Physiological and Behavioural Consequences of Network Breakdown in Brain Injury

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Declaration

I, Michelle Shereen Balaratnam confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.

Signed……..

Abstract

Traumatic brain injury (TBI) is a major public health problem with a huge unmet need for effective long-term care. Advances in MRI technology using diffusion tensor imaging (DTI) have demonstrated structural abnormalities in patients with TBI, often not seen on conventional brain imaging. The structural and neuropsychological consequences are described in existing research. The aim of this thesis is to identify whether there are physiological and behavioural consequences of TBI, which may be contributing to the observed problems in daily activities associated with this condition. This will help to understand the devastating functional impact following TBI, and its neurorehabilitation needs.

This thesis initially develops a study protocol to investigate the physiology in TBI. Initial work explores physiology in thirty four healthy individuals using transcranial magnetic stimulation (TMS) to produce a study protocol that can be used in the patient group. This examined a selection of pathways, including the assessment of callosal physiology using a twin coil TMS method to assess for interhemispheric inhibition. This protocol was used to assess seventeen TBI patients, and compared to healthy controls, and demonstrated that callosal transfer is physiologically different between the two groups. The behavioural consequences of callosal transfer were then explored through the development of a bimanual tapping task in twenty nine healthy participants. The behavioural consequences were then assessed in the same group of TBI patients, and compared to the control group. The TBI patients had comparable mean performance. However, the variability in performance was the main difference between the two groups. The MRI DTI metrics were then investigated in the TBI and control groups. A relationship between the physiology, behaviour and microstructure was then explored. Through this series of investigations this thesis hopes to increase existing understanding of the consequences of brain injury.

Impact Statement

Traumatic brain injury (TBI), is a major public health problem with a huge unmet need for effective long-term care. Despite TBI predicted to be carrying a similar public health burden to stroke in terms of long term care, there is a discrepancy in the level of support for TBI sufferers and stroke sufferers. At present, the extent of the consequences of TBI is underestimated by standard clinical assessments, which may also contribute to this problem. Public awareness of TBI has increased through studies suggesting that TBI in athletes may have effects on neurocognitive function. The structural and neuropsychological consequences following TBI are already described in existing neuroimaging research. Diffusion tensor imaging measures have already been shown to be related to known consequences of TBI, including information processing speed, working memory and motor skills. However, the neurorehabilitation needs following TBI are more extensive than cognitive impairment, and still poorly understood. This thesis evaluates the physiological and behavioural consequences of traumatic brain injury. These areas are less well studied in the TBI literature.

This research is of particular importance to TBI research. Firstly, existing physiological work in TBI has included patients on neuromodulatory medications for mood disturbance or epilepsy. In contrast to other studies, the patients in this study were not on any neuromodulatory medication at the time of testing. Therefore, the physiological findings of abnormal callosal transfer is relevant, and may reflect the nature of the acceleration deceleration shearing injury described to preferentially affect the fibres across the corpus callosum in TBI. The behavioural findings of increased variability could reflect the physical recovery many patients appear to make following TBI, where performance on the whole is comparable to the general population. However, the observed behavioural findings in the TBI group demonstrate that sustained performance is difficult to maintain, resulting in the observed variability of performance.

The findings from these investigations are particularly important as these are chronic TBI patients, and had sustained their injury at least one year prior to testing. In addition, there were no objective abnormalities on clinical assessment at the time of their participation. This demonstrates the complexity of the functional problems encountered after TBI. The differences observed between the patient and healthy control groups provides valuable insight into the difficulty returning to normal physical function following TBI. These studies therefore provide insight into the persistent functional problems following injury. This can therefore be used to develop strategies to address neurorehabilitation needs following TBI.

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Abbreviations

AMT Active motor threshold

ANOVA Analysis of variance

AD Axial diffusivity

CC Corpus callosum

CMAP Compound motor action potential

CS Conditioning stimulus

CT Computed tomography

DTI Diffusion tensor imaging

EMG Electomyography

FA Fractional anisotropy

FDIO First dorsal interosseous

fMRI Functional magnetic resonance imaging

GABA Gamma-aminobutyric acid

IHI Interhemispheric inhibition

IQ Intelligence quotient

ISI Interstimulus interval

LCT Left corticospinal tract

M1	Primary motor cortex
MD	Mean diffusivity
MEP	Motor evoked potential
MRC	Medical research council
MRI	Magnetic resonance imaging
ms	milliseconds
MSO	Maximum stimulator output
mV	millivolt
PTA	Post traumatic amnesia
RC	Recruitment curve
RCT	Right corticospinal tract
RD	Radial diffusivity
RMT	Resting motor threshold
RT	Reaction time
rTMS	Repetitive transcranial magnetic stimulation
SD	Standard deviation
SE	Standard error
SICI	Short intracortical inhibition
TBSS	Tract-Based Spatial Statistics
TS	Test stimulus
TMS	Transcranial magnetic stimulation
UK	United Kingdom

Chapter 1

Introduction

1.1 Introduction to Traumatic brain injury

Traumatic brain injury is defined as, “a traumatically induced injury that contributed to the physiological disruption of brain function” (Malec et al., 2007). It is a public health challenge of vast, but insufficiently recognised proportions (Maas et al., 2017). Head trauma is a leading cause of death and long term disability in young people in the developed world (Thurman et al., 1999, NICE, 2014). More than 50 million people have a traumatic brain injury each year (Maas et al., 2017). It is a major public health problem, with a huge unmet need for effective long-term care (Thurman et al., 1999). The prevalence of stroke and traumatic brain injury is comparable, yet there is less recognition for traumatic brain injury sufferers, and far less research in traumatic brain injury than for stroke (Li et al., 2014). Unlike stroke, traumatic brain injury also predominantly affects the young population aged 15-24 years, and many survivors have a near normal life expectancy (Jennett, 1998, Thornhill et al., 2000). At present, the extent of the consequences of traumatic brain injury are likely to be underestimated by standard clinical assessments, which may contribute to this problem.

Public awareness of traumatic brain injury has increased through studies suggesting that traumatic brain injury in athletes may result in cognitive impairment (De Beaumont et al., 2009). However, prior to this public awareness, cognitive and neuropsychiatric impairment following traumatic brain injury was already recognised (Whitnall et al., 2006). It is believed that cognitive impairment following traumatic brain injury is due to the breakdown of connections between the remote brain regions that determine the cognitive interactions (Mesulam, 1990). Advances in MRI technology have led to the development of diffusion tensor imaging. This has already demonstrated structural abnormalities in patients with traumatic brain injury, often not seen on conventional brain imaging (Mayer et al., 2010, Sharp et al., 2011a). Diffusion tensor imaging measures have already been shown to correlate with known consequences of traumatic brain injury, including poor information processing speed (Niogi et al., 2008), poor working memory (Palacios et al., 2012) and impaired motor skills (Farbota et al., 2012, Leunissen et al., 2013). This has added to the current appreciation that traumatic brain injury is associated with disruption of brain networks, rather than just a consequence of

focal areas of damage (Smith et al., 2003, Sidaros et al., 2008). The structural and neuropsychological consequences following traumatic brain injury are already described in existing neuroimaging research. However, the neurorehabilitation needs are likely to be more extensive and are still poorly understood. Physical difficulties usually relate to clumsiness, uncoordinated muscle contraction, ataxia and imbalance, rather than hemiparesis as encountered in stroke (Greenwood, 2002, Greenwood et al., 2016).

This thesis evaluates the physiological and behavioural consequences of network breakdown that is believed to occur following traumatic brain injury. These areas are less well studied in the traumatic brain injury literature, but improved understanding of this areas will increase insight into the devastating functional impact following injury. This would develop current understanding of neurorehabilitation needs following injury.

1.1.1 Epidemiology

1.4 million people each year attend emergency departments in England and Wales with a recent head injury (NICE, 2014). In the UK, acute brain injury admissions have been found to account for 320,900 bed days in hospitals in England. Although this has only been found to represent 0.64% of all NHS bed days (Department of Health, 2001 a & b), in 2007 the National Institute for Clinical Excellence (NICE) initially estimated that the acute hospital care costs for traumatic brain injury was approximately £1billion annually (NICE, 2007). However, by 2010 the cost of acute hospital care for traumatic brain injury in the United Kingdom increased to £4.1 billion annually (Gustavsson et al., 2011). More recently, it has been estimated that traumatic brain injury costs the global economy approximately 400 billion US dollars annually (Mass et al., 2017). In comparison, the direct cost of acute hospital care in stroke has been estimated as £2.8 billion annually, and informal care, loss of productivity and disability costs costing an extra £4.2 billion (NICE, 2010). It is estimated that up to 1.3 million people in the UK are living with a disability related to traumatic brain injury (Parsonage, 2016).

1.1.2 Causes of Traumatic Brain Injury

Traumatic brain injury is commonly caused by road traffic accidents (RTA), sports injuries, falls, assaults and blast injuries in the military (Butcher et al., 2007, Maas et al., 2008, Faul et al., 2010, Mac Donald et al., 2017). Earlier data from the UK suggested that 70-88% of all people that sustain a traumatic brain injury are male, 10-19% are 65 years and older and 40-50% are children (Jennett, 1998). It is now well recognised that the number of sufferers of traumatic brain injury continues to increase each year. Falls (22-43%) and assaults (30-50%) are the most common cause of a minor head injury in the UK, followed by road traffic accidents (around 25%) (Department of health, 2001a &b). However, the number of elderly people with traumatic brain injury is increasing due to falls (Maas et al., 2017). In the United States approximately 75% of traumatic brain injuries are attributable to mild or concussive events (CDC report, 2003). Road traffic accidents account for a far greater proportion of moderate to severe head injuries. Alcohol has been found to be involved in up to 65% of adult head injuries. It is acknowledged that there are marked regional variations, especially in terms of the head injuries sustained through assault and the involvement of alcohol, but the incidence of penetrating head trauma remains less common (Swann et al., 1981, Vakil et al., 2017).

A brain injury is suspected when there is a period of loss of, or a decreased level of consciousness, any loss of memory for events immediately before or after the head injury, alteration in mental state at the time of the injury (confusion, disorientation, slowed thinking), or neurological deficits (weakness, loss of balance, change in vision, praxis, paresis/plegia, sensory loss, aphasia) that may or may not be transient (NICE, 2014). Formal guidelines for an urgent scan within one hour include Glasgow Coma Score less than thirteen on arrival to the emergency department, less than fifteen two hours after the injury, or if there is a suspected open or depressed skull fracture, post traumatic seizures, focal neurological deficit or more than one episode of vomiting (NICE, 2014). Otherwise, guidelines state that a scan needs to be undertaken within eight hours following injury if the patient is on anti-coagulation.

1.1.3 Consequences of traumatic brain injury

Variation in the clinical manifestations of traumatic brain injury are attributed to the heterogeneity of injury, the pattern and extent of damage (Maas et al., 2017). Although the incidence of traumatic brain injury is high, the incidence of death from head injury is comparatively low. The incidence of death due to traumatic brain injury has been estimated to be 6-10 per 100,000 per annum (Department of Health, 2001). With half of all traumatic brain injury events occurring in people of working age, it is likely that loss of productivity will be greater than in stroke.

Traumatic brain injury has become a topic of public interest over recent years in view of the public burden of the disease in members of the population who are often young and previously well. Because most survivors are young, the burden on public health and social care is thought to be substantial (Sharp, 2008). The pathological features, symptoms and course of traumatic brain injury can be widely different depending on the cause, type or severity of the injury sustained. It has been predicted that traumatic brain injury will become the third leading cause of death and disability in the world by 2020 (Murray et al., 1997). The World Health Organisation (WHO) has therefore identified brain injury, in particular traumatic brain injury as a major public health problem with a huge unmet need for effective long-term treatment (Thurman et al., 1999).

1.1.4 Discrepancy in support for traumatic brain injury sufferers

Despite traumatic brain injury predicted to be carrying a similar public health burden to stroke in terms of long term care, there is a discrepancy in the level of support for traumatic brain injury sufferers and stroke sufferers. Part of the problem may be that conventional methods of assessment, including imaging, do not necessarily visualise structural damage present in the context of traumatic brain injury. In addition, focal structural injury seen on conventional diagnostic imaging does not predict functional outcome after traumatic brain injury, as it is more likely to do in stroke. It has therefore been more difficult to assess or predict the functional implications of traumatic brain injury based on standard assessments. A paucity of traumatic brain injury research has also been highlighted in an observational, cross-sectional study of

all clinical studies in traumatic brain injury and stroke (Li et al., 2014). Li and colleagues showed that the similarity in public health burden in stroke and traumatic brain injury was not reflected in the amount of translational research and evidence based practice available for traumatic brain injury, compared to stroke (Li et al., 2014). It is possible that this observation simply reflects a less overall understanding of traumatic brain injury, where the heterogeneity of disease spectrum in traumatic brain injury would make testing a clinical hypothesis difficult. However, with increasing awareness of the public health burden of traumatic brain injury, this should motivate more research into the understanding and treatment of this condition.

1.2 Historical interest in traumatic brain injury

The recognition of consequences following a head injury is not new. However, in the absence of structural representation of the damage on conventional brain imaging, it has previously been difficult to conclude that the observed consequences have an organic basis. In the past, what was understood about penetrating and non-penetrating traumatic brain injury came from clinical assessment, surgical findings during operative intervention and post mortem analysis of the deceased. Work by the surgeon Pott in the 1700s led to the understanding that the consequences of brain injury arose from injury to the brain tissue. This contradicted the views of the time that consequences from a brain injury required visible structural damage to have occurred, such as fracture of the skull (Jennett, 1975).

The consequences of brain injury can be catastrophic. In the history of medicine, one of the best known cases describing such consequences following a penetrating traumatic brain injury was the case of Phineas Gage in 1848. This gentleman sustained a penetrating traumatic brain injury after a three foot pointed rod penetrated his skull during his work as a railway construction foreman. Prior to the incident he was described to be a mild-mannered, conscientious man. However, after the injury he was noted to have marked personality and antisocial behavioural problems which persisted until his death (Butler, 2004). This was particularly relevant as he otherwise survived the incident with no residual motor, sensory or speech deficit. His clinical case encouraged discussion on cerebral localisation at the time,

and not the consequences of penetrating traumatic brain injury. At the time of Gage's injury, the phrenologists of the day proposed the "organs of benevolence and veneration" had been destroyed. However, this was shunned by orthodox medicine at the time. It was only after further debate that his body was exhumed and studied later in the 19th century. It was then that John Harlow attributed the changes to Gage's character following the injury to selective brain damage. It was this further analysis that recognised that the cerebral regions affected by the injury spared the movement and language areas of the brain (Butler, 2004). Devastating consequences following historical non-penetrating brain injury are less famous in the existing literature. However, the term concussion or *commotio cerebri* has been used to describe the effects of injuries to the brain without fracture of the skull since the sixteenth century (Denny – Brown et al., 1941). Gama was reported to state, "fibres as delicate as those of which the organ of mind is composed are liable to break as a result of violence to the head" in 1835. Gama's studies involved a brain model for concussion, consisting of black threads embedded in glass flasks with jelly. The to and fro movement of the threads observed after the glass flask was moved led to the proposal that concussion was due to vibration of the brain (Strich, 1956). Extensive degeneration of the white matter was also seen post mortem in a patient who was in a "sleep-like" state for eight months in case described by Rosenblath in 1899 (Strich, 1956). It is possible that any additional cases around that time were felt to have a non-organic basis, and therefore were not as extensively evaluated.

There have been some interesting conclusions drawn from studies earlier last century. Turner and Eden reported that cortical contusion could be demonstrated in cases where the brain injury was not associated with amnesia (Turner and Eden., 1941). Munro commented on the role of loss of consciousness on the severity of the injury, stating that no significant cerebral injury occurs without some degree of unconsciousness (Munro, 1938). Denny – Brown and colleagues' work with concussion broadened this further by defining head injury as, "such injury to the skull as might directly or indirectly damage the brain, even when there is no unconsciousness (i.e. no concussion)" (Denny – Brown et al., 1941). By the 1960s it was more apparent that fatal brain damage could occur without fracture to the skull or visible "blemish" on the scalp, but lacerations and fractures were signs of underlying brain damage (Jennett,

1975). Miller and Jennett undertook further work which recognised that local contusion and laceration of the cortex frequently occurs under a compound depressed fracture in patients who have never been unconscious (Miller and Jennett, 1968, Jennett and Miller, 1972).

1.3 Pathophysiology of Traumatic Brain Injury

1.3.1 Post mortem studies of traumatic brain injury

The spectrum of structural damage that can occur with traumatic brain injury is thought to be vast. Until relatively recently, our understanding of the damage to brain tissue following traumatic brain injury was only available through post mortem studies. Through this analysis of post mortem tissue of deceased patients after blunt head trauma, it has been found that brainstem lesions, acute haemorrhagic lesions, damage to subcortical white matter and thalamus and diffuse axonal injury are common neuropathological findings after head injury (Strich, 1956, 1961, 1970, Jellinger, 1983, 2013, Adams et al., 1999, 2000, Jennett et al., 2001).

Over the years post mortem studies have evaluated diffuse axonal injury in more detail. It has been noted that the diffuse axonal lesions are not always visible macroscopically. Strich acknowledged that although some lesions due to head injury can be seen with the naked eye, many important ones can only be seen with the microscope (Strich, 1970). Oppenheimer's view was that these microscopic lesions are attributed to the accelerations within the brain substance at the time of injury (Oppenheimer, 1968). This has been corroborated by more recent studies. In a series of 434 fatal non-missile head injuries, 24 of the cases would not have been identified without microscopical examination (Adams et al., 1989). Diffuse axonal injury has also been referred to by other names in the historical literature. A further post mortem account by Adams and colleagues referred to their nineteen patients to have diffuse white matter of immediate impact type (Adams et al., 1977). Of those lesions identified macroscopically, it was noted that many lesions demonstrated yellow-orange colouring

consistent with the persistence of haemosiderin. Intracranial haematomas were also evident in 26% of cases. Ischaemic damage was seen in 43% of cases; this was centred on arterial boundary zones in the cerebral hemispheres. The subcortical white matter was noted to be diffusely abnormally pale and granular in texture in 83% of cases. A later series of 49 cases examined one month to eight years after the injury demonstrated that diffuse axonal injury was the commonest structural abnormality, having been identified in 71% of cases (Adams et al., 2000). With increasing survival after injury, Adams and colleagues observed that the bulk of the white matter was reduced and that there was enlargement of the ventricular system (Adams et al., 2000). A further total autopsy series (n=630) of patients in a vegetative state after head injury studied by Jellinger demonstrated the incidence of cortical contusions averaged 73%, diffuse axonal injury averaged 55% and intracranial haemorrhages averaged 68% (Jellinger, 2013).

Diffuse axonal injury is demonstrated histologically by axonal separation, where the axon is torn at the site of stretch and the part distal to the tear degrades. It is associated with myelin destruction (Oppenheimer, 1968). The histological diagnosis was historically based on the presence of axonal bulbs seen in sections stained with haematoxylin and eosin, silver impregnation or immunocytological techniques for neurofilaments (Strich, 1956, Oppenheimer, 1968). In addition, it is accepted that axonal bulbs are not specific to diffuse axonal injury and can occur in any condition where the axon is damaged (Adams et al., 1989). Previously the identification of axonal injury has been dependent on silver staining to identify axonal swellings. As mentioned earlier it is now felt that these staining methods may underestimate axonal pathology (Johnson et al., 2013). Recent advances in immunohistochemistry have enabled the use of an antibody to the precursor protein of beta-amyloid (amyloid precursor protein, APP) (Ikonomovic et al., 2004). This has improved the reliability of identification of axonal injury in brain injury (Gentleman et al., 1993, Sherriff et al., 1994). The presence of amyloid precursor protein can be identified in damaged axons within two hours following injury, and has been demonstrated in patients who die soon after a traumatic brain injury (Roberts et al., 1991, Roberts et al., 1994). These post mortem studies

have therefore concluded that diffuse axonal injury is a more frequent occurrence in head injuries, than was previously appreciated (Gentleman et al., 1995).

The study by Adams and colleagues demonstrated that diffuse axonal injury occurs in the axons of the cerebral hemispheres, the corpus callosum, the brainstem and the cerebellum after traumatic brain injury (Adams et al., 1989). This supports earlier observations by Oppenheimer that softening, without haemorrhage is often seen within the corpus callosum and upper brainstem after a head injury (Oppenheimer, 1968). The presence of diffuse axonal lesions is often associated with cases with the worst clinical prognosis (Adams et al., 1989, Jellinger, 2013). However, myelin destruction and axonal retraction bulbs have also been observed in cases of concussion lasting a few minutes (Oppenheimer, 1968).

The concept of “shearing injury” as a cause of the microscopic abnormalities occurring in the context of an injury is not new. On the basis of her cases lacking pathological lesions usually attributed to anoxia, oedema, vascular disturbances, or fat embolism, Strich proposed that distortion of the brain by mechanical forces causes the neuropathological condition following brain injury (Strich, 1956). She felt that the histological picture was compatible with injury to nerve fibres, causing death and secondary degeneration. Strich acknowledged that there was complete sparing of certain areas, with disproportionate degeneration in others, thus supporting a mechanical basis for the injury. Oppenheimer proposed the effect of direct impact and shearing due to excessive accelerations from the injury causes the microscopic lesions noted post-mortem (Oppenheimer, 1968). Diffuse axonal injury has therefore been concluded to be a significant type of brain damage that can occur as a result of non-missile head injury (Adam et al., 1989).

Many existing studies have been undertaken in cases with relatively short survival. However, in cases with a longer survival, widespread wallerian-type degeneration in subcortical white matter and in descending tracts in the brainstem and spinal cord has also been demonstrated (Strich, 1956). Strich’s neuropathological findings were demonstrated in a series of cases who survived a closed head injury and died five to fifteen months after injury. Their clinical state

was poor, with a quadraparesis and reported severe post-traumatic dementia. However, this study elegantly demonstrates the presence of significant neuropathological findings in a patient group who had not suffered a skull fracture or intracranial haematoma, and remained in a significantly impaired state until death. This study therefore additionally proposes a role for axonal degeneration in cognitive impairment after traumatic brain injury. This complemented existing studies by Holbourn and also work by Pudenz and Sheldon on the possibility of axonal damage being associated with the gross neurological deficits observed at the time of injury (Holbourn, 1943, Holbourn, 1945, Pudenz et al., 1946). The possibility of shearing at the time of injury as an aetiological factor for axonal injury was also supported by subsequent work by Peerless and Rewcastle and also work by Zimmerman (Peerless et al., 1967, Zimmerman et al., 1978).

Although the neuropathological findings with traumatic brain injury are well described, the relationship between the injury (initial head impact) and brain pathology is less well understood. The possibility that axonal damage occurred at the time of head injury was proposed by Strich (Strich, 1956). In her case series of patients who had survived months after injury, she acknowledged the limitations of attempting to date any lesions with accuracy. However, her opinion was that the lesions demonstrated appeared old, quiescent and of the same age, with the exception of some recent haemorrhages. In the context of the reported static neurological impairments for her cohort of patients until their death, she concluded the damage demonstrated occurred at or near the time of the accident (Strich, 1956). However, other post-mortem studies at that time have also proposed the possibility that the axonal damage is a secondary process, arising as the result of hypoxia and ischaemia or brain swelling (Jellinger et al., 1970).

1.3.2 Post mortem studies in non-human primates

Experimental studies in non-human primates have enhanced our understanding of axonal injury further. Axonal damage identical to what has been found to occur in humans can be produced by non-impact angular acceleration of the head (Gennarelli et al., 1982). Disruption

immediately after the injury has been seen in non-human primates subjected to shearing injuries (Maxwell et al., 1993). Further analysis has recognised a secondary process that damages axons (Maxwell et al., 1997). It is thought that the axons undergo a series of events; initially focal swelling of the axons occurs (Maxwell et al., 1995a, Pettus et al., 1996). This is then accompanied by swelling of axonal mitochondria (Maxwell et al., 1995a,b, Pettus et al., 1996), the development of nodal blebs (Maxwell et al., 1991) and focal decrease in internodal axonal diameter. There is then loss of axonal microtubules (Maxwell, 1995a, 1996, Pettus et al., 1996). There is subsequent alteration of the intra-axonal relationship of neurofilaments (Pettus et al., 1996). There is then involution of the internodal axolemma (Maxwell et al., 1995a). This is then followed by the separation of the axolemma from the internal aspect of the myelin sheath (Maxwell et al., 1995a). Axonal swellings then occur (Maxwell et al., 1991), this is followed by myelin intrusions and then axonal separation (Maxwell et al., 1995a). It is thought that the first insult (referred to as primary axotomy) can be identified within 60 minutes of injury, whereas the second insult (referred to as secondary axotomy) requires a minimum of 4 hours to develop (Maxwell et al., 1997). On the basis of the experimental, model three grades of severity of diffuse axonal injury have been defined (Adams et al., 1989); histologically this has been applied to humans.

Despite well-described histopathological findings in post-mortem tissue of humans and non-human primates, the underlying pathophysiology of the variable patient outcome after traumatic brain injury remains poorly understood (Lowenstein, 2009). Although traumatic brain injury can often involve only a single insult, patients can improve or deteriorate after traumatic brain injury (Whitnall et al., 2006). Post-traumatic epilepsy (Denny – Brown, 1943, Annegers et al., 1998) and late cognitive decline (Guo et al., 2000) have also been described. More recent work has demonstrated intracellular deposits of hyperphosphorylated tau, which has a distribution distinct from Alzheimer's disease (McKee et al., 2013). This indicates either an active process, or degenerative change causes the late change in clinical state. Inflammation has been proposed as a possible factor to explain late emerging clinical features. The inflammatory response produced by the traumatic brain injury is well recognised (Maxwell et al., 2006). Animal models suggest the initial insult results in a neuronal injury which disrupts

the blood brain barrier. Microglial cells are then thought to become chronically activated. In animal models, the initial inflammatory process has been shown to persist for at least a year (Smith et al., 1997). In humans, post mortem studies have shown microglial activation many years after traumatic brain injury; sites of activation often coincide with those of neuronal degeneration and axonal abnormality (Gentleman et al., 2004). Microglial activation has been studied in vivo using positron emission tomography (PET) ligand [11C](R)PK11195 (PK). Increased microglial activation has been found to be present up to 17 years after traumatic brain injury. In addition, impaired information processing after traumatic brain injury is associated with greater thalamic microglial activation. This has suggested that the disease process triggers a chronic inflammatory response and evolves over time (Ramlackhansingh et al., 2011).

The resultant temporary changes of conduction in damaged axons are likely to contribute to the transient disturbances of consciousness after a head injury (Adams et al., 2000). The biomechanics of the injury also impacts on the neuropathology seen (Ommaya et al., 1974). The presence of these histopathological abnormalities, even in the post–mortem tissue of individuals with a history of concussion who have died of other causes (such as bronchopneumonia), has provided some evidence of a structural basis to account for persisting consequences after even mild head injury (Oppenheimer, 1968, Blumbergs et al., 1994).

1.4 Clinical considerations in traumatic brain injury

1.4.1 Clinical assessment

Although conventional imaging improved survival through recognition of extracerebral haemorrhage in closed head trauma (Snoek et al., 1979, Zimmerman et al., 1982), current methods of clinical assessment (clinical examination, conventional imaging) of traumatic brain injury have not been able to predict deficit or long term outcome. This is partly due to the difficulty in formulating a robust classification system, and also because underlying structural damage was underestimated by conventional neuroimaging until recently. Neuroimaging

methods have improved, with imaging demonstrating residual damage, even when conventional imaging methods were normal (Sharp et al., 2011a).

Despite this, most guidelines on head injury have focussed on the perspective of acute management, and are neurosurgically orientated (Greenwood, 2002). However, only 5-10% of those admitted in the UK warrant neurosurgical intervention (Jennett, 1996). National guidelines on head injury focus on the urgency for a brain scan (NICE, 2014). There is no mention of post traumatic amnesia in the NICE guidelines, and there are no formal guidelines to help with management of the consequences of traumatic brain injury. With the assistance of Headway, the brain injury association, the Parliamentary paper on acquired brain injury (June 2018) has touched on some of the consequences following traumatic brain injury. However, far more awareness is needed.

1.4.2 Severity of injury and outcome measures

Part of the problem has been the need for a classification system that can assess severity of injury and predict outcome after traumatic brain injury. Until recently, traumatic brain injury has been classified as mild, moderate or severe using the Glasgow Coma Scale, a scale used to assess impaired consciousness of any cause. The three components, consisting of eye opening, verbal response and motor response are summed to produce a total score out of fifteen. It is not a static measure, and regular repeat assessments form part of the acute management and observation during a hospital admission of any cause. Its advantages are that it is relatively quick to assess, and enables repeat assessments as part of the neurological observations through a hospital admission. It is a standard neurological assessment for acute admissions through accident and emergency departments in the UK.

The Glasgow Coma Scale is also used to assess severity of traumatic brain injury; a Glasgow Coma Scale score of 13-15 is defined as mild traumatic brain injury, 9-12 as moderate traumatic brain injury, 3-8 as severe traumatic brain injury (Teasdale et al., 1974). The lower the Glasgow Coma Scale, the more significant the brain injury in hospital attendees (Hetda, 2007). The majority of fatal outcomes will be in sufferers of a moderate traumatic brain injury

(with Glasgow Coma Scale of 9 to 12) or those with severe traumatic brain injury (with a Glasgow Coma Scale less than or equal to 8) (Swann et al., 1999). The Glasgow Coma Scale continues to be used to guide management of traumatic brain injury in the acute setting (NICE, 2003). For example, it forms part of the criteria used to determine the clinical indication for a CT scan (NICE, 2003, 2007, 2014). Intubation is indicated if the Glasgow Coma Scale is less than or equal to 8, as it is felt that this level of consciousness would not be sufficient to maintain a patent airway. The main advantages are that it is already part of acute neurological assessment, widely practised, and assessable by a breadth of medical professional disciplines.

The Glasgow Coma Scale does have limitations in its use in a clinical setting. It may be affected by early sedation or intubation at the scene (Zafonte et al., 1996) or intoxication at the time of injury (Kelly et al., 1997). In one study by Ashkenazi and colleagues, the proportion of patients who required neurosurgical intervention after traumatic brain injury sustained through explosion in the civilian setting, could not be identified by the Glasgow Coma Scale alone (Ashkenazi et al., 2016). In this study, the patients in need of neurosurgical intervention were comparable in both groups (GCS 3-8 versus GCS 9-14) (Ashkenazi et al., 2016). Importantly, the Glasgow Coma Scale alone is also not able to identify which patients would be at risk of complications following traumatic brain injury, where it has been found to be less helpful in predicting late outcome (Katz et al., 1994, Keyser-Marcus et al., 2002).

This has been superseded by other severity indices which have been more predictive of late traumatic brain injury outcome, and include time to follow commands (TFC) and duration of post-traumatic amnesia (PTA). Post traumatic amnesia is defined as, “the length of the interval from injury during which current events have not been stored after the injury” (Russell and Smith, 1961). Practically, this is until the patient is orientated and can recall memories (Nakase-Thompson et al., 2004). The findings from a study by Nakase-Richardson and colleagues indicated that post traumatic amnesia of less than 14 days was classified as moderate traumatic brain injury, post traumatic amnesia of 15-28 days was classified as moderately-severe traumatic brain injury and post traumatic amnesia of 29-70 days was

classified as severe traumatic brain injury (Nakase-Richardson et al., 2011). This has already been shown to correlate highly with prospectively acquired assessments of post traumatic amnesia (McMillan et al., 1996). Post traumatic amnesia in particular has been found to be an important index of the severity of traumatic brain injury (McMillan et al., 1996, Greenwood, 1997, Nakase-Richardson et al., 2011). The study by Nakase-Richardson reported that 67% of individuals with post traumatic amnesia for less than fourteen days returned to productivity within one year (Nakase-Richardson et al., 2011). A post traumatic amnesic period of more than two weeks predicts residual cognitive problems, a period of one month predicts a reduced work capacity, and a period of three months predicts voluntary or subsidised work will be the best outcome (Greenwood, 2002). Longer post traumatic amnesia periods are associated with a greater likelihood of being non-productive at one year following traumatic brain injury (Ponsford et al., 2016).

The Mayo Classification System for traumatic brain injury severity was most recently developed to address the problems of the existing measures of severity: mainly unreliability of traumatic brain injury severity indicators, and missing documentation in the hospital records. It also emphasises the information available, rather than relying on making inferences from missing information. It has been of value in classifying traumatic brain injury severity in research studies, post acutely and for the purpose of planning post-acute clinical care (Malec et al., 2007). The Mayo Classification System determines three main classifications including Moderate-Severe (definite) traumatic brain injury, Mild (probable) traumatic brain injury, and Symptomatic (possible) traumatic brain injury. Multiple criteria are used in each classification, including presence of loss of consciousness, duration of post traumatic amnesia, presence of skull fracture, and evidence of neuro-radiological abnormalities including subdural haematoma, cerebral contusion, and haemorrhagic contusion (Malec et al., 2007). The classification of moderate-severe traumatic brain injury includes the presence of loss of consciousness for 30 minutes or more, post traumatic amnesia of 24 hours of more, and Glasgow Coma Scale in the first 24 hours of less than 13, in the absence of intoxication or sedation. Haematoma, contusion, haemorrhage would also place the traumatic brain injury in the moderate-severe (definite) category. The classification of mild (probable) traumatic brain

injury involves loss of consciousness of less than 30 minutes, post traumatic amnesia of less than 24 hours and the presence of depressed, basilar or lineal skull fracture. A symptomatic (possible) traumatic brain injury would be made on the basis of symptoms of blurred vision, confusion, feeling dazed, dizziness, headache, or nausea (Malec et al., 2007).

The Mayo Classification System for traumatic brain injury severity has its advantages. It utilises a combination of indicators, rather than a single indicator to provide the score. It has been demonstrated to be sensitive and specific in identifying the severity of the traumatic brain injury; using the Mayo Classification system, the estimated sensitivity for identifying moderate-severe traumatic brain injury is calculated to be 89% and the estimated specificity is 98% (Malec et al., 2007). However, there are limitations to the Mayo classification. As mentioned previously, the Glasgow Coma Scale component of the Mayo classification can be influenced by sedation or intubation by emergency staff at the scene (Zafonte et al., 1996) or intoxication (Kelly et al., 1997). The presence of concurrent systemic or psychological shock or fractures can extend the post traumatic amnesic period (Malec et al., 2007). Opioid medication given through the acute period, when undergoing surgery, or to manage clinical anxiety may cause gaps in memory which could mimic post traumatic amnesia. This has already been demonstrated in patients who had not suffered a traumatic brain injury, but had a post-traumatic-like amnesia phenomenon (Kemp et al., 2010).

1.4.3 Clinical consequences of traumatic brain injury

Although it is the case that some sufferers can make a full recovery, it has also been noted that many mild traumatic brain injury patients will suffer long-term disability (Thornhill et al., 2000). Traumatic brain injury can be associated with a wide range of consequences ranging from coma to disabling neuropsychiatric or cognitive problems (Thornhill et al., 2000). The variation in clinical consequences is attributed to the complexity of the brain, the pattern and extent of damage (Maas et al., 2017). The earlier physical, cognitive and behavioural consequences are easily identified in the presence of abnormal awareness. Treatment usually involves a structured environment with reduction of distraction and stimulation. However, the attentional, executive and memory problems usually manifest later and affect almost all

patients (Greenwood, 2002). Traumatic brain injury has been found to result in persistent cognitive impairments, with all levels of injury (Whitnall et al., 2006), but even similar severities of injury result in highly variable outcomes (Lowenstein, 2009). Functional outcome variability after an injury is not just restricted to cognitive function (Whitnall et al., 2006).

Abnormal motor function is also recognised to be one of the more devastating impairments (Willemse-van Son et al., 2007). However, it has been estimated only 10% of patients fail to mobilise long term, even after severe injury (Greenwood, 2002). Neurological deficit tends to reflect the regions of damage. Long term hemiparesis is uncommon, compared to stroke. However, physical difficulties usually relate to clumsiness, uncoordinated muscle contraction, ataxia and imbalance (Greenwood, 2002, Greenwood et al., 2016).

It is now well recognised that traumatic brain injury sufferers experience deficits in daily functional tasks at home and in society, and may not return to their pre-morbid level of functioning. This has previously been attributed to cognitive impairment and can often occur in the setting of a normal clinical examination and brain imaging within normal limits (Lund et al., 2012). Functional therapy assessments have found that this functional impairment can involve a number of factors. Factors already identified include reduced movement, speed and accuracy in children and adolescents following injury (Rossi et al., 1996). This impairment is significant, and there is evidence to suggest it may deteriorate with time. A longitudinal study of 94 military service members with mild concussive injury demonstrated decline, when comparing clinical outcomes in neuropsychological, psychiatric and neurobehavioural measures at 1 and 5 years, compared to control (Mac Donald et al., 2017). Rather than resolution or improvement of deficit with time, this study suggested that there is a progression of functional deficit from traumatic brain injury with time, as evidenced by the worsening outcome measures. Whether this is due to an evolving neurological syndrome causing network breakdown with time, or due to other factors is not clear. However, the authors have recognised that there are significant disparities between the patient and control group in the psychiatric measures, compared to the neurophysiological measures. So it is possible that the psychiatric impact following traumatic brain injury may be confounding some of the described

poor functional outcome. In any case, this just adds to the evidence that the effects of traumatic brain injury on everyday function is not fully understood. It is likely to be multifactorial and is inadequately assessed by standard clinical measures, so inadequate provision will be made for survivors.

Long term, only 20-30% of patients achieve re-employability after a severe injury (Greenwood, 2002). Injury severity according to Glasgow Coma Scale has been linked as a risk factor to unemployment after traumatic brain injury (Doctor et al., 2005). Duration of post traumatic amnesia can also predict the ability to return to employment (Greenwood, 2002). However, in other studies the association between injury severity and employment after injury is not thought to be as significant (Ip et al., 1995, Gollaher et al., 1998). Education level and occupation pre-injury are thought to be factors. In addition, specific residual impairments from the brain injury are felt to be more reflective of ability for employment after the injury. These include poor self-awareness, interpersonal skills, cognitive difficulties and impulsivity (Wehman et al., 2005).

Traumatic brain injury is a heterogeneous condition. Understanding the important effects of traumatic brain injury on network function is still in its infancy. However, the development of additional assessment tools to assess network function following traumatic brain injury will add to our understanding of network breakdown from existing research. This will facilitate classification in the first instance. However, the follow-up of these patients will also facilitate prediction of prognosis. Robust tools to assess the patient following traumatic brain injury and monitor interventions have the potential to lessen this neurorehabilitation burden considerably.

1.4.4 Improving awareness of traumatic brain injury

Interest in, and public awareness of traumatic brain injury has increased through the publication of studies suggesting that traumatic brain injury in professional and amateur athletes may have both acute and chronic effects on neurocognitive function (De Beaumont et al., 2009) and persistent motor abnormalities (De Beaumont et al., 2011). Although estimated to only be approximately 10% of all cases of traumatic brain injury in the states

(Sahler et al., 2012), the incidence of traumatic brain injury in athletes has probably prompted much of the interest in the short and long-term consequences of traumatic brain injury (Thurman et al., 1998). This is probably due to the high media profile of this group, and high profile litigation cases. Concussion and mild traumatic brain injury are often interchanged in the sports literature. Previously, concussion symptoms were often thought to have a non-organic basis due to the absence of structural abnormalities on imaging. However, imaging has evolved and it is now accepted that concussion is part of traumatic injury, and is induced by traumatic forces (Aubry et al., 2002, McCrory et al., 2005, 2013). The Vienna Concussion in Sport Agreement Statement stated concussion is defined as, “a complex pathophysiologic process affecting the brain, induced by traumatic biomechanical forces”. As part of their document, they have acknowledged that concussion results in a graded set of clinical syndromes that may or may not involve loss of consciousness or memory dysfunction. Concussion typically results in a functional disturbance with the rapid onset of short-lived impairment of neurologic function that resolves spontaneously (Aubry et al., 2002). McCrory and colleagues have defined concussion as, “a pathophysiological injury to the brain caused by biomechanical forces, which leads to clinical alterations but no structural damage” (McCrory et al., 2013). Although recognised that many symptoms of concussion resolve over time, it is also recognised that symptoms can be significant and can end the sporting career (Wennberg et al., 2003, Wennberg et al., 2008). This interest has led to prospective case series of concussion cases over sports seasons, to increase understanding of the condition (Benson et al., 2011).

The combination of the increasing awareness in the sports world, with the existing interest in traumatic brain injury has been helpful in increasing the awareness of the need for more brain injury research. In any case, even in non-athletes the cognitive and psychological consequences of traumatic brain injury can be long-lasting and have particular impact on the ability to return to previous educational or employment status (Hoffmann et al., 2002, Engberg et al., 2004, Corrigan et al., 2007, Andelic et al., 2009). There is considerable morbidity associated with this condition.

1.5 Neuroimaging in traumatic brain injury

Computed tomography and magnetic resonance imaging scans taken initially after injury are often normal.

1.5.1 Conventional neuroimaging following traumatic brain injury

Although large haematomas and contusions are identifiable using conventional imaging measures such as computed tomography (CT) and magnetic resonance imaging (MRI), post-mortem analysis has already demonstrated that the damage is often more extensive than demonstrated on conventional structural imaging. In addition, it is recognised that visible areas of structural damage on neuroimaging do not necessarily reflect the neurocognitive outcome (Bigler, 2001, Lee et al., 2008).

Previously, it has not been possible to study the location and extent of diffuse axonal injury in vivo, even using MRI. Animal studies have already revealed that histologically-proven axonal damage can appear as normal on conventional MRI (Mac Donald et al., 2007a,b, Li et al., 2011). There are different techniques to assess traumatic brain injury and network breakdown after traumatic brain injury. Conventional MRI FLAIR sequence is more sensitive for focal brain injury. The gradient echo MRI sequence will detect microbleeds. Diffusion tensor imaging identifies the location of diffuse axonal injury (Arfanakis et al., 2002). Functional MRI evaluates brain activity to allow networks to be identified (Ham et al., 2012).

1.5.2 Diffusion tensor imaging

Diffusion tensor imaging is a MRI–based neuroimaging technique that allows the structural integrity of white matter tracts to be revealed and quantified (Basser et al., 1996). It measures the direction of water diffusion within white matter tracts, where it can map and characterise the three dimensional diffusion of water (Conturo et al., 1999, Mori et al., 1999, Basser et al., 2000). The diffusion of water in biological tissues occurs inside, outside, around and through

cellular structures, caused by random thermal fluctuations (Le Bihan et al., 2001, Alexander et al., 2007). Diffusion tensor imaging is therefore sensitive to the diffusion of water molecules, and hindered by extracellular and intracellular components. The diffusion is isotropic when unobstructed by tissues or barriers. However, diffusion becomes anisotropic when hindered by axons and their myelin sheath. In fibrous tissues including white matter, water diffusion is relatively unimpeded in the direction parallel to the fibre orientation. Axons constrain the diffusion of water so tend to occur in parallel with the direction of the tract. Therefore, quantification of the direction and integrity of axons can be measured with diffusion tensor imaging, thus allowing estimation of the structural integrity of the tract (Basser et al., 1996, Le Bihan et al., 2001, Beaulieu, 2002, Alexander et al., 2007, Assaf et al., 2008, Hellyer et al., 2013). Developmental, aging and pathological processes of the central nervous system have influence on microstructural architecture of affected tissues. Diffusion tensor imaging therefore provides quantitative information regarding the white matter structure (Ham et al., 2012) as it is sensitive to changes at the cellular and microstructural level (Alexander et al., 2007).

The main diffusion tensor imaging measures include fractional anisotropy (FA), mean diffusivity (MD), radial diffusivity (RD) and axial diffusivity (AD) (Alexander et al., 2007). Fractional anisotropy estimates the orientation and coherence of white matter tracts (Le Bihan et al., 2001, Beaulieu, 2002). The molecular diffusion rate Mean Diffusivity is otherwise known as Apparent Diffusion Coefficient (ADC). The directional preference of diffusion is the fractional anisotropy. The axial (diffusion rate along the main axis of diffusion), and radial (rate of diffusion in the transverse direction) diffusivity provide further information on the direction of diffusion (Farbota et al., 2012, Soares et al., 2013). Diffusion in white matter is less restricted along the axon and tends to be anisotropic (directionally-dependent). In the grey matter it is usually less anisotropic. In the cerebrospinal fluid, it is unrestricted in all directions (isotropic) (Pierpaoli et al., 1996 a,b, Song et al., 2002, Hagmann et al., 2006). Based on this assumption, Basser and colleagues modelled the diffusion process by an ellipsoid, which can mathematically be represented by a 3 × 3 symmetric matrix, also known as a tensor (Basser et al., 1996, 2000).

The diffusion of water within tissues is sensitive to differences in the microstructural architecture of cellular membranes (Alexander et al., 2007). Therefore, pathological processes which affect tissue microstructure will be identified with diffusion tensor imaging. Most studies focus on fractional anisotropy measures to ascertain pathological changes in tissue in diffusion tensor imaging (Alexander et al., 2007). Fractional anisotropy is a highly sensitive, but non-specific biomarker of neuropathology and microarchitectural microstructures. Estimates of the eigenvector directions, hence tract directions are susceptible to image noise (thermal and physiological) (Basser et al., 2000, Alexander et al., 2007), artefacts (for example, head motion resulting in misreading), partial volume averaging between tissues in large voxels (i.e. signal mixing of white matter, grey matter and CSF) and regions of crossing white matter tracts; this is unavoidable as many areas have regions of fibre crossing, although the corpus callosum, has fewer crossings (Alexander et al., 2001). Nevertheless, fractional anisotropy is respected as a marker of white matter integrity. However, fractional anisotropy analysis only provides part of the picture; decrease in fractional anisotropy may be due to increase in radial diffusivity and/or decrease in axial diffusivity (Farbota et al., 2012). This has led to the current thinking that the axial and radial diffusivities provide more specific information about diffusion differences, than fractional anisotropy alone.

In the demyelination literature, studies have demonstrated increased radial diffusivity values with or without influence on axial diffusivity in the human and Cuprizone (bis-cyclo-hexanone oxaldihydrazone) mouse model of demyelination in the corpus callosum (Song et al., 2002, Song et al., 2005, Tyszka et al., 2006). Axonal degeneration and loss have been demonstrated through lower axial diffusivity in studies that have focussed on the corpus callosum (Harsan et al., 2006, Sun et al., 2006). However, the mechanism for reduced axial diffusivity in damaged axons is not clear. It has been suggested that build-up of cellular debris from the breakdown of axonal structure, disordered microtubule arrangement and aggregation of filaments impede the movement of water molecules (Sun et al., 2006).

1.5.3 Diffusion tensor imaging following traumatic brain injury

Diffusion tensor imaging has been found to be sensitive to changes following traumatic brain injury (Arfanakis et al., 2002, Huisman et al., 2004, Singh et al., 2010). Lower fractional anisotropy and axial diffusivity has been demonstrated within the first few hours after injury in an experimental mouse model of traumatic brain injury (Mac Donald et al., 2007a, b). Song and colleagues have demonstrated these early changes are reflective of axonal damage in an additional experimental mouse model (Song et al., 2003). The current thinking is that this is followed by an evolving injury, that includes inflammatory changes (tissue oedema) and demyelination. The lower fractional anisotropy is thought to persist with time, the radial diffusivity then increases and plateaus. The axial diffusivity is felt to be more dynamic, as reflected in the diffusion tensor imaging metrics (Mac Donald et al., 2007b, Sidaros et al., 2008).

Diffusion tensor imaging is therefore thought to provide a sensitive measure of changes in white matter structure after traumatic brain injury. Studies to date have shown diffusion tensor imaging to be able to quantify white matter integrity when conventional imaging is normal (Mayer et al., 2010, Sharp et al., 2011a). Diffusion tensor imaging abnormalities have also been found to correlate with patterns of cognitive impairment (Salmond et al., 2006, Levin et al., 2008, Kumar et al., 2010, Kinnunen et al., 2011, Jilka et al., 2014). Diffusion tensor imaging is also often abnormal after mild traumatic brain injury, which enables study of this group of patients (Inglese et al., 2005, Sharp et al., 2011a). Abnormal tissue microstructure has been found to alter the pattern of water diffusion within white matter following diffuse axonal injury, resulting in abnormal diffusion measures in several brain regions of soldiers following mild traumatic brain injury (Baxter et al., 2013). A study by Mayer and colleagues also demonstrated that diffusion tensor imaging was found to be better at identifying patients from controls than the results from neuropsychology tests (Mayer et al., 2010). Diffusion tensor imaging can also be used to evaluate progress over time with patients with traumatic brain injury (Sharp et al., 2011a, Cole et al., 2018). Diffusion tensor imaging measures from interval imaging have been demonstrated to correlate with improvement in neuropsychological

measures with time. Longitudinal studies have also demonstrated the progressive loss of brain volume with time following traumatic brain injury (Cole et al., 2018).

A meta-analysis of 13 studies of mild traumatic brain injury by Aoki and colleagues evaluated the reported diffusion tensor imaging differences further and demonstrated a significant fractional anisotropy decrease and significant increase in the mean diffusivity in the splenium of the corpus callosum post traumatic brain injury, but only a marginal reduction in fractional anisotropy in the body of the corpus callosum, and no significant change in the fractional anisotropy in the genu of the corpus callosum, compared with controls (Aoki et al., 2012). A meta-analysis of 100 studies has also demonstrated the areas of fractional anisotropy abnormality in traumatic brain injury are more widespread than in the other diffusion tensor imaging metrics (Hullkower et al., 2013). Diffusion tensor imaging therefore provides an elegant method of assessing axonal damage in traumatic brain injury in vivo, which previously would have only been identified post-mortem.

