error post intervention (0.002935) when compared to pre-intervention (0.003605) measures (mean difference = 0.000670, *P* = 0.002, η^2 = 0.45).

In the ‘No vision’ trial there was no main effect of trial (*P* = 0.30, η^2 = 0.07), visit (*P* = 0.76, η^2 = 0.01) or interaction effect (*P* = 0.16, η^2 = 0.10) when analysing the amateur boxers RMSE. Amateur boxers exhibited a main effect of time (*P* = <0.001, η^2 = 0.68), with post-hoc comparison analysis revealing RMSE increased over the ten seconds, all seconds were significantly different (*P* = <0.05) to one another apart from second 9 and 10 (*P* = 0.31).

	CON	PAD	BOX
Reaction Time			
'Full vision'	P = 0.31, η^2 = 0.07	P = 0.16, η^2 = 0.11	P =0.21, η^2 = 0.09
'No vision'	P = 0.97, η^2 = 0.01	P =0.82, η^2 = 0.01	P =0.20, η^2 = 0.09
Rate of Rise			
'Full vision'	P = 0.83, η^2 = 0.01	P = 0.75, η^2 = 0.06	P = 0.93, η^2 = 0.01
'No vision'	P = 0.79, η^2 = 0.01	P = 0.99, η^2 = 0.01	P = 0.98, η^2 = 0.01
Percentage Overshoot			
'Full vision'	P = 0.26, η^2 = 0.08	P = 0.32, η^2 = 0.06	P = 0.21, η^2 = 0.09
'No vision'	P = 0.60, η^2 = 0.03	P = 0.35, η^2 = 0.05	P = 0.30, η^2 = 0.07

Table 5.6 Statistical output from 3 x 2 repeated measures ANOVA of pre- and post-trial visuomotor control metrics. Data presented as *P* values, partial eta squared values.

5.3.6 Neurocognitive Function

Verbal fluency scores increased after each trial compared to pre-trial measures (P <0.01, η^2 = 0.76, respectively), however there was no main effect of trial (P = 0.52, η^2 = 0.03, or trial by time interaction effect (P = 0.08, η^2 = 0.14).

Executive function scores increased after each trial compared to pre-trial measures (P = <0.01, η^2 = 0.56), however there was no main effect of trial (P 0.17, η^2 = 0.10), or trial by time interaction effect (P = 0.88, η^2 = 0.01).

There was a main effect of trial in the delayed memory scores (P <0.01, η^2 = 0.38). In the boxing trial delayed memory scores were significantly lower (mean difference = 2, P = 0.001, η^2 = 0.38) compared to control and padwork (P = 0.003, η^2 = 0.29) (BOX = 6, CON = 8, PAD = 8). There was no interaction effect for orientation, concentration, or immediate memory (P >0.19, η^2 = 0.09).

5.3.7 Balance

A significant effect of trial was observed on balance performance ($P < 0.01$, $\eta^2 = 0.41$), with post-hoc pairwise analysis revealing a significant increase in balance error in the boxing trial (BOX = 3) when compared to control (CON = 1)($P < 0.01$, $\eta^2 = 0.37$) and padwork trials (PAD = 1) ($P < 0.01$, $\eta^2 = 0.29$). However, there was no trial by time interaction effect ($P = 0.19$, $\eta^2 = 0.10$).

5.4 Discussion

The noteworthy findings of this study are that three rounds of boxing altered phase during 0.05 Hz SSM, but no other indices of cerebral autoregulation or cerebrovascular reactivity. No alterations were observed in visuomotor performance, and metrics of cognitive function were not altered by boxing apart from a poorer performance in delayed memory.

The present study found CA was altered, specifically phase during SSM at the 0.05 Hz frequency, after boxing. The 0.05 Hz frequency of SSM indicates that myogenic properties involved in CA may be altered, as opposed to sympathetic properties. With high coherence detected in this study (>0.90) reflecting input-output linearity and alterations in $P_{ET}CO_2$ likely inadequate to change MCAv (Smirl *et al.*, 2015a), the observed alterations suggest a delayed response of changes in CBF in relation to fluctuation in MAP. This extends the narrative of Wright et al (2018) who found a reduced phase 72 hrs after sport-induced concussion when compared to pre-season measures.