Further to the demonstrated diffusion tensor imaging findings following traumatic brain injury, it is thought that the resultant damage to brain networks and disruption of their structural connectivity as a result of diffuse axonal injury underlies the problems encountered by traumatic brain injury sufferers (Sharp et al., 2011a). It is thought that subsequent damage to brain connectivity is the cause of poor clinical outcome after traumatic brain injury (Adams, 1982, Bernabeu et al., 2009, De Beaumont et al., 2009, Gentry et al., 1988a, Greenwood, 2002, Medana et al., 2003, Sharp et al., 2011b). This has therefore unlocked the potential to study the consequences of diffuse axonal injury in white matter tracts after traumatic brain injury.

1.6 Causes of diffuse axonal injury

As mentioned earlier, diffuse axonal injury, the generalised form of traumatic axonal injury, has been identified in post-mortem tissue as a consequence of traumatic brain injury for many years (Strich, 1961, Adams et al., 1977, Adams, 1982, Adams et al., 1989). Current thinking is that it results from shearing of nerve fibres due to sudden acceleration, deceleration, often combined with rotational forces (Strich, 1961, Adams, 1982, Arfanakis et al., 2002, Meythaler et al., 2001, Huisman et al., 2004). The pathology of diffuse axonal injury in humans is characterised histologically by widespread damage to the axons of the brainstem, parasagittal white matter of the cerebral cortex, corpus callosum, and the grey-white matter junctions of the cerebral cortex (Gentry et al., 1988a, Parizel et al., 1998, Meythaler et al., 2001, Scheid et al., 2003). The presence of diffuse axonal injury within the corpus callosum has been associated with a worse clinical prognosis after traumatic brain injury (Gentry et al., 1988a).

1.6.1 Biomechanical and biochemical contributions to diffuse axonal injury

The corpus callosum is one of the areas identified on diffusion tensor imaging to be affected by diffuse axonal injury in traumatic brain injury. It is thought that the main mechanism of traumatic brain injury involves biomechanical forces (Gennarelli et al., 1993, Ghajari et al., 2017). Earlier post mortem studies proposed an effect of direct impact and indirect (shearing) factors on the development of the neuropathological findings seen after traumatic brain injury (Oppenheimer, 1968). More recently, computer models and animal studies findings have demonstrated that acceleration, deceleration and rotational motions are the three main biomechanical forces in closed head injury (Gennarelli et al., 1993, Cole et al., 2018). Histological analysis has demonstrated that axonal damage is more prevalent in regions where biomechanical strain associated with injury are most likely; these regions of axonal damage affect multiple areas along the axon (Gaetz et al., 2004). Ommaya and Gennarelli proposed that traumatic brain injury followed a centripetal hypothesis, where the sudden rotational forces cause shearing strains and stress which result in functional decoupling of nerve fibres. The depth of functional decoupling depends on the rotational acceleration of the brain. With more

rotation-acceleration, the likelihood of mechanical injury to fibres increases (Ommaya et al., 1974). This has led to the hypothesis that the peripheral structures of the brain would potentially be more prone to damage with less severe trauma, while deeper structures would require more force to be damaged.

Early experimental models suggested that the actual axonal damage occurred because rotational forces sheared axons by stretching them beyond tolerance (Holbourn, 1943, Strich, 1956, 1961). However, more recent work in the rat has suggested that axons have been shown to tolerate being stretched up to 65% without breaking (Smith et al., 1997). Further post mortem studies of patients who had suffered severe head injury supported this finding (Adams et al., 1989). This implies that the biomechanical force of the injury cannot solely account for the extent of damage associated with diffuse axonal injury.

Recent evidence suggests that traumatic brain injury is not a single brain insult; it is more likely to trigger a cascade of events within the brain that are associated with a clinical syndrome associated with progression. There are biochemical changes following injury, where the stretching of the axon membrane at the time of injury is thought to result in an extensive release of neurotransmitters, resulting in a massive ionic flux and cascade of toxic substances associated with the cell soma and within the axon (Povlishock and Christman., 1995, Gennarelli et al., 1998), which results in secondary cell death. It is thought that this stretching, even when insufficient to cause shearing, results in an increase in intracellular calcium which results in injury to axons; lesser degrees of injury-induced calcium flux causes temporary injury, greater degrees are thought to cause greater damage (Gennarelli et al., 1998). This supports the hypothesis that the process of diffuse axonal injury is the product of both biomechanical and biochemical changes that occur, resulting in generalised degeneration of white matter (Adams, 1982). In the context of what is known about the biomechanical and biochemical events following traumatic brain injury, it is possible that such injury would make the longer tracts more susceptible to diffuse axonal injury, than the localised tracts.

1.7 Consequences of network breakdown in brain injury

1.7.1 Cognitive impairment

Traumatic brain injury is known to be associated with persistent cognitive impairments, with all levels of injury (Whitnall et al., 2006). Although the overall IQ and visuospatial skills are relatively preserved following traumatic brain injury, memory, attention and executive skills constitute the residual neuropsychological deficits, and may not be identified in simple cognitive assessments (Greenwood, 2002). Increase in appreciation of this problem has led to more specific neuropsychological evaluation, and more recently diffusion tensor imaging. The deficits can range from impairment of attention and information processing speed to executive dysfunction and memory impairment (Stuss et al., 1999, 2003, Bonnelle et al., 2011, Sharp et al., 2011a,b). Compared with healthy controls, cognitive impairment assessed with formal neuropsychological measures have also been demonstrated in patients with mild traumatic brain injury (Balanger et al., 2005, Shores et al., 2008).

In the healthy brain a predominantly right-lateralised frontoparietal network is thought to be utilised during attention-requiring tasks (Paus et al., 1997). Momentary attentional lapses have been found to be associated with the failure to suppress activity in the posterior cingulate cortex and precuneus (Weissman et al., 2006). In contrast, during periods of attentionally demanding tasks, there is reduced activation of what is termed the default mode network in the normal brain (Buckner et al., 2008). The default mode network consists of the posterior cingulate cortex, precuneus and parts of the ventromedial prefrontal cortex. Their interaction is thought to be important in regulating the focus of attention (Hampson et al., 2006, Leech et al., 2011). The ability to regulate default mode network activity is thought to be a part of normal brain function. However, damage results in impairment of sustained attention (Malhotra et al., 2009).

The attentional deficits observed after traumatic brain injury could plausibly arise from diffuse axonal injury affecting respective areas, disrupting the networks involved in attention. Studies to date investigating attentional impairment after traumatic brain injury have already

demonstrated increased activation of the frontoparietal attentional network (Levine et al., 2006, Scheibel et al., 2007). Work by Bonnelle and colleagues has already suggested that the presence of diffuse axonal injury disrupts distributed brain networks (Bonnelle et al., 2011). Other work has also suggested that the observed activated networks in moderate to severe traumatic brain injury may not be operating efficiently, in view of the cognitive impairment observed in these cases (Scheibel et al., 2007).

Longitudinal analysis by Bonnelle and colleagues has demonstrated that increased activation of the default mode network occurs over time (Bonnelle et al., 2011). Therefore, the increase in activation patterns has been interpreted as a compensatory response to the injury. Using diffusion tensor imaging, structural disconnection within the default mode network correlates with levels of sustained attention, where low functional connectivity within the default mode network has been found to predict impairment in sustained attention after traumatic brain injury (Bonnelle et al., 2011). This has been demonstrated to be independent to the presence of focal brain injury, so is thought to reflect damage to the white matter tracts. Further work has supported a compensatory increase in functional connectivity within the default mode network and a variable degree of structural disconnection that modulates the change in network function following brain injury (Sharp et al., 2011b). Sharp and colleagues have also demonstrated a relationship between changes in functional connectivity after traumatic brain injury, cognitive impairment and white matter damage (Sharp et al., 2011b). In a simple button press task in the MRI scanner, patients were slower and more variable in their responses on the task. However, they were able to perform it accurately. This has led to the proposal that increased deactivation within the default mode network in patients following traumatic brain injury is associated with a requirement for greater cognitive effort to maintain accurate task performance (Sharp et al., 2011b).

1.7.2 Other considerations regarding network breakdown

This is only part of the research into the cognitive consequences following traumatic brain injury. Cognitive research following traumatic brain injury continues to develop in the literature. This has been assisted by the advances in imaging techniques, which have demonstrated

microstructural damage which was not identified using conventional imaging. However, cognitive consequences following traumatic brain injury is likely to be one component of the long term consequences following traumatic brain injury. To our knowledge, little is still known regarding the physiological and behavioural consequences (in relation to bimanual coordination) of traumatic brain injury.

1.8 Investigating physiological consequences using Transcranial Magnetic Stimulation

Transcranial magnetic stimulation is a non-invasive method of assessing brain connectivity and is a common research tool in neurology. It has been used as a research tool since 1985 in both healthy subjects and in patients with neurological impairment, with no reported long-term sequelae and is regarded as a safe technique (Barker et al., 1985, Tassinari et al., 2003, Rossi et al., 2009).

During transcranial magnetic stimulation a coil, typically in a figure of eight formation is laid on the scalp of the subject. A rapidly changing electrical current produces a rapidly changing magnetic pulse, perpendicular to the brain's surface that penetrates the scalp and skull to reach the brain. This in turn causes an electrical field parallel to the cortical surface sufficient to cause axonal depolarisation. This leads ultimately to the motor evoked potential (Day et al., 1989). Studies by Amassian and colleagues have revealed many components of the motor evoked potential which can be observed by epidural recordings or by measuring single motor unit recordings with needle electrodes (Amassian et al., 1989). The motor evoked potential consists of a short latency direct wave (D-wave), followed by several indirect waves (I-waves) of longer latency (Di Lazzaro et al., 1998b, Hanajima et al., 1998). The D-wave is thought to result from direct depolarisation of the initial axon segment of the corticospinal neuron. The subsequent I-waves following the D-wave occur sequentially with a periodicity of approximately 1.5ms. This is thought to reflect the delay required for synaptic discharge. Therefore, the first I-wave (I1) is thought to be generated through the depolarisation of the axon synapsing directly onto the corticospinal neuron, whilst the subsequent I-waves are

thought to require local polysynaptic circuits. In human participants, the I-waves have been found to be elicited using relative low transcranial magnetic stimulation intensities (Amassian et al., 1989).

Transcranial magnetic stimulation has already been used to provide physiological information in patients with a variety of motor disorders, for example, stroke, multiple sclerosis, dystonia and spinal cord injury (Boniface et al., 1991, 1994, Davey et al., 1999, Murase et al., 2004, Talelli et al., 2006, Ward et al., 2006, Sandbrink, 2008, Chen et al., 2008, Kern et al., 2011, Quartarone, 2013, Kojovic et al., 2013). It has helped understand the physiological consequences of these conditions, and has the potential to develop interventions to assist with their management. Transcranial magnetic stimulation therefore has the potential to provide information about the physiological consequences of traumatic brain injury, which is an area currently poorly understood.

1.8.1 The human cortical motor output

The human cortical motor output starts at the pyramidal cells originating in layer V of the primary motor cortex. These cells contribute approximately 40% of the axons making up the corticospinal tract (Jane et al., 1967). Some directly provide monosynaptic projections to the alpha motor neurons of the spinal cord; 90% decussate at the level of the medulla. Projections to the primary motor cortex are also received from the premotor cortex (Dum et al., 1991). In addition, there also exist significant projections from other brainstem regions (Schieber, 2007). The primary motor cortex receives numerous projections from the thalamus and other areas of the cortex. The complex integration of these inputs is required for the fine control of the movement.

1.8.2 Assessment of corticospinal excitability using transcranial magnetic stimulation

Stimulation of the primary motor cortex with transcranial magnetic stimulation evokes a twitch in the contralateral hand muscles, which can be recorded by surface electromyography as the motor evoked potential (Day et al., 1989). Motor threshold is the lowest stimulus intensity

necessary to evoke a motor evoked potential in the target muscle. It can be measured with the muscle at rest (resting motor threshold, RMT) or during voluntary activation (active motor threshold, AMT). It is thought to depend on the excitability of axon membranes which are activated by transcranial magnetic stimulation, as well as the excitability of the synapses that cause discharge of corticospinal neurons (Ziemann et al., 1996, Rosler et al., 2008). Resting motor threshold also reflects the excitability of motor neurons in the spinal cord (Mann et al., 1996). Active motor threshold is less dependent on synaptic excitability since all neurons in the pathway are near their firing threshold and readily discharged by any synaptic input. Motor threshold is commonly increased in neurological diseases that affect the corticospinal tract, such as multiple sclerosis and stroke (Boniface et al., 1991, 1994, Rossini et al., 1998).

Motor recruitment curves are created by measuring motor evoked potential amplitudes across a range of stimulus intensities (Ridding and Rothwell, 1997). As the stimulus intensity is increased, motor evoked potentials of increasing amplitude are obtained until a plateau level is reached (Devanne et al., 1997). For the first dorsal interosseous the relationship is sigmoidal (Devanne et al., 1997). It is thought to reflect the recruitment of the components of the corticospinal tract, the motoneuron pool and spinal interneuronal relays (Burke et al., 1994), and the resulting curve is thought to reflect the functional integrity of the corticospinal tract (Devanne et al., 1997). The plateau of the curve is thought to reflect the number of corticospinal fibres that can be accessed by transcranial magnetic stimulation as well as the effectiveness of their connections in the spinal cord. The gradient of the curve has therefore been found to give an indication of how readily corticospinal output can be recruited to the target muscle. This depends on the height of the plateau as well as on the distribution of excitability within the population and the distance of the excitable elements from the site of stimulation. It is steeper in active than relaxed muscle because activation increases the excitability of all output elements making them easier to recruit as the intensity is increased. In healthy subjects, it has been suggested that muscles with larger cortical representation have steeper recruitment pattern than muscles with a smaller cortical representation (Devanne et al., 1997, Chen et al., 1998, Davey et al., 1999).

The gradient of the recruitment curve is reduced in diseases where there is damage to the corticospinal tract, such as stroke and multiple sclerosis. Much of this is thought to be caused by a reduced plateau. However, since defining the plateau in disease can be difficult if not impossible with available stimulators, gradient of the recruitment curve is often used as a surrogate marker of corticospinal tract integrity (Devanne et al., 1997, Ridding and Rothwell, 1997, Boroojerdi et al., 2001).

1.8.3 Assessment of interhemispheric inhibition with transcranial magnetic stimulation

It is thought that the two hemispheres communicate to execute bimanual movement. Ferbert and colleagues used an elegant transcranial magnetic stimulation technique to investigate the callosal connections between the motor areas of each hemisphere (Ferbert et al., 1992). This involved applying a conditioning stimulus over the motor cortex of one hemisphere prior to a test stimulus to the opposite hemisphere. They found that if the conditioning stimulus preceded the test by more than 8ms, it reduced the amplitude of the motor evoked potential. The duration of the effect depended on the intensity of the conditioning stimulus and lasted for up to 50ms (Ferbert et al., 1992). Subsequent studies have demonstrated this effect to be absent in patients with corpus callosum agenesis or partial callosotomy (Meyer et al., 1995, Woiciechowsky et al., 1997) and disrupted in patients after stroke (Butefisch et al., 2008) and multiple sclerosis (Boroojerdi et al., 1998, Wahl et al., 2011). However, interhemispheric inhibition has been demonstrated in patients with purely subcortical lesions that interrupt the corticospinal tract (Boroojerdi et al., 1996). The effect is therefore thought to be mediated via the corpus callosum, although there are other factors involved. Further evidence in favour of a predominantly callosal origin for interhemispheric inhibition has been provided in three patients with no abnormality of the motor system. These patients had epidural spinal cord electrodes installed for relief of intractable pain (Di Lazzaro et al., 1999). Recordings of the descending spinal volleys directly from the epidural space of the cervical spinal cord showed a reduction in the size of some of the I-waves by a prior conditioning stimulus to the opposite hemisphere (Di Lazzaro et al., 1999).

The interval for interhemispheric inhibition in the Ferbert protocol has supported existing research of transcallosal transfer. Motor cortical stimulation has been shown to evoke an electroencephalogram potential over the contralateral motor cortex with an onset latency of 8.8 -12.2ms (Cracco et al., 1989). Differences in the onset latency of jerks in patients with cortical myoclonus is also approximately 10ms (Wilkins et al., 1984, Brown et al., 1991).

Although inhibition is the primary effect, it is usually possible to demonstrate an early period of facilitation at interstimulus intervals of 6-8ms (Hanajima et al., 2001). Interhemispheric inhibition has been found to include an early phase at relatively short interstimulus intensities (8 - 10ms) and then a later phase (30 - 50ms) (Chen et al., 2003, Kukaswadia et al., 2005). These phases are thought to be mediated by different neuronal circuits (Chen et al., 2003, Chen, 2004). However, both are thought to involve GABA-mediated neurotransmission (Daskalakis et al., 2002). However, a study by Irlbacher and colleagues showed that interhemispheric inhibition with long interstimulus intervals was enhanced after the use of Baclofen (GABA-B agonist), thus indicating the long phase was mediated by GABA-B receptors (Irlbacher et al., 2007). However, the neurotransmitter system mediating the short phase has not been established (Irlbacher et al., 2007). A further study by Udapa and colleagues has also supported that different cortical neurons mediate the inhibitory interactions in the two phases (Udapa et al., 2010).

1.8.4 Assessment of intracortical excitability using transcranial magnetic stimulation

Inhibitory and facilitatory interactions in the cortex occurring within the primary motor cortex can be studied by delivering two transcranial magnetic stimulation pulses, a subthreshold conditioning stimulus with a suprathreshold test stimulus, through the same coil. This is referred to as paired-pulse transcranial magnetic stimulation (Kujirai et al., 1993). Inhibitory and excitatory circuits are stimulated simultaneously, and varying the intensity of the conditioning stimulus allows each to be studied in more detail (Chen et al., 1998). Test motor evoked potentials are suppressed (short interval intracortical inhibition, SICI) at interstimulus intervals of 1-4ms, if the conditioning stimuli have an intensity of 60-80% active motor

threshold. It is followed by a period of facilitation (intracortical facilitation, ICF) at interstimulus intervals of 6-25ms. Experiments by Hanajima and colleagues on single motor unit discharges in activated muscle suggested that although overt inhibition is only seen with interstimulus intervals less than 5ms, it actually may persist up to 20ms “underneath” the later period of intracortical facilitation. If the conditioning stimulus intensity is increased, inhibition deepens but then falls off, in a “u-shaped” relationship (Hanajima et al., 2001). It is thought that at intensities lower than 80% of the active motor threshold, the conditioning stimulus activates low threshold inhibitory neurons; above 80% of the active motor threshold, the stimulus begins to activate facilitatory inputs, which are probably the same as those that produce I-waves at higher intensities (Ziemann et al., 1998).

Short intracortical inhibition is thought to be due to an interaction within the motor cortex (Nakamura et al., 1997, Di Lazzaro et al., 1998a,b). Since GABA-A agonists enhance short intracortical inhibition, it is thought to be predominantly mediated by GABA-A receptors (Ziemann et al., 1996, 2003). This produces relatively short lasting inhibition.

Excitatory glutamatergic interneurons within the primary motor cortex and N-methyl-D-aspartate (NMDA) receptors have been found to influence intracortical facilitation (Ziemann, 2003). The NMDA receptor antagonists Dextromethorphan and Memantine decrease the excitability of the motor cortex by blocking the binding of glutamate. A study by Ziemann demonstrated that the drug abolished intracortical facilitation (Ziemann et al., 1998). Memantine has been found to enhance intracortical inhibition, but reduce intracortical facilitation (Schwenkreis et al., 1999). This has given some support to the theory that intracortical facilitation is, in part, mediated by glutamatergic neurons. Intracortical facilitation is also reduced by Lorazepam, an intermediate acting benzodiazepine, which enhances the effects of the neurotransmitter GABA at the GABA-A receptor, and is abolished by ethanol (Ziemann et al., 1995, 1996, 2004).

1.8.5 Transcranial magnetic stimulation in traumatic brain injury

In contrast to the transcranial magnetic stimulation literature for stroke, there are comparatively fewer studies that have investigated the physiological consequences after traumatic brain injury with transcranial magnetic stimulation. In addition, the rate of increase in the number of studies in traumatic brain injury over the last 10 years is only a third, compared to the increase in studies in stroke (Li et al., 2014).

1.8.6 Corticospinal excitability

Chistyakov and colleagues investigated thresholds and reported significant elevations in motor threshold of abductor pollicis brevis muscle in traumatic brain injury, concluding that there was a reduction in corticospinal excitability following traumatic brain injury (Chistyakov et al., 1998, 2001). Similar findings of elevated motor thresholds have also been supported by Nardone and colleagues and Lapitskaya and colleagues (Nardone et al., 2011, Lapitskaya et al., 2013). However, the studies by the three groups investigated traumatic brain injury acutely, and the follow-up study by Chistyakov and colleagues demonstrated normalisation of the motor thresholds at three months following injury (Chistyakov et al., 1998). Chistyakov and colleagues only investigated mild to moderate traumatic brain injury, with a Glasgow Coma Scale greater than 9 on presentation. Therefore, their findings cannot be generalised to the whole traumatic brain injury population. A further factor to consider when interpreting these findings is the use of neuromodulatory drugs and their impact on physiology; the patients in this study were on Phenytoin. Phenytoin is associated with increasing motor thresholds (Ziemann, 2004). Nardone and colleagues' findings of increased resting motor threshold were only found in the subgroup of patients with excessive daytime sleepiness, and not those without (Nardone et al., 2011). The findings by Lapitskaya and colleagues were in the context of a larger cohort of patients with brain injury (which included stroke, subarachnoid haemorrhage, traumatic brain injury) and the use of neuromodulatory medication (Lapitskaya et al., 2013).

Studies by Fujiki and colleagues, Takeuchi and colleagues, Jang and colleagues, Bernabeu and colleagues and Tallus and colleagues, have investigated cortical excitability at least six months after injury (Jang et al., 2005, Fujiki et al., 2006, Takeuchi et al., 2006, Bernabeu et

al., 2009, Tallus et al., 2012). Findings demonstrated no significant difference in the motor thresholds of traumatic brain injury patients (Fujiki et al., 2006, Takeuchi et al., 2006) or amplitude of motor evoked potential (Jang et al., 2005). However, Bernabeu and colleagues found motor thresholds were increased in traumatic brain injury; this was more pronounced where there was a degree of motor impairment (Bernabeu et al., 2009). Motor thresholds reflect the excitability of axon membranes and synapses responsible for the discharge of corticospinal neurons. Therefore, increased motor thresholds would reflect physiological impairment of the corticospinal tract. Interestingly, a study by Tallus and colleagues demonstrated an elevation in motor threshold in mild traumatic brain injury on average 5 years after injury. Although there was a large variation in the sampled group, the physiological abnormalities demonstrated were in the context of normal neuroimaging in the patients investigated (Tallus et al., 2012).

Abnormal recruitment was noted in the study by Bernabeu and colleagues, who reported narrower recruitment curves compared to controls (Bernabeu et al., 2009). In their study, they reported the slope of the recruitment curve was narrower (i.e. less steep) compared to the healthy counterparts, thus suggesting that the recruitment of corticospinal neurons responsible for the motor evoked potential was impaired (less excitable) in their patient group, compared to the healthy controls. The slope of the recruitment curve is often reduced in conditions where there is damage to the corticospinal tract, and is used as a surrogate marker of corticospinal tract integrity (Devanne et al., 1997, Ridding and Rothwell, 1997, Boroojerdi et al., 2001). Thus, a less steep recruitment curve in the study by Bernabeu suggests impaired corticospinal tract integrity in their TBI group.

1.8.7 Interhemispheric inhibition

At the time of designing the studies in this thesis, no studies had investigated interhemispheric inhibition in traumatic brain injury acutely. A study by Takeuchi and colleagues in the chronic phase (mean duration post traumatic brain injury 22 months) had reported less interhemispheric inhibition in traumatic brain injury patients compared to controls (Takeuchi et al., 2006). In this study, motor thresholds were not elevated in the traumatic brain injury group.

This would be in keeping with the findings by Chistyakov and colleagues who have reported normalisation of motor thresholds three months following injury (Chistyakov et al., 1998).

1.8.8 Intracortical inhibition

Lapitskaya and colleagues demonstrated impaired intracortical inhibitory measures in their group of patients with disorders of consciousness (Lapitskaya et al., 2013). However, the findings of this study may also have been affected by the inclusion of patients with additional structural abnormalities of the brain (the presence of hydrocephalus in 7/19) and the heterogeneity of the group studied (including stroke, subarachnoid haemorrhage and traumatic brain injury). It is also difficult to interpret the findings in the context of the use of neuromodulatory drugs at the time of testing (selective serotonin receptor antagonist use in 2/19 drugs not specified, Methylphenidate use in 2/19, Sodium Valproate and Clonazepam combination in 2/19). Methylphenidate and some selective serotonin receptor antagonists are known to affect physiological parameters (Ziemann, 2004). This makes the results more difficult to interpret.

At the time of developing this study, only one study had investigated short intracortical inhibition in the chronic phase after a single brain injury (Fujiki et al., 2006). This study investigated short intracortical inhibition, intracortical facilitation (ICF) and short latency afferent inhibition (SAI) to investigate motor cortex excitability in patients with diffuse axonal injury with memory impairment. Although the findings suggested that short intracortical inhibition was less pronounced in the traumatic brain injury group than controls, these results were not statistically significant (Fujiki et al., 2006). However, these patients were not on any neuromodulatory medication at the time of investigation (although Donepezil was investigated as part of the study). In addition, the patients had no sensory or motor deficit at the time of testing.

The studies to date that have examined the physiological function of traumatic brain injury patients have been few. Where findings have been observed, they have not been reproduced in other studies. In addition, studies have included patients on neuromodulatory medication

(Chistyakov et al., 1998, 2001, Bernabeu et al., 2009, Lapitskaya et al., 2013). It is therefore difficult to interpret their findings in the context of the use of drugs which are known to affect transcranial magnetic stimulation parameters. In the studies by Takeuchi and colleagues and Fujiki and colleagues, the patients were not on such medications, however, there are no studies to directly compare findings (Takeuchi et al., 2006, Fujiki et al., 2006). Some of the previous traumatic brain injury studies were undertaken early in the traumatic brain injury disease course (less than six months post injury) and included patients with residual hemiparesis (Chistyakov et al., 1998, Bernabeu et al., 2009).

1.9 Investigating behavioural consequences following traumatic brain injury

1.9.1 Impairment of co-ordinated movements after traumatic brain injury

Activities of daily living such as dressing and feeding, require bimanual coordination (Bangert et al., 2010). Age-related decline in bimanual coordination is well described, and depends on the type of bimanual task performed (Stelmach et al., 1988, Swinnen et al., 1998, Bangert et al., 2010, Fling et al., 2011a). Older adults have been found to show slower movement execution and breakdown in coordination, particularly in asymmetric movements (Stelmach et al., 1988, Swinnen et al., 1998). Agenesis of the corpus callosum, or acquired damage to the pathways impairs bimanual motor performance, especially in asynchronous tasks (Serrien et al., 2001). It has been mentioned that persistence of clumsy and slow performance is common after traumatic brain injury especially in tasks where collaboration and coordination between the hands is required, although the underlying reasons for this are not well understood (Stuss et al., 1989, Caeyenberghs et al., 2011a).

1.9.2 Bimanual control in the existing literature

Bimanual control has already been studied through existing literature. In humans, self-paced (internally guided) bimanual tapping has been found to involve projections from the supplementary motor area to the anterior corpus callosum (Roland et al., 1980, Stephan et al., 1999). This has been corroborated in non-human primates, where lesions to the mesial frontal

cortex have been found to impair self-initiated movement (Kazennikov et al., 1998). The response to an external stimulus has been speculated to arise from visuospatial projections to the corpus callosum, thus proposing bimanual coordination involves the anterior and posterior corpus callosum (Eliassen et al., 2000). More recent literature has corroborated the complexity of bimanual coordination, with a widespread brain activation pattern identified on functional neuroimaging during the execution of such bimanual movements, which includes the primary sensorimotor cortex, supplementary motor area, premotor cortex, cingulate motor cortex, lateral premotor cortex, basal ganglia, inferior parietal cortex, cerebellum and subcortical structures (Sadato et al., 1997, Jancke et al., 2000a, Kermadi et al., 2000, Deiber et al., 2001, Immisch et al., 2001, Debaere et al., 2004, Witt et al., 2008). It is thought that interhemispheric transfer through the corpus callosum plays a major part in the coordination of this integrated behaviour, mediated through these excitatory and inhibitory transcallosal communications between the motor cortices. The transcallosal fibres are thought to connect homologous cortical regions of the right and left hemispheres (Aboitiz, 1992). Such bimanual coordination relies on precise timing, which requires efficient interhemispheric communication (Caeyenberghs et al., 2011a). Good bimanual control is therefore thought to be dependent on efficient interhemispheric communication through the corpus callosum, although other areas are involved. As previously mentioned, MRI studies have already demonstrated the corpus callosum to be particularly vulnerable to traumatic brain injury (Aoki et al., 2012, Farbota et al., 2012, Hulkower et al., 2013, Hellyer et al., 2013). Assessing bimanual coordination across the corpus callosum would therefore be a suitable method of evaluating behaviour in the traumatic brain injury group.

1.9.3 Bimanual movement following callosotomy

The role of the corpus callosum in the coordination of bimanual movement has been explored through studies on patients who have previously, or have partial or complete callosotomy, for example, as part of management for intractable epilepsy. Previous work in functional imaging has demonstrated the complexity of networks involved in bimanual movement. Therefore, the assessment of bimanual coordination in callosotomy patients has provided an elegant means of determining which aspects of bimanual coordination are dependent on the corpus callosum.

Bimanual coordination deficits have been demonstrated in callosotomy patients (Tuller et al., 1989, Ivry et al., 1999, Eliassen et al., 2000, Kennerley et al., 2002, Sternad et al., 2007). However, callosotomy patients demonstrate the ability to maintain a degree of bimanual synchronous movement in finger tapping tasks. Synchronous movement in a repetitive tapping task has been shown to be less variable than unimanual movement in callosotomy patients, thus proposing a subcortical mechanism for simultaneous movement (Tuller et al., 1989, Ivry et al., 1999). However, asymmetrical movement has been consistently found to be impaired (Franz et al., 1996, Eliassen et al., 2000, Kennerley et al., 2002). This has led to the proposal that more complex bimanual coordination, such as asymmetric bimanual movement, may rely on interhemispheric control. However, the work by Eliassen and colleagues also demonstrated a three times increase in variability of reaction time in the simultaneous movement in callosotomy patients, compared to healthy controls (including non-surgical patients who have epilepsy), thus proposing some callosal interaction is needed for a bimanual simultaneous movement. The study by Eliassen and colleagues also demonstrated that the average performance in the reaction time task that tested simultaneous bimanual movement and unimanual movement was comparable between the two groups, despite the differences in variability between the patient and control groups. (Eliassen et al., 2000). Inferences from studies of this size (n = 3 callosotomy patients) are difficult, however, they provide evidence that bimanual coordination involves the corpus callosum. Later work by Kennerley and colleagues on a different group of callosotomy patients (n = 3) explored the relationship between the coordination of bimanual movement and the corpus callosum further. Using an elegant combination of tasks that involved symmetrical and asymmetrical circle drawing, and a discrete tapping task, increased variability of the asynchronous tapping and asymmetric circle drawing was observed. Their findings concluded synchronisation of hands in continuous movements reflect communication across the corpus callosum (Kennerley et al., 2002).

1.9.4 Bimanual movement in older adults

Work by Stelmach and colleagues demonstrated that older adults exhibited breakdown in coordination and slower movement execution times than younger adults, especially in the asymmetric condition (Stelmach et al., 1988). Work in cohorts of older patients has

demonstrated age-related bimanual coordination difficulties (continuous and discrete movements) relate to a self-reported executive dysfunction questionnaire (Bangert et al., 2010, Fling et al., 2011b). In addition, a relationship was demonstrated between the most difficult bimanual circling condition and a measure of working memory (Bangert et al., 2010). It was proposed that this was because older adults had difficulty recruiting executive strategies, and demonstrates the complexity of the networks involved in bimanual coordination. The same study demonstrated the "bimanual advantage", where the variability of the simultaneous bimanual task was lower than the unimanual task. This was found to be preserved in the older adults. The increase in variability with increasing callosal interaction suggests the corpus callosum is involved in the difference in variability in bimanual coordination between older and younger adults (Bangert et al., 2010).

Further work by Fling and colleagues comparing older to younger adults has demonstrated that older adults are disproportionately impaired at bimanual tasks compared to younger adults (Fling et al., 2011a). The tapping conditions described in Fling's work demonstrated a range of interhemispheric interactions (Fling et al., 2011a). The interhemispheric interactions assessed were simultaneous bimanual (low interhemispheric interaction), right hand unimanual (moderate interhemispheric interaction), left hand unimanual (moderate interhemispheric interaction), right leads left bimanual (high interhemispheric interaction), left leads right bimanual (high interhemispheric interaction). Bimanual coordination in further work has also been related to structural integrity of the corpus callosum, with better microstructure being related to better performance (Fling et al., 2011a, 2012b). However, the group have also suggested that there is a fundamental shift with age in these relationships as the opposite relationship was evident in the younger group. It has therefore been proposed that a poorer callosal tract structure causes an alteration in the balance of interhemispheric excitation and inhibition, thus causing the age-related changes observed (Fling et al., 2011a).

Studies utilizing diffusion tensor imaging-based fibre tractography have demonstrated the relationship between callosal microstructural integrity and task performance in healthy subjects (Johansen-Berg et al., 2007). However, work by Johansen-Berg and colleagues in a

slightly different task, which involved an asynchronous thumb opposition movement, demonstrated a strong relationship between corpus callosal microstructure and simultaneous bimanual tapping, which is otherwise believed to employ the lowest levels of interhemispheric inhibition (Johansen-Berg et al., 2007). A relationship between fractional anisotropy of the anterior corpus callosum and bimanual finger opposition movements in patients with multiple sclerosis has also been demonstrated (Bonzano et al., 2008). This raises the exciting possibility of establishing a relationship between structure and function.

1.9.5 Bimanual movement following traumatic brain injury

Work by Caeyenberghs and colleagues in adult patients with traumatic brain injury has demonstrated elevated movement time, with poorer performance in daily living tasks (unilateral tasks - pick up and move a jar, pick up a pitcher and pour water into a glass, handle coins, and bimanual tasks – open a jar and take a spoon of coffee, unlock a lock, open a pill container, write on an envelope and stick on a stamp, shuffle and deal playing cards, put a scarf around the neck), reduced peg insertions (Purdue Pegboard Test) and slower responses in switching directions in a circular motion task in patients following traumatic brain injury, compared to controls (Caeyenberghs et al., 2011a). When separating the corpus callosum into seven regions (prefrontal, premotor/supplementary motor, primary motor, primary sensory, parietal, temporal and occipital), they have also demonstrated elevated radial diffusivity and lower fractional anisotropy in three regions of the corpus callosum (prefrontal, primary sensory and parietal region), with a strong relationship with a bimanual circular movement switching task (Caeyenberghs et al., 2011a). Work by the same group in adolescents, has demonstrated correlations between measures of white matter integrity and motor tasks, ball skills and cerebellum, manual dexterity and cerebellum, balance and corticospinal tract. However, these relationships were not replicated in their control group. In the presence of reduced fractional anisotropy in these respective areas, these findings suggest that poor white matter integrity is related to poorer performance following traumatic brain injury (Caeyenberghs et al., 2011b).

From these studies, it has been concluded that performance in such tapping tasks is linked to the interhemispheric interactions across the corpus callosum. Therefore, assessing bimanual

coordination would provide a behavioural measure involving the corpus callosum, and therefore increase understanding regarding the behavioural consequences following traumatic brain injury.

1.10 Thesis overview

To summarise, this thesis investigates the physiological and behavioural consequences of traumatic brain injury. These areas are less well studied in the traumatic brain injury literature, but will help understand the devastating functional consequences encountered by sufferers following their injury. This, in turn, would help to identify and address its neurorehabilitation needs. Through this series of investigations, I have developed simple studies in healthy participants, which have then been used to investigate the patient group. Through these studies, I have looked to identify the physiological abnormalities encountered after traumatic brain injury. I have then used the findings from the physiological study to identify the area of interest for the behavioural study. Specifically, I hypothesised that:

1. Traumatic brain injury would be associated with impaired physiological function of the long white matter pathways, rather than the short (intracortical) pathways. This would reflect the nature of the shearing injury. This is discussed in more detail in Chapter 4.
2. Traumatic brain injury would be associated with poorer performance in a reaction time task. I expected this to manifest in impaired reaction time and increased variability of performance. This is discussed in more detail in Chapter 6.
3. Traumatic brain injury would be associated with abnormal diffusion tensor imaging structural metrics to reflect the diffuse nature of the injury. This is discussed in more detail in Chapter 7.

In Chapter 2, I introduce the methodological techniques implemented to investigate the physiological and behavioural consequences of brain injury.

In Chapter 3, I investigate a selection of white matter tracts using transcranial magnetic stimulation in 34 healthy participants. I identified potential areas of interest to study, in the

context of common areas affected by traumatic brain injury, and determined whether these pathways could be tested with validated protocols using transcranial magnetic stimulation.

In Chapter 4, I investigate the physiology of the white matter pathways in 17 patients with traumatic brain injury, and compare these results to healthy controls. Furthermore, I use these findings to determine an area which could be explored in more detail from a behavioural perspective.

In Chapter 5, I investigate how a behavioural measure could be assessed, in light of the physiological findings from the previous traumatic brain injury study. I then develop a study on bimanual coordination, which is tested on 29 healthy participants.

In Chapter 6, I use findings from Chapter 5 to undertake a study which is tested on the 17 traumatic brain injury patients, and compared to the healthy controls.

In Chapter 7, I assess the basic diffusion tensor imaging metrics for the traumatic brain injury group, and compare to healthy controls.

In Chapter 8, I explore a relationship between physiology and microstructure, and behaviour and microstructure.

Finally, in Chapter 9, I discuss the implications of this work and insight from the different lines of enquiry presented in this thesis.

1.11 Acknowledgement of contributions

I gratefully acknowledge Dr M Hamada's guidance in pre-programming the scripts for the required transcranial magnetic stimulation protocols used in Chapters 3 and 4. I thank Dr J Galea for programming the MATLAB protocols used in Chapters 5 and 6. I thank Dr S Jilka and Dr L Li who retrieved the brain images which had been pre-acquired from the Imperial College traumatic brain injury database, and ran through the programmed scripts to obtain the raw diffusion tensor imaging metrics used in Chapters 7 and 8.

This thesis intends to increase our limited understanding of the consequences of network breakdown in brain injury. This in turn will help to develop further work to improve assessment tools and targeted neurorehabilitation for long term care for these patients.

Chapter 2

General Methods

2.1 Introduction

This chapter outlines the information about the participants for the studies included in this thesis. It introduces the methodological techniques implemented in this thesis to investigate the physiological and behavioural consequences of brain injury. It also summarises the analytical tools utilised in the studies presented in this thesis.

2.2 Participants

2.2.1 Healthy participants

The healthy participants for all studies (presented in Chapters 3, 4, 5, 6, 7, 8) were recruited from a database of healthy volunteers from UCL Institute of Neurology and Imperial College. The healthy participants for the transcranial magnetic stimulation (TMS) study (Chapter 3) and the behavioural study (Chapter 5) were two different groups of healthy participants, although some people participated in both studies. The group of healthy controls for the studies presented in Chapters 4, 6, 7, 8 were selected on the basis of their participation in the existing MRI DTI research with Imperial College, and absence of contraindications for TMS studies. The healthy control group was matched for sex, with the patient group. All healthy participants had no history of significant medical or psychiatric illness. In particular, they had no known history of head injury. They had no known physical disability. They were not on any regular medication.

2.2.2 Patient recruitment

All patients had sustained an impact injury to the brain on one occasion (from a road traffic accident, sports injury or assault) causing a loss of consciousness. Patients were all recruited at least one year following their injury. Patients for the traumatic brain injury physiological study

(Chapter 4), traumatic brain injury behavioural study (Chapter 6) and structural analysis (Chapters 7 and 8) were part of a database of traumatic brain injury patients recruited from brain injury units across London. These patients were already part of the Imperial College database, so had already participated, or were also going to participate in traumatic brain injury research as part of the MRI study conducted by Imperial College. The centres were Charing Cross Hospital, St Mary's Hospital, The National Hospital for Neurology and Neurosurgery, the Royal Free Hospital and the Regional Neurological Rehabilitation unit at the Homerton Hospital. These patients had either been directly referred to the respective trauma centre following their injury, or to Neurology clinics in the respective centres. Recruitment of traumatic brain injury patients to the Imperial College database was coordinated through Neurology Out-patient clinics where these individuals were being treated for ongoing neurological symptoms. All patients had been treated conservatively after their traumatic brain injury and had no neurosurgical intervention as part of the management of their brain injury. They were on no neuromodulatory (such as antidepressant or anticonvulsant) medications at the time of participating in the studies undertaken in this thesis. The same group of patients participated in the traumatic brain injury transcranial magnetic stimulation study (Chapter 4), traumatic brain injury behavioural study (Chapter 6) and their structural imaging was used for analysis (in Chapters 7 and 8).

2.2.3 Patient Characteristics

This is summarised in Table 2.1.

Table 2.1: Patient Characteristics

No	Age* (in years)	Sex	Mode of injury	TBI Classification**	Duration since injury (years)***	Duration since DTI imaging (years) ****	Imaging following injury*****	Other Medical History	Medication
1	40	M	Sports accident	Moderate - severe	6.4	3.25	CT: cerebral oedema and contusion	None	None
2	60	M	Fall	Mild (probable)	2.7	1.1	MRI: No significant findings	Raised cholesterol	Simvastatin
3	36	F	Road traffic accident	Mild (probable)	1.3	1.1	MRI: No significant findings	None	None
4	22	M	Sports accident	Mild (probable)	1.3	1.1	MRI: No significant findings	None	None
5	41	F	Road traffic accident	Moderate - severe	6	2	MRI: Diffuse axonal injury and frontal contusion	None	None
6	46	F	Road traffic accident	Mild (probable)	2	1.1	MRI: No significant findings	None	None
7	60	M	Fall	Mild (probable)	4.8	1.1	No imaging at time of injury.	Raised cholesterol	Simvastatin
8	37	M	Road traffic accident	Moderate - severe	4.8	0.5	CT: No significant findings	None	None
9	47	M	Assault	Moderate - severe	3.6	0.25	CT: Global sulcal effacement. No definite fracture	None	None
10	38	M	Assault	Moderate - severe	2.1	1.1	CT: Skull fracture	None	None
11	53	M	Road traffic accident	Moderate - severe	5.7	3.6	MRI: Frontal contusion, diffuse axonal injury, skull fracture	None	Ramipril, Amlodipine
12	25	F	Road traffic accident	Moderate - severe	1.4	0.92	CT: brain swelling	None	None
13	57	F	Road traffic accident	Mild (probable)	1.1	0.92	MRI: No significant findings	Diabetes, Hypertension, Raised cholesterol	Metformin, Gliclazide, Atenolol, Lisinopril, Aspirin, Bendroflumathiazide, Vitamin D, Simvastatin
14	49	F	Road traffic accident	Moderate - severe	7.5	0.33	CT: small left temporoparietal contusion	None	None
15	37	M	Road traffic accident	Moderate - severe	1.5	0.1	CT: Bifrontal contusion. Skull fracture	Hypertension	Amlodipine, Losartan, Lansoprazole
16	39	M	Road traffic accident	Moderate - severe	5	2.75	MRI: diffuse axonal injury	None	None
17	51	F	Road traffic accident	Moderate - severe	5.7	4	MRI: Punctate haemosiderin deposits consistent with the effects of diffuse axonal injury	None	None

*age at time of testing
**based on The Mayo Classification System for traumatic brain injury
***duration since injury at the time of TMS/behavioural testing
****duration between DTI imaging and TMS/behavioural testing
*****most detailed imaging modality around time of injury

2.3 Classification of traumatic brain injury severity

The Mayo Classification System for traumatic brain injury severity was used to classify the traumatic brain injury for each patient. This is assessed on the basis of multiple criteria for each classification, including lowest acute Glasgow Coma Scale score in the first twenty four hours, presence and duration of post traumatic amnesia, presence of skull fracture, and evidence of neuro-radiological abnormalities including intracerebral haematoma, subdural haematoma, epidural haematoma, cerebral contusion, and haemorrhagic contusion, penetrating injury (dura penetrated), subarachnoid haemorrhage, brainstem injury (Malec et

al., 2007). This system has already been developed to classify traumatic brain injury and determine the severity of the injury for the purpose of planning post-acute clinical care (Malec et al., 2007).

The Glasgow Coma Scale score and duration of post – traumatic amnesia was already recorded on the Brain injury database held at Imperial College, where it was available. These measures had already been acquired from the available recorded assessments from the original injury, post traumatic amnesia in the post – injury period, and retrospective self-reported assessment of memory loss in the immediate period following the injury. The duration of post traumatic amnesia recorded on the existing database had been defined as the interval between the injury and the patient regaining continuous memory for day to day events. Existing research had already demonstrated that this correlates highly with prospectively acquired assessment of post traumatic amnesia (McMillan et al., 1996). The MRI criterion used for classification were also already recorded on the database, and had been obtained through the evaluation of the imaging by a single experienced clinical neuroradiologist, who had extensive experience in neuroimaging after traumatic brain injury.

The Mayo Classification System determined three main classifications including Moderate - Severe (definite) traumatic brain injury, Mild (probable) traumatic brain injury, or Symptomatic (possible) traumatic brain injury. The classification of moderate - severe traumatic brain injury for a patient required the presence of one or more of the following criteria: loss of consciousness for 30 minutes or more; post traumatic anterograde amnesia of 24 hours or more; lowest Glasgow Coma Scale full score in the first 24 hours of less than 13, in the absence of intoxication or sedation; one or more of intracerebral haematoma, subdural haematoma, epidural haematoma, cerebral contusion, and haemorrhagic contusion, penetrating injury (dura penetrated), subarachnoid haemorrhage, brainstem injury. Although, death due to traumatic brain injury would also place a patient in the moderate to severe category, it was naturally not relevant to these research studies. The classification of mild (probable) traumatic brain injury required none of the above criteria to apply, but the presence

of one or more of the following criteria: loss of consciousness of less than 30 minutes; post traumatic amnesia of less than 24 hours; the presence of depressed, basilar or lineal skull fracture with intact dura. A symptomatic (possible) traumatic brain injury required none of the above criteria to apply, and at least one of the following symptoms to be present: blurred vision, confusion, feeling dazed, dizziness, focal neurological symptoms, headache, or nausea (Malec et al., 2007).

2.4 Institutional and ethical approval

The information sheets and protocols used in the studies at the Institute of Neurology site were reviewed and ethical approval was obtained from the South London Research Ethics Committee. Research and Development approval was obtained from UCL R&D department. The MR data that was accessed as part of this thesis had been obtained under the ethical approval of the Hammersmith Research Ethics Committee.

For all studies, participants gave written consent. All studies were performed in accordance with the Declaration of Helsinki. Participants were allowed to decline further participation at any point of the study. They were also assured that if they declined further participation, it would not have impact on their future medical care.

2.5 Clinical examination of traumatic brain injury patients

A neurological clinical examination was undertaken by a neurologist (MB) on initial meeting. This determined whether there was any evidence of subtle neurological deficit, such as a visual field defect or inattention. The muscle power was assessed using the Medical Research Council (MRC) muscle power grading system, sensory testing and coordination were also

assessed. The purpose of this assessment was twofold, for a baseline assessment in the event of a medical emergency during the testing sessions. In addition, the neurological assessment was to determine whether there were any deficits that would need taking into account when interpreting the respective results of the investigations.

2.6 Medical Emergency Procedure

There were safety protocols in place with a responsible First Aid officer. An additional physician was always within the TMS department during established testing sessions to provide additional medical help, in the event of an emergency situation.

2.7 Methodological techniques implemented in this thesis to investigate the physiological and behavioural consequences of brain injury

2.7.1 Electromyography (EMG)

Participants were seated on a comfortable chair. For all experiments, the first dorsal interosseous (FDIO) muscle was used. Surface EMG from the first dorsal interosseous of the respective hand being tested, was recorded using silver disc surface electrodes in a belly to tendon montage. The negative electrode was placed over the muscle belly of the first dorsal interosseous muscle. The positive electrode was placed over the first metacarpophalangeal joint. The ground electrode was placed at the dorsum of the wrist.

The raw EMG signal was amplified with a D360 amplifier (DigitimerD360; Digitimer Ltd, UK). Each signal response was sampled at 5 kHz and digitized using a laboratory interface (CED

1401 laboratory interface; Cambridge Electronic Design, Cambridge, United Kingdom). They were collected using SIGNAL software (Cambridge Electronic Design, Cambridge, United Kingdom). This was stored for data analysis.

The measure of EMG signals in all TMS studies was peak to peak amplitude of MEP of individual trials using a customised script for Signal software and exported to Excel. Cursors were manually placed at the peaks and the amplitude was measured by a pre-programmed customised script. For each measurement, the raw MEP value was obtained. In addition, the peak to peak amplitude of the conditioned MEP was also normalised against the peak to peak amplitude of the test MEP. Within each experimental condition, outliers (defined as > 2 standard deviations from the mean) were removed.

The compound motor action potential (CMAP) was obtained by supramaximal peripheral stimulation of the ulnar nerve at the wrist using the Digitimer Peripheral Stimulator. Bipolar surface electrodes were used with the cathode pointing distally. The maximal compound motor action potential was recorded by gradually increasing the stimulus intensity until the CMAP amplitude showed no further increase. This was recorded for off-line analysis. Further details are available in the relevant chapter.

2.7.2 Transcranial Magnetic Stimulation (TMS)

Transcranial magnetic stimulation was performed with one or two (for the single-pulse and interhemispheric inhibition paired-pulse protocols, respectively) Magstim 200^2 stimulators (Magstim Company, Dyfed UK). For both the interhemispheric inhibition (IHI) protocols, two figure of eight coils were used. For the short interval intracortical inhibition (SICI) protocol transcranial magnetic stimulation was performed with two Magstim 200^2 stimulators connected to a coil through a Bistim module. The stimulator(s) for the respective experiments were connected to the respective figure of eight coil with a 70mm internal wing diameter.

For all TMS experiments each participant was seated comfortably in an armchair. They were asked to remain as still as appropriate. They were advised to indicate to the investigator if the experiment was uncomfortable. The investigator held the stimulator coil(s) standing behind the seated participant. The coil was placed tangentially to the scalp. The handle pointed postero-laterally at a 45^{o} angle to the sagittal plane, which is a standard coil orientation for standard transcranial magnetic stimulation studies. This induces a posterior - anterior current in the brain. This coil orientation was chosen to enable the lowest motor threshold to be achieved with the induced electrical current flowing approximately perpendicular to the line of the central sulcus (Werhahn et al., 1994, Sakai et al., 1997).

Prior to determining the mean threshold, the “motor hotspot” was located with the coil on the M1 contralateral to the examined limb. The coil was then moved at 0.5cm intervals in the anterior-posterior and then medio - lateral direction around the presumed motor hand area of the cortex of both hemispheres. The starting point for the presumed area was 5cm lateral and 1cm anterior to the mid – point between the nasion to inion, and tragus to tragus. This was to determine the optimal position (the “motor hotspot”) for activation of the respective first dorsal interosseous muscle. The site where stimulation of a slightly suprathreshold intensity consistently produced the largest and stable MEPs in the corresponding first dorsal interosseous muscle was referred to as the “motor hotspot”. This position was marked with a red marker pen by drawing a crescent following the anterior bifurcation of the coil and a straight line indicating the orientation of the coil. This was to assist with consistently repositioning the coil through the experimental procedures.

Resting motor threshold (RMT) was defined as the minimum stimulus intensity that produced a MEP of at least 50μV in 5 out of 10 consecutive trials (Rossini et al., 1994). Active motor threshold (AMT) was determined using voluntary tonic contraction at 10% of maximal voluntary contraction (MVC) and was defined as the minimum intensity to produce a MEP of at least 200μV in at least 5 out of 10 trials (Rossini et al., 1994). 1mV MEP was defined as the minimal

stimulator intensity to produce a 1mV MEP in at least 5 out of 10 trials. Motor thresholds were expressed as a percentage of maximum stimulator output (MSO). All TMS protocols used were accordance with published safety recommendations (Wassermann, 1998) and have been discussed further in the relevant chapters.

2.7.3 Behavioural experimental technique

Participants in both the healthy participant study and the TBI studies (Chapters 5 and 6) performed a finger tapping experiment to assess reaction time in a variety of conditions. Participants sat facing a computer screen positioned approximately 30 cm away. They were instructed to rest their left and right index fingers on the two buttons that corresponded to the respective finger, and to maintain this position throughout the task. For each block, this reaction time task required the participants to respond to the presentation of a visual cue on a screen by pressing the corresponding button with the required index finger, in response to the cue. The cue was presented on a desktop computer running MATLAB (MathWorks, USA). In each study, participants performed finger-tapping conditions, defined by the conditions documented in the studies of interhemispheric communication in young and older adults by Bangert and colleagues and Fling and colleagues (Bangert et al., 2010, Fling et al., 2011a).

Participants were first introduced to the testing equipment and trained prior to formal testing. This allowed participants to familiarise themselves with the different tapping conditions, how they were presented on the computer screen and which button corresponded to which finger, left index and right index respectively. Each participant was taught how to execute the bimanual finger movement, required for the two out of phase states (left following right, right following left), where the fingers were required to move 180^0 out of phase. This was through a demonstration by the assessor, with the participant then demonstrating the movement. They were then given feedback.

Participants were initially presented with a written instruction on the command screen at the start of each training block, which confirmed the action required (Figure 2.1). This was reinforced with verbal instruction of what the testing block involved, to ensure understanding. The verbal instruction was read from a pre-prepared instruction sheet, to ensure consistency of instruction to the participants. Participants were asked to focus on a fixation cross (+) in the centre of the computer screen. They were presented with a “get ready” (!) icon. 200 ms after the presentation of the “get ready” (!), the cue (a square) would then appear on the screen, indicating that they should press the corresponding key. For example, during the left hand unimanual tapping, the square would appear on the screen at the participant’s left. The cue would present ten times in each testing block at a target rate of 1Hz. Participants were instructed to not use the fixation cross (+) or exclamation mark (!) as a cue to tap, they were to only use the square as the cue to tap. They were also asked to react (i.e. not tap in synchrony) to the cue, as a measure of responsivity to the cue. There was a brief pause between each testing block, prior to initiating the next testing block. The formal testing session was then commenced.

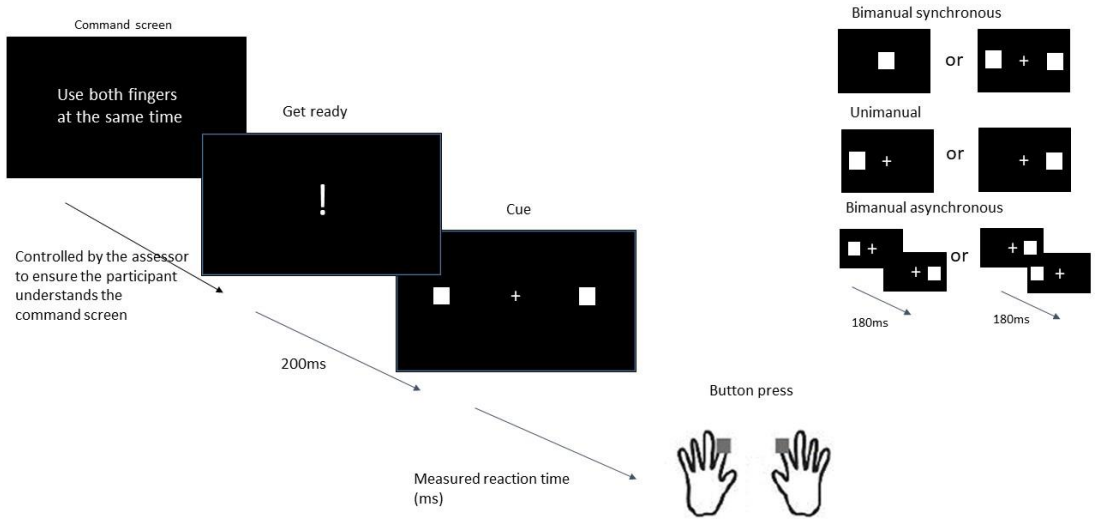


Figure 2.1 Reaction time task

The formal testing has been discussed in further detail in the relevant chapters.

2.7.4 Clinical imaging

The MRI data for the control and patient groups were obtained as part of the larger TBI study at Imperial College, and under the ethical approval of the respective study. The participants in the control and patient groups had already participated in, or were going to participate in TBI MRI research with the team at Imperial College.

The MRI images were obtained using a Phillips Intera 3.0 - Tesla MRI scanner using a phased – array head coil, and sensitivity encoding with an undersampling factor of 2. Using an 8-array head coil and sensitivity encoding (SENSE) with an under sampling factor of 2 (as described in Hellyer et al., 2013). For each participant, diffusion-weighted volumes with gradients applied in 16 non-collinear directions were collected in each of four DTI runs. This resulted in a total of 64 directions. The following parameters were used: 73 contiguous slices, slice thickness=2mm, field of view 224mm, matrix 128×128, voxel size 1.75×1.75×2mm^3, b value=1000 and four images with no diffusion weighting (b=0s/mm^2).

The sequences collected included standard high - resolution T1 imaging. This assessed for evidence of structural abnormality, such as focal brain injury. Gradient echo (T2*) sequences were obtained to identify evidence of microbleeds. This is a marker of diffuse axonal injury (Scheid et al., 2003). The same senior consultant neuroradiologist had reviewed all study MRI scans and provided the reports for the imaging to ensure consistency in the interpretation of the scans.

This has been discussed further in the relevant chapter.

2.8 Statistical analysis

Data was analysed and statistical procedures were conducted using the statistical package SPSS version 19.0 for Windows; SPSS Inc. Numerical data were represented as mean ± SE

unless otherwise stated. For the report of the statistical differences, the significance threshold of $\alpha = 0.05$ was used. Normality of data distribution was assessed with the Kolmogorov – Smirnov test. Analysis of variance (ANOVA) was performed where appropriate. Where assumptions of sphericity were violated (where Mauchley's test $P < 0.05$), the Greenhouse – Geisser correction was applied.

Independent or paired t tests were performed when appropriate. Levene's test was used to verify that there was no significant difference in the variances of the populations from which the data samples had been drawn. All t tests were two-tailed, unless otherwise stated.

Correlation analysis was undertaken where appropriate to investigate relationships between diffusion tensor imaging metrics; physiological measures and callosal metrics; behavioural measures and callosal metrics, where appropriate.

Details of the respective t tests, ANOVA and correlation analyses are described in more detail in the respective results sections of each chapter.

Chapter 3

The Physiology of White Matter Pathways in Healthy Individuals

I led this study and was responsible for study design, recruitment, experimental work, analysis and interpretation of the study. Scripts used were standard protocol programmed by Dr M Hamada.

3.1 Introduction

Transcranial magnetic stimulation is a non-invasive method of assessing brain connectivity and is a common research tool in neurology. It has been used as a research tool since 1985 in both healthy individuals and in patients with neurological impairment (Barker et al., 1985). To date, there are no reported long-term serious consequences with transcranial magnetic stimulation. It has an established reputation as a safe technique to assess physiology within established safety guidelines (Rossi et al., 2009, Tassinari et al., 2003). Transcranial magnetic stimulation research has already provided insight into the physiological changes occurring in neurological conditions, such as stroke (Boniface et al., 1994, Murase et al., 2004, Talelli et al., 2006, Ward et al., 2006), dystonia (Quartarone, 2013, Kojovic et al., 2013), multiple sclerosis (Boniface et al., 1991 and 1994, Kern et al., 2011) and spinal cord injury (Davey et al., 1999). Transcranial magnetic stimulation has therefore demonstrated potential in helping understand the physiological consequences of neurological conditions and developing interventions to assist in clinical recovery. Therefore, it has the potential to provide information about the physiological consequences of traumatic brain injury.

Stroke and traumatic brain injury are the most common causes of focal and diffuse acquired brain injury (Greenwood et al., 2016). It is now recognised that the prevalence of stroke and traumatic brain injury is comparable. However, there is less recognition for traumatic brain injury sufferers, and far less research in traumatic brain injury than in stroke (Li et al., 2014). Attesting to this, the increase in the number of studies in traumatic brain injury over the last ten years is only a third of that in stroke (Li et al., 2014). The use of transcranial magnetic stimulation in stroke has already provided useful physiological information about the condition (Boniface et al., 1994, Murase et al., 2004, Talelli et al., 2006, Ward et al., 2006). It has already been used to obtain longitudinal physiological information following stroke (Swayne et al., 2008). In addition, the use of more complex transcranial magnetic stimulation protocols has enabled the investigation of interventions. For example, the effect of theta burst has already been investigated in healthy participants and stroke (Ackerley et al., 2010, Vernet et al., 2014,

Talelli et al., 2012). In contrast to the wealth of transcranial magnetic stimulation literature for stroke, there are very few studies that have investigated the physiological consequences after traumatic brain injury with transcranial magnetic stimulation.

The plethora of research studies using transcranial magnetic stimulation to investigate stroke suggests that it is relatively well tolerated in this patient group. However, a variety of clinical and behavioural consequences are encountered following traumatic brain injury. Therefore, at the time of developing this study, it was uncertain whether this patient group would even tolerate transcranial magnetic stimulation. Therefore, prior to assessing the physiological consequence of traumatic brain injury, a study protocol was developed by investigating white matter tracts in a large cohort of healthy individuals. The intention of this study was to develop a study protocol that could be transferred to the traumatic brain injury patient group. First, areas of potential interest to study physiologically in the patient group were identified, and the feasibility of their assessment by transcranial magnetic stimulation ensured. Second, as this patient group was also relatively new to transcranial magnetic stimulation, an effort was made to develop a physiological study that would be simple and tolerated by the patient group, yet still able to provide useful information with regards to the physiology of the brain following traumatic brain injury. Diffuse axonal injury has been seen to affect predominantly parasagittal white matter of the cerebral cortex, corpus callosum, and the pontinemesencephalic junction (Gentry et al., 1988a,b, Parizel et al., 1998, Meythaler et al., 2001, Scheid et al., 2003).

The corpus callosum is known to be particularly vulnerable to traumatic brain injury (Gentry et al., 1988a). Physiological transfer across the corpus callosum can be assessed with transcranial magnetic stimulation. Using two magnetic stimulation coils, the effects of a conditioning stimulus applied to the motor cortex of one hemisphere on the motor evoked potential elicited by a test stimulus to the opposite hemisphere can be studied as a measure of transcallosal physiology (Ferbert et al., 1992, Hanajima et al., 2001). It is proposed that the conditioning stimulus activates excitatory transcallosal fibres, which project to local inhibitory GABAergic neurons in the contralateral motor cortex (Chen, 2004, Daskalakis et al., 2002).

The interval for interhemispheric inhibition in the Ferbert protocol has supported existing research of transcallosal transfer; motor cortical stimulation has been shown to evoke an electroencephalogram potential over the contralateral motor cortex with an onset latency of 8.8 -12.2ms (Cracco et al., 1989). Differences in the onset latency of jerks in patients with cortical myoclonus are also approximately 10ms (Wilkins et al., 1984, Brown et al., 1991). For the purpose of assessing the physiology of the callosal pathways in traumatic brain injury, the interhemispheric inhibition elicited by the Ferbert protocol is thought to originate at a cortical level, mediated through transcallosal fibres (Ferbert et al., 1992). This has been challenged. However, this has also been supported by the study by Boroojerdi and colleagues who demonstrated no significant difference in interhemispheric inhibition in patients who have suffered a subcortical stroke involving the corticospinal tract, but sparing the corpus callosum (Boroojerdi et al., 1996).

These existing studies, and the established accessibility of the transcallosal pathways to transcranial magnetic stimulation investigation, made it a natural choice to include an investigation of callosal transfer in this physiological study of traumatic brain injury patients. Further to this, and to maximise the information we would be able to obtain in this relatively new patient group to transcranial magnetic stimulation research, measures of corticospinal excitability and short intracortical inhibition were also included in this study.

The purpose of this study is to investigate the physiology of white matter pathways in healthy individuals. However, transcranial magnetic stimulation studies often involve small numbers; this study aimed to incorporate a large group of participants in order to obtain robust information of physiological values that can be extrapolated to the general population. This is particularly relevant since there are reports of inter-individual variability of some common transcranial magnetic stimulation protocols. This is an interesting observation, as findings in transcranial magnetic stimulation studies are already widely used to characterise differences between patient groups and healthy volunteers in established research. For example, Wassermann found that inter-individual variation in short-interval intracortical inhibition (SICI)

was surprisingly variable with 6 of 53 people having facilitation rather than inhibition (Wassermann, 2002). Therefore, as this study incorporates a larger group of participants than standard transcranial magnetic stimulation studies, this area was also explored in this study.

3.2 Study design

3.2.1 Participants

Thirty four healthy participants consisting of 19 males and 15 females were recruited from a database of healthy volunteers from the UCL Institute of Neurology and Imperial College. None of the participants had a history of neurological or psychiatric illness, drug or alcohol abuse. They were not on any regular medication.

All participants were introduced to the study informally, prior to being given the information sheet outlining the details of the study. A health questionnaire was then undertaken to ensure that they were medically stable and were suitable for a transcranial magnetic stimulation study. Participants with contraindications to transcranial magnetic stimulation including 1) magnetic implants, pacemaker, 2) previous history of other head trauma, 3) past or current history of other neurological or psychiatric illness, 4) presence of intracerebral haemorrhage, 5) ingestion of psychotropic, sedative or anticonvulsant medication, 6) major comorbidities or clinically unstable participants, 7) participants unable to cooperate with the study session, including significant language impairments, 8) not able to give informed consent, 9) pregnancy, were not included in the study (Wassermann, 1998, Rossi et al., 2009).

An additional physician was always in the TMS laboratory to provide additional medical help, in the event of an emergency situation.

3.2.2 Institutional and ethical approval

The information sheets and protocols were reviewed and ethical approval was obtained from the Ethics research committee.

Participants gave written consent prior to participating in the study, and were given the option to withdraw from the study at any time. All studies were performed in accordance with the Declaration of Helsinki.

3.3 Experimental Methods

3.3.1 Physiological measures assessed using transcranial magnetic stimulation

The techniques for electromyographic (EMG) measurement are provided in the General Methods (Chapter 2). Transcranial magnetic stimulation was delivered using MagStim 200^2 stimulator (Magstim Co., Dyfed, United Kingdom) connected to a figure of eight coil with an internal wing diameter of 70 mm. The handle was pointing postero-laterally. EMG recordings were made using silver disc surface electrodes in a belly to tendon montage (as described in Chapter 2).

The location of the “motor hotspot” in the left hemisphere was determined (see the method described in Chapter 2). This was defined as the site where stimulation evoked the optimal motor evoked potential in the right first dorsal interosseous. This area was marked with a red pen, to assist with consistent placing of the coil. The procedure was then repeated for the “motor hotspot” in the right hemisphere.

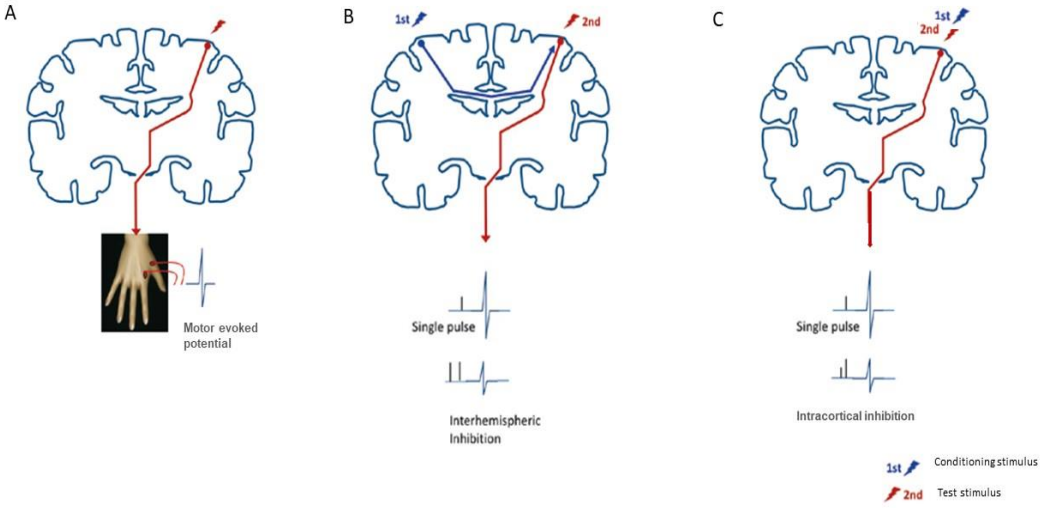


Figure 3.1 Schematic representation for TMS measurements of motor cortical physiology:

A Measuring corticospinal tract excitability by eliciting a motor evoked potential (MEP) from the primary motor cortex

B Using two TMS coils to measure interhemispheric inhibition. For the measurement of interhemispheric inhibition, the test stimulus was delivered to the primary motor cortex contralateral to the target muscle having been preceded by a suprathreshold conditioning stimulus. The interstimulus intervals investigated were 6ms, 8ms, 10ms, 12ms, 15ms, 20ms, 30ms, 50ms (modified from Ferbert et al., 1992).

C Using paired pulse stimulation through the same coil to measure intracortical inhibition. For the measurement of short intracortical inhibition, a subthreshold conditioning stimulus was delivered prior to the suprathreshold test stimulus at a variety of interstimulus intervals. The interstimulus intervals investigated were 2ms, 2.5ms, 3ms, 4ms, 6ms, 10ms, 15ms (modified from Kujirai et al., 1993).

(Diagrams adapted from Swayne, 2017).

1) Corticospinal tract integrity was first assessed by measuring the i) motor thresholds and ii) the resting recruitment curve of motor evoked potential (MEP) size versus stimulus intensity in the first dorsal interosseous.

i) The resting motor threshold (RMT) was obtained by determining the minimum stimulus intensity that produced a motor evoked potential (MEP) of at least 50μV in 5 out of 10 consecutive trials (Rossini et al., 1994). The transcranial magnetic stimulation was initiated at a subthreshold intensity of stimulation, with the coil placed over the "motor hotspot" (as in Figure 3.1A). The stimulator intensity was then gradually increased in steps of 5% maximum stimulator output (MSO) until stimulation consistently evoked MEPs with the desired peak to peak amplitude. The stimulator intensity was then gradually lowered by 1% MSO steps until the required 5 out of 10 trials were recorded. A similar method was adopted to obtain the active motor threshold (AMT). However, the transcranial magnetic stimulation was initiated at much lower stimulator intensity than the RMT. The AMT was determined using voluntary tonic contraction at 10% of maximal voluntary contraction (MVC). Initially, the participant was asked to maximally activate the first dorsal interosseous and the waveform produced represented the MVC. Cursors were then placed peak to peak, the value for 10% of that maximal contraction was calculated, and the two cursors were placed corresponding to that value. The participant was then asked to maintain tonic contraction within the cursors, and the AMT was determined using a similar method to the RMT. The AMT was defined as the minimum intensity to produce a MEP of at least 200μV in at least 5 out of 10 trials (Rossini et al., 1994). 1mV MEP was obtained by measuring the minimal stimulator intensity required to produce a 1mV MEP in at least 5 out of 10 trials. Motor thresholds were expressed as a percentage of MSO.

These values were recorded and used to calculate 110%, 120%, 130% of the RMT in the left hemisphere. 90%, 100%, 110%, 120% of the RMT was calculated for the right hemisphere. 60%, 70%, 80%, 90% of the AMT was then calculated for the left hemisphere. These values were then used to programme the software for the respective subsequent experiments.

ii) The resting recruitment curve was obtained by measuring the peak to peak amplitude of the MEP at intensities of 110%, 120% and 130% of the RMT in the left hemisphere. This range was chosen to maintain patient compliance. Ten responses were obtained for each trial, as this was common practice. Each trial was undertaken and then the peak to peak amplitude data was averaged for each state tested. This is a measure that has been used successfully in previous investigations to relate function of corticospinal connections to patterns of brain activation after stroke (Ward et al., 2006).

In addition to the raw MEP amplitude, the average MEP was additionally corrected by the compound motor action potential CMAP (MEP/CMAP) (Rossini et al., 1994). This was calculated as the evoked MEP amplitude related to the CMAP amplitude, and can be altered by central or peripheral disease of the corticomotor pathway. The ratio between the transcranial MEP amplitude and the maximal distally evoked CMAP (MEP/CMAP) is therefore felt to reflect the central mechanisms contributing to the MEP amplitude. This was also calculated as it reduces variability within participants caused by individual differences in peripheral MEP amplitude (Rossini et al., 1994).

Measurement of the CMAP involved a supramaximal stimulus being applied to the distal peripheral nerve (in this case, the ulnar nerve) supplying the target muscle using bipolar surface electrodes with the cathode pointing distally. The maximal CMAP was recorded by gradually increasing the stimulus intensity until the CMAP amplitude showed no further increase. This was recorded for off-line analysis. The peak to peak amplitude of the maximal peripheral CMAP was then used to calculate the MEP/CMAP ratio.

2) Interhemispheric inhibition - Interhemispheric inhibition was studied using a twin coil approach similar to that described by Ferbert and colleagues (Ferbert et al., 1992).

The test coil was placed over the predetermined “motor hotspot” of the left hemisphere (as shown in Figure 3.1B). The conditioning coil was placed over the predetermined “motor hotspot” of the right hemisphere. This is because the effect of the conditioning stimulus is maximal when it is applied over the hand area of the motor cortex, and decreases when the stimulus is moved medial or lateral (Ferbert et al., 1992). The test stimulus was 1mV MEP, and a value for each participant had already been obtained as part of the baseline measurements. The first experiment investigated the interstimulus interval, where the test stimulus was delivered to the primary motor cortex contralateral to the target muscle (first dorsal interosseous of the right hand) having been preceded by a suprathreshold (120% RMT) conditioning stimulus. The script was preprogramed to deliver the conditioning stimulus at a variety of interstimulus intervals. Ten responses, which were randomly intermixed, were obtained for each condition. In addition, 20 test (unconditioned) responses alone were recorded. The interstimulus intervals investigated were 6ms, 8ms, 10ms, 12ms, 15ms, 20ms, 30ms, 50ms (modified from Ferbert et al., 1992). The protocol by Ferbert and colleagues was modified in this way to cover both periods of short interhemispheric inhibition (S-IHI between 8–12 ms) and long interhemispheric inhibition (L–IHI between 30–40ms) with an additional interstimulus interval at either extreme, to account for any physiological variations that may be encountered when the patient group is later investigated (Chapter 4).

The second experiment investigated transcallosal recruitment. Using a customised script for signal software, the data was processed and the interstimulus interval at which the peak of inhibition occurred was then obtained and recorded for each individual. This interstimulus interval value in milliseconds (ms) was then inputted into the software as the constant interstimulus interval for the next experiment. Thus, the interstimulus interval was individualised for each participant. The conditioning stimulus was therefore the variable factor in the subsequent experiment to investigate transcallosal recruitment. In this experiment, a range of conditioning stimulus intensities was investigated (90% RMT, 100% RMT, 110% RMT, 120% RMT). Ten responses which were randomly intermixed, were obtained for each condition and their peak to peak amplitude recorded.

3) Short intracortical inhibition - Intracortical circuits (inhibitory and facilitatory interactions in the cortex) occurring within the M1 of the motor cortex were studied by delivering two transcranial magnetic stimulation pulses to the motor hotspot in the left hemisphere (as shown in Figure 3.1C). The transcranial magnetic stimulation was performed with two Magstim 200^2 stimulators connected to a single coil through a Bistim module. The values for the subthreshold conditioning stimulus (80% AMT) and suprathreshold test stimulus (1mV MEP) were obtained through the baseline measurements and programmed into the respective MagStim 200^2 stimulator for this experiment. For the first experiment, the measurement of short intracortical inhibition interstimulus interval was investigated. The subthreshold conditioning stimulus was delivered prior to the suprathreshold test stimulus at a variety of interstimulus intervals (2ms, 2.5ms, 3ms, 4ms, 6ms, 10ms, 15ms) (modified from Kujirai et al., 1993). Ten responses were obtained for each conditioned state. In addition, 20 responses for test (unconditioned) stimulus alone were obtained. The conditions were randomly intermixed.

In the second experiment, short intracortical fibre recruitment was investigated. Using a customised script for signal software, this data was also processed and the interstimulus interval at which the peak of inhibition occurred was then obtained and recorded for each individual. This interstimulus interval value in milliseconds (ms) was then inputted into the software as the constant interstimulus interval for the next experiment. Thus, the interstimulus interval was individualised for each participant. The conditioning stimulus was therefore the variable factor in the subsequent experiment to investigate short intracortical inhibition recruitment. For this experiment, the test intensity was set at 1mV MEP from the pre-acquired baseline measurements. A range of conditioning stimulus intensities (60% AMT, 70% AMT, 80% AMT, 90% AMT) were investigated. Ten trials from each state were intermixed randomly and their peak to peak amplitude measured. In addition, 20 responses for test (unconditioned) stimulus alone were obtained.

3.3.2 EMG data analysis

The measure of EMG signals in all TMS studies was peak to peak amplitude of MEP of individual trials using a customised script for Signal software and exported to Excel. Cursors were manually placed at the peaks and the amplitude was measured by a pre-programmed customised script. For each measurement, the raw MEP value was obtained. In addition, the peak to peak amplitude of the conditioned MEP was also normalised against the test MEP peak to peak amplitude. Within each experimental condition, outliers (defined as > 2 standard deviations from the mean) were removed.

3.3.3 Data and Statistical analysis

For the measures of corticospinal excitability, paired t tests were used to compare resting motor threshold (RMT) in the left and right hemisphere, and compare active motor threshold to resting motor threshold. 1mV MEP/RMT x 100 was calculated to obtain the intensity required to evoke a 1mV MEP relative to RMT. For each participant and stimulus intensity, the single trial peak to peak motor evoked potential amplitudes were averaged. In addition to the raw motor evoked potential amplitude, the average motor evoked potential was additionally corrected by the compound motor action potential CMAP (MEP / CMAP). One-way analysis of variance (ANOVA) was undertaken with factors “STIMULUS INTENSITY” (110% RMT, 120% RMT, 130% RMT) using absolute motor evoked potential values. A further one-way analysis of variance was undertaken with factors “STIMULUS INTENSITY” (110% RMT, 120% RMT, 130% RMT) using the absolute motor evoked potential values corrected by the CMAP, as there is evidence to suggest that this reflects the central mechanisms contributing to the motor evoked potential amplitude (Rossini et al., 1994).

For the measures of interhemispheric inhibition, one-way analysis of variance (ANOVA) was undertaken with factors “TIME” (6ms, 8ms, 10ms, 12ms, 15ms, 20ms, 30ms, 50ms) using absolute motor evoked potential values. For each individual and time point, the peak to peak motor evoked potential amplitudes were additionally averaged at each trial and normalised to

the unconditioned motor evoked potential amplitude measured at baseline. A further one-way analysis of variance was undertaken with factors “TIME” (6ms, 8ms, 10ms, 12ms, 15ms, 20ms, 30ms, 50ms) and normalised motor evoked potential amplitude values. For transcallosal recruitment, a one-way analysis of variance (ANOVA) was undertaken with factors “STIMULUS INTENSITY” (90% RMT, 100% RMT, 110% RMT, 120% RMT) using absolute motor evoked potential values, and repeated using normalised motor evoked potential values.

For the measures of short intracortical inhibition, one-way analysis of variance (ANOVA) was undertaken with factors “TIME” (2ms, 2.5ms, 3ms, 4ms, 6ms, 10ms, 15ms) using absolute motor evoked potential values, and repeated using normalised motor evoked potential values. For short intracortical inhibition recruitment, one-way analysis of variance (ANOVA) was undertaken with factors “STIMULUS INTENSITY” (60% AMT, 70% AMT, 80% AMT, 90% AMT) using absolute motor evoked potential values, and repeated using normalised motor evoked potential values.

Correlation analysis was undertaken between interhemispheric inhibition 10 (IHI 10), interhemispheric inhibition 30 (IHI 30), short intracortical inhibition 2.5 (SICI 2.5) and motor evoked potential recruitment. Further correlation analysis was undertaken between SICI 2.5 and IHI 10, SICI 2.5 and IHI 30, IHI 10 and IHI 30. Correlations were calculated by Pearson linear correlation.

Paired t tests were performed where required. Before conducting the relevant t – test, Levene’s test was first used to verify that there were no significant differences in the variances of the populations being tested. Normality of all the data sets was tested with Kolmogorov – Smirnov tests. For the isolated time points that deviated from normal, parametric statistics have been used throughout to avoid inconsistent treatment of the data at different time points (as in Swayne et al., 2008). For the report of the statistical differences, the significance threshold of

α = 0.05 was used. Where assumptions of sphericity were violated (where Mauchley's test P < 0.05), the Greenhouse – Geisser correction was applied. Post–hoc t test, Bonferroni corrected were used where appropriate. Statistical procedures were conducted using the statistical package SPSS version 19.0 for Windows; SPSS Inc. All data are given as mean ± standard error of the mean unless otherwise stated.

3.4 Results

A set of baseline data was obtained from 34 healthy participants consisting of 19 males and 15 females aged between 22 and 52 years (mean ± SE 31.1 ± 1.14). All results are mean ± SE unless otherwise stated.

3.4.1 Corticospinal excitability – Thresholds

Figure 3.2 shows the average thresholds obtained for resting motor threshold (RMT) (both hemispheres), active motor threshold (AMT) (left hemisphere) and 1mv MEP (left hemisphere) in the healthy participant group. Average RMT in the left hemisphere was (45 ± 1.66% maximal stimulator output (MSO)) and the right hemisphere (48 ± 1.73% MSO) ($t = -2.60$, $df = 33$, $p = 0.01$). As expected, given that the threshold is lower during contraction than at rest (Day et al., 1989), AMT was lower than RMT (left hemisphere: 41 ± 1.48% MSO) ($t = 5.53$, $df = 33$, $p < 0.05$). The 1mv MEP was elicited at 119 ± 1.5% RMT.

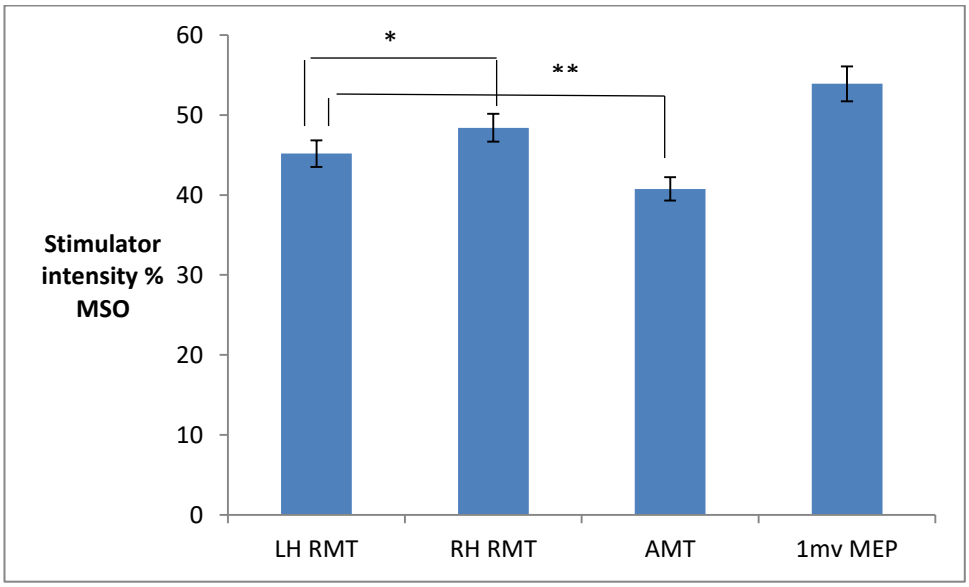


Figure 3.2: Corticospinal excitability – Thresholds. Resting motor threshold (RMT) in the left hemisphere (LH RMT), right hemisphere (RH RMT), active motor threshold in the left hemisphere (AMT) and the intensity required to evoke a 1mv MEP from the left hemisphere. Data presented as mean (+/- SE).

3.4.2 Corticospinal excitability – recruitment curve (RC)

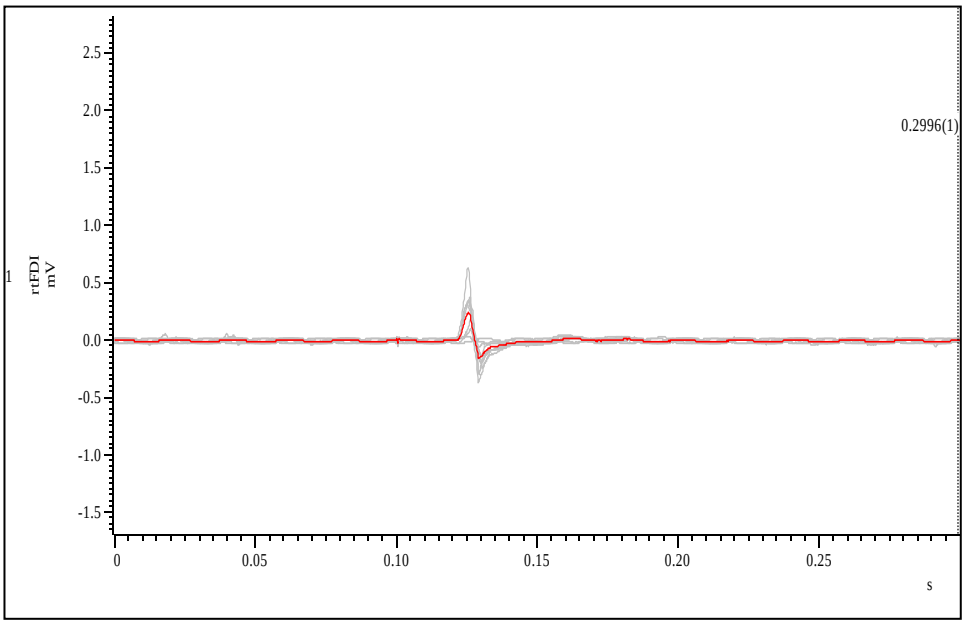
Table 3.1 gives the individual data for the raw and normalised MEPs in each participant, together with the calculated coefficient of variation (CV) for each measurement point. It is possible that some of this variation was due to the wide range in CMAP amplitudes between individuals. However, normalising to CMAP amplitude had little effect on the CV, which was approximately 40% for both sets of data. Thus, planned future analyses or correlations with behavioural and MRI data can be performed on either data set. MEPs were measured as peak-to-peak amplitude since their waveform remained relatively simple and biphasic over the range of intensities used (Fig 3.2a). As described by others (Devanne et al., 1997), the RC was relatively linear between 110- 130% RMT whether measured in terms of raw data (Fig 3.2b) or normalised to each individual's peripheral compound motor action potential (CMAP) (Fig 3.2c). However, the average data conceals a high variability between individuals.

	MEP Stimulus intensity				MEP/CMAP		
Participant number	110%	120%	130%	CMAP	110%	120%	130%
1	0.646	1.312	2.387	14.304	0.045	0.092	0.167
2	0.084	0.494	1.972	17.280	0.005	0.029	0.114
3	0.781	1.495	2.091	19.648	0.040	0.076	0.106
4	0.364	0.312	0.790	22.227	0.016	0.014	0.036
5	0.445	1.326	2.787	23.527	0.019	0.056	0.118
6	0.268	0.739	2.360	16.045	0.017	0.046	0.147
7	0.395	0.455	1.885	23.894	0.017	0.019	0.079
8	0.400	0.963	1.063	15.885	0.025	0.061	0.067
9	1.400	1.712	2.269	15.256	0.092	0.112	0.149
10	0.264	0.701	1.219	13.257	0.020	0.053	0.092
11	0.563	0.405	0.824	14.230	0.040	0.028	0.058
12	0.609	0.978	1.094	12.886	0.047	0.076	0.085
13	0.599	1.112	1.389	17.861	0.034	0.062	0.078
14	0.569	1.088	2.006	12.991	0.044	0.084	0.154
15	0.728	1.149	1.596	16.000	0.046	0.072	0.100
16	0.779	1.046	2.117	21.752	0.036	0.048	0.097
17	0.579	1.242	1.565	13.901	0.042	0.089	0.113
18	0.567	0.822	1.862	19.622	0.029	0.042	0.095
19	0.606	1.480	2.044	19.342	0.031	0.077	0.106
20	0.587	1.227	1.466	11.548	0.051	0.106	0.127
21	0.311	1.456	2.574	15.256	0.020	0.095	0.169
22	0.699	0.772	0.901	12.446	0.056	0.062	0.072
23	0.895	1.558	2.136	16.157	0.055	0.096	0.132
24	0.637	1.089	1.340	19.831	0.032	0.055	0.068
25	0.768	0.923	1.534	15.471	0.050	0.060	0.099
26	0.393	1.083	1.199	12.433	0.032	0.087	0.096
27	0.624	0.703	1.045	13.648	0.046	0.051	0.077
28	0.377	1.107	2.562	16.164	0.023	0.069	0.158
29	1.137	2.342	2.405	19.342	0.059	0.121	0.124
30	0.559	0.819	1.285	13.147	0.043	0.062	0.098
31	0.441	1.847	2.882	16.704	0.026	0.111	0.173
32	0.661	0.835	1.074	13.658	0.048	0.061	0.079
33	0.866	0.609	2.187	20.000	0.043	0.030	0.109
34	0.465	0.547	1.333	17.897	0.026	0.031	0.074
Mean	0.590	1.051	1.742	16.577	0.037	0.066	0.106
SD	0.251	0.442	0.598	3.351	0.017	0.028	0.034
SE	0.043	0.076	0.103	0.575	0.003	0.005	0.006
COV	0.426	0.421	0.343	0.202	0.449	0.420	0.323

Table 3.1: Corticospinal excitability - Recruitment curve

Individual data for the raw and normalised MEPs in each participant, together with the calculated coefficient of variation (CV) over all individuals for each measurement point.

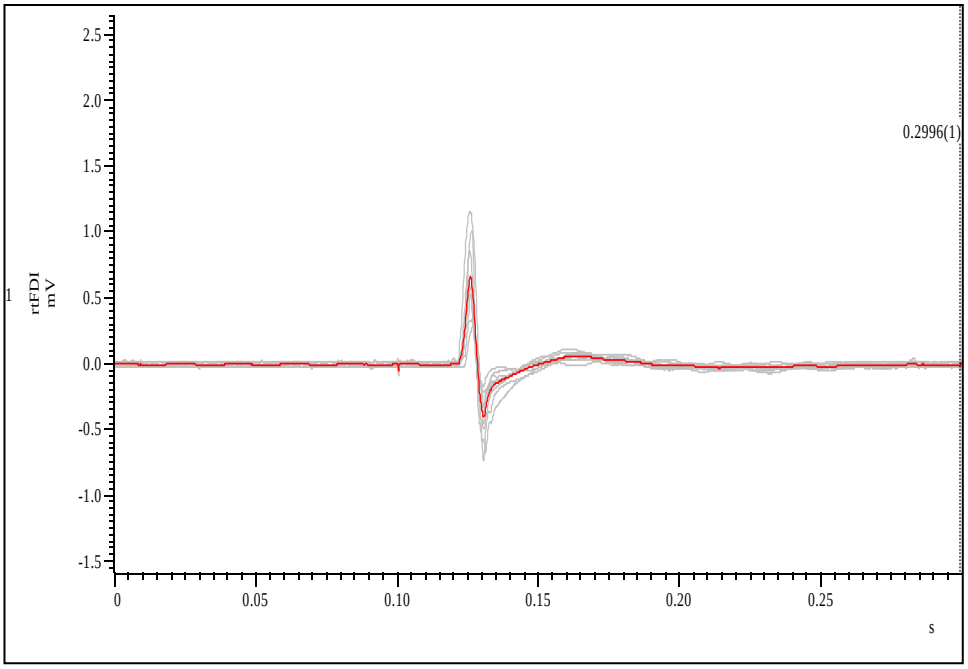
110%



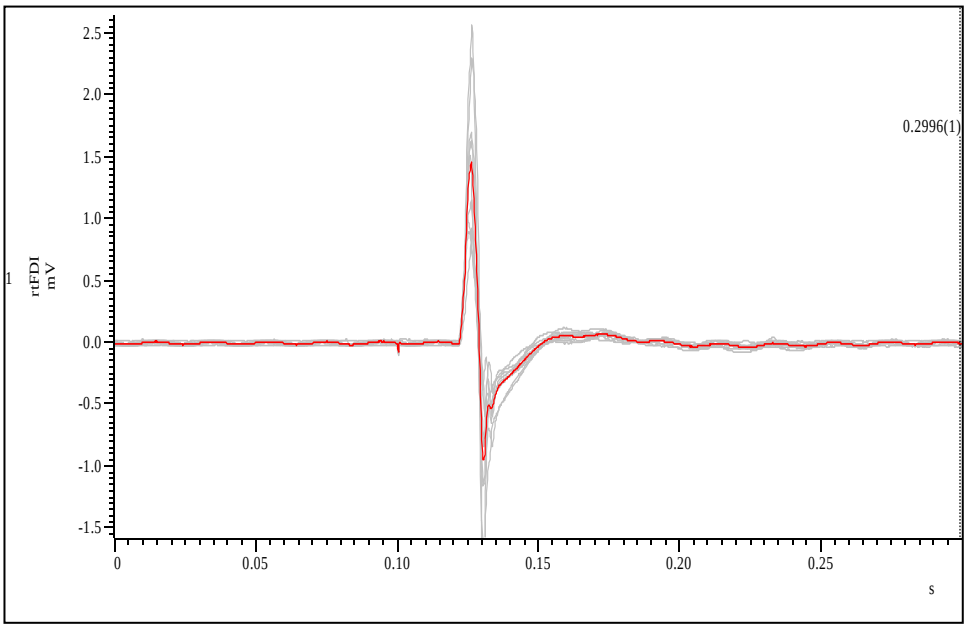
↕ 0.5 mv

↔ 50 ms

120%



130%



CMAP

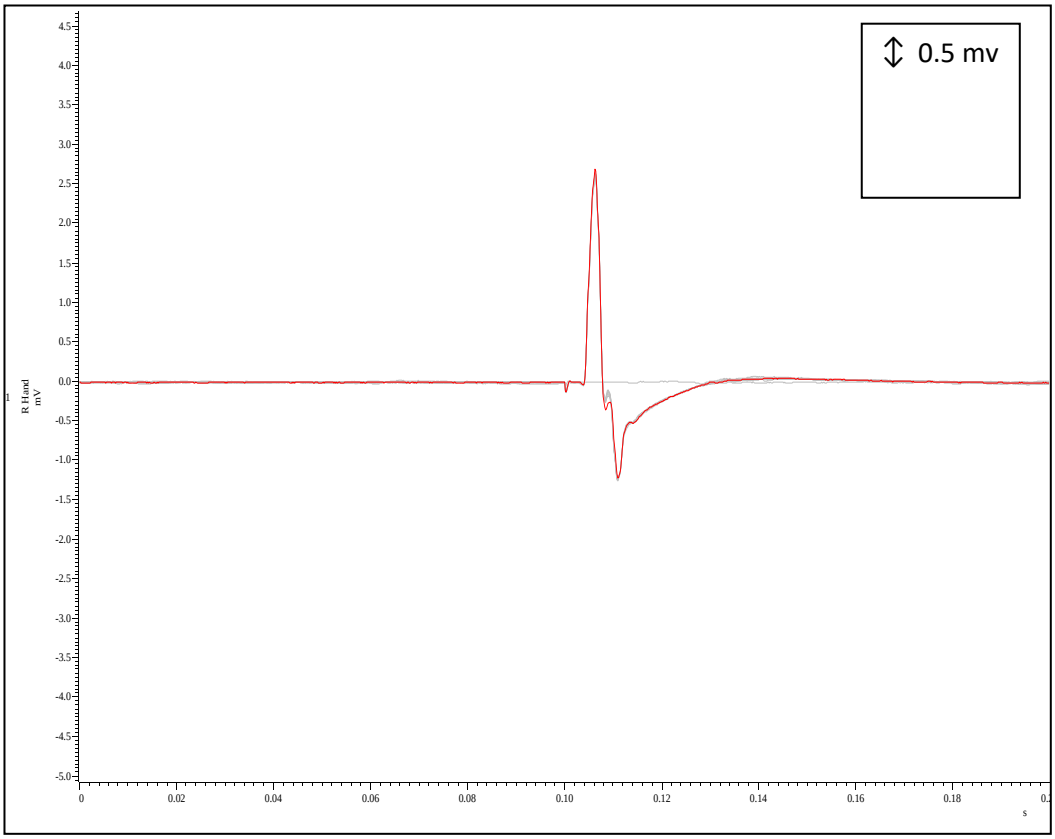


Figure 3.3 a) Corticospinal excitability – recruitment curve Example of raw data from a representative individual at each stimulus intensity and the corresponding compound motor action potential (CMAP).