An interesting finding from our study is that alterations in phase in the systolic part of the cardiac cycle may be proportional to both the total number of head impacts, and the accumulated acceleration of such impacts. Previous work by Smirl et al (2018) has revealed that both mean and diastolic phase components operate in a similar manner after SSM whereas, systolic components exhibit a greater pressure buffering capacity in healthy controls. This is thought to reflect a protective mechanism against cerebral haemorrhage due to over perfusion. In addition to this, Burma et al (2020) found that systolic phase is altered 4hr after a HIIT workout suggesting a reduced buffering capacity. More pertinent to this study, Wright et al (2018) found that athletes who had suffered an SRC exhibited altered systolic phase at the 0.10 Hz

frequency of SSM. The nature of the head impacts was different, with Wright and colleagues focussing on confirmed concussive events sustained during American Football and Ice Hockey, however the magnitude of the head impacts might be similar. Typical head accelerations of 60 ± 24*g* (Miller *et al.*, 2007) and 27 ± 12*g* (Rousseau, 2014) have been reported in American Football and Ice Hockey, respectively, whilst 58 ± 13*g* has been reported in Olympic boxing (Walilko *et al.,*2005). Accumulated acceleration delivered to the head in the current study over three rounds of amateur boxing averaged 290 ± 135*g* whilst the average acceleration per punch was 7.43 ± 1.14*g*, although it is acknowledged that there is uncertainty regarding how such results are calculated from the triaxial accelerometer used.

It is important to highlight that we used three rounds of boxing in the current study, which is the official length of a competitive amateur boxing bout. However, it may not be reflective of a typical sparring session, which often surpass this length and may occur more than once in a single week. To this end, further research could explore the effects of multiple sparring sessions across the week and how this accumulates throughout a month which may be more representative of typical of both amateur and professional boxing training. It would also be interesting to observe how long phase is altered after boxing, as this was only assessed ~30 min post in the present study. The aforementioned Wright et al (2018) paper identified that alterations in phase are present at 72 hours post-concussion and persisted for 2 weeks, without concomitant changes in other dCA metrics. After 1 month the detriment to phase measures to sport-induced concussion had subsided when compared to preseason measures. Further research is needed to investigate the associated time course of CA alterations observed after sub-concussive blows to head through amateur boxing, and whether there is a period of increased vulnerability during a second sparring session.

The present study found no evidence of alteration in cerebrovascular reactivity following three rounds of amateur boxing. Given that we adopted the same breath-holding and hyperventilation protocols which have been shown to be sensitive to concussion (Len *et al*., 2011), this potentially indicates that cerebrovascular reactivity is acutely unaltered following the sub-concussive impacts sustained in amateur boxing. In contrast, Bailey et al 2013 demonstrated that impairments in CVR are

observed in boxers with a typical competitive history of 13 years, and that this is only related to the total exposure to boxing, rather than technical KOs. This indicates that such impairment following sub-concussive head impacts might be a chronic, rather than acute process. However, we cannot discount that this discrepancy is a function of our CVR methods. Bailey et al 2013 adopted 3 minutes of 5% CO_2: 21% O_2 gas inspiration instead of repetitive breath-holding in order to induce hypercapnia. Whilst the use of hypercapnic stimuli are not without their issues (Burley *et al.*, 2020), our data indicate that the BH protocol failed to increase $P_{ET}CO_2$ in a manner which would be expected with traditional CO_2 breathing challenges. Furthermore, only ~65% of the MCAv response to the BH protocol might relate to elevations in arterial CO_2 (Przybyłowski *et al.*, 2003). Thus, our BH protocol likely failed to isolate the reactivity to a similar CO_2 stimulus. However, CVR to breath holding has been shown to be sensitive to sub-concussive head impacts sustained over the course of a season in female footballers (Svaldi et al. 2017). Additionally, we observed no alterations in CVR following hyperventilation, which did elicit profound changes in $P_{ET}CO_2$ (average change -12.8 ± 8.0 mmHg) and MCAv (average change -24.9 ± 13.7%). Therefore, these data indicate that CVR alterations might not be apparent immediately after three rounds of amateur boxing

Impaired balance has long been referred to as a symptom and consequence of long term participation in boxing, associated with the repetitive concussions and head impacts (Martland, 1928; Riemann & Guskiewicz, 2000). Stiller and researchers found that sparring exposure (frequency & intensity) was associated with altered balance performance in professional boxers (Stiller *et al.*, 2014). Balance was not acutely impaired following boxing in the present study. This is not in line with our observation in Chapter 4, which may suggest that the arousal of competition rather than a no-audience sparring session may impact balance performance, but we cannot discount the nature of the received head impacts might have been different in some way.