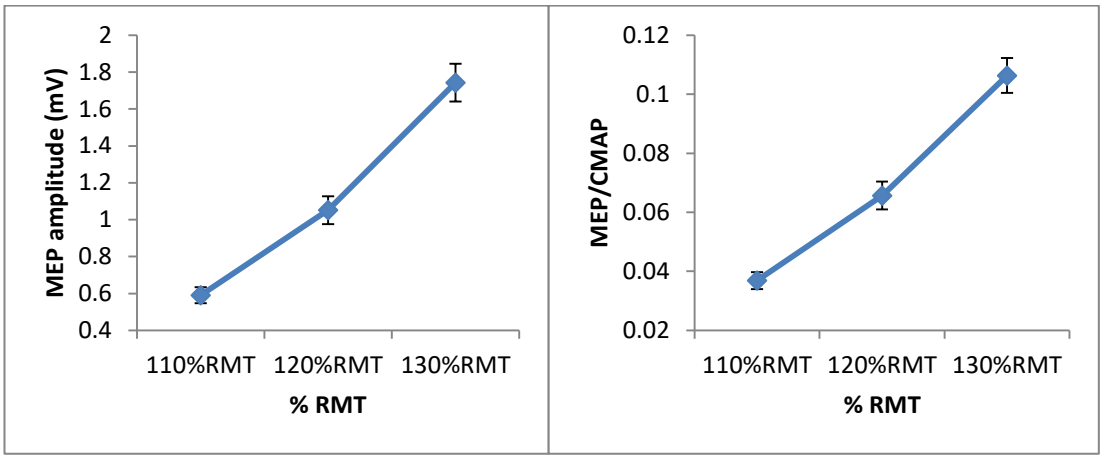


Figure 3.3 Corticospinal excitability – recruitment curve. b) Raw MEP size (peak to peak MEP amplitude: mean ± SE in mV) (left panel). This shows the average MEP amplitude at each stimulus intensity. c) MEP/CMAP at each stimulus intensity (right panel)

3.4.3 Interhemispheric inhibition

Figure 3.4 plots the mean (± SE) time course of interhemispheric inhibition (3.4a: raw data; 3.4b: normalised to baseline MEP given alone) in all participants. A one-way ANOVA showed a significant main effect of time on both the raw MEP values ($F(4.48, 147.7) = 5.61$, $P < 0.05$) and normalised data ($F(4.92, 162.2) = 5.12$, $P < 0.05$). As previously reported, there appears to be two peaks of inhibition at 10ms (short latency interhemispheric inhibition (SIHI)) and 30ms (long latency interhemispheric inhibition (LIHI)). Although not proven statistically, this is in keeping with known literature, where interhemispheric inhibition has been found to include an early phase at relatively short interstimulus intervals (8-12ms) and then a later phase (30-40ms) (Chen et al., 2003, Kukaswadia et al., 2005). These phases are thought to be mediated by different neuronal circuits, and a study by Irlbacher and colleagues demonstrated that interhemispheric inhibition at long interstimulus intervals was enhanced after the use of Baclofen, which is a GABA-B agonist (Chen et al., 2003, Chen, 2004, Irlbacher et al., 2007, Udapa et al., 2010). This suggests that the long phase could be mediated by GABA-B receptors, whereas the short phase is yet to be identified.

The effect of changing the conditioning stimulus intensity on amount of interhemispheric inhibition is illustrated in Figure 3.5a (raw) and b (normalised). There is a significant main effect of intensity on raw ($F(3, 99) = 14.2$, $P < 0.05$) and normalised data ($F(2.36, 77.99) = 10.9$, $P < 0.05$). Inhibition became significant from conditioning stimulus intensities > 110% RMT (paired t test conditioned v. control MEP: $P < 0.01$).

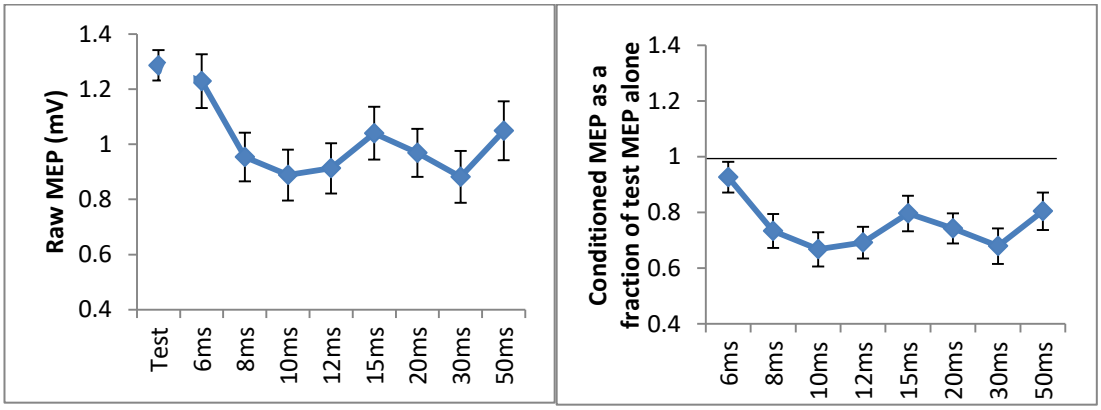


Figure 3.4 Transcallosal connections: effect of interstimulus interval (ISI) on interhemispheric inhibition a) Raw data (peak to peak MEP amplitude: mean +/- SE in mV). This shows the average amplitude of the MEP at each ISI. The conditioning impulse was set at 120% RMT. The leftmost point plots the amplitude of the unconditioned (test) MEP alone: the remaining points plot the MEP amplitude when preceded by a conditioning stimulus at each ISI (left panel) b) Normalised data: conditioned MEP response plotted as a fraction of the size of the test response at each ISI. The horizontal line at 1 indicates the baseline value (right panel).

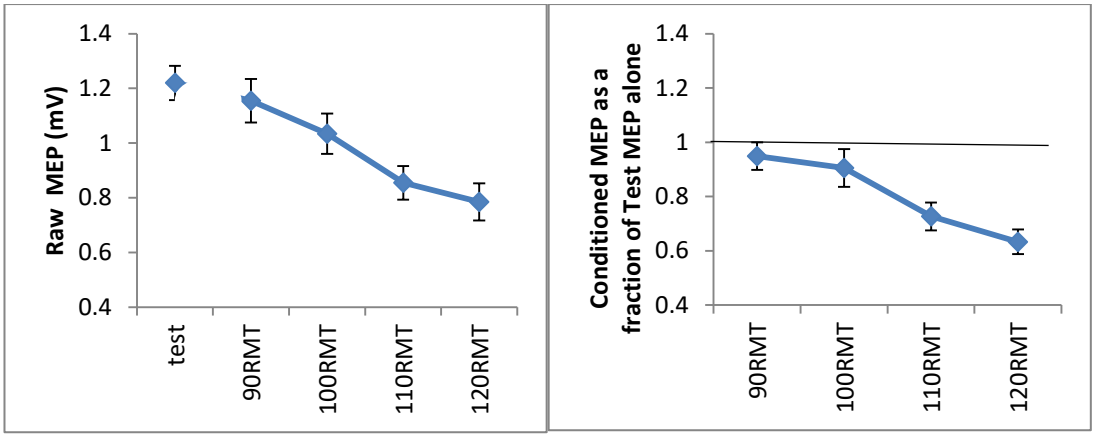


Figure 3.5 Transcallosal connections: effect of conditioning stimulus intensity on interhemispheric inhibition with an ISI at individualised peak of inhibition, as determined by the previous experiment a) Raw data (peak to peak amplitude of MEP) show the average amplitude of the MEP at each conditioning intensity. The leftmost point plots the amplitude of the unconditioned (test) MEP alone - the remaining points plot the MEP amplitude when preceded by a conditioning stimulus at each intensity (left panel) b) Normalised data: conditioned response plotted as a fraction of the size of the test response alone at each stimulus intensity. The horizontal line at 1 indicates the baseline value (right panel).

3.4.4 Intracortical inhibition

Figure 3.6a (raw) and b (normalised) show mean (± SE) time course of short interval intracortical inhibition in the healthy participants. A one-way ANOVA showed a significant main effect of time on raw MEP values ($F(2.97, 98.1) = 31.9$, $P < 0.05$) and normalised data ($F(3.10, 102.3) = 32.6$, $P < 0.05$) with a peak of inhibition at 3ms.

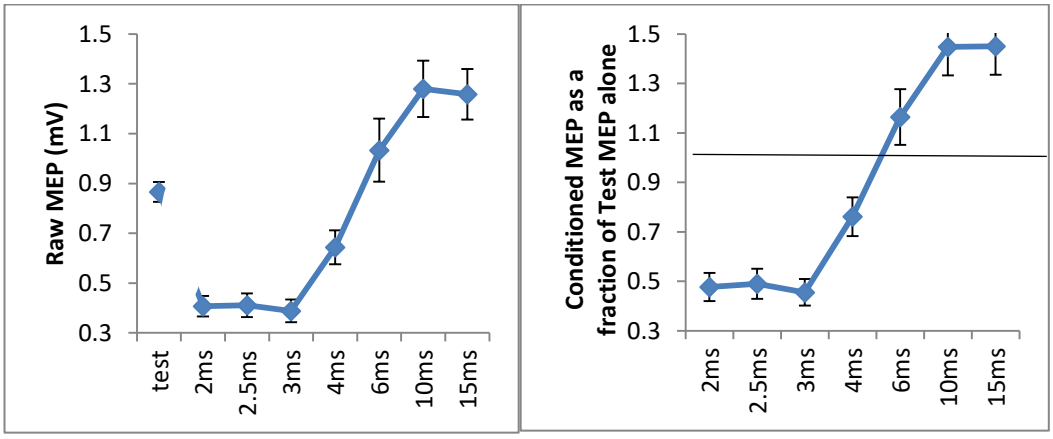


Figure 3.6 Intracortical connections effect of interstimulus interval on short intracortical inhibition a) Raw data (peak to peak amplitude of MEP) showing the average MEP amplitude

at each interstimulus interval. The conditioning stimulus intensity was set at 80% AMT. The leftmost point plots the amplitude of the unconditioned (test) MEP alone: the remaining points plot the MEP amplitude when preceded by a conditioning stimulus at each ISI (left panel). b) Normalised data. Response plotted as a fraction of the size of the test response alone at each ISI. The horizontal line at 1 indicates the baseline value (right panel).

The effect of adjusting the conditioning stimulus intensity is shown in Figure 3.7a (raw) and b (normalised). A one-way ANOVA showed a significant main effect of intensity on the raw ($F(1.62, 53.5) = 20.0$, $P < 0.05$) and normalised data ($F(1.86, 59.4) = 33.3$, $P < 0.05$). As reported by Orth and colleagues (Orth et al., 2003), inhibition became significant from a conditioning intensity of 70% AMT ($P < 0.05$).

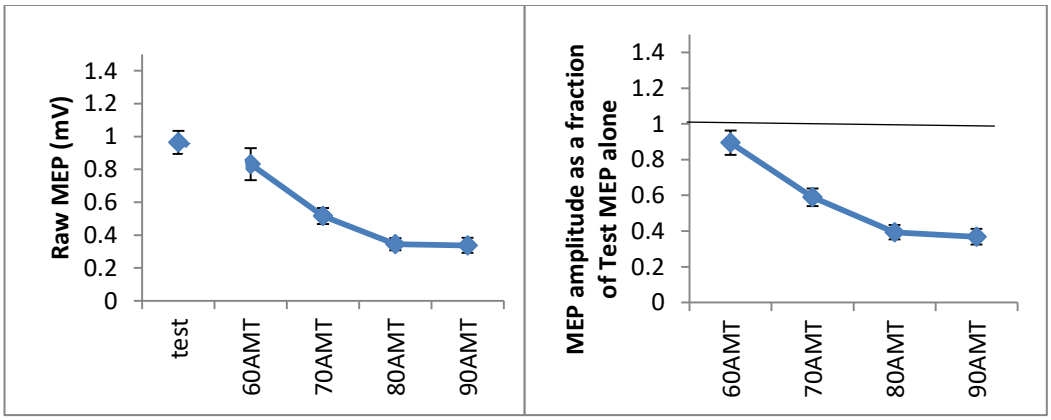


Figure 3.7 Intracortical connections: effect of conditioning stimulus intensity on short interval intracortical inhibition a) Raw data (peak to peak amplitude of MEP). Effect of conditioning stimulus intensity at individual's peak ISI from the previous experiment. Average amplitude of the MEP obtained at each conditioning intensity. The leftmost point plots the amplitude of the unconditioned (test) MEP alone: the remaining points plot the MEP amplitude when preceded by a conditioning stimulus at each intensity (left panel) b) Normalised data. Conditioned response plotted as a fraction of the size of the test response alone at each conditioning intensity. The horizontal line at 1 indicates the baseline value (right panel).

3.4.5 Variation in physiological measures

The variation of short intracortical inhibition, interhemispheric inhibition and MEP recruitment curves were assessed in more detail. For interhemispheric inhibition and short intracortical inhibition, the size of conditioned responses is expressed as (test/conditioned) *100% (i.e. the more positive the number the greater the inhibition). Figure 3.8a plots the range of interhemispheric inhibition and short intracortical inhibition values in all 34 individuals. The distribution of data is slightly skewed because of the ceiling effect at 100% (maximum inhibition when the conditioned response is eliminated) and has a tail towards negative values (i.e. facilitation). Approximating the data to a normal distribution gives a mean ± SD for IHI 10, IHI 30 and SICI 2.5 of 33.23 ± 38.85, 32.07 ± 37.15, 50.97 ± 35.04 respectively. As a rough guide this would predict that a sample size of 25 would be needed to detect a difference of 25% in the mean of two populations (e.g. healthy versus patients). Interhemispheric inhibition and short intracortical inhibition produced facilitation in around 10% of individuals.

Figure 3.8b summarises the recruitment data for IHI and SICI. It shows the mean inhibition in each individual over the four different conditioning intensities for the individualised peak of inhibition (IHI 90% RMT, 100% RMT, 110% RMT, 120% RMT; SICI 60% AMT, 70% AMT, 80% AMT, 90% AMT). The larger the value, the more effectively a conditioning stimulus recruits an inhibitory effect. The spread of data values is smaller for SICI but not IHI 10, than in fig 3.7a where only one intensity of conditioning stimulus was used (IHI 10 = 19.64 ± 22.56; SICI = 43.86 ± 20.85). The last scatter plot (Fig 3.7c) shows recruitment curve data for the MEP expressed in a similar way after normalising the MEP amplitude to the CMAP in each individual (0.07 ± 0.02).

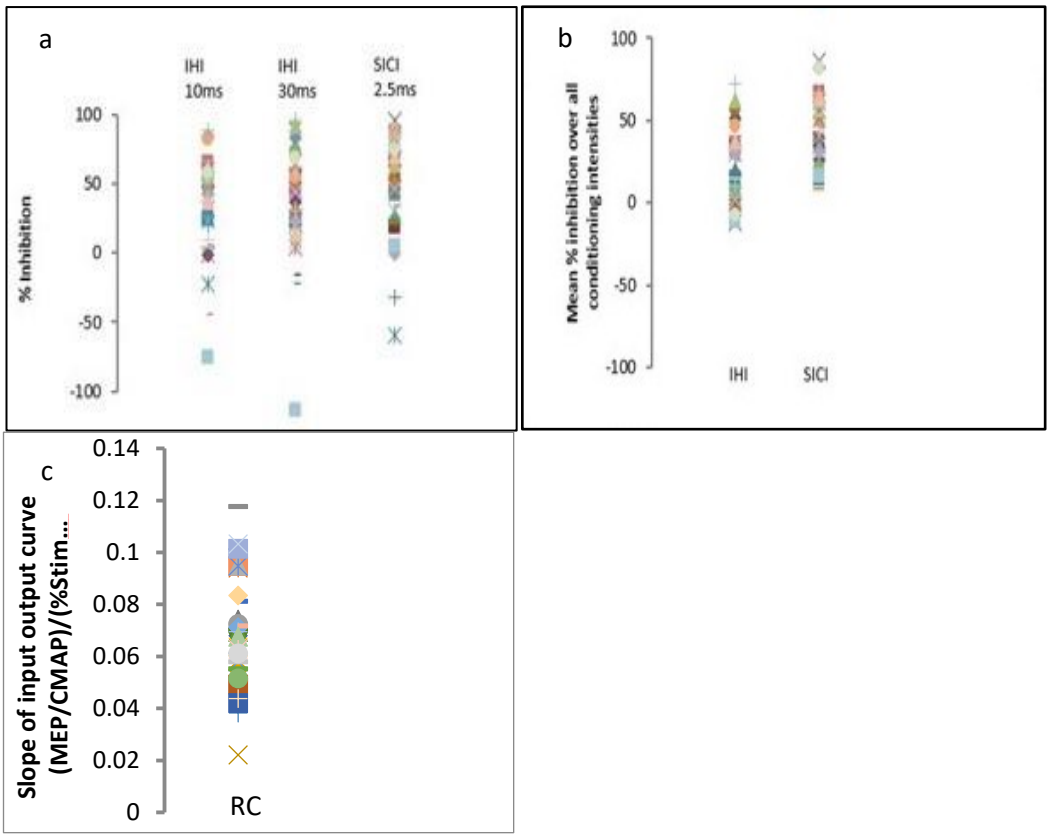


Figure 3.8 Range of values: interhemispheric inhibition, short intracortical inhibition and recruitment data a**)** Scatter plot of individual % inhibition at IHI 10ms, IHI 30ms and SICI 2.5ms (single intensity conditioning stimulus. Note that each individual is plotted with the same colour and symbol in each graph b) Mean % inhibition over four different intensities of conditioning stimulus (CS) for interhemispheric inhibition (CS 90%, 100%, 110%, 120%) and SICI (CS 60%, 70%, 80%, 90%). c) Scatter plot of individual average slopes of MEP I/O curves. MEP amplitude was normalised to individual CMAP.

Correlation analysis was undertaken between IHI 10, IHI 30, SICI 2.5 and MEP recruitment. There were no significant relationships between MEP recruitment and the other three measures, consistent with previous suggestions that recruitment of corticospinal neurons responsible for the motor evoked potential is separate from the recruitment of neurons responsible for short intracortical inhibition and interhemispheric inhibition (Ziemann et al., 1996; Chen et al., 2003). However there was a weak correlation between SICI 2.5 and IHI 10 ($r = 0.44$, $p < 0.05$; Figure 3.7d) and a moderate correlation between IHI 10 and IHI 30 ($r = 0.72$, $p < 0.05$; Figure 3.7e) but no significant correlation between SICI (2.5ms) and IHI 30 (30ms).

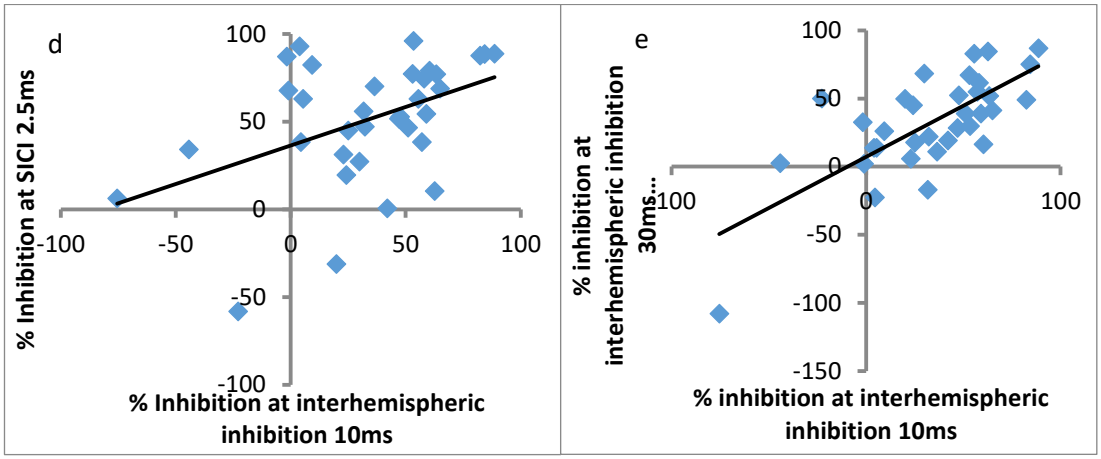


Figure 3.8 Range of values: interhemispheric inhibition, short intracortical inhibition and recruitment data d**)** Correlation between % inhibition at interhemispheric inhibition 10ms versus % inhibition at SICI 2.5ms. e) Correlation between % inhibition interhemispheric inhibition 10ms versus % inhibition at interhemispheric inhibition 30ms.

3.5 Discussion

The results in this healthy cohort were comparable to those reported in the literature. This is reassuring for a number of reasons. First, it reassures us that the experimental techniques employed to investigate the physiology of these pathways produce results comparable to the established literature. This provides confidence, from an experimental point of view, that any physiological differences observed in the patient group are genuine physiological differences, rather than variations in experimental technique. In addition, the healthy participants tolerated the study well. The general verbal feedback was positive about the experience. This reassured the suitability of this protocol in assessing physiology of the selection of tracts in a patient study. Finally, an important observation from this study, which included a larger number of participants than usual transcranial magnetic stimulation studies, is that this study revealed quite a large variation in physiological measures between individuals.

3.5.1 Corticospinal excitability

The significant difference demonstrated between the resting motor threshold (RMT) of the left and right hemispheres is consistent with what has been reported in the literature (Navarro et al., 2009, Maeda et al., 2000). The RMT has been described to be higher than the active motor threshold (AMT) (Devanne et al., 1997, Hallett, 2000); this is thought mainly to reflect increased excitability of spinal motor neurons during contraction. This was reproduced in our study.

3.5.2 Recruitment curve

Given that over the range of stimulus intensities investigated the motor evoked potentials remained relatively simple and biphasic, the amplitudes were measured as peak-to-peak values. Higher intensities may have led to more complex waveforms, but were avoided by limiting intensity to 130% RMT. As expected, the amplitude increased with intensity (Devanne

et al., 1997). However, as shown in Table 3.1 which gives the raw MEP data for all individuals, there is a very large variability in the MEP amplitude from person to person. Normalising to CMAP amplitude had little effect on the CV which was approximately 40% for both sets of data. Thus, planned future analyses or correlations with behavioural and MRI data can be performed on either data set.

3.5.3 Interhemispheric inhibition

In the primary motor cortex, interhemispheric inhibition can be assessed non-invasively using either a paired–pulse protocol or the ipsilateral silent period. Interhemispheric inhibition (IHI) is a physiological measure of transcallosal transfer (Ferbert et al., 1992). A suprathreshold conditioning stimulus is applied over the primary motor cortex of one hemisphere (in the case of the studies in this thesis, the right hemisphere) followed by the test stimulus delivered to the primary motor cortex contralateral to the target muscle (in this case, the left hemisphere). It is proposed that the conditioning stimulus activates excitatory transcallosal fibres, which project to local inhibitory GABAergic neurons in the contralateral motor cortex (Daskalakis et al., 2002, Chen, 2004). In the context of the studies in this thesis, the effect of IHI was determined by the MEP amplitude in the first dorsal interosseous. The effect of IHI manifests as a reduction in the amplitude of the motor evoked potential in the measured muscle.

This leads to two corresponding periods of inhibition, short latency and long latency interhemispheric inhibition. The ipsilateral silent period assesses transcallosal physiology by applying the stimulus to the motor cortex ipsilateral to the test hand. The target muscle is activated voluntarily and there is a brief interruption of the ongoing muscle activity. This interruption of voluntary muscle activity is thought to reflect transcallosal inhibition mediated by the M1 opposite to that maintaining the contraction (Meyer et al., 1995). Although the ipsilateral silent period has been examined as a method of assessing transcallosal physiology in some of the existing traumatic brain injury literature, it is often felt to be unreliable as the detection of onset and offset is difficult to determine. It addition, it requires a strong contraction from the participant, which may be difficult in the patient group. This, in turn, may have deterred

the patient group from participating in the rest of the study. Lastly, outside of active motor threshold, all other measures were obtained at rest. Both these methods reflect interactions between the motor cortices, and are accepted techniques to assess callosal physiology. However, their effects appear to be mediated by different neuronal populations (Chen et al., 2003). Despite these concerns, on reflection, had I assessed both measures, the addition of the ipsilateral silent period would have provided additional complementary information of callosal transfer.

As noted by others, interhemispheric inhibition appeared to consist of two components depending on the interstimulus interval between conditioning and test stimuli (Chen et al., 2003, Kukaswadia et al., 2005). An initial inhibition began at approximately 8ms, reaching a peak at 10-12ms, and was followed by a later peak at around 30-40ms. These have previously been termed short and long interhemispheric inhibition respectively (Chen et al., 2003; Kukaswadia et al., 2005). As described originally by Ferbert and colleagues, the threshold for producing interhemispheric inhibition was approximately 110% RMT; inhibition increased monotonically as the intensity of the conditioning stimulus was raised (Ferbert et al., 1992). There was variation in the amount of inhibition between individuals, which is discussed later.

The conditioning stimulus was applied over the right M1, and inhibited the motor evoked potential generated by the test stimulus applied over the left M1 at interstimulus intervals in keeping with known literature (Ferbert et al., 1992, Gerloff et al., 1998). The measurement of an MEP in the non test hand (left hand) was outside the scope of the experiments presented in this thesis. However, conceivably the conditioning stimulus alone could cause an MEP in the contralateral hand. Ferbert and colleagues initially proposed that interhemispheric inhibition occurred at a cortical level through transcallosal connections (Ferbert et al., 1992). Normal interhemispheric inhibition has already demonstrated in patients with subcortical stroke (Boroojerdi et al., 1996). Studies have also failed to demonstrate the presence of interhemispheric inhibition in patients with corpus callosum agenesis or partial callosotomy, which lends support to this hypothesis (Meyer et al., 1995, Woiciechowsky et al., 1997).

Spinal influences will undoubtedly be involved in the generated MEP. However, in support of the inhibition occurring at a non-spinal level, the duration at which the inhibition–effect occurs is very short, and would be in keeping with closer proximity to the test impulse than a spinal origin. In addition, the time interval with which the inhibitory effect occurs would be supportive of existing literature of communication across the corpus callosum, for example, motor cortical stimulation has been found to evoke an electroencephalogram potential over the contralateral motor cortex with an onset latency of 8.8-12.2ms (Cracco et al., 1989). In addition Di Lazarro and colleagues have explored whether the inhibitory effect occurs at a spinal level in their group of patients with cervical cord electrodes. In their study of patients, albeit a small number (n = 3), they demonstrated that the delivery of a test stimulus alone generated three I-waves in the spinal cord. However, during the IHI protocol, with simultaneous measurement of the EMG recording in the hand muscle and the spinal electrode recordings, it was found that with the delivery of the conditioning stimulus, the resultant MEP response in the hand muscle reflected that of known literature. However, the expected I-wave response from the spinal electrodes, demonstrated some inhibition in I_3 with minimal or no inhibition in I_2 and I_1. (Di Lazarro et al., 1999). This additionally provides further support that the IHI effect occurs outside of the spinal level.

3.5.4 Intracortical inhibition

The time course of short interval intracortical inhibition was similar to that described previously (Kujirai et al., 1993) with maximum suppression at 2-3ms. As described by Orth and colleagues, short intracortical inhibition became evident when the intensity of the conditioning stimulus was greater than 70% AMT (Orth et al., 2003); using the standard 80% AMT conditioning stimulus gives an approximately 50% suppression at interstimulus interval = 2-3 ms. Finally, there was considerable variation in the depth of short intracortical inhibition between individuals, which is discussed later.

3.5.5 Variation in physiological measures

The variation of short intracortical inhibition, interhemispheric inhibition and MEP recruitment curves across the participant group was important to acknowledge. Although there are existing studies on day to day variation of individuals in a population, variation across a group is less reported in the literature. However, it does have implication on the reliability of physiological measures. Approximately 40 – 50% of variability appears to be due to individual factors (Wassermann, 2002). Wassermann found that inter-individual variation in SICI was surprisingly variable with 6 of 53 people having facilitation rather than inhibition (Wasserman, 2002). Between subject variation is reported in the ipsilateral silent period (Orth et al., 2004). The study by De Gennaro and colleagues also demonstrates within subject variability and acknowledges between subject variability (De Gennaro et al., 2003). However, to the best of my knowledge, there are no formal reports of variability in MEP recruitment. The sample size in this study is larger than most (n = 34). This finding emphasises the variation of standard TMS measures in healthy individuals, and the importance in acknowledging this when making inferences regarding differences between patient groups and healthy volunteers.

There was no significant relationship between MEP recruitment and IHI 10, IHI 30 and SICI 2.5. This is consistent with previous suggestions that recruitment of corticospinal neurons responsible for the MEP is quite separate from the neurons responsible for short intracortical inhibition and interhemispheric inhibition. Therefore a lack of relationship in their physiological function is not surprising (Ziemann et al., 1996, Chen et al., 2003). However, there was a weak correlation between SICI 2.5 and IHI 10, and a moderate correlation between IHI 10 and IHI 30, but no significant correlation between SICI 2.5 and IHI 30. The relationship between IHI 10 and IHI 30 was unexpected, since Chen and colleagues failed to find evidence for a relationship between early and late phases of interhemispheric inhibition. However, their studies were measured at slightly different timings to those used here (IHI 8 and IHI 40) (Chen et al., 2003, Kukaswadia et al., 2005). It is possible that there are subtle differences in the mechanisms at these different timings. Alternatively, it may be that the relationship only becomes evident with the larger number of individuals used in the present study. If so, it may imply that they share some common mechanism: for example they could involve activity in an

overlapping set of transcallosal neurons that activate short and long interval inhibition within the test hemisphere. The relationship between SICI 2.5 and IHI 10 was weak, given that they are thought to involve separate, although interacting inhibitory neurons (Sanger et al., 2001). One possible common factor that may be common to both of them is recruitment of late I-waves by the test pulse. Both forms of inhibition target the late I-waves, so that is it possible that individuals with large late I-waves will have good inhibition to both interventions.

3.6 Conclusions

Results in the healthy cohort mostly reproduced that reported in the literature. However, this study also demonstrated a variation in interhemispheric inhibition and recruitment in individuals. Variation in short intracortical inhibition has previously been described (Wasserman et al., 2002). This may be due to this study investigating a larger group than standard transcranial magnetic stimulation studies. This data has demonstrated the range of values for short intracortical inhibition, interhemispheric inhibition and MEP recruitment in young healthy adults. The techniques used are standard measures for the study of electrophysiological pathways. Therefore, these findings illustrate the variation across the general population and emphasises the care required in comparisons of transcranial magnetic stimulation parameters between healthy individuals and those with neurological disorders.

The next step would be to ascertain the physiology of long and short tract pathways in the brain after traumatic brain injury. This is the focus of the next chapter.

Chapter 4

Physiological Consequences of Traumatic Brain Injury

I led this study and was responsible for study design, recruitment, experimental work, analysis and interpretation of the study. Scripts used were standard protocol programmed by Dr M Hamada.

4.1 Introduction

In the previous chapter (Chapter 3), a physiological study was developed to assess white matter pathways in a group of healthy participants. The main purpose of this study was to produce a protocol that would be simple and well-tolerated in a patient group that was relatively new to transcranial magnetic stimulation research. The study protocol was designed in the knowledge that the corpus callosum is thought to be particularly vulnerable to diffuse axonal injury in traumatic brain injury (Gentry et al., 1988a, Parizel et al., 1998, Meythaler et al., 2001, Scheid et al., 2003). The protocol examined a selection of pathways, including the assessment of callosal physiology using a twin coil transcranial magnetic stimulation method to assess interhemispheric inhibition (Ferbert et al., 1992). In summary, the study detailed in Chapter 3 not only provided an appreciation of the normal physiology in a large cohort of healthy individuals, but set the foundations for the protocol used to examine the brain physiology in patients with traumatic brain injury.

Previous to the study detailed in this chapter, there were limited studies detailing the physiological consequences following traumatic brain injury. Not only studies examining the physiological function of traumatic brain injury patients were scarce, but several limitations in the design of those studies precluded the drawing of firm conclusions with regards to the physiological changes following traumatic brain injury. First, existing traumatic brain injury studies have been taken in the early stage following the injury (Chistyakov et al., 1998, Nardone et al., 2011). Second, studies have also included patients on neuromodulatory medication, which is known to affect cortical excitability measures (Chistyakov et al., 1998, 2001, Bernabeu et al., 2009, Lapitskaya et al., 2013). Finally, some patients had residual neurological deficit, such as a hemiparesis on clinical examination (Chistyakov et al., 2001, Bernabeu et al., 2009).

The initial objectives were that of safety and tolerability, in a relatively new patient group to transcranial magnetic stimulation research. The healthy participants who participated in the study in the previous chapter (Chapter 3) provided positive feedback with regards to comfort and tolerability of the protocol. Nevertheless, at the time of designing this study, it was uncertain whether a traumatic brain injury would require higher stimulus thresholds because of the nature of the injury.

Traumatic brain injury is understood to physically involve shearing and disruption of the axons due to the acceleration and deceleration motion (Strich, 1961, Oppenheimer, 1968, Adams, 1982, Meythaler et al., 2001, Arfanakis et al., 2002, Huisman et al., 2004, Gajawelli et al., 2013). Therefore, I propose that the physiological function of the long white matter pathways (corticospinal tract measures, interhemispheric inhibition across the corpus callosum) is more likely to be affected by this process than the more localised intracortical pathways. This would likely manifest as impaired corticospinal excitability, evidenced by higher motor thresholds and impaired recruitment of corticospinal fibres. Likewise, the interhemispheric inhibition across the corpus callosum is also likely to be impaired, as this structure would be particularly vulnerable to the shearing motion in traumatic brain injury. Measurements pertaining to the intracortical pathways (SICI), are likely to be similar in the traumatic brain injury group and the controls.

4.2 Study design

4.2.1 Participants

The healthy participants who acted as controls for this study were recruited from a database of healthy volunteers from the UCL Institute of Neurology and Imperial College. Seventeen healthy participants consisting of 10 male and 7 females were recruited. See details in chapter 2.

Patients recruited from brain injury units across London were listed in a database of traumatic brain injury. The participating units were Charing Cross Hospital, St Mary's Hospital, The National Hospital for Neurology and Neurosurgery, the Royal Free Hospital and the Regional Neurological Rehabilitation unit at the Homerton Hospital. Seventeen participants consisting of 10 males and 7 females were recruited. All patients had sustained an impact injury to the brain on only one occasion (from a road traffic accident, fall, sports injury or assault) causing a loss of consciousness. All these patients had been treated conservatively after their traumatic brain injury, and had not required neurosurgical intervention as part of their management. All patients had no additional history of significant medical or psychiatric illness. These patients had already participated in traumatic brain injury research as part of an MRI study conducted by Imperial College.

Prior to recruitment, the safety of this relatively new patient group to transcranial magnetic stimulation research needed careful consideration. A major concern related to the potential risk of seizure induction. Of note, seizure induction has been described in repetitive transcranial magnetic stimulation protocols, although most of these seizures occurred prior to the definition of safety limits (Wassermann, 1998, Rossi et al., 2009). Even though the planned study protocol did not intend to use repetitive transcranial magnetic stimulation, it is important to acknowledge Rossi and colleagues did report the occurrence of two seizures in single pulse protocols following the Wasserman guidelines (Rossi et al., 2009). However, both events occurred under specific circumstances. In the first case, the seizure might have resulted from the concomitant use of Olanzapine, which can potentially reduce the seizure threshold (Haupts et al., 2004). In the second case, the patient's family history of epilepsy and the concomitant use of anti-psychotic medication may have contributed to the seizure (Tharayil et al., 2005).

To minimise the risk of seizure induction, the structural imaging of each patient on the database was considered prior to inviting participants to the study. The neuroimaging of each patient on the database had already been reported by a senior neuroradiologist, with experience in traumatic brain injury imaging. This information was available on the database

and carefully considered. In addition to transcranial magnetic stimulation safety criteria, traumatic brain injury patients were not invited if their risk of first seizure was greater than the risk of first seizure after cerebral infarction (Hauser et al., 1993, Annegers et al., 1998, Bladin et al., 2000). As a consequence, traumatic brain injury patients were recruited at least one year following their injury.

A member of the Imperial College team introduced all participants to the study informally, and verbal consent was obtained for further contact regarding this study. This was followed up with a telephone conversation from the host team (MB) outlining the nature of the study. A health questionnaire was then undertaken to ensure that each participant was medically stable and was suitable for a transcranial magnetic stimulation study prior to recruitment. With the participant's consent a formal invitation and information sheets were sent to the participant outlining the details of the study. A time and date were agreed for a further follow-up telephone conversation, allowing the participants time to read the information and discuss with their next of kin. The participants then confirmed whether they wanted to take part in the study.

Patients with traumatic brain injury meeting the following criteria were enrolled: 1) a diagnosis of traumatic brain injury, 2) age between 16 and 80 years, 3) clinically stable, 4) no significant premorbid neurological or psychiatric illness, 5) capable of giving informed consent for participation. Patients with contraindications to transcranial magnetic stimulation including 1) magnetic implants, such as a pacemaker, 2) previous history of other head trauma, 3) past or current history of other neurological or psychiatric illness, 4) presence of intracerebral haemorrhage, 5) ingestion of psychotropic, sedative or anticonvulsant medication, 6) major comorbidities or clinically unstable patients, 7) patients unable to cooperate with the study session, including significant language impairments, 8) not able to give informed consent, 9) pregnancy, were excluded (Wassermann, 1998, Rossi et al., 2009).

Twenty patients out of a possible 115 on the database were eligible to be included in this study; out of these, 17 patients agreed to participate. The patients had injuries secondary to road traffic accidents 64%, assaults 12%, falls 12% or a single sports injury 12%. Based on the Mayo classification system for traumatic brain injury severity, eleven had a moderate – severe injury and six were mild (probable) cases of traumatic brain injury (Malec et al., 2007). All patients were in the chronic phase following their traumatic brain injury (mean 3.72 years, range 1.1 – 7.5 years).

A second physician was always within the TMS laboratory to provide additional medical help, in the event of an emergency situation.

4.2.2 Institutional and ethical approval

The information sheets and protocols were reviewed and ethical approval was obtained from the Ethics research committee. Further details are outlined in chapter 2.

As described above, participants gave written consent prior to participating in the session. All studies were performed in accordance with the Declaration of Helsinki, and participants were given the option to withdraw from the study at any time.

4.3 Experimental Methods

4.3.1 Physiological measures using transcranial magnetic stimulation

The methods pertaining to the electromyography recording, assessing the location of the "motor hotspot" and assessment of the resting motor threshold, active motor threshold and 1mV MEP are detailed in the General Methods chapter (Chapter 2). The methods pertaining to the assessment of the respective pathways are detailed in the previous chapter (Chapter 3). In summary, measures were obtained pertaining to:

1) Corticospinal tract integrity - this was assessed by measuring the i) motor thresholds and ii) the resting recruitment curve of motor evoked potential (MEP) size versus stimulus intensity in the first dorsal interosseous. The stimulus intensities (110%, 120%, 130% RMT) were chosen to maintain patient compliance with the study.

2) Interhemispheric inhibition - Interhemispheric inhibition was studied using a twin coil approach similar to that described by Ferbert and colleagues (Ferbert et al., 1992).

The interstimulus intervals investigated were 6ms, 8ms, 10ms, 12ms, 15ms, 20ms, 30ms and 50ms (modified from Ferbert et al., 1992). These intervals were chosen in order to investigate those known to be associated with inhibition, SIHI 8ms, and LIHI 40 ms (Chen et al., 2003). However, short interstimulus intervals between 6-12ms have also been demonstrated to cause inhibition (Ferbert et al., 1992, Di Lazzaro et al., 1999. Daskalakis et al., 2002). Therefore, the range of the interstimulus intensities investigated was widened slightly, as the effect of traumatic brain injury on callosal transfer was not known at the time of designing the study. Ten randomly intermixed responses were obtained for each condition, as this was the practice at the time of starting this study. In addition, 20 test responses were recorded. As the heterogeneity of brain injury types was anticipated, the individual's peak of inhibition was used to investigate transcallosal recruitment.

3) Short intracortical inhibition - Intracortical circuits (inhibitory and facilitatory interactions in the cortex) occurring within the M1 of the motor cortex were studied by delivering two transcranial magnetic stimulation pulses using the same coil. A range of interstimulus intervals (2ms, 2.5ms, 3ms, 4ms, 6ms, 10ms, 15ms) was investigated (modified from Kujirai et al., 1993). Ten responses were obtained for each conditioned state, and 20 responses for test stimulus. Conditions were randomly intermixed. As the heterogeneity of brain injury types was anticipated, the individual's peak of inhibition was used to investigate intracortical recruitment.

4.3.2 EMG data analysis

The measure of EMG signals in all TMS studies was MEP peak to peak amplitude of individual trials using a customised script for Signal software and exported to Excel. Cursors were manually placed at the peaks and the amplitude was measured by a pre-programmed customised script. For each measurement, the raw MEP value was obtained. In addition, the peak to peak amplitude of the conditioned MEP was also normalised against the peak to peak amplitude of the test MEP. Within each experimental condition, outliers (defined as > 2 standard deviations from the mean) were removed.

4.3.3 Data and Statistical analysis

For the measures of corticospinal excitability in the traumatic brain injury group, paired t tests were used to compare resting motor threshold (RMT) in the left and right hemisphere, and compare active motor threshold (AMT) to RMT. 1mV MEP/RMT x 100 was calculated to obtain the intensity required to evoke a 1mV MEP relative to RMT. For each participant and stimulus intensity, the single trial peak to peak motor evoked potential amplitudes were averaged. In addition to the raw motor evoked potential amplitude, the average motor evoked potential was additionally corrected by the compound motor action potential (CMAP) (MEP/CMAP).

For the comparison of corticospinal excitability between the traumatic brain injury group and healthy controls, independent t tests were used to compare RMT, AMT and the intensity required to elicit a 1mV MEP between the two groups. Comparison of resting mean threshold (RMT) between the two groups was additionally undertaken with “HEMISPHERE” as within subject factor and “GROUP” as between subject factor. For each participant and stimulus intensity, the single trial peak to peak MEP amplitudes were averaged and corrected by the CMAP (MEP/CMAP). A two-way repeated measures analysis of variance (ANOVA) was undertaken with factors “STIMULUS INTENSITY” (110% RMT, 120% RMT, 130% RMT) as the within subjects variable and “GROUP” (traumatic brain injury, healthy control) as the between subjects variable, using absolute motor evoked potential values corrected to the compound motor action potential.

For the measures of interhemispheric inhibition, a two–way repeated measures analysis of variance (ANOVA) was undertaken with factors “TIME” (6ms, 8ms, 10ms, 12ms, 15ms, 20ms, 30ms, 50ms) as the within subjects variable and “GROUP” (traumatic brain injury, healthy control) as the between subjects variable, using motor evoked potential values normalised to the unconditioned motor evoked potential amplitude. For transcallosal recruitment, a two-way repeated measures analysis of variance (ANOVA) was undertaken with factors “STIMULUS INTENSITY” (90% RMT, 100% RMT, 110% RMT, 120% RMT) as the within subjects variable and “GROUP” (traumatic brain injury, healthy control) as the between subjects variable using normalised motor evoked potential amplitude values.

For the measures of short intracortical inhibition, two-way repeated measures analysis of variance (ANOVA) was undertaken with factors “TIME” (first analysis (2ms, 2.5ms, 3ms, 4ms, 6ms) and then second analysis (10ms, 15ms)) as the within subjects variable and ”GROUP” (traumatic brain injury, healthy control) as the between subjects variable, using normalised motor evoked potential values. For short intracortical inhibition recruitment, two-way repeated measures analysis of variance (ANOVA) was undertaken with factors “STIMULUS INTENSITY” (60% AMT, 70% AMT, 80% AMT, 90% AMT) as the within subjects variable and

"GROUP" (traumatic brain injury, healthy control) as the between subjects variable, using normalised motor evoked potential values.

Paired t tests were performed where appropriate in the traumatic brain injury group. Independent t tests were undertaken for group comparisons. Before conducting the relevant t test, Levene's test was first used to verify that there were no significant differences in variance of the populations being tested. Normality of all the data sets was tested with Kolmogorov – Smirnov tests. For the isolated time points that deviated from normal, parametric statistics have been used throughout to avoid inconsistent treatment of the data at different time points (as in Swayne et al., 2008). For the report of the statistical differences, the significance threshold of $\alpha = 0.05$ was used. Where assumptions of sphericity were violated (where Mauchley's test $P < 0.05$), the Greenhouse – Geisser correction was applied. Post–hoc t test, Bonferroni corrected were used when appropriate. Where patients declined participation in subsequent TMS experiments, the experiments they completed were included in the analysis. Therefore corticospinal tract integrity measures n = 17, interhemispheric inhibition IHI ISI n = 16, IHI CS n = 14, SICI ISI n = 15, SICI CS n = 15. Statistical procedures were conducted using the statistical package SPSS version 19.0 for Windows; SPSS Inc. All data are given as mean ± standard error of the mean unless otherwise stated.

4.4 Results

The patient study consisted of 10 males and 7 females aged between 22 and 60 years (mean ± SE 43.3 ± 2.69). The control group contained 10 males and 7 females aged between 22 and 52 years (mean ± SE 30.5 ±1.75). All patients had sustained their traumatic brain injury at least one year prior to testing. All values are expressed as mean ± SE unless stated otherwise. To allow comparisons between groups, I have used normalised data throughout this section, except for the description of motor thresholds below.

4.4.1 Corticospinal excitability – Thresholds

Figure 4.1 shows the average thresholds obtained for resting motor threshold (RMT) (both hemispheres), active motor threshold (AMT) (left hemisphere) and 1mv motor evoked potential (MEP) (left hemisphere) in the a) control (red) and b) traumatic brain injury (blue) group. Comparison of resting mean threshold (RMT) between the two groups with “HEMISPHERE” as within subject factor and “GROUP” as between subject factor demonstrated an effect of “HEMISPHERE” ($F(1, 32) = 5.72$, $P < 0.05$) but no effect of “GROUP” ($F(1, 32) = 0.75$, $P = 0.39$) and no “HEMISPHERE” x “GROUP” interaction ($F(1, 32) = 4.12$, $P = 0.051$). This means that a significant difference in the resting motor excitability of the two hemispheres was demonstrated. However, a difference in the resting motor threshold between the two groups was not demonstrated. There was no statistically significant difference between the two groups in the mean AMT left hemisphere ($t = 0.51$, $df = 30$, $p = 0.61$) or the intensity required to elicit a 1mV MEP ($t = 0.15$, $df = 32$, $p = 0.88$).

In the traumatic brain injury group, as in the healthy participant data previously described, RMT was lower in the left hemisphere (45 ± 1.82% MSO) than right hemisphere (52 ± 3.64 % MSO) ($t = -0.37$, $df = 16$, $p = 0.02$). AMT was lower than RMT (left hemisphere: 40 ± 1.57% MSO) ($t = 2.81$, $df = 14$, $p = 0.01$). The 1mv MEP was elicited at 120 ± 2.60 % RMT.

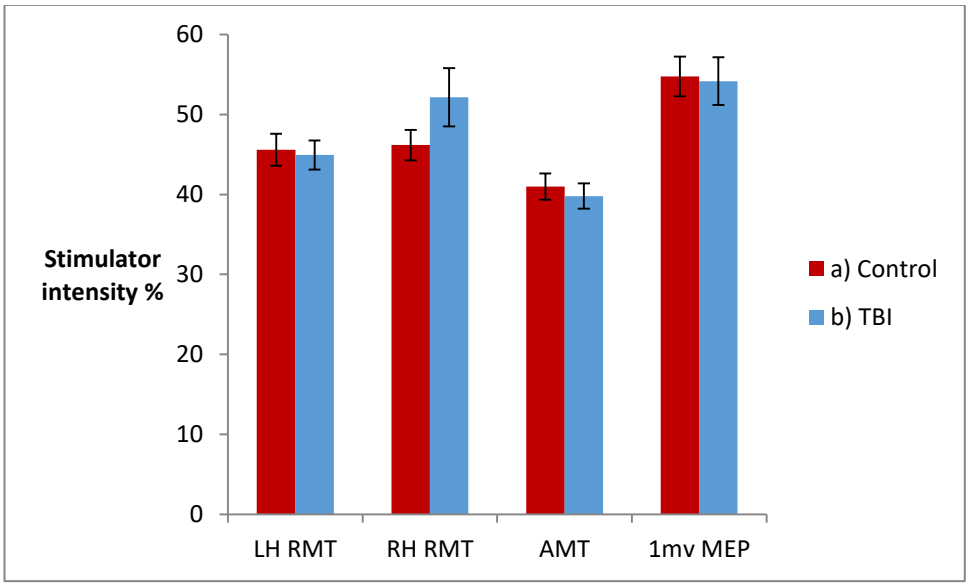


Figure 4.1: Corticospinal excitability: thresholds in control (red) and traumatic brain injury (blue) groups. The plots show the resting motor threshold (RMT) in the left hemisphere (LH RMT), right hemisphere (RH RMT), active motor threshold in the left hemisphere (AMT) and the stimulus intensity required to elicit a 1mv MEP from the left hemisphere. Data are mean +/- SE.

4.4.2 Corticospinal excitability – recruitment curve (RC)

Motor evoked potentials were measured as peak to peak amplitude since their waveform remained relatively simple and biphasic over the range of intensities used (Fig 4.2a) in the traumatic brain injury group. The recruitment curve was relatively linear between 110 - 130% RMT whether measured in terms of raw data (Fig 4.2b left) or normalised to each individual's peripheral CMAP (Fig 4.2b right). As with the healthy participants, the average data conceals a high variation between individuals. Table 4.1 illustrates the individual data for the raw and normalised MEPs in each participant together with the coefficient of variation (CV) at each measurement point.

As noted in the preceding chapter (Chapter 3) on healthy participants, variation in the recruitment curves is unaffected by normalising the data to peripheral CMAP (Fig 4.2c). Figure 4.2c demonstrates the comparison between the control and traumatic brain injury groups. Figure 4.2d illustrates the variability of the individual responses in the two groups. A two-way analysis of variance (ANOVA) showed that although there was a main effect of "STIMULUS INTENSITY" ($F(2, 64) = 19.7$, $P < 0.05$) on the motor evoked potential, there was no effect of "GROUP" ($F(1, 32) = 0.35$, $P = 0.56$) and no "GROUP" x "STIMULUS INTENSITY" interaction ($F(2, 64) = 0.23$, $P = 0.79$), indicating that a difference in corticospinal excitability between the two groups was not demonstrated.