Typically, both active and retired boxers, who have sustained concussions exhibit decreased performance in fronto-temporal outcomes such as impairments in memory, information processing and attention (A. H. Roberts, 1969; Johnson, 1969; Neubuerger, Sinton & Denst, 1959; Thomassen *et al.*, 1979). However, many studies continue to report an absence or negligible effects of sub-concussive head impacts

on NF (Mainwaring *et al.*, 2018; Miller *et al.*, 2007; Stojsih *et al.*, 2010; Hart *et al.*, 2017; Moriarity *et al.*, 2004; Belanger, Vanderploeg & McAllister, 2016). In the present study, there were no changes to any NF measures. NF increased after boxing in chapter 4, however this was not replicated in the present study. It is plausible that the absence of any identifiable neurocognitive dysfunction, could be a result of the extent of the sub-concussive trauma induced over 3 rounds, was not great enough to exhibit an acute neuropsychological decline, yet prevented the post-boxing increase observed in Chapter 4. However, it is more likely that such deleterious changes are only detectable following chronic exposure to head impacts. Additionally, the competitive nature of boxing in Chapter 4 (i.e. in the presence of a crowd) was not replicated here, which might explain the lack of improvement in these outcomes.

Visuomotor performance error was not altered following boxing in the present study. Similar isometric hand grip tasks are known to be governed by broadly distributed neural network system, which may be vulnerable to associated neuropathological manifestations of sport-induced concussions (Raikes, Schaefer & Studenka, 2018; Vaillancourt, Mayka & Corcos, 2006). Recently, Raikes and colleagues (2018) added that impaired visuomotor function is indeed associated with individuals who have suffered sport-induced concussions, particularly the ability to regulate force complexity which is also exhibited by neurodegenerative disease populations such as PD (Vaillancourt, Slifkin & Newell, 2001). More specifically, a recent report revealed that amateur boxers present with dampened motor control following a single session of boxing practice (Di Virgilio *et al.*, 2019). Our study was unable to detect such a change, which suggests that such alterations may be reserved to concussion, rather than sub-concussive head impacts. Though the results of the present study do not replicate the previous chapters results conditions (chapter 4) as the boxers in the previous study were under competition pressure and likely had greater nerves, adrenaline and arousal.

In reference to amateur boxers, presently there is no evidence of visuospatial or information processing dysfunction in comparison to age-matched controls (Lesiakowski, Zwierko & Krzepota, 2013; Butler, 1994) and there was no observable acute changes in force decay post-boxing when compared to other trials and visits. Therefore, sub-concussive head impacts sustained during the three round boxing

intervention may not have been substantial enough to distress the visuomotor memory systems governing the visuomotor control performance. Further research is needed to identify whether sub-concussive head impacts through amateur boxing acutely effects visuomotor performance when compared to an age-matched control.

5.5 Limitations

These data further the available literature in this field, and the within-measures design and inclusion of a boxing trial without head impacts (PAD) is a considerable methodological strength. However, there are some limitations in our design which should be acknowledged. Firstly, the inclusion criteria outlined that participant's only had to have at least one year boxing experience. This meant that boxers were not standardised to skill or experience level, but only weight category and sex. One result of this approach is that some of the bouts were resounding victories and other individuals failing to land a collection of punches. Our approach of quantifying the number and accumulated acceleration of head impacts received attempted to mitigate this variation. However, determining the acceleration of the head (rather than the glove of the puncher) would be a superior measure. Furthermore, utilising devices which can quantify the direction of the force (i.e. rotational vs linear acceleration of the skull) would provide further valuable insight (Zhao, Ruan & Ji, 2015; Stemper *et al.*, 2015).

Another shortfall of this study is that it was unable to detect an effect of sex, as only 5 females were recruited (20 ± 1 years, 164 ± 6 cm, 68 ± 8 kg, 25.2 ± 2.5 kg/m^2). These differences are proposed to be determined by disparities in neck stability, as well as having a lower biomechanical threshold for concussion in females (McGroarty, Brown & Mulcahey, 2020). Within boxing, neck strengthening exercises are common to increase neck stability and isometric strength. Research suggests overall neck strength is a significant predictor of concussion in high school athletes, with every 1 pound increase in neck strength results in a 5% decrease in risk of concussion (Collins *et al.*, 2014). Additionally, we know that females may experience greater brain microtrauma than men following sub-concussive head impacts (Rubin *et al.*, 2018). Further research should investigate whether 3 rounds of amateur boxing results in greater alteration to CA in females, when compared to age-matched male amateur boxers.

A shortcoming of using TCD ultrasound to measure CA is that it quantifies the velocity of blood, rather than flow. To deduce CBF from the MCAv one must suppose velocity is equal to flow with the premise that the isonated vessel diameter does not change. Previous literature has found that when $P_{ET}CO_2$ is within 8 mmHg of eucapnia, the diameter of MCA is relatively constant (Ainslie & Hoiland, 2014; Verbree *et al.*, 2014; Coverdale *et al.*, 2014). In the present study, all squat–stand protocols were completed at eucapnia, suggesting the data obtained is an accurate representation of CBF and was not influenced by changes in carbon dioxide.

5.6 Conclusion

Using a multi-discipline approach, this study assessed acute alterations in brain health parameters after three rounds of amateur boxing on university amateur boxers, when compared to a non-head impact high intensity exercise and a seated control trial. This study demonstrates a delayed cerebral autoregulatory response to orthostatic stress without concomitant changes in cerebrovascular reactivity after amateur boxing. We observed no change to balance or any other measures of NF or visuomotor performance after three rounds of boxing. This is the first study of its kind and highlights that alterations in indices of cerebrovascular integrity are possible following sub-concussive head impacts. Further work is needed to quantify the time course of the CA changes, and to see if boxers would be more vulnerable to a second boxing session in the following days (which reflects typical modes of practice).

Chapter 6: Synthesis

The aims of this thesis were as follows:

Thesis aim 1: To explore the acute effect of 3 rounds of amateur boxing on various cerebral health parameters.

Thesis aim 2: If the brain function was impaired, to identify if the magnitude of impairment related to the amount of force sustained/number of punches during the boxing trial.

Thesis aim 3: To explore whether visuomotor performance is impaired alongside alterations in cerebral autoregulation, cerebrovascular reactivity, cognitive function, and balance.

It was hypothesised that a) critical aspects of cerebrovascular function, namely cerebral autoregulation (CA), cerebrovascular reactivity (CVR), neurocognitive function (NF) and balance would be impaired after boxing and b) the magnitude of functional impairment would be related to the extent of head impacts sustained during the boxing trial (sparring). It was hypothesised also that c) boxing would impair PGVP and NF in university amateur boxers.

The data presented in this thesis identify that although boxers presented with impaired PGVP during the visual feedback task after three rounds of competitive boxing in Chapter 4, when compared to control and pad-boxing trial measures these differences were no longer significant (as described in Chapter 5). In addition, amateur boxers present with a delayed CA response to orthostatic stress following three rounds of amateur boxing. Indeed, this altered autoregulatory response was related to the extent (total amount and accumulated acceleration) to which the amateur boxers were exposed to head impacts. This alteration in CA was despite a maintenance of CVR.

These data need to be considered in a wider context; active amateur boxers likely exceed this exposure to head impacts included in this thesis as they may routinely perform multiple rounds of sparring on a weekly basis, throughout a season. In addition, amateur boxing at university is commonly completed in multi-day, multi-fight tournament, meaning individuals could be subjected to over 10 rounds in as little as 3 days. This poses a pertinent question, would multiple sparring sessions completed

in the same week lead to an increase in the magnitude of the effect presented in this thesis, and if there was an alteration present, what would the time-course of this alteration and its recovery look like?

The inclusion criteria for participation within this thesis (chapter 5) was at least 1 year of boxing experience, but this is far from representative of a well-established amateur boxing career. Bailey et al (2013) reported impaired haemodynamic function in professional boxers with an average of 12 years of boxing experience when compared to controls. While the present thesis tested an intervention response to a single bout of boxing and didn't test the participants against an age-related control population, it might would be interesting to determine whether an interaction effect between prior history and these acute responses might exist. In other words, are younger/less experienced boxers at less risk of acute changes than their more experienced peers. Future research could also explore whether the population difference between professional boxers and age- and sex-matched controls (reported by Bailey et al 2013) are also observed in *amateur* boxers with over 10 years' experience.

A further way to develop the data presented in this thesis would be to consider whether any acute post-boxing alterations in CA are altered by specific participant characteristics. Evidence is available which indicates that females may experience greater microtrauma than men following similar head impacts (Rubin *et al.*, 2018) – possibly due to differences in neck strength. This could also be considered in light of weight categories in boxing, given that force is mass x acceleration. Additionally, it is important to consider whether prior experience plays a role in protecting against changes post boxing. A more experienced boxer might presumably receive fewer blows to the head, and have developed greater neck strength. Thus, a less experienced boxer might be more vulnerable. On the other hand, a more experienced boxer might will already boxed throughout such a period of vulnerability. Whether chronic exposure to head impacts moderates the acute response to boxing is unknown. The current study was not designed to explore this, and this idea is worth further study. Any such study would also benefit from the use of validated accelerometers which are able to quantify linear and rotational acceleration of the head – i.e. instrumented mouth guards. We attempted to quantify the head impacts in Chapter 5, albeit by measuring acceleration of the punching fist – rather than that

received at the head. However, these devices were unable to provide the raw, triaxial acceleration output for us to use, and instead provided a simple “punch force” metric. The use of instrumented mouth guards in other sports for head impact/ concussion monitoring has grown in recent years. Given that boxing provides a unique opportunity to understand the acute changes to head impacts, such technology would provide valuable insight for future study.