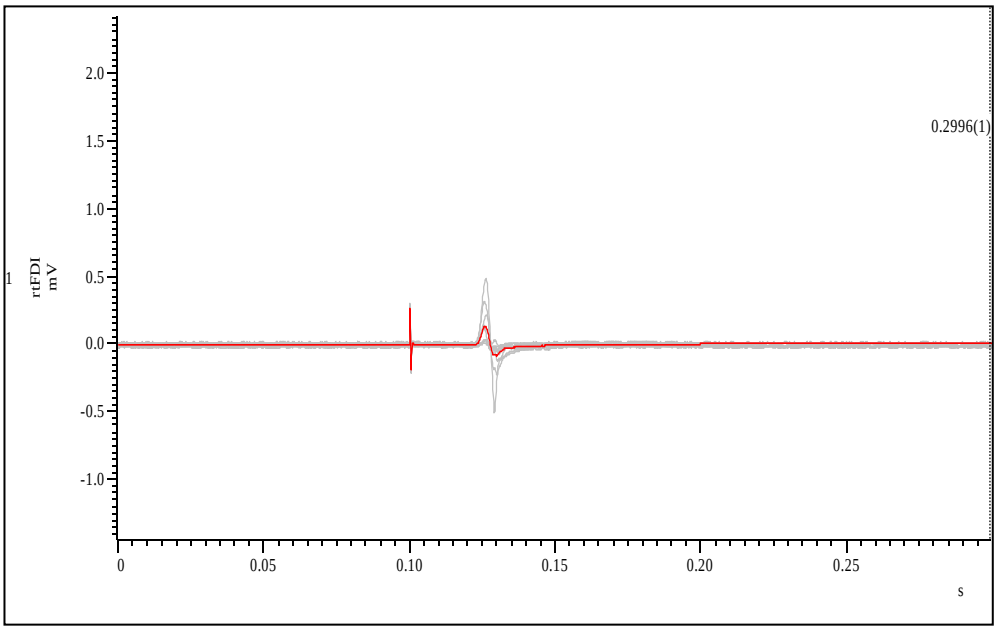
TBI					MEP/CMAP		
TBI no.	110%	120%	130%	CMAP	110%	120%	130%
1	0.305	1.205	1.718	18.856	0.016	0.064	0.091
2	0.878	1.680	1.113	17.977	0.049	0.093	0.062
3	1.029	1.254	1.709	12.146	0.085	0.103	0.141
4	1.103	3.824	3.868	14.215	0.078	0.269	0.272
5	0.570	1.315	2.209	13.412	0.043	0.098	0.165
6	0.320	0.857	1.392	16.676	0.019	0.051	0.083
7	0.282	0.859	1.758	17.161	0.016	0.050	0.102
8	0.504	0.746	1.674	14.646	0.034	0.051	0.114
9	0.433	0.917	2.190	17.809	0.024	0.051	0.123
10	0.524	1.182	1.393	20.231	0.026	0.058	0.069
11	0.954	1.535	3.017	16.901	0.056	0.091	0.178
12	0.612	0.451	0.717	20.186	0.030	0.022	0.036
13	0.700	1.041	0.719	19.705	0.036	0.053	0.036
14	0.405	0.597	1.711	18.497	0.022	0.032	0.093
15	0.261	0.840	2.049	15.854	0.016	0.053	0.129
16	0.372	0.902	2.681	16.759	0.022	0.054	0.160
17	0.661	1.278	1.231	18.279	0.036	0.070	0.067
Mean	0.583	1.205	1.832	17.018	0.036	0.074	0.113
SD	0.270	0.747	0.806	2.346	0.021	0.055	0.059
SE	0.065	0.181	0.196	0.569	0.005	0.013	0.014
COV	0.462	0.620	0.440	0.138	0.576	0.738	0.523

Table 4.1 Individual data for the raw and normalised MEPs in each patient, together with the calculated coefficient of variation (CV) over all individuals for each measurement point.

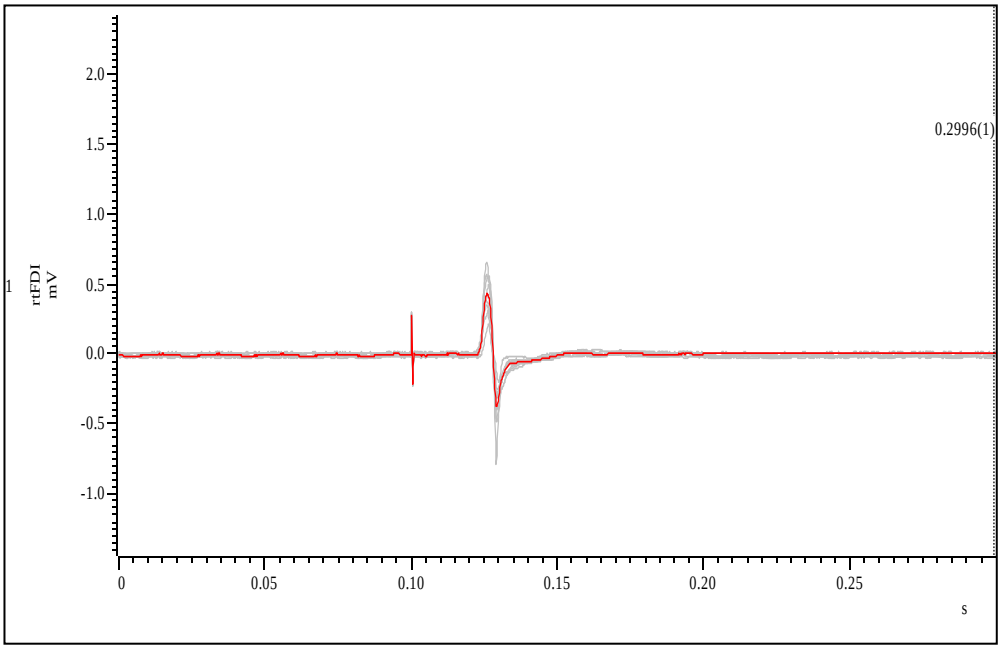
110%

↕ 0.5 mv

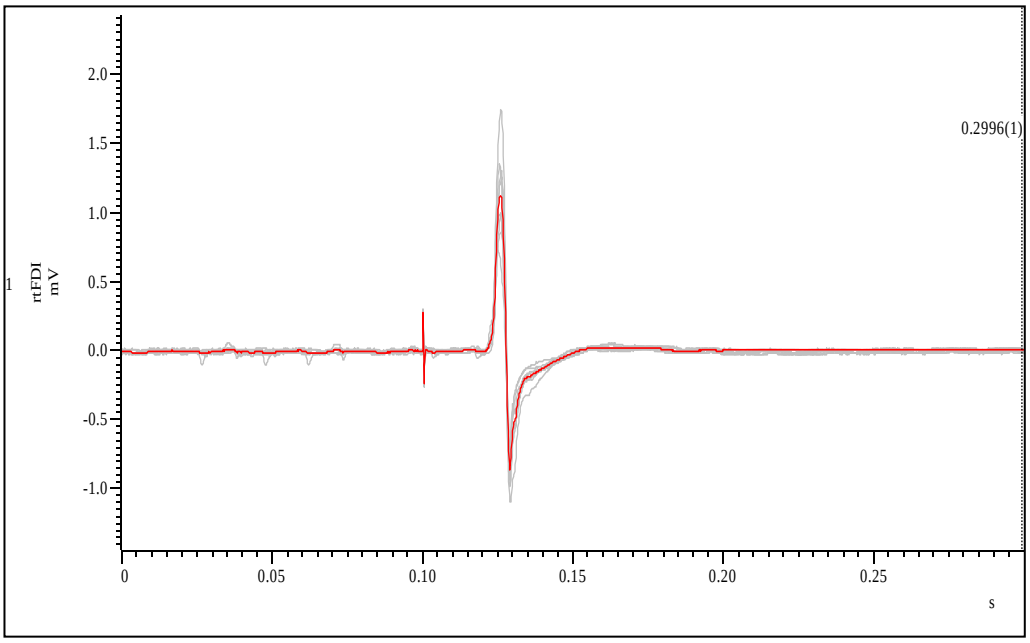
↔ 50 ms



120%



130%



CMAP

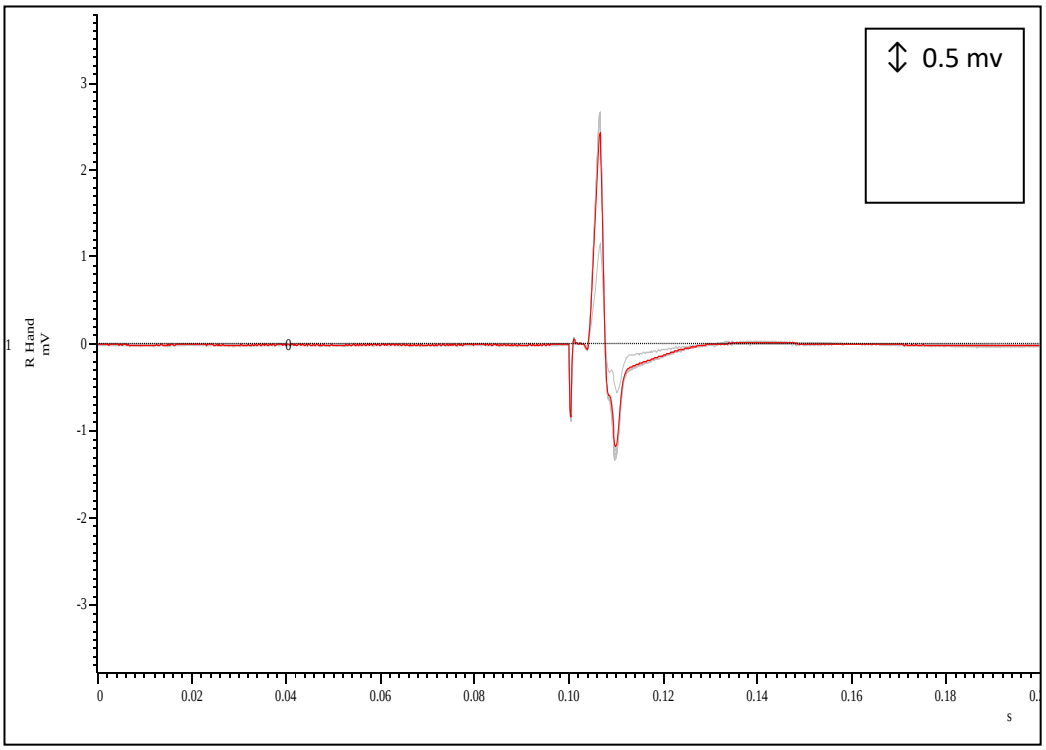


Figure 4.2 a) Raw data from one representative patient with traumatic brain injury showing the MEP at different stimulus intensities. The compound motor action potential (CMAP) is also shown.

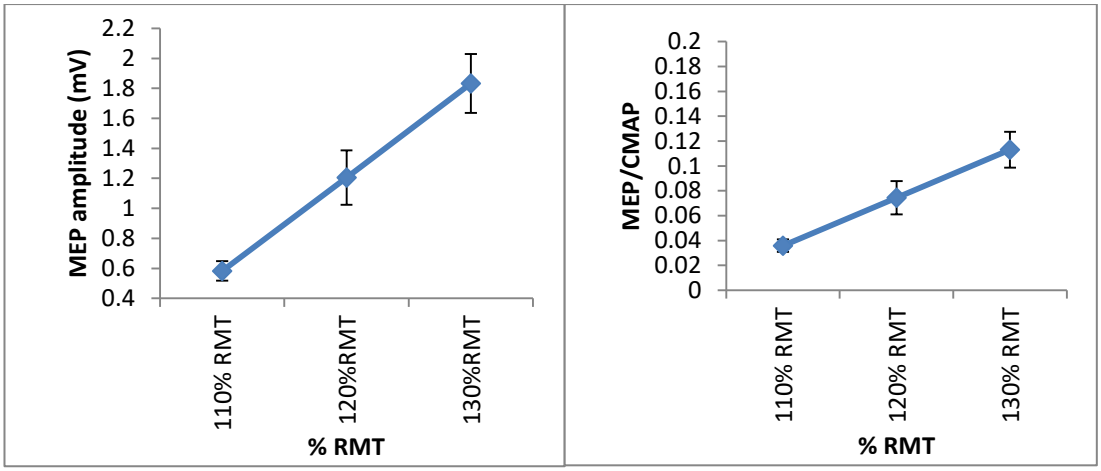


Figure 4.2 b) Effect of stimulus intensity on amplitude of MEP in the TBI group. Left panel: raw data (MEP peak to peak amplitude); right panel: normalised to each patient's peripheral CMAP in the TBI group

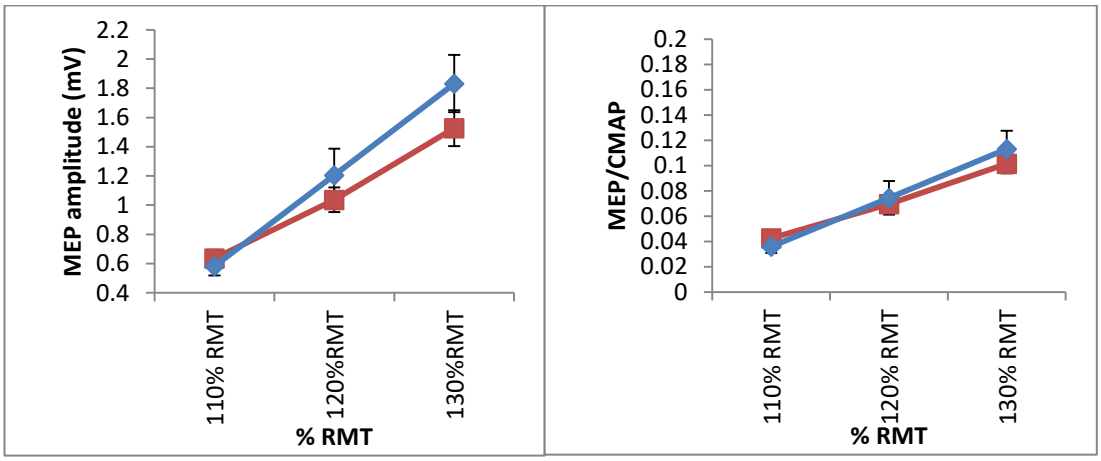


Figure 4.2 c) As in (4.2b), with healthy control group data superimposed (red points and connecting line)

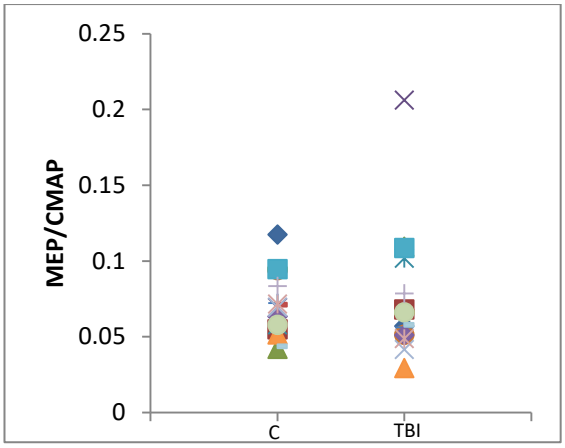


Figure 4.2 d) Scatter plot showing the variability of MEP/CMAP response in the control (C) and traumatic brain injury (TBI) groups. Each point plots the average response of the three intensities (110% RMT, 120% RMT, 130% RMT) in each individual.

4.4.3 Interhemispheric inhibition (IHI)

The time course of interhemispheric inhibition is plotted separately and combined in the control and traumatic brain injury groups in Figs 4.3a (raw and normalised) and 4.3b (comparison of traumatic brain injury and controls). The variation of individual responses in the control and traumatic brain injury groups at an interstimulus interval of 10ms is illustrated in 4.3b (right panel). Both groups show the expected two phases of inhibition with peaks at interstimulus intervals (ISI) of 8-12ms and 30-40ms (short interhemispheric inhibition SIHI and long interhemispheric inhibition LIHI, respectively). A two-way ANOVA with “INTERSTIMULUS INTERVAL” and “GROUP” as factors showed a significant main effect of interstimulus interval ($F(7, 21) = 5.10$, $P < 0.05$) on the motor evoked potential and a significant “GROUP” X “INTERSTIMULUS INTERVAL” interaction ($F(7, 21) = 2.33$, $P = 0.03$) but no effect of “GROUP” ($F(1, 30) = 0.053$, $P = 0.82$). This indicates that the effect of the interstimulus interval on the motor evoked potential (i.e. the amount of inhibition) produced is different between the traumatic brain injury group and the controls. Although the plots suggest a difference in interhemispheric inhibition between the two groups at 10ms and 12ms, there were no statistically significant differences between traumatic brain injury and controls at any single time point after post hoc comparisons.

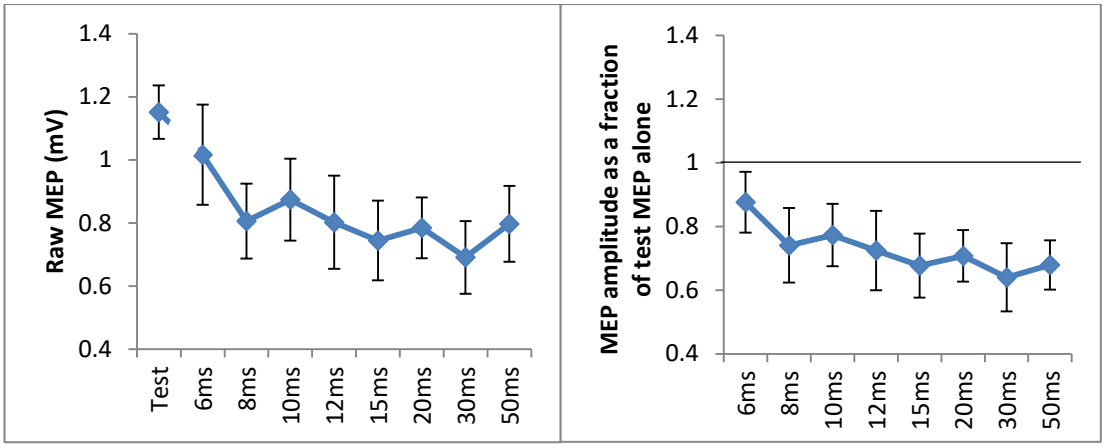


Figure 4.3 a) Transcallosal connections. Interhemispheric inhibition time course. Interhemispheric inhibition time course in TBI patients: left panel, average MEP amplitude at each time point. The leftmost point plots the amplitude of the unconditioned (test) MEP alone: the remaining points plot the MEP amplitude when preceded by a conditioning stimulus at each ISI. Right panel, data normalised to the size of the test response alone at each time point. The horizontal line at 1 indicates the baseline value.

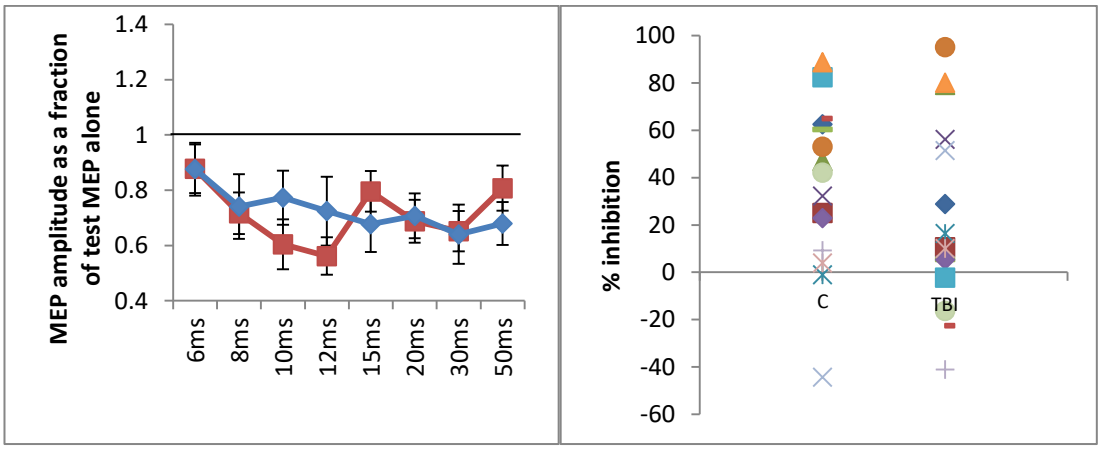


Figure 4.3 b) Transcallosal connections. Interhemispheric inhibition time course. Left panel: normalised time course as in (a, right panel) above, with healthy control data superimposed (red line and points). Right panel: scatter plot of individual IHI values at ISI = 10ms in controls (C) and TBI groups. Note, increasing inhibition in this graph is plotted upwards; facilitation of MEP is expressed as negative values. Therefore interhemispheric inhibition = 0% indicates no inhibition, and interhemispheric inhibition = 100% indicates complete inhibition.

The effect of changing the intensity of the conditioning stimulus on the amount of interhemispheric inhibition is plotted separately and combined in the control and traumatic brain injury groups in Figures 4.4a (raw and normalised), 4.4b (comparison of the two groups). A two-way ANOVA with “GROUP” and “STIMULUS INTENSITY” as factors showed a significant effect of intensity ($F(3, 78) = 26.4$, $P < 0.05$) but no effect of “GROUP” ($F(1, 26) = 0.10$, $P = 0.75$) or “GROUP” x “STIMULUS INTENSITY” interaction ($F(3, 78) = 2.26$, $P = 0.09$). This indicates that, unlike the time course of inhibition, a difference in the recruitment of transcallosal fibres between the two groups was not demonstrated.

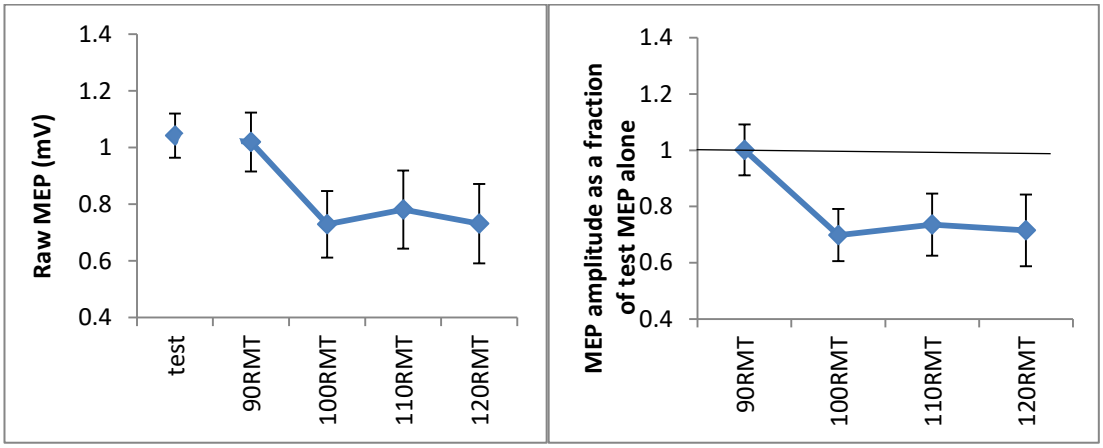


Figure 4.4 a) Interhemispheric inhibition: effect of conditioning stimulus intensity at fixed ISI (peak of inhibition at previous experiment) TBI patients: left panel, average MEP amplitude at each time point. The leftmost point plots the amplitude of the unconditioned (test) MEP alone: the remaining points plot the MEP amplitude when preceded by a conditioning stimulus at each intensity. Right panel, same data plotted as a fraction of the size of the test response alone at each conditioning intensity. The horizontal line at 1 indicates the baseline value. Inhibition became significant from conditioning stimulus intensities > 100% RMT ($P < 0.05$).

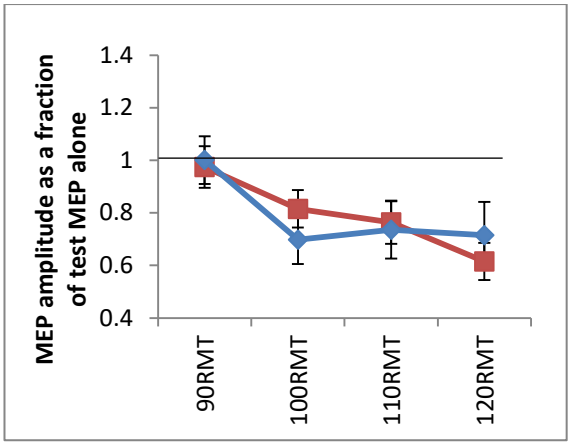


Figure 4.4 b) Interhemispheric inhibition: effect of conditioning stimulus intensity at fixed ISI (peak of inhibition at previous experiment) Same data as in right panel of (a) above but with healthy control data superimposed (red points and connecting line).

4.4.4 Intracortical pathways: Short interval intracortical inhibition (SICI)

Figures 4.5a (raw and normalised), 4.5b left panel (comparison of the two groups) plot the time course of short intracortical inhibition separately and combined in the two groups. Figure 4.5a (right panel) illustrates the variability of individual response in the two groups at an interstimulus interval of 2.5ms. A two-way ANOVA with factors "GROUP" and "INTERSTIMULUS INTERVAL" for (2, 2.5, 3, 4ms) showed the expected effect of "INTERSTIMULUS INTERVAL" ($F(2.34, 65.5) = 8.16$, $P < 0.05$) but no effect of "GROUP" ($F(1, 28) = 3.19$, $P = 0.09$) and no "GROUP" X "INTERSTIMULUS INTERVAL" interaction ($F(2.34, 65.5) = 0.07$, $P = 0.96$). A two-way ANOVA with factors "GROUP" and "INTERSTIMULUS INTERVAL" for (10 and 15 ms) showed no effect of "INTERSTIMULUS INTERVAL" ($F(1, 28) = 0.66$, $P = 0.42$), "GROUP" ($F(1, 28) = 0.98$, $P = 0.33$) and no "GROUP" X "INTERSTIMULUS INTERVAL" interaction ($F(1, 28) = 0.35$, $P = 0.56$). Thus, like the recruitment curves, but unlike interhemispheric inhibition, an effect of short intracortical inhibition time course was not demonstrated between the traumatic brain injury and controls groups.

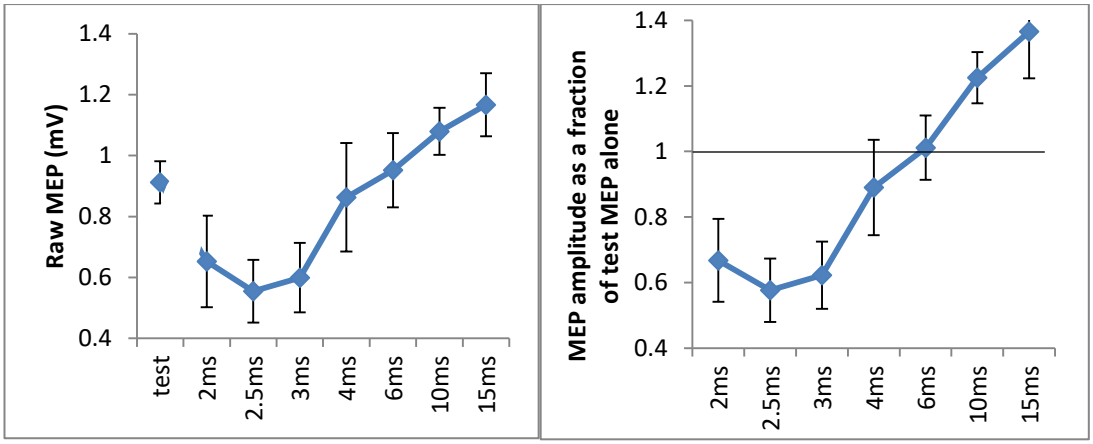


Figure 4.5 a) Intracortical connections: time course of short interval intracortical inhibition: SICI time course in TBI patients: left panel, average MEP amplitude at each time point. The leftmost point plots the amplitude of the unconditioned (test) MEP alone: the remaining points plot the MEP amplitude when preceded by a conditioning stimulus at each ISI. Right panel, time course plotted as a fraction of the size of the test response alone at each ISI. The horizontal line at 1 indicates the baseline value.

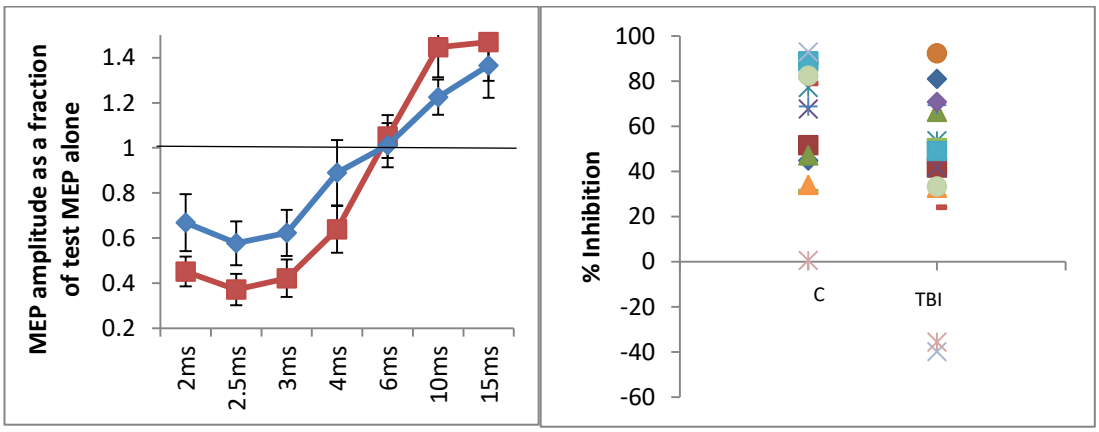


Figure 4.5 b) Intracortical connections: time course of short interval intracortical inhibition: Left panel: normalised time course as in (a) (right panel) above, with healthy control data superimposed (red line and points). Right panel: scatter plot of individual SICI values at ISI = 2.5 ms in control (C) and TBI groups. Note, increasing inhibition in this graph is plotted upwards; MEP facilitation is expressed as negative value. Therefore inhibition = 0% indicates no inhibition, and inhibition = 100% indicates complete inhibition.

This was also reflected in the short intracortical inhibition recruitment curves (Figs 4.6 a, b). A two-way ANOVA with “GROUP” and “STIMULUS INTENSITY” as factors showed the expected effect of conditioning intensity ($F(2.17, 60.7) = 33.4$, $P < 0.05$), “GROUP” ($F(1, 28) = 9.89$, $P < 0.05$), but no “GROUP” x “STIMULUS INTENSITY” interaction ($F(2.17, 60.7) = 0.71$, $P = 0.50$). This means that the effect of increasing stimulus intensity on the amount of inhibition in the short intracortical pathways was different between the two groups, even though a difference in the effect of the interstimulus interval on the amount of inhibition was not demonstrated. As with the healthy participant data, inhibition in the traumatic brain injury group became significant at a conditioning intensity from 70% AMT ($P < 0.05$).

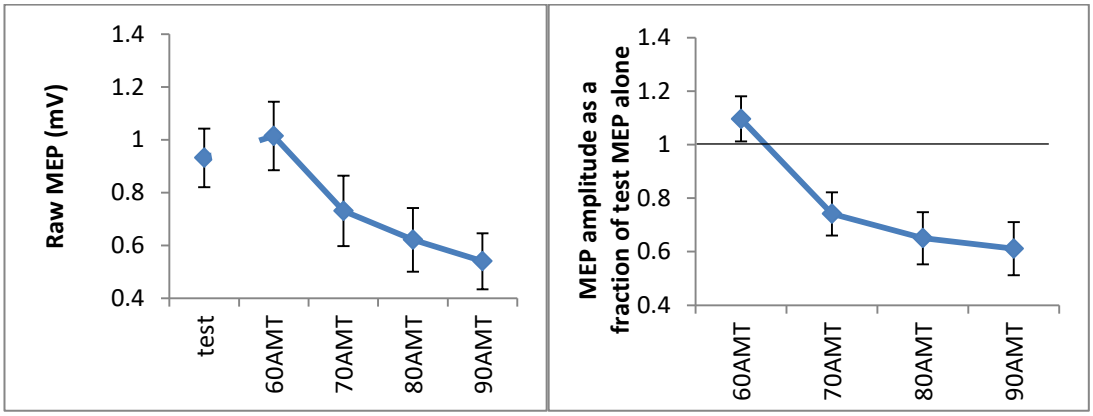


Figure 4.6 a) Short interval intracortical inhibition: effect of conditioning stimulus intensity at fixed ISI (peak of inhibition from previous experiment) Data from TBI patients: left panel, average MEP amplitude at each intensity of the conditioning stimulus. The leftmost point plots the amplitude of the unconditioned (test) MEP alone: the remaining points plot the MEP amplitude when preceded by a conditioning stimulus at each intensity. Right panel, same data plotted as a fraction of the size of the test response alone at each conditioning intensity. The horizontal line at 1 indicates the baseline value.

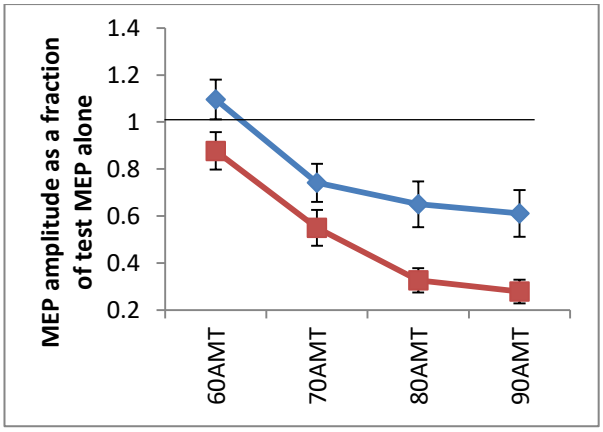


Figure 4.6 b) Short interval intracortical inhibition: effect of conditioning stimulus intensity at fixed ISI (peak of inhibition from previous experiment) as in (a) (right panel) above with healthy control data superimposed (red points and line).

4.5 Discussion

The current study provides physiological information on the white matter pathways after traumatic brain injury. The main finding from this study is that physiological transfer across the corpus callosum is different between the patient group and control. There were no significant differences in measures of corticospinal excitability (motor thresholds, recruitment curves) or intracortical inhibition (effect of interstimulus interval on amount of intracortical inhibition) in the traumatic brain injury patients, compared to the controls. This provides support that pathways across the corpus callosum are physiologically affected after traumatic brain injury. This data also revealed a range of responses in the patient group, which can be explored further in the correlation analysis later in the thesis. This study is also in patients who are not on any neuromodulatory medication. In addition, this particular cohort of patients had no physical clinical neurological deficit at the time of testing.

4.5.1 Patient group

Patients were recruited from a traumatic brain injury database covering a number of centres in London. Because of the perceived risk of seizure in this relatively new group of patients to transcranial magnetic stimulation, strict adherence to transcranial magnetic stimulation safety guidance influenced our patient selection (Wassermann, 1998, Rossi et al., 2009). Out of a possible 115 traumatic brain injury patients on the database, only 20 patients were eligible to be included in our study. Only 17 agreed to participate. The main reasons for exclusion from the study were either due to the use of neuromodulatory medication or history of intracerebral haemorrhage. A study size of this magnitude is not unusual for transcranial magnetic stimulation studies. However, compared to larger MRI studies, the traumatic brain injury cohort investigated in this study is relatively small. This means care must be exercised in extrapolating conclusions from this study to the larger traumatic brain injury population.

As a result of this inclusion criteria, the participants in this study included all patients with mild, moderate to severe traumatic brain injury who were eligible and who agreed to participate. All of the patients had sustained an impact injury to the brain on one occasion (from a road traffic accident, sports injury or assault) or falls causing a loss of consciousness, rather than a history of repeated injuries over many years, such as the type of injuries experienced by boxers and footballers. Therefore, this simplifies any inferences from this study, and it does not have to take into account the effect of a single head injury versus the effects of multiple head injuries over a period of time (such as experienced in sports). On the other hand, the fact that the patient group were heterogeneous to traumatic brain injury-type, severity and neuroimaging results would inevitably reduce the statistical power of comparisons with the healthy controls, and is a limitation of this study. Also, it is possible that the participants who had sustained their head injury during an assault, may have suffered multiple impacts to the head, even though it was only on one occasion. These limitations, however, are not restricted to this study, and are likely to be common across traumatic brain injury research in general; unlike stroke, where patients can be selected on the basis of a focal lesion, traumatic brain injury is a diffuse brain injury (Greenwood et al., 2016). The patient group who participated in this study is probably

grossly reflective of the heterogeneity encountered in the general traumatic brain injury population. Selection of patients on the basis of a particular structural imaging result or particular type of injury would be ideal, but would require a far larger traumatic brain injury cohort which was not available at the time. However, this may be possible in the future.

In the UK, for all traumatic brain injury severities (mild, moderate to severe), there is a peak in incidence in those between 80 and 90 years of age. However, for those with severe traumatic brain injury, there is a smaller peak between the ages of 20 and 30 years (Lawrence et al., 2016). There was a wide age range in our patient group, between 22 years and 60 years. Although the average age of our group did not fit either of those peaks, it did reflect the fact that whilst stroke is characteristically a disease of “older age”, traumatic brain injury affects a wider age range. Our average age of the patient group was 43 years. The observation has been made from UK data between 2015 and 2016, that the younger group are more likely to sustain the traumatic brain injury from road traffic accidents and assaults, whereas with increasing age there is an increase in injuries sustained after falls under 2 metres (Lawrence et al., 2016). Eleven participants from our patient group had been involved in road traffic accidents. Two participants had been involved in assaults. Two participants had been involved in a single sports-related accident (one horse riding accident, and one participant had been involved in a rafting accident). All of these participants were under the age of 55 years of age at the time of their individual injury. The two participants who had suffered a fall were over 55 years of age at the time of injury. Unfortunately, our patient group did not have the benefit of patients who were over the age of 80 years. However, the injury subtypes with age in this study does reflect the spread of injuries with age in the existing literature (Lawrence et al., 2016).

There have been relatively few neurophysiological studies of traumatic brain injury. Previous work with transcranial magnetic stimulation have mainly examined stimulation threshold, often in patients taking neuromodulatory medications. Neuromodulatory medications can interfere with physiology measured by transcranial magnetic stimulation (Ziemann, 2003, 2004, 2008).

In contrast, the patients included in this study were not taking any neuromodulatory medication. This is therefore a strength of this study. All patients investigated had sustained their injury at least one year prior to testing. The consequences of possible neurophysiological fluctuations in corticospinal excitability in the acute phase should have been minimised, as previously reported (Chistyakov et al., 1998). However, the variation of time post injury was large in our study (mean 44.3 months ± SE 6.29) which may have impacted on the range of physiological values obtained.

Despite the described limitations of this current study, the breadth of neurophysiological parameters assessed by transcranial magnetic stimulation in this present data in patients who were not on any neuromodulatory medication, represents a fairly unique dataset.

4.5.2 Corticospinal excitability

Motor thresholds

No significant difference was found in any of the threshold measures (RMT LH, RMT RH, AMT, 1mV MEP) in the traumatic brain injury group, compared to controls.

Motor threshold analysis in the TBI group has produced mixed results in the existing literature. Some groups have found motor thresholds to be elevated in traumatic brain injury (Chistyakov et al., 1998, Bernabeau et al., 2009, Nardone et al., 2011 and Tallus et al., 2012). However, the traumatic brain injury patients investigated in the studies of Chistyakov and colleagues and Nardone and colleagues were investigated during the acute phase of traumatic brain injury (Chistyakov et al., 1998, Nardone et al., 2011). It is also worth noting that Nardone and colleagues' findings of increased RMT were only found in the subgroup of patients with excessive daytime sleepiness, and not in those without the symptom (Nardone et al., 2011). The study by Bernabeu and colleagues included patients on neuromodulatory medication (Bernabeu et al., 2009). Tallus and colleagues, however, investigated unmedicated patients

with mild traumatic brain injury, on average five years after injury. Their study demonstrated higher thresholds than their healthy participants, thus proposing a chronic physiological consequence following injury (Tallus et al., 2012). The reasons for this finding is unclear; their study only investigated mild traumatic brain injury and it may reflect the nature of the injuries sustained, but this is not described in the paper. In contrast, similar to the current study, there were no differences demonstrated in motor thresholds in a similar group of patients (Takeuchi et al., 2006, Fujiki et al., 2006).

Elevation in motor threshold is well described in the acute period following stroke, and has been demonstrated in a longitudinal study to normalise with time (Swayne et al., 2008). The follow-up study by Chistyakov and colleagues in traumatic brain injury also demonstrated normalisation of the motor threshold after three months (Chistyakov et al., 2001). It is therefore possible that a similar process occurs after traumatic brain injury, but that is beyond the scope of this study. However, as the patients in this current study were all tested at least a year after injury, it is possible that such a mechanism may explain why motor thresholds were similar in patients and controls.

In the traumatic brain injury group, the average left hemisphere RMT was less than the right hemisphere RMT. This is well reported in healthy individuals (Navarro et al., 2009, Maeda et al., 2000), However, in this study the range of motor thresholds in both left and right hemispheres was larger in the traumatic brain injury group than the control group, which is harder to explain. This may reflect the variation in time after injury and the heterogeneity of the individual's damage encountered following their traumatic brain injury.

4.5.3 Recruitment curve

In the current study no statistically significant difference between motor evoked potential recruitment in the traumatic brain injury patients was found, compared with healthy controls.

This was initially a surprising finding, given the previous findings by Bernabeu and colleagues. However, the study by Bernabeu and colleagues only investigated patients with severe traumatic brain injury (Bernabeu et al., 2009) while the current study included patients with all types of traumatic brain injury. In addition, eight of the seventeen patients in the study by Bernabeu and colleagues were on neuromodulatory medication for seizures or mood disturbance. These medications included Valproate, Fluoxetine, Phenytoin and Venlafaxine. This complicates interpretation of the physiological findings for that study, as it is likely that the physiological parameters investigated have been influenced by the medications, and may not be a true representation of the underlying physiology.

The recruitment curve is a further measure of corticospinal excitability. However, I had proposed it should be affected in the traumatic brain injury group as it reflected the physiological function of a long white matter tract. I had initially anticipated that there might be reduced corticospinal excitability in the traumatic brain injury patients, reflecting possible damage to the long descending fibres of the corticospinal tract with the nature of the injury. However, this was not the case in this cohort of traumatic brain injury patients. This may be because the patients in the traumatic brain injury group had no motor neurological deficit at the time of the study. The patients in the study by Bernabeu and colleagues had motor weakness (according to MRC scale) at the time of testing, which may account for their results. This may have been contributory to the comparable physiological response of the traumatic brain injury patient group, with the healthy controls in the current study.

Finally, although there were no significant differences in measures of recruitment between the patient group and controls, graphically, the range of recruitment values was wider in the traumatic brain injury group, compared to the healthy controls. This may reflect the heterogeneity of the traumatic brain injury disease spectrum.

4.5.4 Interhemispheric inhibition

In the breadth of physiological parameters investigated, this study demonstrated that the effect of interstimulus interval on the amount of interhemispheric inhibition (as measured by the evoked motor potential amplitude) was different between the traumatic brain injury patient and control groups. Interhemispheric inhibition is likely mediated predominantly by transcallosal inhibition (Ferbert et al., 1992, Di Lazzaro et al., 1999). This physiological finding does provide physiological support that the physiology of the transcallosal pathways are likely to be affected after traumatic brain injury. This may reflect the proposed shearing nature of the injury. This physiological finding would complement existing imaging and post mortem literature findings that the corpus callosum is particularly vulnerable in traumatic brain injury (Gentry et al., 1988a, Parizel et al., 1998, Meythaler et al., 2001, Scheid et al., 2003).

To the best of my knowledge, only one previous study of interhemispheric inhibition in traumatic brain injury existed at the time of designing this study, which demonstrated reduced interhemispheric inhibition in traumatic brain injury patients, compared to control (Takeuchi et al., 2006). The mean duration post traumatic brain injury was 1.83 years in the study by Takeuchi and colleagues. The study presented in this chapter therefore supports the findings of reduced interhemispheric inhibition demonstrated in the study by Takeuchi and colleagues. However, the current study has additionally demonstrated that the effect on interhemispheric inhibition persists for longer, since the mean duration following traumatic brain injury in this current study was 3.72 years (range 1.1 – 7.5 years).

As previously mentioned, the cortical silent period is also a physiological measure of callosal transfer. It was not measured as part of this current study. There are additional studies in which the cortical silent period has been tested. Bernabeu and colleagues found the cortical silent period to be normal in their patients (Bernabeu et al., 2009). However, as noted above, many of these patients were taking neuromodulatory medication. Tremblay and colleagues have demonstrated elevated ipsilateral silent period in concussed Canadian footballers, compared

to non-concussed footballers, at least one year following the last injury (Tremblay et al., 2011). However, unlike the patient group in the current study, these athletes had suffered at least two impact injuries to the head over a period of time. De Beaumont and colleagues have also demonstrated increases in cortical silent period in concussed athletes, two years and thirty years following impact, compared to non-concussed athletes (De Beaumont 2007, 2009). However, Pearce and colleagues have demonstrated reduced cortical silent period duration in retired Australian footballers who had sustained concussions during their career, and at least two decades prior to testing (Pearce et al., 2014). Interhemispheric inhibition and cortical silent period both reflect interactions between the motor cortices, but are thought to originate by different mechanisms. Interhemispheric inhibition and cortical silent period are therefore considered complementary, rather than equivalent measures (Chen et al., 2003, Davidson et al., 2013). No direct comparisons can be made between these studies and the current study. However, the existing work suggests that the physiological changes to the transcallosal transfer after injury is abnormal, even years after the injury.

In the current study, no single time interval was identified where the demonstrated interaction effect was significant. This may be due to the variety of injuries in the patient group, and/or the duration following injury in our patient group. However, graphically, the time period of reduced interhemispheric inhibition was observed at 10-12ms, which is within the period of short interval interhemispheric inhibition. The time period of long interval interhemispheric inhibition was less different in the two groups graphically. However, this is not surprising as it is thought that interhemispheric inhibition at short and long intervals are likely to be mediated by different mechanisms (Chen et al., 2003).

The recruitment of inhibitory fibres in the traumatic brain injury group, as evidenced by the conditioned recruitment curve was similar in the patient group and healthy control group. It is not immediately obvious why this is the case. With the proposed damage and loss of some callosal axons associated with the condition, it is possible that the net effect produced by the remaining connections resulted in similar recruitment due to compensatory mechanisms in

connectivity after traumatic brain injury. Compensatory alterations in networks in chronic traumatic brain injury have already been described in the cognitive literature following traumatic brain injury (Bonnelle et al., 2011). However, it is also possible the conditioning pulse is not activating the callosal fibres directly, but indirectly i.e. transynaptically, like the corticospinal tract. Thus, there could have been adjustments in the excitability of these inputs to compensate for the smaller number of surviving callosal neurons following injury.

The findings from this particular study could support the hypothesis that traumatic brain injury affects the transcallosal connections preferentially (Gentry et al., 1998a,b). Interhemispheric inhibition measures are dependent on additional factors outside the corpus callosum. Therefore the fact that this study only demonstrated a physiological difference in interhemispheric inhibition, but not the corticospinal excitability measures would support that the demonstrated physiological difference relates to the physiology of the corpus callosum, rather than affected by the physiology of the corticospinal tract. The findings from this study lend themselves to the possibility that the nature of the shearing injury in traumatic brain injury disrupts the physiological balance between the inhibitory and excitatory interneurons across the corpus callosum. A larger study may be able to define this more clearly. The fact that the late period of interhemispheric inhibition, appearing to be less affected in traumatic brain injury, would support its representation of a separate mechanism (Chen et al., 2003).

4.5.5 Intracortical inhibition

In the present study, there was no significant difference in the short intracortical inhibition time course. However, recruitment of short intracortical inhibitory fibres was different in the group of traumatic brain injury patients compared with the healthy control group.

Although there are previous studies investigating intracortical inhibitory circuits, in the study by Lapitskaya and colleagues, only a small number (5/19) investigated had traumatic brain

injury. In that study, two patients were treated with selective serotonin receptor antagonists (drugs not specified), two with Methylphenidate, and two with Sodium Valproate and Clonazepam combination. Methylphenidate and some selective serotonin receptor antagonists (SSRI) are well known to affect physiological parameters (Ziemann, 2004, 2008). Conversely, Citalopram, a commonly prescribed SSRI has been shown to increase short intracortical inhibition (Ziemann, 2003). It is therefore difficult to draw conclusions from this study in the context of the use of the neuromodulatory drugs. However, the study by Fujiki and colleagues investigated short intracortical inhibition in the chronic phase. It did not demonstrate any significant difference in short intracortical inhibition in the traumatic brain injury group (Fujiki et al., 2006). The number of patients (sixteen) was similar to this current study. It is, however, difficult to draw conclusions from the other studies in the context of their concomitant neuromodulatory medication use.

More recent studies have demonstrated reduced short intracortical inhibition in athletes. Pearce and colleagues have demonstrated reduced short intracortical inhibition in retired Australian footballers who had sustained concussions during their career, and at least two decades prior to testing (Pearce et al., 2014). However, Tremblay and colleagues have demonstrated no significant difference in short intracortical inhibition between concussed athletes, compared to non-concussed athletes (Tremblay et al., 2011). It is difficult to draw conclusions from this. The study by Pearce and colleagues demonstrated comparable corticospinal measures (MEP recruitment curve) between the patient group and controls. This implies that the difference is due to reduced local inhibition (rather than damage to the corticospinal tract). Short intracortical inhibition is a measure of intracortical excitability i.e. intracortical circuits that arise within the same cortical area (Kujirai et al., 1993). Transcranial magnetic stimulation works by stimulating cortical inhibitory and excitatory interneurons to a localised area (Rothwell, 1997). The difference demonstrated between the two groups in the recruitment of the intracortical fibres in the current study suggests some difference exists in the physiological function of the more localised pathways, especially as this was in the presence of comparable corticospinal excitability. However, it is less surprising that there was

no difference demonstrated in the short intracortical inhibition time course in the current study as the localised circuit would be less vulnerable to the shearing nature of a traumatic brain injury, than the transcallosal pathways. However, the reported significantly reduced short intracortical inhibition in the sports literature may be explained by repeated impacts in sports injuries being more likely to cause localised damage to the underlying cortex. It is possible this would then render the more localised (intracortical) pathways to be vulnerable, which would in turn affect their underlying physiology.