The acute impairment to visually guided precision grip visuomotor performance presented in chapter 4, suggested the sub-concussive head impacts sustained during an amateur boxing competition compromised the ability to complete a fine motor task. However, when this task was replicated in chapter 5 and compared against both controls and pad-boxing, the impairment was no longer evident. It may be the case that sub-concussive head impacts sustained through three rounds of boxing in a sparring setting without audiences in chapter 5 may not be a substantial enough stimulus to compromise the brains visuomotor ability (Galea *et al.*, 2018; Vaillancourt, Slifkin & Newell, 2001). Future research should look to better assess the magnitude of head impacts experienced in such settings to better shed light on this idea – for example through the use of instrumented mouth guards. Research is also needed to explore whether an extensive career (10+ years) in either amateur or professional boxing may present with similar visuomotor alterations evident in populations with NDs.

There is much further research to be completed before any firm conclusions regarding the relationship between the sport of amateur boxing, sub-concussive head impacts, and cerebrovascular health can be made. Despite this, the findings of this thesis suggest that sub-concussive head impacts may induce an acute window of latency to CBF regulation, specifically during the systolic portion of the cardiac cycle during successive transient fluctuations in blood pressure, which may be reflective of alterations to the myogenic capabilities of the cerebrovasculature. However, it is not known whether these successive periods of systolic vulnerability impact long-term brain function. This is a concern regarding boxer welfare and remains a pertinent area for future research, given the increasing popularity of boxing.

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Appendix 1 – Certificate of ethical approval for study described in chapter 4

College of Life and Environmental Sciences
SPORT AND HEALTH SCIENCES

St. Luke's Campus
University of Exeter
Heavitree Road
Exeter
EX1 2LU
United Kingdom

Certificate of Ethical Approval

Proposal Ref No: 2019/M/24

Title: The acute influence of boxing on fine-motor control and cognitive function in university amateur boxers

Applicants: William Wallis, Dr Bert Bond Dr Genevieve Williams Hamish Lorimer Sanya Manglani

The proposal was reviewed by a Representative on the Committee.

Decision: This proposal has been approved until 12/06/2020

Signature:  Date: 06/12/2019

Name of Ethics Committee Reviewer: Melvyn Hillsdon

Your attention is drawn to the attached paper which reminds the researcher of information that needs to be observed when Ethics Committee approval is given.

Appendix 2 – Informed consent form for study described in chapter 4

Participant Identification Number:

CONSENT FORM

Title of Project: The acute influence of boxing on fine-motor control and cognitive function in university amateur boxers

Name of Researcher: Will Wallis

1. I confirm that I have read the information sheet dated 21/11/19 (v1.0) for the above project. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily ☐
2. I understand that my participation is voluntary and that I am free to withdraw at any time without giving any reason and without my legal rights being affected. ☐
3. I understand that relevant sections of the data collected during the study, may be looked at by members of the research team, individuals from the University of Exeter, where it is relevant to my taking part in this research ☐
4. I understand that taking part involves anonymous video data collection ☐
5. I understand that the video footage will be stored in coded form, and that nobody outside of the research team will be able to access this footage without my express consent. ☐
6. I give permission for these individuals to have access to my records. ☐
7. I understand taking part involves answering a questionnaire ☐
8. I understand that I will take part in a visual search task (Where's Wally?) ☐
9. I understand that I will complete a finger-pinch task led by the researcher ☐
10. I understand that my data will be used for the purposes of an academic project: reports published in an academic publication and grant proposal, media publication, teaching or training materials for use in University activities or public engagement activities. Data will be kept for future analysis ☐
11. I agree to take part in the above study ☐

Name of Participant	Date	Signature
Name of researcher	Date	Signature

Appendix 3 – Participant information sheet for study described in chapter 4

Version 1.0 21/11/2019

Participant Information Sheet

Title of Project: The acute influence of boxing on fine-motor control and cognitive function in university amateur boxers.

Lead Researcher names: Will Wallis

Invitation and brief summary:
Thank you for taking interest in participating in this study. This research is interested in your movement control and cognitive processing before and after suspected concussion.

Purpose of the research:
To determine if your ability to squeeze an object with a specific force with and without visual feedback are linked to symptoms of concussion.

Why have I been approached? You have been invited to this study as you are part of the university of Exeter amateur boxing club. You also participate in activities which might make you susceptible to concussion.

What would taking part involve?
You will be asked to sit in a chair in front of a laptop screen. You will be asked to pick up a force measuring device between your thumb and little finger and squeeze it as hard as you can three times. You will then be challenged to match the force that you create to a target on screen, during a number of repetitions. Sometimes we will remove your ability to see the target force.