4.5.6 Study limitations

The recruitment for this study was limited by transcranial magnetic stimulation safety criteria. Therefore, all patients who were eligible to participate were approached. The patient group therefore consists of the spectrum of traumatic brain injury. Therefore, care must be exercised in extrapolating conclusions from this study to the larger traumatic brain injury population. Further study limitations have been discussed in more detail in the body of the discussion.

4.6 Clinical considerations

The physiological abnormalities demonstrated in this current physiological study could reflect the shearing of callosal fibres thought to underlie traumatic brain injury. Shearing would plausibly disrupt the physiological function of the callosal fibres. It seems most likely that the significant finding in this current study is a detrimental physiological abnormality as a result of damage in the context of the injury. However, it is difficult to confidently conclude at this stage that the abnormalities in interhemispheric inhibition demonstrated in the current study are detrimental. Reduced interhemispheric inhibition is well recognised in a number of disease states, such as stroke and multiple sclerosis (Boroojerdi et al., 1998, Butefisch et al., 2008). However, interhemispheric inhibition is also reduced in musicians, a group of healthy individuals who would have exquisite bimanual coordination, thus raising the possibility that reduced interhemispheric inhibition is due to plasticity changes with intense practice, in order

to be able to play a musical instrument (Ridding et al., 2000, Fling et al., 2012c). Therefore, at this stage of the investigation into traumatic brain injury, there also exists the possibility that the findings are an adaptive change to the physiology across the corpus callosum, following the traumatic brain injury.

4.7 Conclusions

Through the physiological study of a selection of short and long tracts using transcranial magnetic stimulation, this study of chronic traumatic brain injury patients demonstrated that the main difference was different interhemispheric inhibition, where the relationship between the interstimulus interval and amount of inhibition was different in the patient group, compared to the controls. In the absence of abnormalities in the other measures of corticospinal excitability, it seems likely that the abnormalities in interhemispheric inhibition relate to the corpus callosum. It is possible the corpus callosum is more vulnerable than the corticospinal tract due to the orientation of the respective fibres in these tracts. The nature of the acceleration deceleration shearing injury preferentially affecting the fibres across the corpus callosum therefore seems a plausible hypothesis.

The next step is to understand the physiological findings from this study, in the context of the behavioural consequences of callosal transfer following traumatic brain injury. This will be the focus of the next two chapters.

Chapter 5

Measuring Behaviour in Healthy Individuals

I led this study and was responsible for study concept, recruitment, experimental work, analysis and interpretation of the study. The MATLAB (MathWorks, USA) script used was programmed by Dr J Galea.

5.1 Introduction

In the last chapter, the physiology of white matter pathways was examined using transcranial magnetic stimulation in traumatic brain injury patients, and compared to healthy controls. This examined a selection of white matter pathways, including the assessment of corpus callosal physiology using a twin coil transcranial magnetic stimulation method to assess for interhemispheric inhibition. Through this study, the only significant finding was that the traumatic brain injury patient group demonstrated less interhemispheric inhibition, with the effect of the interstimulus interval on the amount of inhibition being different in this patient group, compared to the healthy controls. There were no significant differences in measures of corticospinal excitability (motor thresholds, recruitment curves) or time course of intracortical inhibition (short interval cortical inhibition) in patients, compared to controls. The consequences of traumatic brain injury are thought to be due to diffuse axonal injury (Strich, 1961, Adams et al., 1977, Adams, 1982, Adams et al., 1989). This is understood to be due to shearing of nerve fibres in the context of acceleration, deceleration, and rotational forces on the brain as a result of the injury (Strich, 1961, Adams, 1982, Arfanakis et al., 2002, Huisman et al., 2004, Meythaler et al., 2001). As mentioned in the previous chapter, the findings in the previous study would provide physiological evidence to support the existing post-mortem and imaging studies, and other studies in the traumatic brain injury literature, demonstrating the corpus callosum is particularly vulnerable. Of particular importance to the neurorehabilitation of traumatic brain injury sufferers, the corpus callosum is also particularly relevant to tasks that involve bimanual coordination of the limbs. Deficits in bimanual function are known to occur in patients with lesions of the corpus callosum or partial callosotomies (Tuller et al., 1989, Ivry et al., 1999, Eliassen et al., 2000, Kennerley et al., 2002).

The next stage will investigate the behavioural consequences following traumatic brain injury. Prior to undertaking a study on patients, a suitable measure of behaviour was initially explored. In the context of the physiological findings of abnormal interhemispheric inhibition in the traumatic brain injury group, a behavioural measure of interhemispheric interaction would be a natural choice.

Interhemispheric interactions are utilised in every day function. They are part of functional tasks where the upper limbs or lower limbs are moving out of phase with each other and have already been demonstrated to be significantly greater during unimanual and bimanual out of phase movements, and down-regulated when the limbs perform simultaneous movements using transcranial magnetic stimulation (Stinear et al., 2002, Sohn et al., 2003). Behavioural work by Ivry and colleagues has already demonstrated that tapping is less variable during synchronous bimanual tapping, compared to unimanual tapping (Ivry et al., 1999). This may be due to the fact that bimanual simultaneous movements require less attention than asynchronous movements (Swinnen, 2002).

It has been observed that tasks where collaboration and coordination between the hands is required, such as opening a bottle, driving a car or playing a musical instrument, are often difficult to perform following traumatic brain injury (Caeyenberghs et al., 2011a). From a functional point of view, these tasks require each hand to work independently to undertake the task. The coordination of this hand movement is essential for successful completion of the task (Swinnen, 2002, Carson, 2005). Bimanual control has already been studied through functional neuroimaging. A widespread brain activation pattern, which includes the primary sensorimotor cortex, supplementary motor area, cingulate motor cortex, lateral premotor cortex, parietal cortex and subcortical structures has been identified in the coordination of such movements (Sadato et al., 1997, Jancke et al., 2000a, Kermadi et al., 2000, Deiber et al., 2001, Immisch et al., 2001, Debaere et al., 2004). It is currently proposed that interhemispheric transfer through the corpus callosum plays a major part in the coordination of the hands during the task, and that this is mediated through excitatory and inhibitory transcallosal communications between the motor cortices (Aboitiz, 1992). Since interhemispheric inhibition is physiologically abnormal in this group of traumatic brain injury patients (as shown in the last chapter), bimanual coordination would be an ideal area to examine through a behavioural task.

The focus of this chapter is therefore to develop a behavioural task to assess the callosal transfer in a group of healthy individuals. Such a task would need to examine a range of interhemispheric interactions, whilst being sufficiently simple to undertake by the traumatic

brain injury patient group in a later study. In the callosotomy literature, unimanual and bimanual conditions have been assessed in a variety of methods, including variations on circle drawing tasks and discrete tapping tasks (Tuller et al., 1989, Ivry et al., 1999, Eliassen et al., 2000, Kennerley et al., 2002). In the study by Kennerley and colleagues, no relationship was identified between the unimanual tap and synchronous circle drawing tasks. Both types of task demonstrated increased variability with the asynchronous movement (Kennerley et al., 2002). However, the variability of the discrete tapping task was higher in the corpus callosotomy group, than in the control group.

Finger tapping tasks are commonly used to study behaviour in humans. They have the advantage of being relatively simple, thus making them appropriate in the study of healthy participants and patient groups. Existing studies tend to vary according to the complexity of the task, and the presence or absence of a pacing stimulus. A finger tapping task therefore seems to be a natural choice to assess behaviour for the purpose of this study.

Other considerations when developing this task related to whether the finger tapping should be in response to a stimulus at a predetermined rate (i.e. externally generated). The stimulus itself could be an auditory or visual cue (Sadato et al., 1996, Jancke et al., 2000b). Alternatively, the finger tapping could be performed in the absence of a stimulus (i.e. self-paced or internally-generated). The use of a pacing stimulus has the benefit of ensuring the participants perform the tapping at a predetermined rate. A meta-analysis by Witt and colleagues demonstrated that self-paced tasks require more complex brain activation patterns and cognitive control, involving several frontal and prefrontal regions, including the lateral aspects of the premotor and parietal cortex and dorsolateral prefrontal cortex, than those utilised in the externally-generated tapping tasks (Witt et al., 2008). Interestingly, the same meta-analysis demonstrated that different brain activation pathways are recruited in the presence of a visual stimulus, compared to an auditory stimulus. Since the main objective of this study was to obtain information about callosal transfer, an externally-generated pacing stimulus was chosen. As the deficits in traumatic brain injury are thought to lead to network breakdown, it was decided that the reaction to the cue would provide a measure of

responsivity, rather synchronicity with the cue, which would likely involve more complex networks.

Investigation of bimanual coordination in the existing corpus callosotomy literature has provided some insight into the role of the corpus callosum in bimanual movement. The limitation is that these studies usually involve very small numbers. However, bimanual coordination in older adults has been explored and related to reduced microstructure in the corpus callosum (Fling et al., 2011a). Older individuals demonstrate more breakdown in bimanual coordination and have slower movement times when compared to their younger counterparts (Stelmach et al., 1988, Swinnen, 2002). Older adults are also reported to have difficulties with executive function, processing speed and inhibitory control (Hasher et al., 1988, Salthouse, 1996, Bangert et al., 2010). Therefore it might be reasonable to explore a task format which has been used successfully in older adults, since traumatic brain injury patients often experience similar deficits (Centers for Disease Control and Prevention 2003, Castellanos et al., 2010, Kinnunen et al., 2011). Existing work by Fling and colleagues compared callosal interactions in older adults with younger adults. The tapping conditions described in Fling and colleagues' work demonstrated a range of interhemispheric interactions (Bangert et al., 2010, Fling et al., 2011a). The interhemispheric interactions assessed were simultaneous bimanual (low interhemispheric interaction), right hand unimanual (moderate interhemispheric interaction), left hand unimanual (moderate interhemispheric interaction), right leads left bimanual (high interhemispheric interaction), left leads right bimanual (high interhemispheric interaction). Their work demonstrated that older adults were disproportionately impaired at bimanual tasks compared to younger adults. This was particularly manifest in the task that involved the greatest interhemispheric interaction (bimanual asynchronous condition). Their work concluded that performance in these tapping tasks is linked to inhibitory interhemispheric interactions, and correlated with fractional anisotropy microstructure of the corpus callosum on diffusion tensor imaging. (Fling et al., 2011a), a topic that will be explored later in this thesis (Chapter 8). The behavioural tapping task in this present study was based on the principles detailed above. As results in the behavioural literature are often difficult to reproduce due to differences in methodology, I chose

to initially undertake a larger study involving healthy participants to ensure that this behavioural task would provide reasonable information about the different conditions.

In the context of the existing literature, I expected the average performance of the healthy participants to become slower as the movement complexity increased. I expected this to manifest as an increase in the mean reaction time from the bimanual simultaneous movement through to the bimanual asynchronous movement. I also expected the standard deviation of the mean (i.e. variability) to also increase as the task becomes more complex. Finally, I expected this variability to be most marked in the condition that assessed the highest interhemispheric interaction, as this would be the hardest to achieve.

5.2 Study design

5.2.1 Participants

This study involved healthy participants who had been recruited from a database of healthy volunteers from the UCL Institute of Neurology and Imperial College. 29 healthy participants consisting of 15 males and 14 females were involved in this study. All healthy subjects were on no regular medication. They had no significant past medical history. They had no known uncorrected visual or physical impairments.

5.2.2 Institutional and ethical approval

The information sheets and protocols were reviewed and ethical approval was obtained from the Ethics research committee.

Participants gave written consent prior to participating in the study, and were given the option to withdraw from the study at any time. All studies were performed in accordance with the Declaration of Helsinki.

5.3 Experimental Methods

5.3.1 Behavioural measures

Participants in this behavioural study performed a finger tapping experiment presented on a desktop computer running MATLAB (MathWorks, USA). This was a reaction time task and the order of the presentation of the testing blocks was not randomised between participants. Participants performed five finger-tapping conditions, similar to the conditions used in the studies of interhemispheric communication in young and older adults by Fling and colleagues and Bangert and colleagues (Bangert et al., 2010, Fling et al., 2011a). The details are described in the general methods (Chapter 2). Both studies showed that the following tapping conditions require a range of interhemispheric interactions (Bangert et al., 2010, Fling et al., 2011a):

1) Simultaneous bimanual (low interhemispheric interaction)
2) Left-hand (non dominant) unimanual (moderate interhemispheric interaction)
3) Right-hand (dominant) unimanual (moderate interhemispheric interaction)
4) Left-leads right (LR non-dominant to dominant) bimanual (high interhemispheric interaction)
5) Right-leads left (RL dominant to non-dominant) bimanual (high interhemispheric interaction)

There were some modifications made when the experimental program was developed. This task was a reaction time task, not tapping in synchrony with the cue as performed with the previous studies. This was to assess responsivity to the cue; traumatic brain injury patients often experience slower response time to a cue (Caeyenberghs et al., 2011a). In addition to the conditions described in Bangert and colleagues and Fling and colleagues (in Bangert et al., 2010, Fling et al., 2011a), the bimanual simultaneous condition was examined in two

different ways; first the two cues to tap were simultaneously presented on both sides of the screen, as reported in the previous studies, and second, a single cue to tap was presented in the centre of the screen. In all of the other conditions described in the paper by Fling and colleagues, the cue to tap was presented on the side of the screen that corresponded to the tapping hand. The concern was that if a visual field restriction was present in the patient group, this would impact on their ability to act on a cue presented on either side of the screen. The additional second task was done to confirm that the reaction time and variability of the bimanual simultaneous state (cues presented simultaneously on both sides of the screen) were not significantly higher than when a cue was presented in the middle of the screen.

As mentioned in the general methods, participants sat approximately 30cm in front of the computer screen, and rested their wrists on the bench. Participants were instructed to place their index fingers on the corresponding buttons. Participants were asked to maintain this position throughout the task. Participants were initially presented with a written and then verbal explanation of each condition with training of the press response required. As the study was intended to evaluate interhemispheric communication, rather than processing and retention of complicated information, the language used in all explanations was appropriate to the participant. In addition, time was taken to ensure the information was understood and that the participant felt that they were given sufficient time to process and practise the correct tapping response to the cue.

As seen in Figure 2.1, participants were initially presented with a written instruction on the command screen at the start of each training block, which confirmed the action required. This was reinforced with verbal instruction of what the testing block involved, to ensure understanding. The verbal instruction was read from a pre-prepared instruction sheet, to ensure consistency of instruction to the participants. Participants were asked to focus on a fixation cross (+) in the centre of the computer screen. They were told they would see a “get ready” (!) icon. A cue (square) would then appear 200ms later, indicating that they should

press the corresponding button. For example, during the left hand unimanual tapping, the cue would appear on the left of the fixation cross. In the asynchronous condition, the cue for the second button press (i.e. out of phase finger) was 180ms after the initial cue. Participants were instructed to not use the fixation cross or exclamation mark as a cue to tap, they were to only use the square. They were also asked to react (not tap in synchrony) to the cue, as a measure of responsivity. There was a pause between each testing block. The testing session was then commenced.

The conditions consisted of the five interhemispheric interactions detailed above, with separate conditions for the two bimanual simultaneous states. The 5 different trial types were presented in blocks. Each block consisted of ten assessments (trials). The participant was required to respond to the cue once seen, but not at the expense of accuracy. There was a fixed stimulus onset from the warning sign. This was to enable the task to be a simple task, focussing on interhemispheric communication, rather than more complicated processing of the visual information, and to give the patient group the best chance of performing the task. They were instructed to not try to anticipate, or tap with the cue, in order to simply assess responsivity to the external stimulus (rather than assess an internally generated tapping rate). Each condition was assessed in four blocks through the study. The six conditions were assessed in 24 blocks.

5.3.2 Behavioural analysis and statistics

The measures obtained through the MATLAB program (MathWorks, USA) were saved and exported to excel for analysis. For all responses, reaction time was calculated as the time between the cue onset and the subsequent button press. The measures of motor performance were the mean reaction time at each condition, and variability (standard deviation of the mean reaction time for each condition) which provided insight into the stability of the movement. This was undertaken at each level of interhemispheric interaction. Normality of all the data sets was tested with Kolmogorov – Smirnov tests. Initially, the two bimanual simultaneous conditions

were compared using a paired t test to assess whether there was a significant difference between the two conditions. As mentioned, the two states differed in where the cue was presented on the screen (one cue in the centre of the screen versus two cues presented at the same time on the left and right of the screen). As there was no significant difference in the reaction time or variability between the two conditions, the respective results for both conditions were combined for the two measures of performance (mean reaction time and standard deviation "variability") of the bimanual simultaneous condition. Analysis of variance (ANOVA) was then undertaken with within subjects factor "TAPPING CONDITION" (bimanual simultaneous, unimanual, bimanual asynchronous) and "HAND" (dominant, non dominant) for the mean reaction time and then for the variability. Paired t tests were undertaken between the bimanual simultaneous condition and unimanual condition for the dominant and then repeated for the non-dominant hand to determine whether the "bimanual advantage" could be replicated in this study (Bangert et al., 2010, Fling et al., 2011a).

For each participant, the hand lag was obtained as the difference between the two hands in each tapping trial within the bimanual simultaneous and the two bimanual asynchronous conditions. This was then averaged to obtain a mean hand lag and a between hand lag variability for the respective conditions for each participant. The measures of hand lag (mean hand lag and then variability of the hand lag) in the two bimanual asynchronous conditions were then compared for the group. As there was no significant difference in hand lag between the two conditions, the respective hand lag results were combined. The results for the bimanual simultaneous condition were then compared with the respective bimanual asynchronous hand lag using a paired t test.

For the report of the statistical differences, the significance threshold of $\alpha = 0.05$ was used. Where assumptions of sphericity were violated (where Mauchley's test $P < 0.05$), the Greenhouse – Geisser correction was applied. Post–hoc t test, Bonferroni corrected were used when appropriate. Statistical procedures were conducted using the statistical package SPSS version 19.0 for Windows; SPSS Inc. For the purpose of the reported results, all results

that refer to the L – R condition is the non-dominant to dominant hand. All results that refer to the R – L condition is dominant to non-dominant hand. All data are given as mean ± standard error of the mean unless otherwise stated.

5.4 Results

A set of baseline data was obtained from 29 healthy participants consisting of 15 males and 14 females aged between 22 and 55 years (mean ± standard error 31.1 ± 1.14). All data are mean (± standard error) unless otherwise stated.

5.4.1 Initial comparisons

The two bimanual simultaneous conditions were initially compared with paired t tests in the respective (non-dominant, dominant) hand. There was no statistically significant difference in the average reaction time of the two bimanual simultaneous conditions in both the non-dominant hand ($t = -0.83$, $df = 28$, $p = 0.41$) and the dominant hand ($t = -1.32$, df 28, $p = 0.20$). There was also no statistically significant difference in the variability of the reaction time in both the non-dominant hand ($t = -1.05$, $df = 28$, $p = 0.22$) and the dominant hand ($t = -0.95$, $df = 28$, $p = 0.35$). Therefore the results for the two states in the respective hands were combined for further analysis.

The ANOVA for reaction time with “TAPPING CONDITION” and “HAND” as within subjects factor revealed no effect of “TAPPING CONDITION” ($F (2, 56) = 1.41$, $P = 0.25$). There was an effect of “HAND” ($F (1, 28) = 9.91$, $P < 0.05$). However, there was no “TAPPING CONDITION” X “HAND” interaction ($F (2, 56) = 2.43$, $P = 0.09$). This means that a significant difference was demonstrated in the performance across the conditions between the two hands in the healthy participants. The ANOVA for variability with “TAPPING CONDITION” and

“HAND” as within subjects factor revealed an effect of “TAPPING CONDITION” ($F(2, 56) = 12.1$, $P < 0.05$), “HAND” ($F(1, 28) = 6.88$, $P < 0.05$) and “TAPPING CONDITION” X “HAND” interaction ($F(2, 56) = 5.73$, $P < 0.05$). This means that a significant difference was demonstrated in the variability of performance between the two hands in the healthy participants. This difference between the hands existed in the variability of performance in the bimanual simultaneous condition ($t = 4.36$, $df = 28$, $p < 0.05$), but not the unimanual ($t = 0.51$, $df = 28$, $p = 0.62$) or bimanual asynchronous condition ($t = 0.41$, $df = 28$, $p = 0.69$). As there was a statistically significant difference in variability for the two hands, the data for the hands could not be combined. The dominant and non-dominant hands are therefore assessed separately in the further analysis.

5.4.2 Reaction time at each condition

The average reaction time for the non-dominant hand was relatively stable from the bimanual simultaneous condition (572 ± 8.03) and unimanual condition (567 ± 7.63). However, the mean reaction time when the non-dominant hand was leading the asynchronous condition appeared higher (579 ± 6.96). However, there were no significant main effects of tapping condition in the non-dominant hand ($F(2, 56) = 1.07$, $P = 0.35$). In the dominant hand, the mean reaction time increased with the complexity of the condition; simultaneous condition (553 ± 5.67), unimanual (568 ± 6.41) and when the hand was leading the asynchronous condition (578 ± 13.5). In this case, there was a significant main effect of tapping condition in the dominant hand ($F(2, 56) = 3.37$, $P = 0.04$) (Figure 5.1).

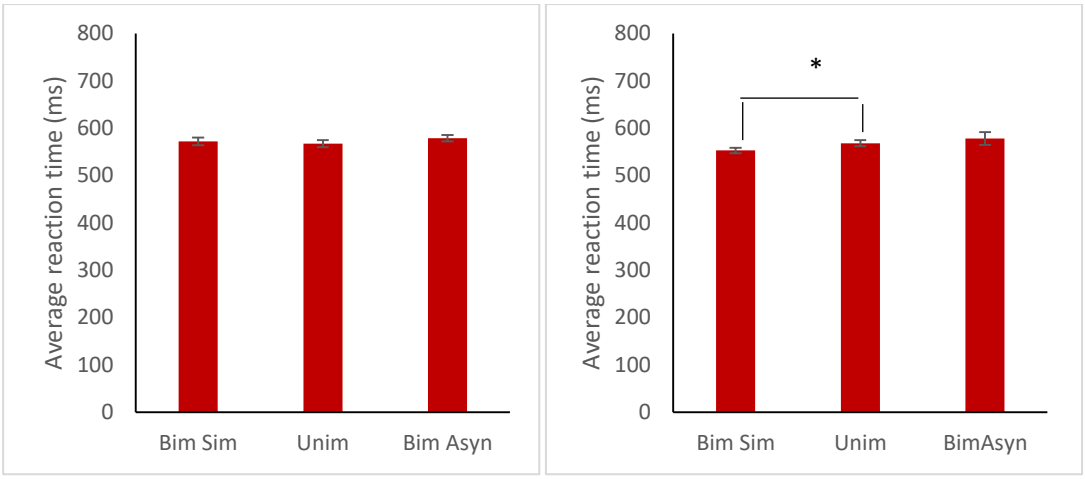


Figure 5.1: Left panel: average reaction time of the non-dominant hand. Right panel: average reaction time of the dominant hand

As expected, there was no statistically significant difference in the reaction time of the bimanual simultaneous condition versus the unimanual condition, in the non-dominant hand (t = 0.54, df = 28, p = 0.59). However, there was a significant difference in the bimanual simultaneous movement versus the unimanual movement in the dominant hand (t = -2.53, df = 28, p = 0.02). There was no statistically significant difference in the reaction time of the unimanual condition versus the leading finger in the bimanual asynchronous condition in the non-dominant (t = -0.71, df = 28, p = 0.98) or dominant hand (t = 0.75, df = 28, p = 0.46).

5.4.3 Variability of each condition

In the non-dominant hand, the variability of the reaction time was slightly higher in the bimanual simultaneous condition (72.0 ± 2.22) than the unimanual condition (68.4 ± 2.39). However, the variability when the non-dominant hand was leading the bimanual asynchronous condition was higher (78.4 ± 2.88). This was associated with a significant main effect of tapping condition on the variability in the non-dominant hand (F (2, 56) = 5.1, P = 0.01). In the dominant hand, the variability of the reaction time increased with the complexity of the condition, with bimanual simultaneous (56.6 ± 3.34), unimanual (66.7 ± 2.52), and when the dominant hand was leading the bimanual asynchronous condition (76.8 ± 3.47). This was associated with a significant

main effect of tapping condition on the variability in the dominant hand (F (2, 56) = 12.5, P < 0.05) (Figure 5.2).

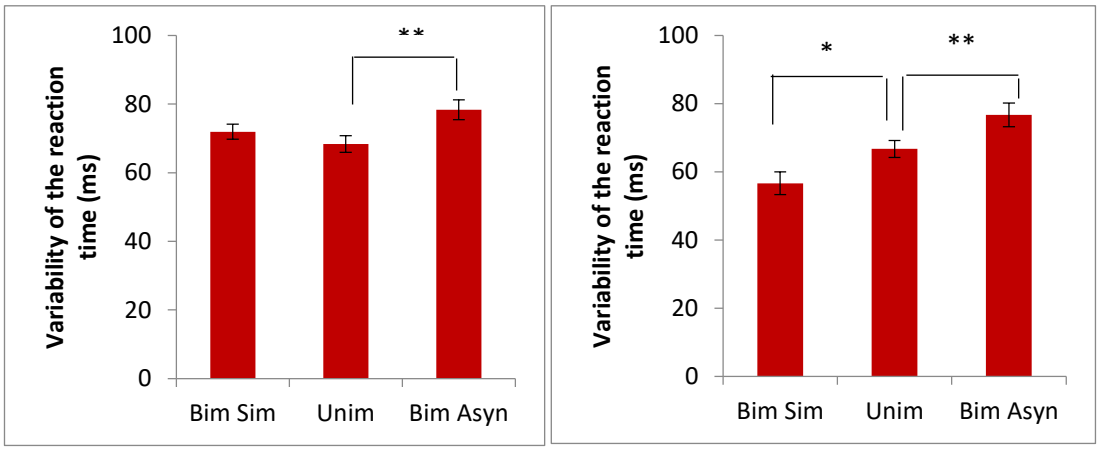


Figure 5.2: Left panel: average variability of the non-dominant hand. Right panel: average variability of the dominant hand

There was no statistically significant difference in the variability of the bimanual simultaneous condition versus the unimanual condition in the non-dominant hand (t = 1.45, df = 28, p = 0.16). However, a statistically significant difference was demonstrated in the variability of the bimanual simultaneous condition versus the unimanual condition in the dominant hand (t = -0.27, df 28, p = 0.01). This demonstrated that the bimanual advantage was demonstrated in the dominant, but not the non-dominant hand. In addition, a statistically significant difference was demonstrated in the variability of the unimanual condition versus the leading finger in the asynchronous condition in the non-dominant (t = -2.82, df = 28, p = 0.01) and dominant hand (t = -2.38, df = 28, p = 0.03).

5.4.4 Between hand lag measures

There was no statistically significant difference between the mean hand lag in the two bimanual simultaneous conditions (t = -0.82, df = 28, p = 0.42), and the two bimanual asynchronous conditions (t = -0.44, df = 28, p = 0.67). This was also the case for the between

hand lag variability in the two bimanual simultaneous conditions (t = -0.44, df = 28, p = 0.67) and the two bimanual asynchronous conditions (t = -1.77, df = 28, p = 0.09). Therefore, these results were combined.

As expected, the combined mean hand lag was lower in the bimanual simultaneous condition (13.0 ± 2.98), compared to the combined mean hand lag in the bimanual asynchronous condition (112 ± 5.63). This was statistically significant (t = -16.9, df = 28, p < 0.05). The combined between hand variability of the hand lag between the dominant and non-dominant hand in the bimanual simultaneous condition (18.5 ± 2.11) was lower than the combined hand lag between the dominant and non-dominant hand in the bimanual asynchronous condition (59.3 ± 4.75). This was statistically significant (t = - 8.31, df = 28, p < 0.05). The mean hand lag (left panel) and standard deviation of the mean hand lag (right panel) are demonstrated in Figure 5.3.

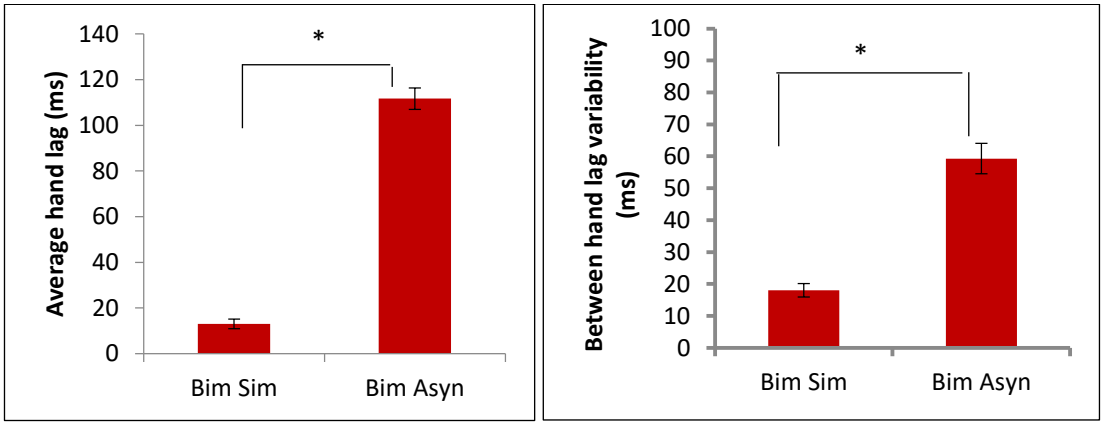


Figure 5.3: Hand lag: Left panel: average hand lag in the bimanual simultaneous and bimanual asynchronous conditions. Right panel: between hand lag variability in the bimanual simultaneous and bimanual asynchronous conditions.

5.5 Discussion

The main purpose of this study was to develop a simple behavioural assessment that would investigate a range of interhemispheric interactions, and could be used to study behaviour across the corpus callosum in a patient group. The principles underlying the investigation of the callosal interactions in this particular behavioural study had been previously reported in two separate cohorts of older and younger adults (Bangert et al., 2010, Fling et al., 2011a). However, as the existing behavioural literature often subtly differ in their methodology, it is often difficult to compare and draw robust conclusions. Therefore, this study was designed to obtain baseline measurements of this behavioural measure in a larger group of healthy individuals. As previously mentioned, the callosal interactions were assessed by bimanual simultaneous tapping (which is thought to reflect low interhemispheric interaction), unimanual tapping (which is thought to reflect moderate interhemispheric interaction), and bimanual asynchronous tapping where the fingers move out of phase with each other (which is thought to reflect high interhemispheric interaction) (Bangert et al., 2010, Fling et al., 2011a). The main findings from this current study were that the performance of the two hands (dominant versus non-dominant) was different across the conditions. Although the average performance (reaction time) across the conditions did not differ between the two hands, the effect of the tapping condition on the variability of the performance was different between the two hands. Both measures of performance (average reaction time and variability) in the dominant hand appeared to deteriorate with the requirements of increasing levels of interhemispheric interaction. However, a similar deterioration was not present in the reaction time, but was evident in the variability of the performance across the conditions of the non-dominant hand. The simultaneous tapping condition was significantly slower in the non-dominant hand, compared to the dominant hand. The variability of the between hand lag in the bimanual asynchronous condition was significantly impaired, compared to the variability of the hand lag when the fingers pressed the button together in the bimanual simultaneous condition. Lastly, the presentation of the cue did not have a statistically significant effect in the performance by

the healthy participants. Of importance, the verbal feedback from this study from this group of healthy participants was reassuring that this would be transferable to a patient study.

5.5.1 The variability of performance when the two hands are compared

There was no significant difference in the average performance, measured by the reaction time, across the conditions when the two hands (dominant and non-dominant) were compared. However, there was a statistically significant difference in the variability of performance across the conditions between the two hands. The results suggest that this difference was only significant between the two hands in the bimanual simultaneous condition, and not the remaining conditions (unimanual and asynchronous). Evaluating the graphs (Figure 5.2), this is because the variability of performance in the dominant hand is significantly more stable (as evidenced by a lower value) than the non-dominant hand in this particular condition. The better performance in the dominant hand may purely reflect hand dominance when both hands are required to perform the same movement at the same time. This would result in the dominant hand having a more consistent and less variable movement, with the non-dominant hand lagging behind. A further consideration concerns the neurological basis of this movement. The work by Fling and colleagues suggests this movement requires the lowest level of interhemispheric interaction (Fling et al., 2011a). This is supported by the evidence that bimanual simultaneous movement has been found to be preserved in patients who underwent corpus callosotomy, thus proposing an origin outside of the corpus callosum for a bimanual simultaneous movement to be executed (Eliassen et al., 1999, Serrien et al., 2001, Kennerley, et al., 2002). Like these other studies, the findings from this current study would support a different neurological basis for bimanual simultaneous movement, compared to that involved with unimanual and asynchronous bimanual movement.

5.5.2 Performance of individual hands with increasing levels of interhemispheric interaction

Both measures of performance appeared to deteriorate with increasing levels of interhemispheric interaction in the dominant hand, but only the variability deteriorated in the corresponding measures in the non-dominant hand. In the dominant hand, the average reaction time increased as the level of interhemispheric interaction increased. This was statistically significant and would be in keeping with the understanding that the level of interhemispheric interactions increases from bimanual simultaneous through to bimanual asynchronous movements (Kennerley et al., 2002, Bangert et al., 2010, Fling et al., 2011a). Further testing demonstrated that this was statistically significant between the bimanual simultaneous tapping movement and the unimanual movement. However, this was not statistically significant when the variability of the unimanual movement was compared with the finger leading the asynchronous movement. This would support existing evidence that the level of interhemispheric interaction increases from bimanual simultaneous movement to unimanual movement. This would also lend support to the previously discussed hypothesis that the unimanual and asynchronous movement rely on callosal interactions, and the bimanual simultaneous movement may have more dependence on non-callosal factors.

However, in the non-dominant hand only the variability of the performance was significantly different across the conditions. The reaction time did not significantly differ across the conditions. This most likely reflects the observed slower reaction times in the non-dominant hand, compared to the dominant hand. However, the non-dominant hand performed with relative consistency between bimanual simultaneous movement and the unimanual movement. The significant difference in variability of movement was evident between the unimanual and asynchronous condition. Therefore, the bimanual advantage was only able replicated in the dominant, but not non-dominant hand in this current study (Ivry et al., 1999, Bangert et al., 2010, Fling et al., 2011a)

It is difficult to propose an explanation for the difference in the hand performance. The studies that have focussed on the behavioural consequences of callosal transfer have tended to focus on the variability of the performance, rather than the reaction time. In the studies by Fling and colleagues and Bangert and colleagues, the range of states investigated was comparable to this current study. However, their studies differed subtly with the current study. The main difference was that the tap was directed to be made in synchrony with the cue in the previous studies. In addition, the study by Bangert and colleagues was slightly more complicated than the current study; the inter-tap interval of the bimanual asynchronous condition was also varied. As previously mentioned, the task in this current study was slightly modified to assess responsivity, rather than an in-built mechanism. In addition, I did not want anticipation to the cue be an additional confounder, as the purpose of this study was to assess callosal interactions. I therefore felt that reacting to the cue would provide more information about the network across the corpus callosum involved to execute the respective finger tap. As mentioned earlier, the findings in this study may therefore be due to this particular group of healthy participants being particularly proficient in using their dominant hand, where the easiest tasks are performed quicker (in this case, where the lowest interhemispheric interaction is associated with a quicker reaction time, than their non-dominant counterpart).

The variability in performance is not likely to be purely dependent on interhemispheric interactions. The presence of an external pacing stimulus is demonstrated to have more influence on performance than the complexity of the task, in a meta-analysis of 38 studies involving a finger tapping task (Witt et al., 2008). This may relate to the brain activation patterns, which are different when the pacing stimulus is visually provided, compared to auditory pacing (Witt et al., 2008). It is also accepted that self-paced bimanual asynchronous tasks are more vulnerable than visually cued bimanual asynchronous tasks (Wahl et al., 2015). However, factors such as tiredness or poor concentration may have also been involved. At the point of completion of each block the assessor (MB) did ensure that the participant felt ready to continue onto the next block. However, such factors were not assessed in more detail at the time of testing.

5.5.3 Between hand lag variability

The between hand lag variability in the bimanual asynchronous condition was significantly impaired, compared to the variability of the bimanual simultaneous hand lag. This was evidenced by the significant difference in the variability of the between hand lag in the asynchronous movement, compared to the hand lag in the bimanual simultaneous movement. This supports the existing work by Bangert, Fling and colleagues (Bangert et al., 2010, Fling et al., 2011a).

The results to this part of the current study are not surprising. It supports the findings earlier that the dominant and non-dominant hand did not perform equally in the bimanual simultaneous movement, hence there was a small hand lag. In addition, the higher variability of the hand lag in the bimanual asynchronous condition means this movement is less stable, than the hand lag in a bimanual simultaneous movement. This is in support of existing literature, where the asynchronous movement is already accepted to be the hardest to maintain (Kennerley et al., 2002, Swinnen, 2002, Bangert et al., 2010, Fling et al., 2011a). In-phase coupling of hand movements needs to be suppressed when coordination is required for asynchronous bimanual movements. Therefore, the complexity of the brain networks required to perform the task increases, and the asynchronous bimanual movement is more complex to execute than the simultaneous bimanual movement (Wahl et al., 2015). This will be of particular importance in the future behavioural study in the traumatic brain injury patients, and the exploration of a relationship between microstructure and behaviour later in the thesis.

5.5.4 The presentation of the cue did not have a significant difference in the performance by the healthy participants

Finally, there was no significant difference in performance, whether the cue was presented on the far sides, or centre of the screen in the bimanual simultaneous conditions. This was not unexpected in a healthy cohort of patients. However, it will provide valuable information with a patient group. In particular, if the performance is better when the cue is in the centre of the

screen, this would cast doubt on the reliability of performance in all the remaining conditions, where the cue is presented on the far sides of the screen. This type of deficit could be due to a visual field defect or visual inattention, so it would be important to ensure that any variability in tapping performance found in the patient group in the next study is not due to visual processing difficulties.

A strength of this study is that it involved 29 healthy participants of a relatively wide age range (range 22 to 55 years). The studies by Caeyenberghs and colleagues involved 17 healthy participants (mean age 25 years), Fling and colleagues 14 (average age 21 years) and Bangert and colleagues 17 (average age 20 years). (Caeyenberghs et al., 2011a, Fling et al., 2011a, Bangert et al., 2010).

5.5.5 Study limitations

There were, however, study limitations. First, the presentation of the trials was not randomised between participants. The main components of this current study were finger taps to assess bimanual simultaneous, unimanual and bimanual asynchronous movement. Although existing literature has validated these taps as representing callosal interactions, execution of such movements involves much larger brain networks than callosal connections (Serrien et al., 2001, Witt et al., 2008). Therefore these results will have to take into account that other factors are also involved in the results obtained. Additional work would include exploring the brain activation patterns activated in these respective conditions, which is outside the scope of this study. Finally, due to subtle differences in methodology, the findings from this study cannot be compared directly to other studies in the literature. However, the principals of the range of interhemispheric interactions are the same as previously reported.

5.6 Conclusions

The current study provides a baseline set of data for the behavioural response in a larger healthy cohort, investigating a range of interhemispheric interactions. These results offer insight into different levels of callosal interactions required to execute bimanual movement.

The next step would be to assess the behaviour involved in corpus callosal transfer in the brain following traumatic brain injury. This is the focus of the next chapter.

Chapter 6

Behavioural consequences of Traumatic Brain Injury

I led this study and was responsible for study concept, recruitment, experimental work, analysis and interpretation of the study. The MATLAB (MathWorks, USA) script used was programmed by Dr J Galea.

6.1 Introduction

The last chapter used the concepts understood from the physiological findings from the earlier traumatic brain injury patient study (Chapter 4) to develop a behavioural (reaction time) study. As previously mentioned, the previous patient study demonstrated that interhemispheric inhibition is physiologically different in this group of traumatic brain injury patients, compared to healthy controls. Interhemispheric inhibition is thought to be mediated by excitatory transcallosal fibres that project onto inhibitory interneurons in the opposite hemisphere, thus involving callosal transfer. Therefore, a protocol was developed to study the behavioural consequences of these interhemispheric interactions. Fling and colleagues had already demonstrated that interhemispheric interactions were related to the microstructure of the corpus callosum in their cohort of older and younger adults (Fling et al., 2011a). The study presented in the last chapter in healthy participants was based on the concepts from this work by Fling and colleagues, and modified to use in our patient group. The results presented in the last chapter provided baseline measures for average reaction time and variability in a range of interhemispheric interactions in 29 healthy individuals. Of importance, the verbal feedback regarding the simplicity of the study was positive, so provided reassurance that this experiment would be suitable for a patient group.

The purpose of this chapter is to use the behavioural measure developed in the last chapter to understand the callosal interactions in traumatic brain injury patients. The same group of traumatic brain injury patients that participated in the previous physiological study were included in this study. The main reason for this was that any inferences on physiology and behaviour made from these two patient studies (Chapter 4 and Chapter 6) reflected the same patient group.

Clumsy and slow performance in bimanual tasks has been observed anecdotally after traumatic brain injury. As traumatic brain injury is understood to involve shearing and disruption of the axons due to the acceleration and deceleration involved in the brain injury, from a behavioural point of view I would expect this to result in a general under-functioning in

performance in the traumatic brain injury group, compared to the healthy controls. This under-functioning in performance would manifest as slower reaction times in all movements investigated, with less stability (i.e. increased variability) in their performance, compared to controls.

6.2 Study design

6.2.1 Participants

The healthy participants who acted as controls for this study were the same participants who participated in the transcranial magnetic stimulation study in Chapter 4. As previously mentioned, they were recruited from a database of healthy volunteers from the UCL Institute of Neurology and Imperial College. Seventeen healthy participants consisting of 10 male and 7 females participated in this study. All healthy subjects were on no regular medication. They were also medically stable. They had also participated in traumatic brain injury research as part of an MRI study conducted by Imperial College, participated in the transcranial magnetic stimulation study and consented to continue with this study.

The patients with traumatic brain injury were the same participants who participated in the transcranial magnetic stimulation study in Chapter 4. As mentioned in the earlier chapter, they were already part of a database of traumatic brain injury patients recruited from brain injury units across London. The units were Charing Cross Hospital, St Mary's Hospital, The National Hospital for Neurology and Neurosurgery, the Royal Free Hospital and the Regional Neurological Rehabilitation unit at the Homerton Hospital. Seventeen participants consisting of 10 males and 7 females were recruited. All patients had sustained an impact injury to the brain on one occasion (such as from a road traffic accident, sports injury or assault) causing a loss of consciousness. All patients had been treated conservatively after their traumatic brain injury, and not required any neurosurgical intervention. These patients had already participated in traumatic brain injury research as part of an MRI study conducted by Imperial

College, participated in the transcranial magnetic stimulation study and consented to continue with this study.

The patients included in this study had injuries secondary to road traffic accidents 64%, assaults 12%, falls 12% or a single sports injury 12%. Based on the Mayo classification system for traumatic brain injury severity, there were eleven moderate – severe and six mild (probable) cases of traumatic brain injury in this traumatic brain injury patient group (Malec et al., 2007). All patients were in the chronic phase following their traumatic brain injury (mean 3.72 years, range 1.1 – 7.5 years).

6.2.2 Institutional and ethical approval

The information sheets and protocols used in the traumatic brain injury study were reviewed and ethical approval was obtained from the Ethics research committee.

For all studies, participants gave written consent prior to participating in the session. All studies were performed in accordance with the Declaration of Helsinki, and participants were given the option to withdraw from the study at any time.

6.3 Experimental Method

6.3.1 Behavioural measures

Participants in the traumatic brain injury study performed a finger tapping experiment presented on a desktop computer running MATLAB (MathWorks, USA). This was a reaction time task, where the participant had to respond to a visual cue. The behavioural task has been detailed in the General Methods (Chapter 2) and in the previous chapter (Chapter 5). Briefly,

participants performed five finger-tapping states, similar to the states used in the studies of interhemispheric communication in young and older adults by Fling and colleagues (Fling et al., 2011a), and Bangert and colleagues (Bangert et al., 2010). Both studies reported these tapping states require a range of interhemispheric interactions (Bangert et al., 2010, Fling et al., 2011a):

1) Simultaneous bimanual (low interhemispheric interaction)
2) Left-hand unimanual (moderate interhemispheric interaction)
3) Right-hand unimanual (moderate interhemispheric interaction)
4) Left-leads right (LR) bimanual (high interhemispheric interaction)
5) Right-leads left (RL) bimanual (high interhemispheric interaction)

As mentioned in the previous chapter, in addition to the conditions described in Bangert and colleagues and Fling and colleagues (Bangert et al., 2010, Fling et al., 2011a), the simultaneous bimanual state was further divided where the cue was a) presented in the centre of the screen, and b) on both sides. This was to assess whether the response to where the cue was placed was different in the patient group.

As the study was intended to evaluate interhemispheric communication in this patient group, rather than processing and retention of complicated information, the language used in all explanations was appropriate to the participant. In addition, prior to data collection, time was taken to ensure the information was understood and that the participant felt that they were given sufficient time to process and demonstrate the correct tapping response.

As previously mentioned, six states were assessed, consisting of the five interhemispheric interactions detailed above. The 5 different trial types were presented in blocks. As in the last chapter, there were two bimanual simultaneous states; one had the cue in the centre of the screen, the other presented two cues on either side of the screen. Each block consisted of ten

assessments (trials) of one condition. The duration of the display of the “command” screen at the start of each testing block was controlled by the assessor. Patients after traumatic brain injury often suffer cognitive consequences with slower information processing speed after their injury (Niogi et al., 2008). Therefore, the MATLAB programme was created so that the “command” screen at the start of each testing block required a button press by the assessor to move onto the next screen (i.e. commencement of the testing block). This was designed to provide time, if required, for repeated verbal reinforcement of the command by the assessor, and to ensure the command was understood. Therefore, the participant was given the best chance of performing the task accurately, if they did have problems with processing information. The participants were allowed as much time as they needed to understand the instruction. When the participant was ready, the assessor (MB) pressed the button to commence the assessment of the respective block.

As mentioned in the general methods (Figure 2.1), participants were instructed to focus on the (uninformative) fixation cross (+). A warning (“!”) was then presented. This was followed by the cue (square) to tap 200ms later. The participant was required to respond to the cue once seen. They were instructed to not anticipate or tap in synchrony with the cue.

6.3.2 Behavioural analysis and statistics

The measures obtained through the MATLAB program (MathWorks, USA) were saved and exported to excel for analysis. For all responses, reaction time was calculated as the time between the cue onset and the subsequent button press. The analysis of motor performance involved the mean reaction time at each condition, and variability (standard deviation of the mean reaction time). This was undertaken at each level of interhemispheric interaction. Normality of all the data sets was tested with Kolmogorov – Smirnov tests.

The performance of the control group (mean reaction time, variability) in the two bimanual simultaneous conditions were initially compared using a paired t test, to assess whether there was a significant difference between the two conditions. As previously mentioned, the two conditions differed in where the cue was presented on the screen (one cue in the centre of the screen versus two cues presented at the same time on the left and right of the screen). There was no significant difference in the control group in the mean reaction time or variability in the respective measures of performance. Therefore, for the next step in the analysis, the results in the control group for the two bimanual simultaneous conditions were combined. The next part of analysis compared performance of the healthy controls in this study (n = 17) with the larger cohort of healthy participants (n = 29) in the previous study (presented in Chapter 5), to ensure reproducibility. The measures of performance (mean reaction time and variability) across the conditions (bimanual simultaneous, unimanual, bimanual asynchronous) were then compared between the healthy participants in the previous study (presented in Chapter 5) and the healthy participants in this current study for each hand, with an analysis of variance (ANOVA) with factors “TAPPING CONDITION” and “HAND” as within subject variable and “GROUP” (healthy participant, healthy control) as the between subjects variable.

The two bimanual simultaneous conditions were then compared in the traumatic brain injury group. Paired t tests were used to compare the measures. However, there was a significant difference demonstrated between the two bimanual simultaneous conditions in the traumatic brain injury patient group. Therefore, the respective results for both conditions were not combined for the bimanual simultaneous condition. Possible reasons for this will be discussed in more detail in the discussion. The bimanual simultaneous condition where the two cues were presented on both sides of the screen at the same time, was used in both the patient group and healthy controls for further analysis, as used in the existing literature (Fling et al., 2011a).

In the control group, paired t tests were undertaken between the bimanual simultaneous condition and unimanual condition for both the dominant and non-dominant hand, in order to

determine whether the “bimanual advantage” could be replicated. This was then repeated for the traumatic brain injury group (Bangert et al., 2010, Fling et al., 2011a).