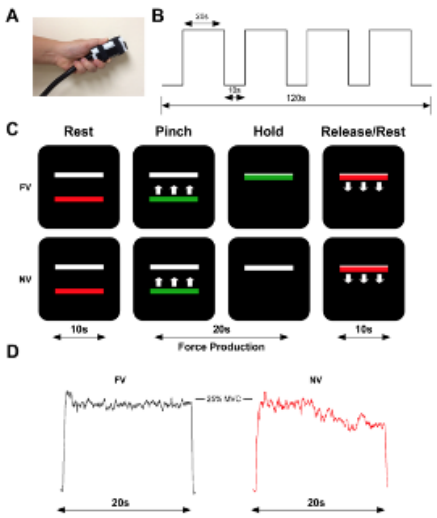

Figure 1. The figure shows **A** The pinch grip dynamometer. The dynamometer is designed to be gripped with the thumb and first finger. **B** shows a typical protocol for pressing the device, where the participant will press for 20 seconds, with a 10 second rest, and repeat this a number of times. **C** shows the interface that guides the participant to press. The white bar is the target force to reach. When the moveable bar is red, the participant rests, when it goes green the task is to match the green bar to the white bar and hold it there. We have a Full Vision (FV) and No Vision (NV) condition where vision is removed and the participant uses proprioception and short term memory to maintain the force (Vaillencourt et al., 2006). A typical data output for FV and NV are shown in **D.** Our metrics quantify key characteristics of these traces.

You will complete a suspected concussion assessment questionnaire which will entail short tasks designed to test a variety of measures. For example, short term memory and word production and quick thinking

What are the possible benefits of taking part?
This study will provide data to support a study exploring whether this pinch-grip task will highlight neurological deficits associated with concussions.

What are the possible disadvantages and risks of taking part?
There are no expected disadvantages of participating in this project. You will take part in your routine boxing session as normal. You are free to withdraw without consequence at any time.

What will happen if I don't want to carry on with the study?
All participants have the right to withdraw from the study at any point without giving any reason. If you want to withdraw from the study at any point please contact wegw201@exeter.ac.uk. If you withdraw from the study, no further data will be collected. However, any previous data will be anonymous and therefore, will not be destroyed by the researcher.

How will my information be kept confidential?
The University of Exeter processes personal data for the purposes of carrying out research in the public interest. The University will endeavour to be transparent about its processing of your personal data and this information sheet should provide a clear explanation of this. If you do have any queries about the University's processing of your personal information may be obtained from the University's Data Protection Officer by emailing dataprotection@exeter.ac.uk or at www.exeter.ac.uk/dataprotection

All data collected will be collected and then stored confidentially and anonymously with each participant being referred to as a number. All data will be collected and stored on a password protected laptop. You will remain anonymous in any reports and publications.

Will I receive any payment for taking part?
There will be no payment for taking part in this study.

What will happen to the results of the study?
The results of this study will be disseminated through a written thesis as part of my Masters assessments.

The information could be presented to the Exeter University Amateur Boxing Club in the following season, and may be disseminated to a scientific audience via publications and conference presentations.

Who has reviewed this study?
This project has been reviewed by the Sport and Health Sciences Research Ethics Committee at the University of Exeter.

Further information and contact details:
If you would like to participate in the study, please read the consent form and complete it.

Thank you for your interest in this project.

Will Wallis

wegw201@exeter.ac.uk

Appendix 4 – Certificate of ethical approval for study described in chapter 5

College of Life and Environmental Sciences
SPORT AND HEALTH SCIENCES

St. Luke's Campus
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Heavitree Road
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EX1 2LU
United Kingdom

Certificate of Ethical Approval

Proposal Ref No: 2019/M/23

Title: Does boxing acutely impair parameters of cerebral health in amateur boxers?

Applicants: Will Wallis, Ollie Smail Dr Genevieve Williams Dr Gavin Buckingham Rosie Andre Hamish Lorimer Sanya Manglani

The proposal was reviewed by a Representative on the Committee.

Decision: This proposal has been approved until 01/12/2020

Signature: Date: 21/11/2019

Name/Title of Ethics Committee Reviewer: Francis Stephens

Your attention is drawn to the attached paper which reminds the researcher of information that needs to be observed when Ethics Committee approval is given.

Appendix 5 – Informed consent form for study described in chapter 5

Participant Identification Number:

CONSENT FORM

Title of Project: **Does boxing acutely impair parameters of cerebral health in amateur boxers?**

Name of Researcher: William Wallis

Please initial box

1. I confirm that I have read the information sheet dated 06/11/19 (v1.0) for the above project. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily.

2. I understand that my participation is voluntary and that I am free to withdraw at any time without giving any reason and without my legal rights being affected.

3. I understand that relevant sections of the data collected during the study may be looked at by members of the research team, individuals from the University of Exeter, where it is relevant to my taking part in this research. I give permission for these individuals to have access to my records.