The performance of the hands was then compared within each group. In the control group, the reaction time across the conditions (bimanual simultaneous, unimanual, bimanual asynchronous) was then compared between dominant and non-dominant hand with an analysis of variance (ANOVA) with factors “TAPPING CONDITION” as within subjects variable and “HAND” (dominant, non-dominant) as the between subjects variable. This was then undertaken for the variability. The same analysis was undertaken for the traumatic brain injury patient group. As there was no significant difference in the performance (mean reaction time or variability) between the two hands in either groups, the results for the dominant and non-dominant hands were combined for the control group and traumatic brain injury group respectively, for further analysis. This has been used in previous behavioural work analysis across the corpus callosum (Fling et al., 2011a).

A two–way repeated measures analysis of variance (ANOVA) was then undertaken with factors “TAPPING CONDITION” (bimanual simultaneous, unimanual, bimanual asynchronous) as within subjects variable and “GROUP” (control, traumatic brain injury group) as the between subjects variable for the mean reaction time and then variability to compare performance between the two groups.

For each participant, the between hand lag was obtained as the difference between the two hands in each tapping trial within the two bimanual simultaneous and the two bimanual asynchronous conditions. Prior to group analysis, this had been averaged to obtain a mean hand lag and a between hand lag variability measure for each participant in the two bimanual simultaneous conditions and the two bimanual asynchronous conditions. Within the control group, paired t tests were undertaken to determine whether there was a significant difference in hand lag in the two bimanual simultaneous conditions. As there were no significant

differences the respective results were combined. This was repeated for the bimanual asynchronous conditions, and combined. This was then repeated for the traumatic brain injury patient group, and combined. A group (traumatic brain injury versus healthy controls) comparison for the respective measures were then made using independent t tests.

Paired t tests were performed where appropriate for comparisons within each respective group. Independent t tests were undertaken for group comparisons. Before conducting the relevant t test, a Levene's test was first used to verify that there were no significant differences in the variances of the populations being tested. For the report of the statistical differences, the significance threshold of $\alpha = 0.05$ was used. Where assumptions of sphericity were violated (where Mauchley's test $P < 0.05$), the Greenhouse – Geisser correction was applied. Post–hoc t test, Bonferroni corrected were used when appropriate. Statistical procedures were conducted using the statistical package SPSS version 19.0 for Windows; SPSS Inc. For the purpose of the reported results, all results that refer to the L – R condition are the non-dominant to dominant hand. All results that refer to the R – L condition are dominant to non-dominant hand. All data are given as mean ± standard error of the mean unless otherwise stated.

6.4 Results

The patient study contained 10 males and 7 females aged between 22 and 60 years (mean ± SE 43.3 ± 2.69). The control group contained 10 males and 7 females aged between 22 and 52 years (mean ± SE 30.5 ± 1.75). All results are mean (± standard error) unless otherwise indicated.

6.4.1 Initial comparisons

6.4.1.1 Comparison of healthy control group with healthy participants

The performance by the control participants (n = 17) in this study was initially compared with the performance of the healthy participants (n = 29) in the previous study (Chapter 5). As this was a novel study, albeit based on the principles of interhemispheric interactions already used in previous studies, this was undertaken to ensure reproducibility between the two studies in healthy people. The ANOVA for reaction time with “TAPPING CONDITION” and “HAND” as within subjects factor, and “GROUP” as between subjects factor revealed no effect of “TAPPING CONDITION” ($F (2, 88) = 2.87$, $P = 0.62$), an effect of “HAND” ($F (1, 44) = 15.7$, $P < 0.05$), but no effect of “GROUP” ($F (1,44) = 0.10$, $P = 0.75$). There was no “GROUP” x “HAND” interaction ($F (1, 88) = 0.03$, $P = 0.86$). There was no “TAPPING CONDITION” X “HAND” interaction ($F (2, 88) = 2.16$, $P = 0.12$) and no “TAPPING CONDITION” x “GROUP” x “HAND” interaction ($F (2, 88) = 0.37$, $P = 0.69$).

The ANOVA for variability with “TAPPING CONDITION” and “HAND” as within subjects factor, and “GROUP” as between subjects factor revealed an expected effect of “TAPPING CONDITION” ($F (2, 88) = 23.5$, $P < 0.05$), “HAND” ($F (1, 44) = 7.72$, $P = 0.01$), but no effect of “GROUP” ($F (1,44) = 1.35$, $P = 0.25$) and no “GROUP” x “HAND” interaction ($F (1, 44) = 0.04$, $P = 0.84$). There was a “TAPPING CONDITION” X “HAND” interaction ($F (2, 88) = 7.9$, $P < 0.05$) but no “TAPPING” x “GROUP” x “HAND” interaction ($F (2, 88) = 0.25$, $P = 0.78$).

There was no statistically significant difference between the two groups in the mean hand lag in the bimanual simultaneous condition ($t = -0.40$, $df = 44$, $p = 0.69$) or bimanual asynchronous condition ($t = -0.55$, $df = 44$, $p = 0.59$). In addition, there was no statistically significant difference between the two groups in the between hand lag variability in the bimanual simultaneous condition ($t = -0.19$, $df = 44$, $p = 0.85$) or the bimanual asynchronous condition ($t = -0.25$, $df = 44$, $p = 0.80$). This provides reassurance that the two studies are comparable.

6.4.1.2 Comparison of the two methods of assessing bimanual simultaneous movement

The two methods for assessing the bimanual simultaneous movement were then compared in the control group. These two conditions differed in the presentation of the cue; whether a single cue was presented in the centre of the screen or whether two cues were presented simultaneously on both sides of the screen. In the healthy control group, there was no statistically significant difference in the reaction time of the non-dominant hand ($t = -1.12$, $df = 16$, $p = 0.28$) or dominant hand ($t = -1.07$, $df = 16$, $p = 0.30$), between the two conditions. There was also no statistically significant difference in the variability of the non-dominant hand ($t = -0.94$, $df = 16$, $p = 0.36$) or dominant hand ($t = -0.53$, $df = 16$, $p = 0.60$) between the two conditions.

When the same assessment was undertaken in the traumatic brain injury group, there was no statistically significant difference in the reaction time of the non-dominant hand ($t = 1.31$, $df = 16$, $p = 0.21$) or dominant hand ($t = 0.51$, $df = 16$, $p = 0.62$). Although there was no statistically significant difference in the variability of performance of the dominant hand ($t = -1.99$, $df = 16$, $p = 0.06$), there was a significant difference in the variability of the performance of the non-dominant hand ($t = -2.43$, $df = 16$, $p = 0.03$). When the cue was presented in the centre of the screen, the variability of the non-dominant hand was higher (112 ± 7.9) than when the two cues were presented simultaneously on either side of the screen (92.2 ± 8.17). The patient group had more difficulty performing this condition accurately, which I did not predict at the time of developing the study. The reasons for this will be considered in the discussion later in this chapter. However, as a result, the two bimanual simultaneous states were not combined for further analysis (as they were in Chapter 5) and the bimanual condition where the two cues were presented simultaneously on either side of the screen was the condition used (as in Fling et al., 2011a).

6.4.1.3 Comparison of bimanual simultaneous and unimanual condition

Previous studies had proposed that the variability of the bimanual simultaneous condition is lower than the unimanual conditions. This is referred to as the “bimanual advantage” (Bangert et al., 2010, Fling et al., 2011a). Both hands were analysed separately. In the control group, there was no statistically significant difference in reaction time between the bimanual simultaneous condition (563 ± 10.8) and the unimanual condition (565 ± 11.9) in the non-dominant hand ($t = -0.145$, $df = 16$, $p = 0.89$) or dominant hand: bimanual simultaneous condition (553 ± 9.27) and the unimanual condition (562 ± 8.01) ($t = -1.39$, $df = 16$, $p = 0.18$). In addition, there was no statistically significant difference in the variability in the non-dominant hand: bimanual simultaneous (74 ± 2.63) and the unimanual condition (69.1 ± 3.10) ($t = 1.48$, $df = 16$, $p = 0.16$). This was also the case for the variability in the dominant hand: bimanual simultaneous (61.6 ± 4.42) and unimanual condition (65.6 ± 3.58) ($t = -0.86$, $df = 16$, $p = 0.40$).

In the patient group, there was a statistically significant difference in the reaction time between the bimanual simultaneous condition (568 ± 13.8) and the unimanual condition (589 ± 13.3) for reaction time in the non dominant hand ($t = 2.35$, $df = 16$, $p = 0.03$). However, this effect was small (-0.37). There was no statistically significant difference between bimanual simultaneous condition (562 ± 15.6) and the unimanual condition (572 ± 13.7) in the dominant hand ($t = -1.0$, $df = 16$, $p = 0.33$). In addition, there was no statistically significant difference in the variability between the bimanual simultaneous condition (92.2 ± 8.17) and the unimanual condition (114 ± 9.15) in the non dominant ($t = -2.10$, $df = 16$, $p = 0.06$) or dominant hand: bimanual simultaneous condition (83.1 ± 8.31) and unimanual condition (93.4 ± 6.98) ($t = -1.06$, $df = 16$, $p = 0.31$).

In summary, no consistent replication of the bimanual advantage was made, where the variability increases between the bimanual simultaneous state and the unimanual state, as reported in previous studies (Bangert et al., 2010, Fling et al., 2011a).

6.4.1.4 Comparison of the performance of the non-dominant versus the dominant hand

When comparing the performance of the non-dominant versus the dominant hand in the control group, there was no statistically significant difference for the reaction time of all tapping conditions, with no effect of “TAPPING CONDITION” ($F (2, 64) = 1.54$, $P = 0.22$), “HAND” ($F (1, 32) = 0.86$, $P = 0.36$) or “TAPPING CONDITION” x “HAND” interaction $(2, 64) = 0.58$, $P = 0.57$). When assessing the variability of the performance between the two hands, there was an effect of “TAPPING CONDITION” ($F (2, 64) = 17.5$, $P < 0.05$), but no statistically significant different effect of “HAND” ($F (1, 32) = 1.90$, $P = 0.18$), or “TAPPING CONDITION” x “HAND” interaction ($F (2, 64) = 2.16$, $P = 0.12$).

When comparing the performance of the non-dominant versus the dominant hand in the traumatic brain injury group, there was no statistically significant difference in the performance between the non dominant and dominant hand for the reaction time of all conditions, with no effect of “TAPPING CONDITION” ($F (1.54, 49.2) = 0.32$, $P = 0.73$), “HAND” ($F (1, 32) = 0.15$, $P = 0.70$) or “TAPPING CONDITION” x “HAND” interaction ($F (1.54, 49.2) = 1.92$, $P = 0.22$). When assessing the variability of the performance between the two hands, there was no statistically significant effect of “TAPPING CONDITION” ($F (2, 64) = 3.10$, $P = 0.052$), “HAND” ($F (1, 32) = 0.91$, $P = 0.35$) or “TAPPING CONDITION” x “HAND” interaction ($F (2, 64) = 2.05$, $P = 0.14$).

As there was no statistically significant effect of “HAND” or “TAPPING CONDITION” x “HAND” interaction in the control group or patient group, the responses for the two hands were combined for the respective conditions in the control group and patient group in the subsequent analysis.

6.4.2 Reaction time analysis

In the control group, as previously demonstrated in the healthy participants in the previous study, the average reaction time was relatively stable from the combined hand bimanual simultaneous condition (558 ± 8.75) to the combined unimanual condition (564 ± 9.27). However, the reaction time of the finger leading the bimanual asynchronous condition was slightly higher (570 ± 7.77).

In the patient group, the average reaction time was relatively stable between the combined hand bimanual simultaneous condition (575 ± 16.7) and the combined hand unimanual condition (570 ± 13.1). There was a slight increase in the average reaction time of the finger leading the bimanual asynchronous condition (578 ± 16.4).

When the two groups were compared, there was no effect of “CONDITION” ($F(2, 64) = 0.76$, $P = 0.47$), “GROUP” ($F(1, 32) = 0.47$, $P = 0.50$) or “CONDITION” x “GROUP” interaction ($F(2, 62) = 0.38$, $P = 0.69$). Therefore there was no significant difference in reaction times between the two groups, as demonstrated in Figure 6.1.

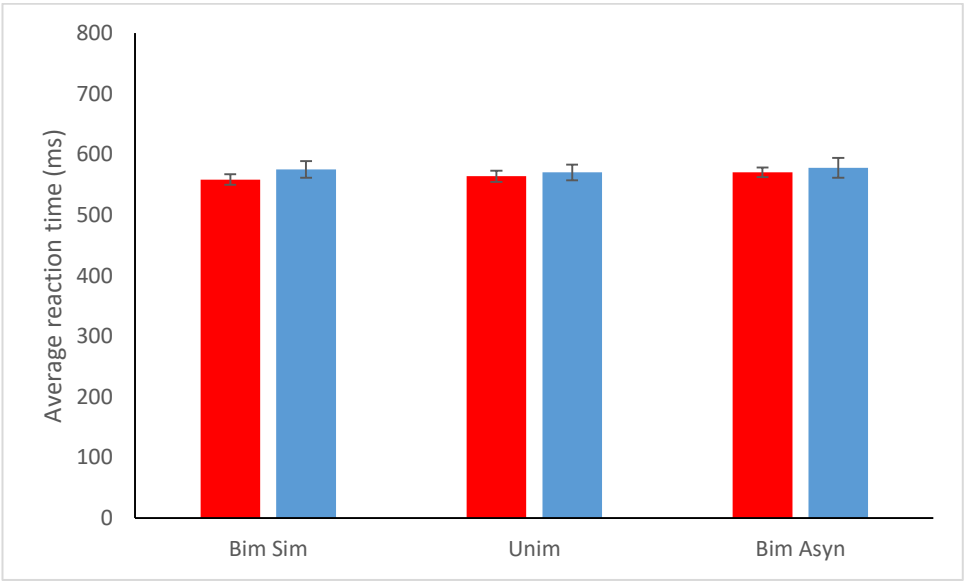


Figure 6.1: Average reaction time of the controls versus the patients. Red: control group, blue: traumatic brain injury patient group

6.4.3 Variability analysis

In the control group, there was relative stability between the combined hand bimanual simultaneous condition (67.8 ± 2.87) and combined hand unimanual condition (67.3 ± 2.78). However, there was a significant increase in variability in the finger leading the bimanual asynchronous condition (83.0 ± 3.10).

In the patient group, all measures were more variable (i.e. less stable), compared to the control group. There was an increase in variability from the combined hand bimanual simultaneous condition (87.7 ± 7.36) to the combined hand unimanual condition (104 ± 6.06). However, the variability of the finger leading the bimanual asynchronous condition was similar (103 ± 5.96).

When the variability of performance was compared between the two groups, there was a statistically significant effect of “CONDITION” ($F(1.53, 48.8) = 6.07$, $P < 0.05$), “GROUP” ($F(1, 32) = 25.9$, $P < 0.05$) but no “CONDITION” x “GROUP” interaction ($F(1.53, 48.8) = 2.36$, $P = 0.12$). Therefore there was a significant difference in the variability of the movement between the controls and patients (Figure 6.2).

This significant difference between the two groups was manifest in all conditions; the bimanual simultaneous ($t = -2.51$, $df = 20.8$, $p = 0.02$), unimanual ($t = -5.46$, $df = 22.4$, $p < 0.05$) and bimanual asynchronous condition ($t = -2.97$, $df = 24.1$, $p < 0.05$).

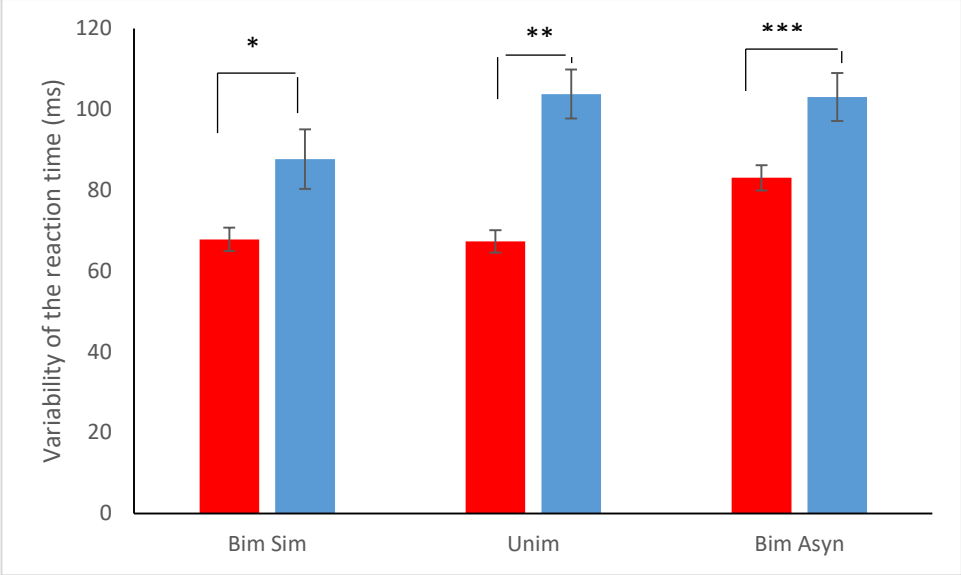


Figure 6.2: Variability of performance in the controls versus the patients. Red: control group, blue: traumatic brain injury patient group

6.4.4 Between hand lag measures

In the healthy control group there was no statistically significant difference in the mean hand lag (t = -1.05, df = 16, p = 0.31) or variability (t = -0.54, df = 16, p = 0.60) between the two bimanual simultaneous conditions. Therefore these results were combined. There was also no significant difference in the mean hand lag (t = 0.63, df = 16, p = 0.54) or variability (t = -1.29, df = 16, p = 0.22) between the two bimanual asynchronous conditions (non-dominant to dominant, dominant to non-dominant). Therefore, these results were combined.

In the traumatic brain injury patient group, there was no statistically significant difference in the mean hand lag (t = 0.63, df = 16, p = 0.54) or the variability (t = 2.0, df = 16, p = 0.06) between the two bimanual simultaneous conditions. Therefore these results were combined. There was also no significant difference in the mean hand lag (t = 0.38, df = 16, p = 0.71) or variability (t = -0.18, df = 16, p = 0.86) between the two bimanual asynchronous conditions (non-dominant to dominant, dominant to non-dominant). Therefore, these results were combined.

When the two groups were compared, there was no significant difference in the mean hand lag of the simultaneous movement ($t = 0.93$, $df = 32$, $p = 0.36$). However, there was a statistically significant difference in the mean hand lag of the asynchronous movement ($t = 5.72$, $df = 32$, $p < 0.05$), with the control group having a slightly higher hand lag than the patient group (Figure 6.3).

There was a statistically significant difference in the between hand lag variability in both the bimanual simultaneous movement ($t = 4.15$, $df = 32$, $p < 0.05$) and bimanual asynchronous movement ($t = 7.38$, $df = 21.5$, $p < 0.05$).

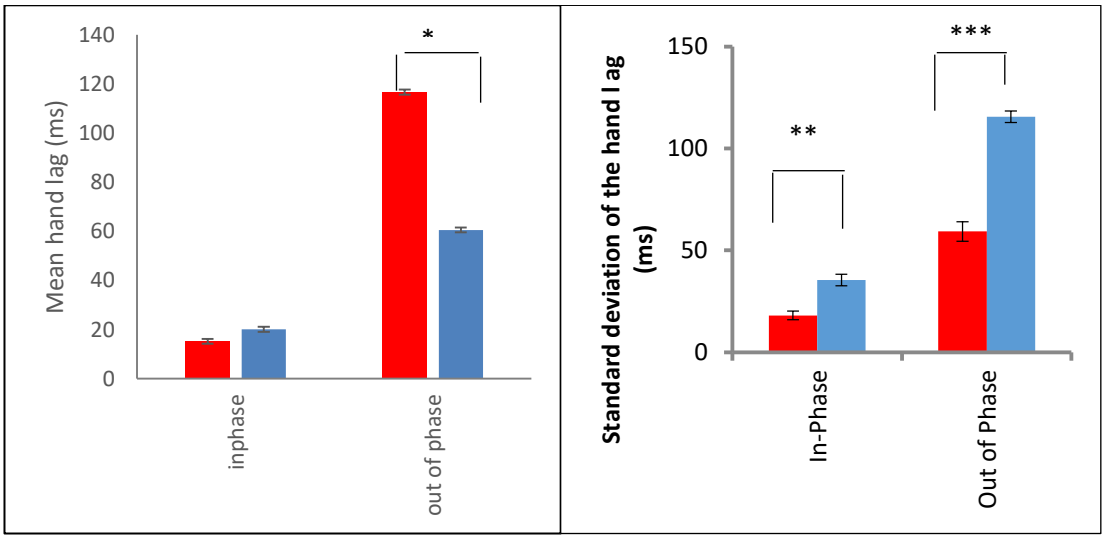


Figure 6.3 Comparison of hand lag between healthy controls and traumatic brain injury patients: Left panel: mean hand lag in the bimanual simultaneous (in-phase) and bimanual asynchronous (out-of-phase) condition. Right panel: Standard deviation of the hand lag in both conditions.

Red, control group. Blue, traumatic brain injury group

6.5 Discussion

The main purpose of this study was to assess the behavioural consequences following traumatic brain injury through a bimanual finger tapping task. This measured a range of interactions across the corpus callosum, which had already been validated in existing studies of two separate cohorts of healthy older adults (Bangert et al., 2010, Fling et al., 2011a). These interhemispheric interactions are required during bimanual movement to prevent interference from the opposite hemisphere (Bangert et al., 2010). This is of relevance, as the corpus callosum is anatomically vulnerable in traumatic brain injury (Gentry et al., 1988a). Tasks where collaboration and coordination between the hands is required, such as opening a bottle, are observed to be difficult in patients after traumatic brain injury (Caeyenberghs et al., 2011a). The present traumatic brain injury behavioural study involved the comparison of 17 patients with a history of traumatic brain injury with 17 healthy controls. These patients were all in the chronic phase following their injury, and had no motor, sensory or visual abnormalities on neurological examination at the time of testing. The main findings from this study were that the average performance in the tapping task was comparable between the two groups. This was evidenced by no significant differences in the mean reaction time between the traumatic brain injury patients and the control group. However, the variability of the performance was significantly different between the two groups. This resulted in higher variability of performance at each level of interhemispheric interaction in the traumatic brain injury group, compared to the control group. There was no consistent significant difference between the performance in the bimanual condition to unimanual condition in either group, which had previously been demonstrated (Bangert et al., 2010, Fling et al., 2011a). There was a significant difference in between hand lag variability in the patient group, compared to the control. The position of the cue did have a significant effect in the bimanual simultaneous condition. Reassuringly, the performance measures from the control group were comparable to the larger cohort of healthy participants in the previous study (Chapter 5). This reinforces the robustness of the behavioural measures obtained from this study. However, the performance of the hands was comparable in each group, which was not the case in the previous study.

6.5.1 Patient group

As previously mentioned, the patients were recruited from a traumatic brain injury database covering a number of centres in London. The recruitment for this behavioural study was dependent on the patients fulfilling safety criteria for transcranial magnetic stimulation (Wassermann, 1998, Rossi et al., 2009). This inevitably reduced the number of patients who could participate. However, it was important to have the same group of patients for the physiological and behavioural studies to improve the validity of any inferences made from the findings. The selection of traumatic brain injury participants to this study therefore included all patients with mild, moderate to severe traumatic brain injury who were eligible and who agreed to participate. A study size of this magnitude is not unusual for behavioural studies. However, it means care must be exercised in extrapolating conclusions from this study to the larger traumatic brain injury population.

As previously mentioned, all the patients had sustained an impact injury to the brain on one occasion (such as from a road traffic accident, fall, sports injury or assault) causing a loss of consciousness, rather than a history of repeated injuries over many years, such as the injuries experienced by boxers and footballers. Therefore, this simplifies interpretation of this study, and it does not have to take into account the effect of a single head injury versus the effects of multiple head injuries. The patient group were heterogeneous to traumatic brain injury-type, severity and neuroimaging results so this would inevitably reduce the statistical power of comparisons with the healthy controls, and is a limitation of this study. However, as previously mentioned, this is likely to be a limitation of traumatic brain injury research in general; traumatic brain injury is a diffuse acquired brain injury (Greenwood et al., 2016). Therefore, selection of patients on the basis of a particular structural imaging appearance, as can often be done in stroke research, is less feasible in the traumatic brain injury population. Recruitment of patients on the basis of Mayo classification severity of brain injury, is possible. However, within each Mayo classification grade underlies heterogeneity in head injury type and imaging appearances. The biomechanics of the injury itself has an impact on the pathology seen (Ommaya et al., 1974). Although more specific recruitment may be possible with more

awareness of the condition and increases in the number of patients participating in research, at present the patient group who participated in this behavioural study is probably grossly reflective of the heterogeneity encountered in the general traumatic brain injury population at this present time.

6.5.2 Comparison of healthy control group with healthy participants

The performance measures from the control group were comparable to the larger cohort of healthy participants in the previous study. The control group in this current study was smaller (n = 17) than the previous study (n = 29). In addition, some participants were common to both studies. However, this does provide reassurance that the conclusions drawn in the smaller group of controls are robust.

6.5.3 Comparison of bimanual simultaneous and unimanual condition

There was no consistent significant difference in performance between the bimanual simultaneous and unimanual condition in the patient or control groups in this current study. A lower variability in the bimanual simultaneous condition compared to the unimanual condition has been previously found in a study of healthy older adults, and is referred to as the bimanual advantage (Bangert et al., 2010, Fling et al., 2011a). This concept has also been used to support the existing research that bimanual simultaneous movements may have a focus outside of the corpus callosum (Ivry et al., 1999).

In the previous study presented in this thesis (Chapter 5), there was a significant difference in the variability of the performance between the bimanual simultaneous condition and unimanual condition in the dominant, but not dominant hand. The difference in the findings of the two studies presented in this thesis (Chapters 5 and 6), compared to the aforementioned studies, may be due to the differences in methodology; the previous studies tapped in synchrony to the cue, this study involved a response to the cue. However, it is also possible that this concept

is difficult to reproduce, or emphasises the complexity of the networks which are involved in executing bimanual movement.

6.5.4 The average performance between the two groups

The mean responses were comparable between the traumatic brain injury and control groups. All conditions had comparable performance, irrespective of proposed level of callosal interaction.

This was against the initial hypothesis of slower reaction times in the context of previously reported studies on bimanual coordination. The study by Fling and colleagues on older adults does not specifically report reaction times at each condition. They focus on the variability of the response for their correlation analysis (Fling et al., 2011a). The study by Kennerley and colleagues did investigate discrete tapping in patients who underwent callosotomy. However, different methods were used to assess this, and measures included velocity of movement and direction of tap (Kennerley et al., 2002). However, an additional study by Eliassen and colleagues describing a separate cohort of patients who underwent callosotomy did investigate simultaneous bimanual, and unimanual movement to a visual cue, and did report that the average responses were comparable to their control group. Their study consisted of epilepsy sufferers and healthy controls (Eliassen et al., 2000). However, post-callosotomy studies involve small numbers (three patients in this case) and it is difficult to make any firm conclusions on the basis of the findings because of the small numbers involved.

There are no studies in the traumatic brain literature that can be directly compared to this study. Increased movement times were observed in the study by Caeyenberghs and colleagues on patients after moderate to severe traumatic brain injury. Their performance did relate to corpus callosal integrity on diffusion tensor imaging. This study was undertaken in patients in the chronic phase after their injury, which is similar to the current study. The patient numbers recruited were also similar. However, assessment of the movement times involved

activities of daily living measures, the number of pegs inserted in the Purdue pegboard test, and response times during a switching task involving bimanual circular movements (Caeyenberghs et al., 2011a). These tasks are far more complex than the bimanual task used in this study, which may account for the increased movement times observed in the study by Caeyenberghs and colleagues. A larger study by Pearce and colleagues included a reaction time assessment on athletes who had suffered at least one concussion (amateur versus elite) and healthy controls (Pearce et al., 2014). The last concussion had been sustained over twenty years prior to testing. This study did report slower response to stimulus in the athlete group, compared to the control group. Similar to the current study, the response was to a visual cue on a screen. However, the reaction to the stimulus was measured from the cue appearing on the screen to releasing the press pad key. The participant was then asked to touch the screen. The task in the study by Pearce and colleagues, is simple. However, the athletes investigated in this study had suffered multiple episodes of concussion in their career, and the authors acknowledged that under-reporting concussion is common in the sports world. The slower response times in this study, may be reflective of the effect of “multiple impacts”, which is not a feature of the patients involved in this current study, all of whom had suffered a single injury.

6.5.5 Performance variability between the two groups

The performance variability was significantly different between the traumatic brain injury patient group and the healthy controls. The traumatic brain injury group demonstrated greater variability through all the conditions investigated, compared to the healthy controls. There also appeared to be a different trend in variability of performance between the two groups, with relative stability between the bimanual simultaneous and unimanual condition in the control group, with more variable performance in the bimanual asynchronous condition. However, the performance of the bimanual simultaneous condition appeared to be less variable than the unimanual and bimanual asynchronous movement in the patient group, which appeared comparable. However, this was not statistically significant when the hands were assessed separately.

The performance variability in the task presented in the current study was consistently increased through the states investigated in the traumatic brain injury group, and was almost double the variability of the healthy participants. The study by Fling and colleagues on older adults did comment on higher variability in the older adults, compared to the younger adults. As previously mentioned, the current study investigated the range of callosal interactions included in the study by Fling and colleagues. However, the increased variability was most evident in the bimanual asynchronous condition (Fling et al., 2011a). Higher variability of performance in the bimanual simultaneous state was observed in the study by Eliassen and colleagues, with the patients who underwent callosotomy demonstrating three times higher variability in simultaneous movement, compared to controls (Eliassen et al., 2000). However, an earlier study on a similar group of patients by Tuller and colleagues demonstrated normal bimanual simultaneous finger tapping, with deficits in bimanual asynchronous finger tapping. This led to the proposal that asynchronous finger tapping depended more on the integrity of the corpus callosum than synchronous finger tapping (Tuller et al., 1989). However, as previously mentioned, the corpus callosotomy literature usually involves small patient numbers. Therefore, although these studies conclude the corpus callosum is integral to simultaneous and asynchronous bimanual movement, it is difficult to make robust inferences on the role of the corpus callosum in preserving normal bimanual movement. Later functional imaging work has demonstrated the complexity of the networks involved (Witt et al., 2008).

In the traumatic brain injury group there appears to be an increase in breakdown of performance with increasing callosal interaction. However, the generalised increased variability of performance in the traumatic brain injury group, compared to the control group, also needs to be considered. This does suggest that additional factors outside the corpus callosum are involved. The frontal cortex has already been implicated in bimanual asynchronous movement in a study assessing a tapping task in a group of patients who underwent excision of the frontal cortex (Leonard et al., 1988). Although the results from the current study may be due to factors such as fatigue, patients with traumatic brain injury often

experience problems with higher cognitive function (Thornhill et al., 2000, Wehman et al., 2005, Kinnunen et al., 2011, Jilka et al., 2014). The study by Bangert and colleagues in older adults demonstrated relationship between asynchronous movement and self-reported executive dysfunction (Bangert et al., 2010). It is conceivable that executive functioning is a factor in this patient group. However, the brain activation patterns in bimanual movement are extensive, and can encompass the primary sensorimotor cortex, supplementary motor area, premotor cortex, cingulate motor cortex, lateral premotor cortex, basal ganglia, inferior parietal cortex, cerebellum and subcortical structures, depending on the nature of the movement (Sadato et al., 1997, Jancke et al., 2000a, Kermadi et al., 2000, Deiber et al., 2001, Immisch et al., 2001, Debaere et al., 2004, Witt et al., 2008). The nature of diffuse axonal injury is, by definition, a diffuse process. Therefore, it is possible that the generalised variability in the patient group through all conditions, is also due (at least in part) to the effects of the diffuse injury to extensive networks involved in bimanual coordination. However, this would only be measured through behavioural studies with functional imaging, and is outside of the scope of this chapter.

6.5.6 Between hand lag variability

The between hand lag variability deteriorated from the bimanual simultaneous condition to the bimanual asynchronous condition in both the traumatic brain injury group and control group. This manifest as an increase in variability between the two conditions in each participant group. As expected the mean hand lag was higher in the bimanual asynchronous condition. However, it was surprising that the asynchronous (trailing finger) was faster in the traumatic brain injury group than the healthy control group.

The overall increase in between hand lag variability in the bimanual simultaneous versus bimanual asynchronous condition has been reported in the study of older adults. Better performance in the bimanual asynchronous condition has been shown to correlate with better white matter integrity in the corpus callosum of older adults, but not in younger adults (Fling et

al., 2011a). Therefore the finding in the current study still supports the importance of interhemispheric interactions in executing bimanual asynchronous movement.

The factors behind the general increase in variability were discussed in the previous section. However, the unexpected result was the lower mean hand lag in the bimanual asynchronous condition in the patient group, compared to the control. It is possible that anticipation or impulsivity contributed to this. Although differing in methods, previous studies have demonstrated that asynchronous coordination is susceptible to become synchronous with repeated movements (Tuller et al.,1989, Swinnen, 2002, Johansen-Berg et al., 2007).

6.5.7 Comparison of the performance of the non-dominant versus the dominant hand

There were no significant differences in performance between the non-dominant and dominant hand in either group. This was not the case in the larger healthy participant group in the previous study (Chapter 5). This was probably due to some participants in the previous study being more functional with their dominant hand. Existing literature on musicians have demonstrated non-musicians show greater difference between hand performance, compared to musicians (Fujii et al., 2010). For the purpose of these studies, handedness was a self-reported measure by the participants. On reflection, a formal measure of handedness may have been helpful.

6.5.8 Influence of cue presentation in the performance of traumatic brain injury participants

Finally, there was a statistically significant difference in the performance of traumatic brain injury patients, when the single cue was presented in the centre of the screen, compared to the two cues being presented on the far sides, in the two bimanual simultaneous conditions. In the previous study (Chapter 5), I mentioned that it would be important to ensure that there

was no significant difference between the two cue presentations in the patient group. The concern was that the presence of slower responses or increased variability when the two cues were presented on either side of the screen in this condition, would cast doubt on the reliability of performance in all the remaining conditions, where the cue is presented on the far sides of the screen. My prediction was that this would be due to involvement of the visual networks resulting in inattention when the patient would face two cues on the sides of the screen. However, this was not the case in the patient group; however, there was a significant difference in the variability of the responses, with slower responses being made by the non-dominant hand when the cue was presented in the centre of the screen.

This finding is obviously the opposite of the previously made hypothesis. However, there are various reasons to be considered. Firstly, the patients did not have a central field defect. Secondly, they was a button press in response to the cue, but this response was more variable than when the cue was presented on the sides of the screen. Thirdly, this effect was not present in the healthy participants (larger study in previous chapter, or the controls in this chapter). On further consideration, the fixation cross and “get ready” icons were presented in the centre of the screen in all conditions. There was a 200ms delay between the “get ready” (!) icon and the cue (square). In the bimanual simultaneous state where the difference was found, the cue appeared in the same part of the screen as the fixation cross and “get ready” icon. It is therefore possible that this finding was due to failure/slowing of the visual processing networks (visual pathways, cognitive control) in the patient group, so it was difficult to differentiate the change from the “get ready” icon to the cue in that short time, as the response was less variable when the cue was presented in a different part of the screen. Slower processing could be due to higher cognitive function dysfunction, and has been recognised in traumatic brain injury (Kinnunen et al., 2011). Given that this was not evident in the healthy participants, this reinforces the possibility of network breakdown in the visual processing pathways as the reason for this observation, but further evaluation is outside the scope of this thesis.

6.5.9 Study limitations

As mentioned in the earlier discussion, it is likely that the corpus callosum is only part of the complex network involved in bimanual movement. Brain activation patterns in bimanual tasks are extensive, and vary with the complexity of the task, as well as the presence of the cue (Witt et al., 2008). This study did not involve functional imaging to evaluate brain activation patterns in this task, but the findings support a breakdown in the networks involved in carrying out this particular behavioural task in the patient group, even though it is likely that the corpus callosum is only part of that network.

Bimanual tasks also require higher cognitive functions to execute the task. The results in this current study suggest additional factors were involved outside of the corpus callosum to account for the differences in variability through all the conditions, even at low levels of interhemispheric interaction. The study in older adults by Bangert and colleagues demonstrated a relationship between asynchronous intermanual timing and executive dysfunction on a self-reported questionnaire (Bangert et al., 2010). These factors would likely have affected the performance of the traumatic brain injury group in this study. However, this was not assessed formally.

6.6 Clinical considerations

It was difficult to conclude whether the physiological differences in interhemispheric inhibition in the traumatic brain injury group described in Chapter 4 were due to a pathological process, rather than an adaptive response to the injury. As previously mentioned, musicians who had started their musical training before the age of 7 years also demonstrated reduced interhemispheric inhibition in a study by Ridding and colleagues (Ridding et al., 2000). With the high levels of bimanual coordination needed to play musical instruments that require both hands, it was also possible that the findings in the previous physiological study (Chapter 4) could represent an adaptive change to the physiology following traumatic brain injury.

However, as mentioned in Chapter 4, a pathological process seemed a more likely explanation in light of the hypothesis that shearing of the callosal fibres occurs in traumatic brain injury (Greenwood, 2002). The findings of this behavioural study add support to the hypothesis that the physiological findings presented in Chapter 4 are due to a pathological process, rather than an adaptive physiological process. Importantly, this study was conducted in the same group of patients, which undoubtedly strengthens this conclusion. Future work could therefore target improving bimanual coordination recovery with known methods to increase interhemispheric inhibition, such as GABA- agonist medication (for example, Baclofen) or methods to modulate the pathways such as repetitive transcranial magnetic stimulation.

In patients with traumatic brain injury, breakdown of bimanual coordination will inevitably have implications in every day functional tasks. This could possibly result in the need for more attention and concentration for consistent performance throughout a task. Although performance, on average, is being maintained (as demonstrated in the task required for this study), consistence of performance is not being maintained. This, in turn, could contribute to the fatigue and psychological consequences (such as anxiety) commonly experienced by patients who have had a traumatic brain injury. These factors will also affect motor performance, and have the potential to result in a vicious cycle. From this study, the variability of performance is evident, even though this particular group of patients were tested at least a year after their actual injury.

6.7 Conclusions

The traumatic brain injury patients studied here were in the chronic phase following their injury, and had no motor or sensory deficit on neurological examination at the time of testing. Although their performance was, on average, comparable to their healthy counterparts, the variability of that performance was impaired. The consequences of this would impact on day to day function, especially in tasks which require sustained performance. Importantly, the

variability of the performance and its consequences are likely to contribute to the “hidden disability” associated with traumatic brain injury, impacting on the ability to return to normal function after injury.

This study suggests that variability in performance is encountered in traumatic brain injury, and is a more sensitive marker than reaction time. It also opens an avenue of enquiry for future functional imaging studies to understand the complex brain networks that contribute to this variability. In addition, it identifies areas to target (such as drug studies, and methods to modulate the pathways with rTMS methods) to assess whether improving interhemispheric inhibition benefits recovery of bimanual coordination after injury. Although there are undoubtedly additional cognitive processes involved, better understanding will also assist physical rehabilitation and recovery for this patient group.

The patient studies presented in this thesis (Chapter 4 and Chapter 6) revealed abnormalities in the physiology and behaviour associated with the corpus callosum, which is a structure commonly affected by traumatic brain injury (Gentry et al., 1988a, Adams et al., 1989, Parizel et al., 1998, Meythaler et al., 2001, Scheid et al., 2003). The next step would be to analyse the microstructure for this particular group of traumatic brain injury patients. This is the focus of the next chapter.

Chapter 7

Microstructural analysis

The images used in this study were pre-acquired, processed and stored on the existing Imperial College traumatic brain injury diffusion tensor imaging database. Dr S Jilka and Dr L Li retrieved the required images and ran the script to obtain the diffusion tensor imaging metrics used in this study. I was responsible for the analysis of the extracted DTI metrics, the concept of the more detailed analysis of the corpus callosum and interpretation of the study findings.

7.1 Introduction

In chapters 4 and 6 the physiology and behaviour of a group of patients who have sustained a traumatic brain injury have been investigated, and compared to healthy participants. These experiments have focussed on the corpus callosum physiology and related behaviour, given that this is a structure commonly affected by traumatic brain injury (Gentry et al., 1988a, Adams et al., 1989, Parizel et al., 1998, Meythaler et al., 2001, Scheid et al., 2003). The main findings through these two studies were that the traumatic brain injury group has different callosal physiological transfer, compared to the healthy participants. There was evidence of abnormal interhemispheric inhibition in the patient group, which indicated that the physiological relationship between interstimulus interval on the amount of interhemispheric inhibition produced was different between the patient and healthy participant groups. Interhemispheric inhibition is a physiological measure to assess the callosal connections between the motor areas of each hemisphere (Ferbert et al., 1992). In the absence of group differences in the other investigated measures of corticospinal excitability (motor thresholds, recruitment curves) and intracortical physiology (short intracortical inhibition time course), these findings support the hypothesis that the mechanism of injury in traumatic brain injury (proposed acceleration, deceleration and rotational motions from impact and shearing of fibres) renders the corpus callosum physiology particularly vulnerable to damage. This shearing of callosal fibres presumably damages axons and disrupts their physiological function. The subsequent behavioural study on the same group of patients demonstrated increased variability of performance in a tapping task in the traumatic brain injury group, compared to the healthy participants. Interestingly, the average performance through all conditions in the tapping task was comparable between the two groups. The difference between the two groups was observed in the variability of the hand lag of the asynchronous bimanual condition, compared to the simultaneous bimanual condition. This asynchronous bimanual movement has been validated in previous studies to be associated with the highest level of interhemispheric interaction in two cohorts of adults (Bangert et al., 2010, Fling et al., 2011a). Clumsy and slow performance, particularly in motor tasks where coordination between hands is required, has

been observed after traumatic brain injury (Chaplin et al., 1993, Rossi and Sullivan 1996). The behavioural study presented in this thesis therefore supports the hypothesis that the callosal interactions are affected in traumatic brain injury, although it is likely other factors are also involved in bimanual movement. Together, these studies provide further valuable information regarding the physiological and behavioural reasons behind problems with bimanual coordination observed after traumatic brain injury, and its considerations to aid neurorehabilitation.

Structural abnormalities following traumatic brain injury include decreased white matter integrity throughout the brain (Sidaros et al., 2008, Rutgers et al., 2008, Caeyenberghs et al., 2011a, 2011b, Fling et al., 2011a, Hellyer et al., 2013, Hulkower et al., 2013). It is recognised that visible areas of structural damage on conventional neuroimaging do not necessarily reflect the clinical deficit, and that contusion load is often a poor predictor of cognitive deficit following traumatic brain injury (Bigler, 2001, Lee et al., 2008). The presence of diffuse axonal injury is an important factor in clinical outcome, but is not always evident on conventional neuroimaging (Adams, 1982, Arfanakis et al., 2002, Medana et al., 2003). Diffusion tensor imaging measures the directional coherence of water diffusion in vivo, and is sensitive to damage to white matter integrity following traumatic brain injury (Huisman et al., 2004, Bendlin et al., 2008). Existing literature has specifically demonstrated microstructural abnormalities on diffusion tensor imaging in traumatic brain injury, when conventional imaging is normal (Arfanakis et al., 2002, Mayer et al., 2010, Sharp et al., 2011a). Therefore, diffusion tensor imaging is thought to provide a sensitive measure of changes in white matter structure after traumatic brain injury (Arfanakis et al., 2002, Huisman et al., 2004, Mac Donald et al., 2007a,b). Abnormal diffusion tensor imaging metrics have also been demonstrated after mild traumatic brain injury, and enables further evaluation of this group of patients, attesting to the usefulness of this imaging modality (Sharp et al., 2011a). The study by Mayer and colleagues also demonstrated that diffusion tensor imaging was found to be superior to neuropsychology tests at distinguishing patients from controls (Mayer et al., 2010). Abnormalities in diffusion tensor imaging metrics have been demonstrated in the presence of concussive (Cubon et al., 2011) and non-concussive head injury in athletes (Bazarian et al., 2012). The latter is particularly interesting

as it suggests that the impact of a head injury, even when not accompanied by clinical symptoms, can be associated with microstructural damage. This provides further evidence that diffusion tensor imaging is more sensitive than the existing clinical diagnostic tests (such as conventional imaging and psychological tests) in evaluating patients after traumatic brain injury. Therefore, to complement the physiological and behavioural studies presented in this thesis, the natural next line of enquiry would be to assess the white matter integrity of the traumatic brain injury patients and controls who have participated in the previous studies in this thesis. As these studies demonstrated significant differences pertaining to the corpus callosum in the patient group compared to control, the main area of interest would be the microstructure of the body of the corpus callosum.

Most existing diffusion tensor imaging studies in the literature focus on fractional anisotropy measures (Alexander et al., 2007, Farbota et al., 2012, Hulkower et al., 2013). Fractional anisotropy estimates the orientation and coherence of white matter tracts by estimating the extent to which the diffusion process is anisotropic, or rather directionally constrained (Le Bihan et al., 2001, Beaulieu, 2002). Higher fractional anisotropy is associated with better white matter integrity (Alexander et al., 2007). As previously mentioned, this means fractional anisotropy is a highly sensitive, albeit a non-specific biomarker of neuropathology and microarchitectural microstructures. Additional imaging metrics provide more information regarding the direction and magnitude of water diffusion. These are the radial and axial diffusivity. Radial diffusivity is an average of diffusion along two minor axes, and axial diffusivity describes diffusion along the principal axis (Hulkower et al., 2013). Mean diffusivity is also commonly reported, and is a measure of the total direction-independent diffusion within a voxel, thus providing less information about the directional coherence of water diffusion. Diffusion tensor imaging does have its limitations; estimates of tract directions are susceptible to image noise (thermal and physiological) (Basser et al., 2000, Alexander et al., 2007), artefacts (for example head motion resulting in misreading), partial volume averaging between tissues in large voxels (i.e. signal mixing of white matter, grey matter and CSF) and regions of crossing white matter tracts; this is unavoidable as many areas have regions of fibre crossing. However, the corpus callosum, the focus of this analysis, has fewer crossings (Alexander et

al., 2001). Nevertheless, fractional anisotropy is considered a marker of white matter integrity in traumatic brain injury, since its first reported study in 2002 (Arfanakis et al., 2002).

As mentioned, a higher fractional anisotropy is associated with a more coherent microstructure (Basser et al., 1996, Alexander et al., 2007). Consequently, lower fractional anisotropy is considered to represent alterations in white matter microstructure consistent with the diffuse axonal injury in traumatic brain injury (Sharp et al., 2011a, 2011b, Shenton et al., 2012, Farbota et al., 2012, Hellyer et al., 2013, Jilka et al., 2014). However, fractional anisotropy analysis only provides part of the picture. Therefore, it would also be helpful to obtain additional metrics to be able to make inferences regarding the nature of the impaired white matter integrity in traumatic brain injury; a lower fractional anisotropy may be due to higher radial diffusivity and/or lower axial diffusivity (Farbota et al., 2012, Soares et al., 2013). Lower radial diffusivity is associated with better microstructure and healthy myelination (Sullivan et al., 2010). Lower axial diffusivity is associated with axonal damage (Song et al., 2002). Therefore, analysis will initially focus on fractional anisotropy metrics, and then explore the axial and radial diffusivity metrics.

The purpose of this chapter is therefore to assess the microstructure following traumatic brain injury, using the diffusion tensor imaging metrics for the traumatic brain injury group who participated in the physiological and behavioural studies already undertaken, and compare to healthy controls. Taking into consideration previous traumatic brain injury literature, I hypothesise that the fractional anisotropy metric will be lower in the traumatic brain injury group, compared to the healthy controls, in line with current traumatic brain injury literature.

7.2 Study design

7.2.1 Participants

The pre-acquired scans for the same healthy participants who acted as controls for the previous studies were accessed. They had already been recruited from a database of healthy volunteers from the UCL Institute of Neurology and Imperial College. As previously mentioned, this consisted of seventeen healthy participants, consisting of 10 males and 7 females. All healthy subjects were on no regular medication. They had also participated in more extensive traumatic brain injury research as part of an MRI study conducted by Imperial College.

The pre-acquired MRI scans for the same traumatic brain injury patients who participated in the traumatic brain injury studies in Chapter 4 and Chapter 6 were accessed. As previously mentioned, this consisted of seventeen traumatic brain injury participants, consisting of 10 males and 7 females. All patients had sustained an impact injury to the brain on one occasion (from a road traffic accident, fall, sports injury or assault) causing a loss of consciousness. All patients had been treated conservatively after their traumatic brain injury and not required any neurosurgical intervention. These patients had already participated in the traumatic brain injury research as part of an MRI study conducted by Imperial College.

The patients had injuries secondary to road traffic accidents 64%, assaults 12%, falls 12% or a single sports injury 12%. Based on the Mayo classification system for traumatic brain injury severity, there were eleven moderate – severe and six mild (probable) cases of traumatic brain injury in this patient group (Malec et al., 2007). Glasgow Coma Scale score was recorded in fourteen of the patients (mean 9.36 ± 1.47). Average length of post traumatic amnesia was recorded in sixteen of the patients (mean 12.1 ± 3.89 days). All patients were in the chronic phase following their traumatic brain injury (mean time since injury 3.72 years, range 1.1 – 7.5 years).

7.2.2 Institutional and ethical approval

The information sheets and protocols had already been reviewed and ethical approval was obtained from the respective Ethics research committee. For all studies, participants had already given written consent for the MRI to be obtained and stored for the Imperial College MRI study. However, additional written consent was obtained for these images to be accessed for the purpose of this study. All studies were performed in accordance with the Declaration of Helsinki.