4. I understand that taking part involves an ultrasound probe will being positioned at the side of my head and I will be asked to squat and stand multiple times.

5. I understand that taking part involves taking part in 20 second breath hold tests.

6. I understand that taking part involves participating in 3 rounds of boxing.

7. I understand that taking part involves completing 20 second hyperventilation tests.

8. I understand that taking part involves a finger grip force transducer test.

9. Computer-based reading tests for neurovascular coupling and tasks to assess cognitive function.

10. I consent for my boxing condition to be video recorded for punch analysis

11. I understand that I will wear punch trackers and an accelerometer under my gloves during the boxing trial

12. I understand that taking part involves wearing an Actiheart ECG monitor + GENEActiv accelerometer overnight

13. I understand that my data will be used for the purposes of:

Written thesis and inclusion in an archive for a period of up to *10* years ☐

Shared with other researchers for use in future research projects

Potential publication in an academic publication

Teaching or training materials for use in University activities

14. I agree to take part in the above study. ☐

Name of Participant	Date	Signature
Name of researcher taking consent	Date	Signature

Appendix 6 – Participant information sheet for study described in chapter 5

Participant Information Sheet
(06/11/19) **V1.0**

Title of Project: Does boxing acutely impair parameters of cerebral health in amateur boxers?

Researcher name: William Wallis

Invitation and brief summary:
Thank you for showing an interest in our study. Here is some information regarding the nature of our investigation. A member of our research team will run through this sheet with you and answer any further questions. Please do ask the researcher for clarity if you're unsure or ask any questions you may have.

Purpose of the research:
Our goal is to build on the knowledge we acquired last year continue to discover information surrounding the acute response of some potentially important markers of brain health after a boxing session. Cerebral blood vessel function has been shown to be impaired in experienced boxers who have trained for at least 13 years. This impairment is interesting because evidence suggests it increases the risk of future brain disorders. We want to test whether impaired markers of brain health namely cerebrovascular autoregulation, cerebrovascular reactivity and cognitive function are seen after acute periods of boxing.

Why have I been approached?
As the study is looking specifically at head impacts we have approached you via the University of Exeter boxing club. For participant safety, we also require that our participants have **at least one year of boxing experience.**

What would taking part involve?
Participation in this study will involve completing one preliminary visit followed by three visits to non-invasively assess some measures of brain health pre and post in a counterbalanced order.
1. Padwork
2. Boxing
3. Control

The boxing trial will follow the same format which you will be used to during competition and training: 3 rounds of 3 minutes, each separated by 1 minute of recovery. A scorecard of the bout will be kept. In order to validate the amount of punches you have sustained during the rounds the boxing bout will be recorded on video cameras. In addition, you will wear
punch trackers (PIQ) + accelerometer in your wraps to gain a
metric of the force of punches delivered to the head.
The padwork trial will match these timings, but instead of
boxing an opponent, you will be landing punches on their
padded hands.
In the control trial you will remain seated for the same duration
of the 3 rounds and intervals.
This allows us to compare between boxing and receiving
blows to the head (using the scorecard) on our markers of brain health and will take place on St Luke's campus during your normal training sessions.
Following the testing on St Luke's, you will then wear a chest-worn heart-rate sensor overnight and the same accelerometer in order to generate a measure of heart rate variability and sleep quantity and quality. The following morning you will meet with a researcher to return the sensor and you will perform a delayed recall shopping list task.

Preliminary visit (approximately 45 minutes):
- Familiarisation of study procedures, collection of baseline characteristics (height, body mass, boxing history, age and concussion history) and collection of baseline cognitive function

- Cognitive function is assessed using a series of short manual tasks, balance tasks and word recall.

Testing visits (each approximately 45 minutes pre boxing/padwork/control, and 65 minutes afterwards):

Collection of pre boxing/padwork cerebral health parameters: cerebral autoregulation cerebrovascular reactivity, neurovascular coupling, cognitive function, pinch-grip-force-transducer:

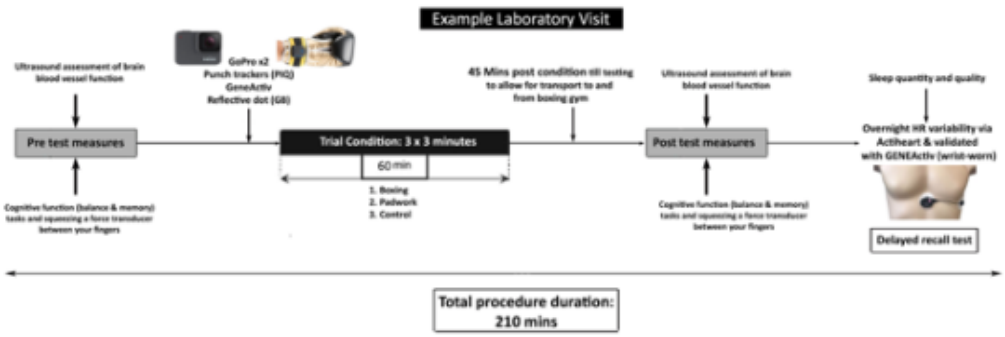
All measures used are via non-invasive means of ultrasound as seen on the diagram below. Cerebral autoregulation will be assessed using two 5-minute squat-stand protocols. Cerebral blood velocity will be assessed using transcranial Doppler ultrasound which is an ultrasound probe that will be placed on your temple, and beat to beat blood pressure will be measured by a monitor that sits over the finger. Cerebrovascular reactivity will be measured following 5, 20 second breath holds while lying down followed by 40 seconds of rest (normal breathing). After 2 mins of recovery (normal breathing), you will perform 20 seconds hyperventilation at 36 breaths per minute. After 40 s of recovery, this hyperventilation protocol will be repeated 5 times.

Neurovascular coupling will be determined by looking at the changes in cerebral blood flow velocity in the posterior cerebral artery in response to 5 cycles of 40 seconds of a visual search task and 20 seconds of eyes closed. This will also be measured using the transcranial Doppler setup.
In addition, you will take part in a finger grip force transducer task where you will be asked to squeeze a handheld device to maintain a constant pressure that will be shown on the screen for 20 seconds, rest for 10 seconds and repeat 3 times. This will be completed once with sight and once without.

After your boxing/padwork session you will return to the laboratory and the tests described above will be repeated. We will also test for cognitive function. Finally, you will be asked to wear an ECG monitor which sticks to your sternum to record your heart rate overnight. In addition, you will be asked to wear a wrist-worn accelerometer in order to give us data on sleep quality and quantity. A researcher will organise to meet up the following morning to retrieve this monitor from you and complete a single delayed recall (cognitive function task).

This protocol will be repeated during a second and a third and final visit pre and post boxing/padwork/control – so that you complete all three trials as a participant.

The testing protocol is represented in the diagram below.



What are the possible benefits of taking part?

By taking part you will be able to retrieve detailed personal data on your punch demographics. Using the modern punch tracking devices, we can harvest speed, power and intensity data.
This study will allow us to better understand the repercussions of boxing training over an acute time period on our brain health.

What are the possible disadvantages and risks of taking part?

University of Exeter amateur boxing club is part of the England boxing, the level 2 coach who is qualified to supervise the boxing sessions due to his first aid training will be supervising the boxing session. Outside of the risks of boxing, the study poses minimal risks.

What will happen if I don't want to carry on with the study?
If at any point you wish for data collection to stop or to withdraw from the study, then it will stop immediately without having provided any reason. Participation is NOT compulsory, however before you commit we do ask that it should be considered carefully

How will my information be kept confidential?
The University of Exeter processes personal data for the purposes of carrying out research in the public interest. The University will endeavour to be transparent about its processing of your personal data and this information sheet should provide a clear explanation of this. If you do have any queries about the University's processing of your personal data that cannot be resolved by the research team, further information may be obtained from the University's Data Protection Officer by emailing dataprotection@exeter.ac.uk or at www.exeter.ac.uk/dataprotection

Research data stored in paper form, such as your age, weight, height, address and medical information will be stored in a locked filling cabinet. You will be assigned a unique study ID and experimental data will be stored using this unique ID number on a password protected computer for analysis purposes. Data will only be accessed by the members of the research team. Research data will be retained intact for a period of ten years from the date of collection. Should you withdraw from the study, your research data will be destroyed, and subsequently your data will not be included in any publications. All data will remain completely confidential at all times.

What will happen to the results of this study?
Individuals who take part in this study will receive a report of the study's findings, as well as their own individual data should they ask for it. The data will be disseminated in a written dissertation and poster presentation as part of my dissertation assessments.

Who is organising and funding this study?
Physiology research team consisting of Head supervisor Dr Bert Bond, Co-supervisor Dr Genevieve Williams and MRes student William Wallis.

This project has been reviewed by the Sport and Health Science Research Ethics Committee at the University of Exeter (Reference Number ……).

Further information and contact details
If you would like some further information, please contact Will Wallis so we can arrange a meeting. If you do wish to take part in this study, you will be asked to complete an informed consent form to confirm that you understand and are happy to participate in this study.

For Study related enquiries please contact Will Wallis by email: wegw201@exeter.ac.uk

If you would like to raise a concern or complain about the study, you can write to Gail Seymour who will investigate your concern.

Research Ethics and Governance Manager
g.m.seymour@exeter.ac.uk, 01392 726621

Thank you for your interest in this project