7.3 Experimental Methods

7.3.1 Magnetic resonance imaging

Traumatic brain injury patients and healthy participants had standard T1 MRI to assess for evidence of focal brain injury and gradient echo imaging to identify any evidence of microbleeds, a marker of diffuse axonal injury (Scheid et al., 2003). A senior consultant neuroradiologist reviewed all study MRI scans.

7.3.1.2 Diffusion tensor imaging

The MRI data for the control and patient groups had already been obtained as part of the larger traumatic brain injury study at Imperial College. Images had been obtained in a Phillips Intera 3.0 Tesla MRI scanner, using an 8-array head coil and sensitivity encoding (SENSE) with an under sampling factor of 2. Diffusion-weighted volumes with gradients applied in 16 non-collinear directions were collected for each participant in each of four diffusion tensor imaging runs. This resulted in a total of 64 directions. The following parameters were used: 73 contiguous slices, slice thickness=2mm, field of view 224mm, matrix 128×128, voxel size $1.75\times1.75\times2mm^3$, b value=1000 and four images with no diffusion weighting ($b=0s/mm^2$).

7.3.1.3 Diffusion tensor imaging processing

Diffusion tensor imaging processing was undertaken using the method as described by Hellyer and colleagues (Hellyer et al., 2013). Through this method, diffusion weighted images were registered to the b = 0 image by affine transformations to minimise distortion due to motion and eddy currents. Using the Brain Extraction tool (BET) from the FMRIB Software library image processing toolbox, the images were brain extracted (Smith, 2002, Smith et al., 2004, Woolrich et al., 2009). Using the Diffusion Toolkit, fractional anisotropy, mode anisotropy and mean diffusivity maps were generated, and images for the eigenvalues (λ1, λ2 and λ3) representing the magnitude of diffusion in the three principal directions were obtained (Behrens et al., 2003, Ennis et al., 2006). Radial diffusivity was then derived from the eigenvalues (RD = λ2+λ3/2), and axial diffusivity from the eigenvalue (AD = λ1). Further processing of these images was performed using the pre-processing stages of Tract-Based Spatial Statistics (TBSS) to skeletonise the white matter (Smith et al., 2006). Firstly, this involved the creation of the fractional anisotropy images by fitting a tensor model to the raw diffusion data using the FMRIB Diffusion Toolbox, and then brain-extracted using the brain extraction tool (BET) (Smith, 2002). This fractional anisotropy data for all subjects was then aligned into a common space using the FMRIB nonlinear registration tool, which uses a b-spline representation of the registration warp field. The mean fractional anisotropy image was created and thinned to create a mean skeleton that represents the centres of all tracts. Each participant's aligned fractional anisotropy data was then projected onto this skeleton. Thus, the white matter skeleton produced by the TBSS constrained the analysis of white matter to the central parts of the white matter tracts. This resulted in only the core of the tract being sampled, whilst excluding peripheral areas of the respective fibre tract that are known to show pronounced interindividual variability (Jilka et al., 2014). This has been acknowledged to produce an incomplete assessment of white matter (Bonnelle et al., 2011). However, it has already been acknowledged to have the advantage of minimising partial volume errors, and constraining the sampling of white matter damage has already been shown to more accurately estimate white matter integrity after traumatic brain injury (Squarcina et al., 2012, Hellyer et al., 2013). Once the MRI data was pre-processed using this customised method, the fractional

anisotropy, mean diffusivity, axial diffusivity and radial diffusivity data for all participants was then exported to excel for analysis.

The white matter skeleton produced by TBSS constrained the analysis of the white matter to the central parts of the white matter tracts, and consisted of twenty three white matter tracts. This script separates the corpus callosum into three sections. These represent the genu, body and splenium of the corpus callosum. The callosal motor fibres connect the primary motor cortices (M1 area) of the two hemispheres. Although the tract based spatial statistics method used above obtained imaging metrics for the body of the corpus callosum, existing literature has suggested that the callosal motor fibres in humans run through the posterior body of the corpus callosum (Meyer et al., 1998, Hofer et al., 2006, Zarei et al., 2006, Wahl et al., 2007). For the purpose of the planned correlation analysis with the physiological and behavioural measures in the next chapter, the diffusion tensor metrics for this region were also obtained. Tractography work using a combined fMRI/DTI procedure by Wahl and colleagues found that the mean Talairach co-ordinates of the hand corpus callosal motor fibres was located at 0, -14.3, 17.6 mm (x,y,z) in a group of healthy participants (Wahl et al., 2007). This mask (referred to as the Wahl mask) was reproduced using the principles reported in the study by Wahl and colleagues, as it was a validated representation of the callosal motor fibres. Their work had already demonstrated significant correlation with interhemispheric inhibition in healthy individuals and had also been used in additional behavioural work by Fling and colleagues. (Wahl et al., 2007, Fling et al., 2011a).

The team produced the script which identified the DTI metrics from this mask (Figure 7.1). To elaborate, this was undertaken by initially producing an individualised tract for each participant using FSL, which extended from one primary motor cortex (M1) to the M1 on the other side, via the corpus callosal motor fibres. The anatomical landmark for the M1 hand representation on either side was as defined in Yousry et al., 1997. The tract traversed through the corpus callosum, via the mean Talairach coordinates of the hand callosal motor fibres as reported in the paper by Wahl and colleagues, which was identified as the average location where the

fibres connecting the hand regions of the primary motor cortex passed through the corpus callosum (Wahl et al., 2007). This was then averaged to obtain a group mask which was constrained within the boundaries of the corpus callosum, in a similar manner as described in the study by Wahl and colleagues. This group mask was then applied to the skeletonised corpus callosum for each participant; the value obtained represented the skeletonised corpus callosum which overlaps with the mask. This was therefore a region of interest- based follow-up to the TBSS method used in the earlier part of the chapter. These results were then exported to excel for analysis.

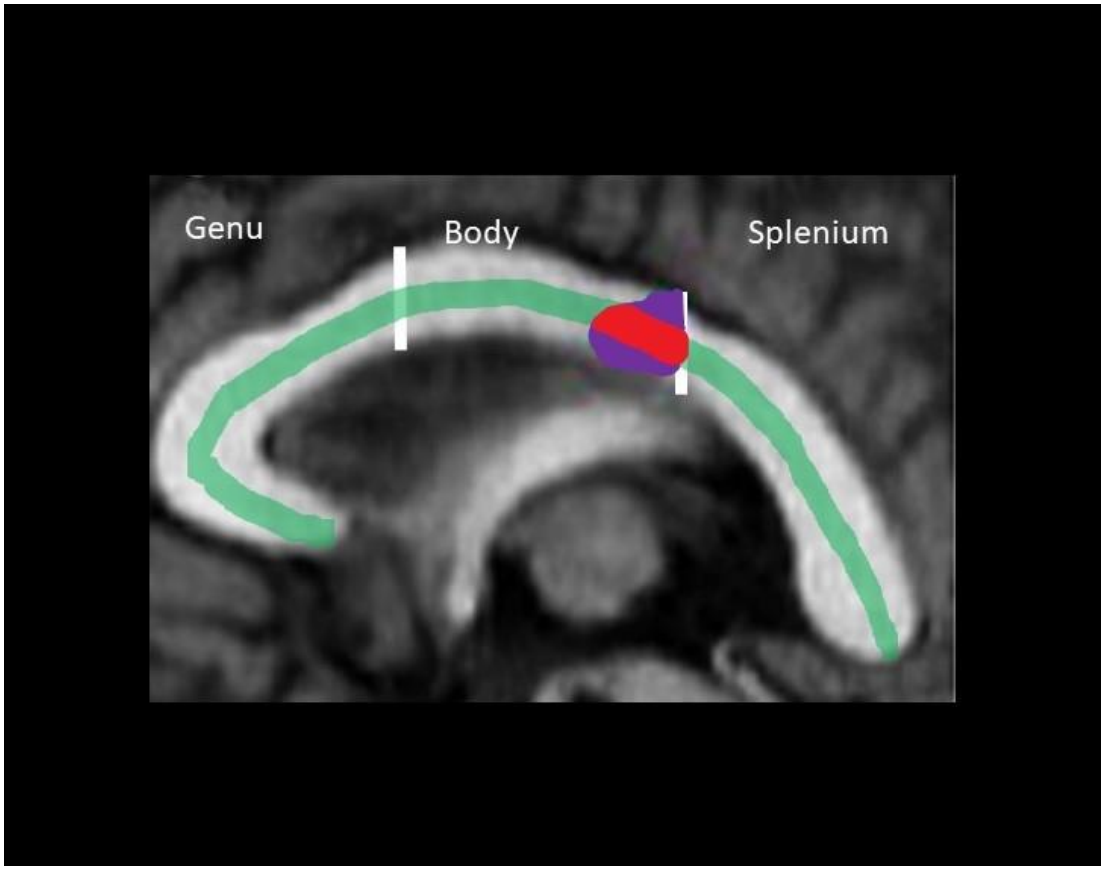


Figure 7.1: A schematic representation of the mask (purple) constrained to the corpus callosum. The TBSS skeleton (green) through the corpus callosum is superimposed onto this diagram for illustration. The DTI value obtained was the central part of the tract (red) that overlapped with the mask. Adapted from Wahl et al., 2007.

7.3.2 Data and Statistical analysis

The white matter skeleton produced by the TBSS consisted of twenty three white matter tracts that are used by the Imperial College team in their cognitive traumatic brain injury research. The tracts are anterior thalamic projections (right and left), cingulum cingulate (right and left), cingulum hippocampus (right and left), corticospinal (right and left), inferior fronto-occipital fasciculus (right and left), inferior longitudinal fasciculus (right and left), superior longitudinal fasciculus (right and left), superior longitudinal fasciculus temporal projection (right and left), uncinate fasciculus (right and left), corpus callosum body, genu and splenium, forceps major and forceps minor. Initial statistical analysis was performed on the whole brain white matter tract DTI output to demonstrate whether there was generalised disruption of the white matter tracts as previously described (Kinnunen et al., 2011). This analysis was initially undertaken for the fractional anisotropy metrics for the twenty three tracts, and two–way repeated measures analysis of variance (ANOVA) was undertaken with factors “WHITE MATTER TRACT” as the within subjects variable and “GROUP” (traumatic brain injury, healthy control) as the between subjects variable. For the report of the statistical differences, the significance threshold of $\alpha = 0.05$ was used. Where assumptions of sphericity were violated (where Mauchley’s test $P < 0.05$), the Greenhouse – Geisser correction was applied. Twenty three regions were assessed and Bonferroni corrected for multiple comparisons. This was then repeated for the radial diffusivity and axial diffusivity metrics for the same tracts.

There was evidence of widespread disruption of white matter in the traumatic brain injury group, which included the tracts relevant to this thesis; the body of the corpus callosum and corticospinal tracts. However, subsequent analysis focused on these specific tracts to explore the cause for the difference in fractional anisotropy in the traumatic brain, compared to the healthy controls. Correlation analysis was performed to confirm a relationship between the corpus callosal measure from the Imperial DTI metrics of the corpus callosum body and the region of interest approach using the coordinates from Wahl and colleagues. The metrics from this mask was then used for further analysis (Wahl et al., 2007). Correlation analysis was

undertaken to determine whether there was a relationship between the DTI metrics of the corpus callosum.

Normality of all the data sets was tested with Kolmogorov – Smirnov tests. For the report of the statistical differences, the significance threshold of $\alpha = 0.05$ was used unless otherwise stated. Statistical procedures were conducted using the statistical package SPSS version 19.0 for Windows; SPSS Inc. All data are given as mean ± standard error of the mean unless otherwise stated.

7.4 Results

For the purpose of comparison, the MRI data was obtained from the same 17 healthy participants and 17 traumatic brain injury patients from the physiological and behavioural studies presented in this thesis. As mentioned, the control group contained 10 males and 7 females aged between 22 and 52 years (mean ± SE 30.5 ±1.75). The patient group contained 10 males and 7 females aged between 22 and 60 years (mean ± SE 43.3 ± 2.69).

7.4.1 Diffusion tensor imaging measures

Comparison of the DTI metrics in the traumatic brain injury patient group and controls demonstrated that the majority of the white matter microstructure was abnormal in the patient group. The difference between the groups was significant for fractional anisotropy and radial diffusivity. Although differences were also observed with axial diffusivity, this was not statistically significant.

The comparisons between the groups was initially undertaken for fractional anisotropy. Average fractional anisotropy was lower in all the white matter tracts in the traumatic brain

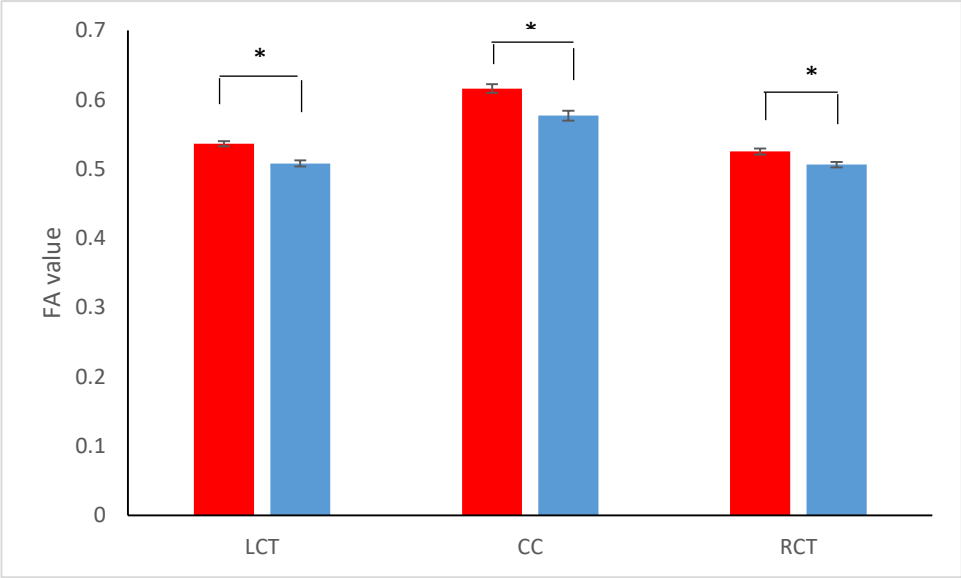
injury group, compared to controls. A two–way repeated measures analysis of variance (ANOVA) with “FA WHITE MATTER TRACT” as within subjects factor and “GROUP” (traumatic brain injury, control) as between subjects factor demonstrated a main effect of “FA WHITE MATTER TRACT” ($F(6.73, 215) = 892$, $P < 0.05$), “GROUP” ($F(1, 32) = 17.7$, $P < 0.05$) and “FA WHITE MATTER TRACT” x “GROUP” interaction ($F(6.73, 215) = 2.80$, $P = 0.01$). This meant that the effect of the integrity of the white matter tract on the corresponding fractional anisotropy value was different between the two groups. This was statistically significant in the corpus callosal fibres (body, genu), corticospinal tracts and intrahemispheric fibres (uncinate fasciculi, inferior and superior longitudinal fasciculi, inferior fronto-orbital fasciculi, cingulum).

A comparison between the traumatic brain injury patient group and healthy controls was also undertaken for the radial diffusivity metrics. The average radial diffusivity was higher in all white matter tracts in the traumatic brain injury group, compared to controls. A two–way repeated measures ANOVA with “RD WHITE MATTER TRACT” as within subjects factor and “GROUP” (traumatic brain injury, control) as between subjects factor demonstrated a main effect of “RD WHITE MATTER TRACT” ($F(6.08, 195) = 24.2$, $P < 0.05$), “GROUP” ($F(1, 32) = 5.08$, $P = 0.03$) but no “RD WHITE MATTER TRACT” x “GROUP” interaction ($F(6.08, 194.6) = 1.07$, $P = 0.38$).

A comparison of axial diffusivity in the white matter tracts was then undertaken. The average axial diffusivity was higher throughout all white matter tracts in the traumatic brain injury group compared to controls. However, a two–way repeated measures ANOVA with “AD WHITE MATTER TRACT” as within subjects factor and “GROUP” (traumatic brain injury, control) as between subjects factor did not demonstrate a main effect of “AD WHITE MATTER TRACT” ($F(4.87, 151) = 0.97$, $P = 0.44$), “GROUP” ($F(1, 32) = 2.27$, $P = 0.14$) or “AD WHITE MATTER TRACT” x “GROUP” interaction ($F(4.87, 151) = 1.43$, $P = 0.22$).

7.4.2 Analysis of specific white matter tracts

The fractional anisotropy was then evaluated in more detail in the left corticospinal tract, body of the corpus callosum and right corticospinal tract of the traumatic brain injury patient group and controls. The mean fractional anisotropy was lower in the traumatic brain injury group compared to control (see Figure 7.2 and Table 7.1). Independent t test demonstrated a significant difference in the fractional anisotropy measure between the traumatic brain injury group and healthy control in the left corticospinal tract ($t = -5.02$, $df = 32$, $p < 0.05$), body of the corpus callosum ($t = -2.63$, $df = 32$, $p = 0.01$) and right corticospinal tract ($t = -3.23$, $df = 32$, $p = 0.03$).

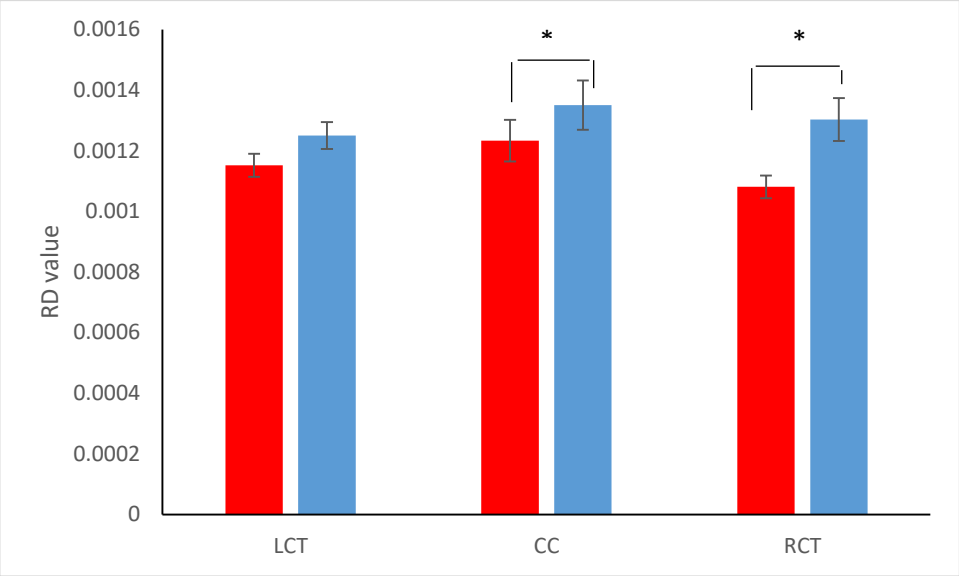


LCT: Left corticospinal tract; CC: Body of corpus callosum; RCT: Right corticospinal tract

Figure 7.2. Fractional anisotropy in TBI and control group. Red represents the control group; blue represents the TBI group

The radial diffusivity was then evaluated between the two groups in the same structures (see Table 7.1). The traumatic brain injury group had higher radial diffusivity than the control group. Independent t test demonstrated this to be significant in the body of the corpus callosum ($t = 2.75$, $df = 26$, $p = 0.01$) and right corticospinal tract ($t = 2.76$, $df = 24.3$, $p = 0.01$). The radial

diffusivity was also higher in the left corticospinal tract in the patient group, but this was not statistically significant (t = 1.67, df = 32, p = 0.11) (Figure 7.3).



LCT: Left corticospinal tract; CC: Body of corpus callosum; RCT: Right corticospinal tract

Figure 7.3. Radial diffusivity in TBI and control group. Red represents the control group; blue represents the TBI group

As mentioned earlier, there was no significant difference in axial diffusivity in the whole brain microstructure. However, for exploratory purposes the axial diffusivity was then evaluated between the two groups (see Table 7.1). The traumatic brain injury group had higher axial diffusivity measures, but this was not statistically significant in the left corticospinal tract (t = 1.80, df = 20.0, p = 0.09), the body of the corpus callosum (t = 0.77, df = 32, p = 0.45) or the right corticospinal tract (t = 0.81, df = 19.4, p = 0.43).

Structure	Fractional anisotropy Control	Fractional anisotropy Traumatic brain injury	Radial diffusivity Control ($x10^{-3}$)	Radial diffusivity Traumatic brain injury ($x10^{-3}$)	Axial diffusivity Control ($x10^{-3}$)	Axial diffusivity Traumatic brain injury ($x10^{-3}$)
CC	0.62 ± 0.03	0.58 ± 0.03	1.23 ± 0.28	1.35 ± 0.33	1.40 ± 0.70	2.20 ± 1.40
LCT	0.54 ± 0.02	0.51 ± 0.02	1.20 ± 0.20	1.20 ± 0.20	1.65 ± 0.38	2.15 ± 1.08
RCT	0.53 ± 0.02	0.51 ± 0.02	1.11 ± 0.20	1.30 ± 0.30	1.68 ± 0.28	1.86 ± 0.21

Table 7.1 Average diffusion tensor imaging values: CC Body of the corpus callosum; LCT Left corticospinal tract; RCT Right corticospinal tract (mean ± SD)

As the callosal motor fibres of the body of the corpus callosum are more relevant to the physiological and behavioural measures already obtained in this current group of patients, a correlation analysis was then undertaken to confirm a relationship between the corpus callosum body white matter tract (TBSS) versus callosal motor fibres (Wahl mask). As anticipated, this demonstrated a significant positive correlation between the two measures in the control subjects ($r = 0.99$, $p < 0.05$) and traumatic brain injury group ($r = 0.99$, $p < 0.05$).

The relationship between the metrics in the callosal motor fibres (co-ordinates by Wahl and colleagues) was then explored (Wahl et al., 2007). As mentioned in the study by Wahl and colleagues, this located the callosal motor fibres to the posterior body of the corpus callosum (i.e. a more localised area than the TBSS tract). Interestingly, this demonstrated a strong correlation between the fractional anisotropy and radial diffusivity corpus callosal measures ($r = -0.93$, $p < 0.05$), but no significant correlation between the fractional anisotropy and axial diffusivity corpus callosal measure ($r = - 0.24$, $p = 0.35$) (Figure 7.4). This demonstrated that

there was a strong relationship between the fractional anisotropy and radial diffusivity measures in the corpus callosum, but not the fractional anisotropy and axial diffusivity measures. This suggests that the callosal radial diffusivity was driving the fractional anisotropy in the traumatic brain injury group, rather than the axial diffusivity.

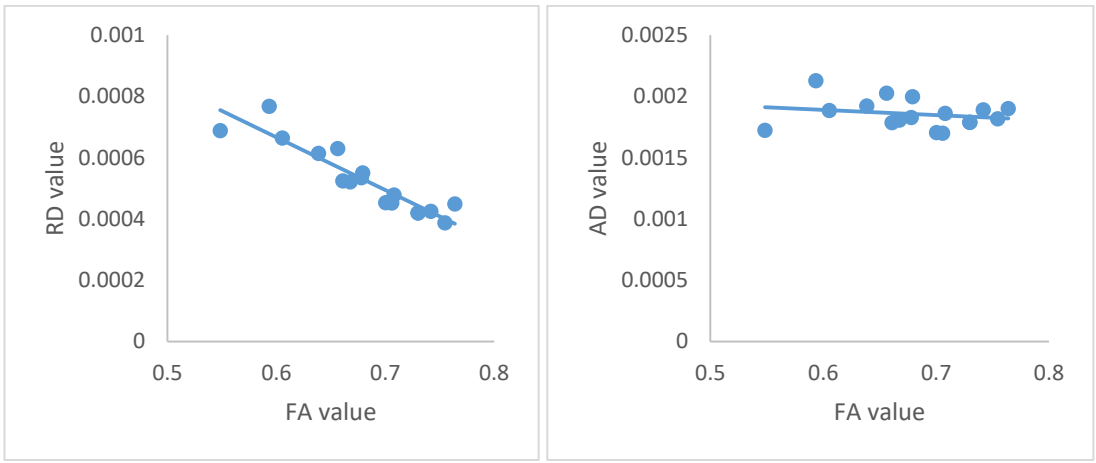


Figure 7.4 Relationship between callosal motor fibres FA value and RD value (left panel) and callosal motor fibres FA value and AD value (right panel) in the traumatic brain injury group

However, similar relationships were also demonstrated in the respective metrics in the fractional anisotropy and radial diffusivity of the callosal motor fibres ($r = -0.93$, $p < 0.05$) and fractional anisotropy and axial diffusivity callosal motor fibres ($r = -0.06$, $p = 0.83$) in the control group (Figure 7.5).

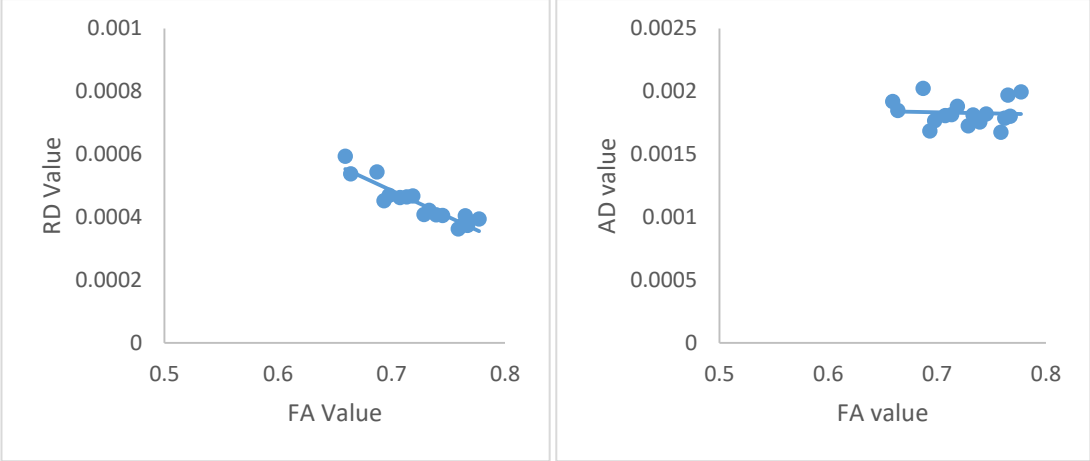


Figure 7.5 Relationship between callosal motor fibres FA value and RD value (left panel) and callosal motor fibres FA value and AD value (right panel) in the healthy participants

7.5 Discussion

The main findings from this study were that there was evidence of differences in white matter integrity in this group of chronic patients with traumatic brain injury, compared to the control group. The fractional anisotropy was lower, and the radial diffusivity was higher in the traumatic brain injury group. The axial diffusivity was higher in the traumatic brain injury group, but this was not statistically significant. Of importance to the theme of this thesis, the body of the corpus callosum demonstrated significantly lower fractional anisotropy and higher radial diffusivity, compared to the control group. The lower fractional anisotropy appeared to be driven by the radial diffusivity of the callosal motor fibres, rather than the axial diffusivity. There were no white matter tracts that showed significant higher fractional anisotropy, lower axial or radial diffusivities in the patient group, compared to the control group.

7.5.1 Patient group

The patient group was recruited from the traumatic brain injury database covering a number of centres in London. The same patients had participated in the physiological and behavioural

studies presented earlier in this thesis. Because of the importance in investigating the same group of patients, the selection of the patients for this microstructural analysis was dependent on transcranial magnetic stimulation safety criteria (as discussed in Chapter 4) (Wassermann, 1998, Rossi et al., 2009). Therefore, out of a possible 115 traumatic brain injury patients on the database, only 20 patients were eligible to be included in our study. Only 17 agreed to participate. The main reasons for exclusion from the study were either due to the use of neuromodulatory medication or history of intracerebral haemorrhage. As previously mentioned, compared to larger MRI studies, the traumatic brain injury cohort investigated in this study is relatively small. This emphasises the fact that care must be exercised in extrapolating conclusions from this study to the larger traumatic brain injury population.

The recruitment of traumatic brain injury participants to this study therefore included all patients with mild, moderate and severe traumatic brain injury who were eligible and who agreed to participate. The difficulty in obtaining a study cohort who are homogenous in terms of their injury is well recognised in traumatic brain injury research (Hulkower et al., 2013). However, in this current study, all of the patients had sustained an impact injury to the brain on one occasion (from a road traffic accident, fall, sports injury or assault) causing a loss of consciousness, rather than a history of repeated injuries over many years, such as the injuries experienced by boxers and footballers. Therefore this simplifies any inferences from this study, and it does not have to take into account the effect of a single head injury versus the effects of multiple head injuries (such as that occur in sports). However, as previously mentioned, it is possible that the participants who had sustained their head injury during an assault, may have suffered multiple impacts to the head, even though the injury occurred on one occasion. The patient group had suffered a variety of injuries. It is recognised that biomechanical forces and subsequent brain deformation following impact will vary, depending on the type of injury. Computational modelling of accelerations in the model of the road traffic accident, fall and American football have demonstrated distinct patterns of injury and strain in the three groups (Ghajari et al., 2017). This has indicated that the nature of the injury is likely to influence the pattern of subsequent brain injury. Therefore, the fact that the patient group in this current

study were heterogeneous to traumatic brain injury-type, severity and neuroimaging results would inevitably reduce the statistical power of any comparisons made with the healthy controls, and is a further limitation of this study. However, as previously mentioned, this is likely to be a limitation of traumatic brain injury research in general.

In the UK, for all traumatic brain injury severities (mild, moderate to severe), there is a peak in incidence in those between 80 and 90 years of age. However, for those with severe traumatic brain injury, there is a smaller peak between the ages of 20 and 30 years (Lawrence et al., 2016). There was a wide age range in our patient group, between 22 years and 60 years, which was reflective of traumatic brain injury affecting a wide age range. Our average age of the patient group was 43 years. A further limitation for the analysis was that the traumatic brain injury group and controls were not precisely matched for age. Age-related changes to brain structure are well described, and abnormal microstructural values of the corpus callosum (lower fractional anisotropy, higher radial diffusivity) have also been observed in healthy older adults (Schulte et al., 2005, Fling et al., 2011a). However, when the statistics were re-run with age as a covariate, there was no interaction with age (data not shown).

Diffusion tensor imaging is exquisitely sensitive to damage following traumatic brain injury. Lower fractional anisotropy and axial diffusivity have been observed within a few hours of injury in an experimental model of traumatic brain injury (Mac Donald et al., 2007a, b). It is accepted that the low fractional anisotropy persists over time, however, axial diffusivity is more dynamic following injury (Sidaros et al., 2008). All patients investigated in this study had sustained their injury at least one year prior to testing. The consequences of possible fluctuations in microstructure in the acute phase should therefore have been minimised. However, although all patients were studied over a year following injury, the variation of time following injury (mean 44.3 months ± SE 6.29) and the time between the testing and pre-acquired imaging (mean 16.2 months ± SE 3.36) were both large. This may impact on the trend of microstructural values obtained for each individual.

There are, however, no studies of traumatic brain injury where physiology, behaviour and microstructure have been undertaken on the same group of traumatic brain injury patients. Therefore, despite the limitations of this study, this present data represents a unique dataset.

7.5.2 White matter disruption associated with traumatic brain injury

For the purpose of this study, analysis initially focussed on fractional anisotropy metrics. As previously mentioned, this appears to be the most sensitive diffusion tensor imaging measure for detecting microarchitectural changes in tissue (Song et al., 2002, Song et al., 2005, Tyszka et al., 2006, Alexander et al., 2007). In this study, there was evidence of lower fractional anisotropy of the callosal fibres (body, genu), corticospinal tracts and intrahemispheric fibres (uncinate fasciculi, inferior and superior longitudinal fasciculi, inferior fronto-orbital fasciculi and cingulum) in the traumatic brain injury group, compared to the controls. This is in support of existing literature. Kinnunen and colleagues who used a similar method in TBSS analysis, demonstrated that in traumatic brain injury the majority of white matter showed evidence of disruption, in inter-hemispheric fibres, intra-hemispheric association fibres, corticopontine, corticospinal tracts, the fornices, thalamic projections, forceps, internal capsule and corona radiata (Kinnunen et al., 2011). This was most evident for fractional anisotropy and mean diffusivity, with less extensive differences in axial diffusivity and radial diffusivity. The study by Hellyer and colleagues demonstrated that measures of fractional anisotropy were lower in patients, and radial diffusivity was higher in patients, compared to controls (Hellyer et al., 2013). However, the fractional anisotropy metric in the study by Hellyer and colleagues was found to be the most accurate in classifying areas of diffuse axonal injury, compared to the other diffusion tensor imaging metrics. Fractional anisotropy was therefore used to identify patients with a high likelihood of diffuse axonal injury when conventional imaging was normal. However, the pattern of diffusion tensor imaging metrics was also acknowledged to be complex after traumatic brain injury (Hellyer et al., 2013). In addition, a meta-analysis of 100 studies has also demonstrated the areas of fractional anisotropy abnormality in traumatic brain injury are more widespread than in the other diffusion tensor imaging metrics (Hullkower et al., 2013). The data from this present study therefore corroborates what is known from the existing

literature. Although the group of patients were only tested in the chronic phase following injury, this current study indicates that disruption of white matter integrity following a single traumatic brain injury persists following the injury.

7.5.3 The microstructure of the body of the corpus callosum is vulnerable following traumatic brain injury and this persists years after the injury

The microstructure of the corpus callosum is particularly important to the theme of this thesis. This current study demonstrated that the fractional anisotropy was significantly lower in the traumatic brain injury patient group, compared to the control group.

Microstructural abnormalities in the corpus callosum following traumatic brain injury are well recognised (Bendlin et al., 2008, Wang et al., 2008, Kinnunen et al., 2011, Caeyenberghs et al., 2011a, Farbota et al., 2012, Hellyer et al., 2013, Gajawelli et al., 2013), with a variety of diffusion tensor imaging methodologies and analysis in the available literature. They are thought to relate to the vulnerability of the corpus callosum to the shearing of nerve fibres due to sudden acceleration, deceleration, and rotational forces, as the primary connecting fibre structure between the two hemispheres (Gajawelli et al., 2013). In the evaluation of corpus callosum metrics in the chronic phase following traumatic brain injury, the fractional anisotropy has been found to be lower in the patient group compared to controls (Bendlin et al., 2008, Kinnunen et al., 2011, Caeyenberghs et al., 2011a, Farbota et al., 2012). The meta-analysis of one hundred papers by Hulkower and colleagues revealed the corpus callosum to be the most common location of abnormal fractional anisotropy by region of interest analysis (genu, splenium of the corpus callosum, posterior limb of internal capsule and corpus callosum body), by tractography and whole brain analysis (Hulkower et al., 2013). However, the meta-analysis revealed that the genu and splenium of the corpus callosum tended to be more investigated, than the body of the corpus callosum, which was assessed in the current study (Arfanakis et al., 2002, Huisman et al., 2004, Inglese et al., 2005, Hulkower et al., 2013). Higher fractional anisotropy values have been demonstrated in the posterior corpus callosum 72 hours following

concussion, and correlate with measures of post-concussive symptoms (Bazarian et al., 2007). Although additional diffusion tensor imaging metrics were not evaluated in more detail by Bazarian and colleagues, it was concluded that these findings were due to axonal injury and axonal swelling. In any case, evidence to date supports the understanding that diffusion tensor imaging metrics are dynamic in the early stages of injury (Bazarian et al., 2007, Wilde et al., 2008, Henry et al., 2011). However, variations in the biomechanical forces of injury and type of injury will inevitably also contribute to differences between existing studies.

In this study, the callosal motor fibres of the hand area demonstrated lower fractional anisotropy in the patient group, compared to the control group. On reflection, it would have been far superior to obtain the fMRI metrics for the callosal motor fibres for each participant in the current study. However, the mask that was used for the callosal motor fibres in this current study had been described by Wahl and colleagues using a combined fMRI and diffusion tensor imaging approach (Wahl et al., 2007). The study supports the hypothesis that compromise to the microstructure of the callosal motor fibres occurs following traumatic brain injury. This may contribute to the functional difficulties that are observed following injury.

Although the findings of lower fractional anisotropy has already been described in existing literature, it is inferred from animal studies that this is usually due to diffuse axonal injury (Van de Looij et al., 2012, Fan et al., 2013). However, reduced fractional anisotropy values and elevated mean diffusivity values are also reported in haemorrhage due to intraparenchymal haemorrhage, haemorrhagic infarcts and haemorrhagic brain tumours (Haris et al., 2006, Kusano et al., 2009). Unfortunately, there is no comment on the other imaging metrics in these studies. The longitudinal study by Farbota and colleagues demonstrated that lower fractional anisotropy in several tracts was persistent four years following injury (Farbota et al., 2012). However, improvement in fractional anisotropy has been observed with time in an animal model of traumatic brain injury (Rubovitch et al., 2011). However, this would require a longitudinal study to be evaluated further.

7.5.4 The radial diffusivity in the corpus callosum was higher in the traumatic brain injury group

This study demonstrated that the radial diffusivity was significantly higher in the traumatic brain injury patient group, compared to the control group. Although axial diffusivity was higher in the traumatic brain injury group, it did not reach statistical significance.

Changes in radial diffusivity in the absence of changes in axial diffusivity are attributed to changes in myelin structure (Beaulieu, 2002, Song et al., 2002). The study by Farbota and colleagues demonstrated that longitudinal changes to the microstructure of the genu of the corpus callosum, as evidenced by lower fractional anisotropy, was driven by changes in radial, and not axial diffusivity, and this is consistent with other studies (Sidaros et al., 2008, Kumar et al., 2010, Farbota et al., 2012). The study by Caeyenberghs and colleagues in chronic traumatic brain injury patients demonstrated higher radial diffusivity and axial diffusivity (Caeyenberghs et al., 2011a). The findings in this current study may reflect the duration between injury and testing; Mac Donald and colleagues demonstrated lower fractional anisotropy and higher axial diffusivity within the first few hours of cortical contusion in an experimental model. The axial diffusivity normalised at four weeks, in the presence of a persistently lower fractional diffusivity, and radial diffusivity consequently increased (Mac Donald et al., 2007a,b). The results demonstrated in the current study could therefore reflect the time following injury.

7.5.5 The lower fractional anisotropy in the callosal motor fibres of the corpus callosum appears to be driven by higher radial diffusivity

The current study demonstrated strong correlations between fractional anisotropy and radial diffusivity, but not fractional anisotropy and axial diffusivity in the callosal motor fibres in both the traumatic brain injury group and healthy controls. The posterior mid-body of the corpus callosum (i.e. where the callosal motor fibres are situated) consists of larger diameter, myelinated and less densely packed fibres (Aboitiz, 1992, Hofer et al., 2006, Wahl et al., 2007,

Hubers et al., 2012). Therefore, this would explain the relationship in the healthy controls. However, in the context of the association with the demonstrated lower fractional anisotropy in the traumatic brain injury group, the strong relationship between fractional anisotropy and radial diffusivity in the callosal motor fibres is interesting when attempting to determine the factors involved in diffuse axonal injury.

Higher radial diffusivity in the corpus callosum following traumatic brain injury is recognised in the literature and has been attributed to thinning of myelin or demyelination (Kraus et al., 2007, Ewing-Cobbs et al., 2008, Farbota et al., 2012, Caeyenberghs et al., 2011a). Studies have demonstrated increased radial diffusivity values with or without influence on axial diffusivity in the human and Cuprizone (bis-cyclo-hexanone oxaldihydrazone) mouse model of demyelination in the corpus callosum (Song et al., 2002, Song et al., 2005, Tyszka et al., 2006, Sun et al., 2006, Alexander et al., 2007). This suggests radial diffusivity is influenced by myelin in white matter. The presence of a stronger relationship between radial diffusivity and fractional anisotropy in the callosal motor fibres found in the current study may purely reflect the larger diameter myelinated fibres (conversely the fibres in the genu and splenium of the corpus callosum are thinner and largely unmyelinated) (Aboitiz, 1992). Taking this into consideration, the observed differences in fractional anisotropy in the patient group compared to the control group are more likely to be driven by changes to myelination. Therefore, it is possible that the higher radial diffusivity and lower fractional anisotropy in the corpus callosum of the traumatic brain injury group is due to abnormalities in myelination following injury.

These results suggest that the pathology underlying diffuse axonal injury is dependent on the balance between demyelination and axonal loss. However, relating the microstructural findings from this current study to the neuropathological findings in diffuse axonal injury is more challenging. Reflecting on histological analysis, diffuse axonal injury is demonstrated histologically by axonal separation, where the axon is torn at the site of stretch and the part distal to the tear degrades. Therefore, the histological diagnosis is based on the presence of axonal bulbs seen in sections stained with haematoxylin and eosin, silver impregnation or

immunocytological techniques for neurofilaments, although it is accepted that axonal bulbs are not specific to diffuse axonal injury and can occur in any condition where the axon is damaged (Adams et al., 1989). Diffusion tensor imaging studies posit that axonal degeneration results in reduced axial diffusivity, whereas demyelination increases radial diffusivity with/without changing axial diffusivity. In this study, the fractional anisotropy level was lower in the body of the corpus callosum and corticospinal tracts, thus indicating a difference in microstructure of these tracts in the traumatic brain injury group versus the control group. However, the radial diffusivity values were significantly higher in the corpus callosum, right corticospinal tract but not left corticospinal tract in the traumatic brain injury group, when compared to the control group. The cuprizone mouse model of demyelination suggests that changes in axial diffusivity are dynamic, transient, and potentially resolve by the time the radial diffusivity begins to increase (Sun et al., 2006). This is supported in additional histological analysis studies, where the primary injury consists of areas of axotomy, and additional areas of stretch injury without complete axotomy, seen within 25 minutes of injury and peaking within two days after injury (Povlishock et al., 1995). Amyloid precursor protein can be identified in axons within two hours (Ikonomovic et al., 2004). Myelin sheath integrity, however, changes during the evolution of diffuse axonal injury. Unmyelinated or thinly myelinated axons in the corpus callosum appear to be more vulnerable to stretch injury (Reeves et al., 2005). Demyelination is not seen in the hyperacute phase, but is evident one week following injury and progresses for up to one year (Ludwin et al., 1990, Bramlett et al., 2003). Therefore, this suggests that the process of demyelination may be a secondary consequence of the biochemical release following the initial injury. All the patients in this study were tested at least one year following injury, with a wide range of duration following injury. Therefore, it is not surprising that the axial diffusivity was not lower in this patient group. The presence of higher radial diffusivity in the patient group, and a stronger relationship between radial diffusivity and fractional anisotropy in the callosal motor fibres suggests that the pattern of diffuse axonal injury in the chronic phase following injury is more in keeping with demyelination. However, elucidating the nature of diffuse axonal injury following traumatic brain injury is likely to be more complex than simple axonal degeneration or abnormalities of myelination.

7.5.6 Limitations

Limitations pertaining to the traumatic brain injury group have been discussed earlier in this section. The size of the sample (n = 17) is a limitation of this study. However, the patient group was selected on the basis of strict transcranial magnetic stimulation safety criteria. A further limitation of the study is the b- value of 1000s/mm^2. This is a common value in clinical settings. However, this b–value may result in extracellular water diffusion dominating the signal, and a higher b–value may provide more accurate information of metrics (Le Bihan et al., 2001). However, the number of gradient directions used in this study was 64, where gradients were applied in 16 directions in four runs. Many existing studies use fewer gradients; the study by Wahl and colleagues used 12 directions, and the study by Fling and colleagues used 6 (Wahl et al., 2007, Fling et al., 2011a). An increasing number of gradient directions improves the reliability of white matter measurements (Gianelli et al., 2009). Therefore, this was a strength in this study. The white matter skeleton produced by the TBSS constrained the analysis of white matter to the central parts of the white matter tracts. This resulted in only the core of the respective tract being sampled, whilst excluding peripheral areas of the fibre tract that are known to show pronounced interindividual variability (Jilka et al., 2014). This has been acknowledged to produce an incomplete assessment of white matter (Bonnelle et al., 2011). However, it has already been acknowledged to have the advantage of minimising partial volume errors. Constraining the sampling of white matter damage has already been shown to more accurately estimate white matter integrity after traumatic brain injury (Squarcina et al., 2012, Hellyer et al., 2013).

7.6 Conclusions

This study found significant differences in the microstructure of the corpus callosal fibres (body, genu), corticospinal tracts and intrahemispheric fibres (uncinate fasciculi, inferior and superior longitudinal fasciculi, inferior fronto-orbital fasciculi, cingulum). The results of the diffusion tensor imaging metrics are in line with published literature. With particular relevance to the theme through this thesis, there is lower fractional anisotropy in the left and right corticospinal tract and body of corpus callosum of the traumatic brain injury group, compared to control. Longitudinal follow-up of the diffusion tensor imaging metrics would allow further information to be obtained, to determine whether there is progression of these abnormalities found in this study, or resolution. However, this was outside the scope of this study. What is interesting is that these changes are present in this current patient group, despite a wide variation of time since their single injury (12 months to 5 years) and spectrum of traumatic brain injury severity. In addition, there are differences in fractional anisotropy in the corticospinal tract measures, despite no clinical motor deficit at the time of testing.

The next step would be to evaluate whether there is a relationship between physiology, behaviour and structure. That is the focus of the next chapter.

Chapter 8

Determining a relationship between physiology, behaviour and microstructure

I was responsible for the concept, analysis and interpretation of this study. The images were pre-acquired from the Imperial College traumatic brain injury database. Dr S Jilka and Dr L Li obtained the brain images and used standard pre-programmed scripts to obtain the raw DTI metrics which were used in the correlation analysis.

8.1 Introduction

Diffuse axonal injury, also known as traumatic axonal injury, is a consequence of traumatic brain injury (Strich, 1961, Adams et al., 1977, Adams, 1982, Adams et al., 1989). It results from injury to the brain which causes shearing of nerve fibres due to sudden acceleration, deceleration, often combined with rotational forces (Strich, 1961, Adams, 1982, Arfanakis et al., 2002, Huisman et al., 2004, Meythaler et al., 2001). The patient studies presented in this thesis have involved physiological measures and behavioural measures that share one common substrate, the corpus callosum. The corpus callosum is particularly relevant to bimanual coordination, where deficits are observed in patients with lesions of the corpus callosum or partial callosotomies (Eliassen et al., 2000, Kennerley et al., 2002). The corpus callosum is the largest fibre bundle in the brain and has been demonstrated to be particularly vulnerable to the shearing of nerve fibres due to sudden acceleration, deceleration, and rotational forces involved in traumatic brain injury, as the primary connecting fibre structure between the two hemispheres (Strich, 1961, Adams, 1982, Huisman et al., 2004, Gajawelli et al., 2013). The ensuing damage is progressive, with longitudinal studies demonstrating diffuse atrophy of the corpus callosum in children who have suffered traumatic brain injury (Levin et al., 2000). The presence of diffuse axonal injury of the corpus callosum has been associated with a worse clinical prognosis after traumatic brain injury (Gentry et al., 1988a). Thus, it is reasonable to explore whether this primary site of anatomical damage is the main contributor to the behavioural and physiological abnormalities demonstrated through the experiments presented in this thesis.

The interest behind determining relationships between physiology and structure across the corpus callosum has been explored in the past (Wahl et al., 2007, 2008). Wahl and colleagues explored this in healthy individuals. Their main finding was that interhemispheric inhibition is significantly correlated with the microstructure of callosal fibres connecting the homologous M1 region of the motor cortex (Wahl et al., 2007). Using an elegant combined functional magnetic resonance imaging fibre tracking procedure, the callosal motor fibres were initially

identified in the posterior body and isthmus of the corpus callosum in twelve healthy participants. This region was assessed by diffusion tensor imaging, and fractional anisotropy metrics for this region were obtained for each individual. The amount of interhemispheric inhibition was then measured using the established paired pulse transcranial magnetic stimulus protocol by Ferbert and colleagues (Ferbert et al., 1992). The amount of inhibition was reported to correlate significantly with the microstructure of the callosal motor fibres in this group of healthy participants (Wahl et al., 2007). Therefore, a similar approach has the potential to provide information regarding any relationship between physiology and microstructure in this current group of traumatic brain injury patients and controls.

The interest behind determining a relationship between behaviour and structure across the corpus callosum has also been explored (Fling et al., 2011a, Gooijers et al., 2014). The work by Fling and colleagues compared older to younger adults, and demonstrated that older adults were disproportionately impaired at bimanual tasks compared to younger adults. This particularly manifest in the task that involved the greatest interhemispheric interaction. The work also demonstrated that better performance on the bimanual task was related to better corpus callosum microstructural integrity (Fling et al., 2011a). As mentioned in chapters 5 and 6, the tapping conditions described in Fling's work demonstrated a range of interhemispheric interactions (Fling et al., 2011a). Work by Johansen-Berg and colleagues has also demonstrated a significant relationship between callosal microstructure and motor performance in unimanual and bimanual tasks. However, their work also demonstrated a strong relationship between callosal microstructure (connecting the M1 region of the motor cortex) and the bimanual simultaneous tapping task; the state which is understood to require the lowest level of interhemispheric inhibition.

In light of these findings, the next stage would be to complete analysis by identifying whether there are any significant relationships between the physiological and behavioural measures of callosal transfer with structure of the callosal motor fibres, in both the traumatic brain injury group, and control.

8.2 Methods

The physiological, behavioural and structural measures for the same control and traumatic brain injury patients who had participated in the traumatic brain injury studies in Chapter 4 and Chapter 6 were accessed. As previously mentioned, this consisted of seventeen traumatic brain injury participants consisting of 10 males and 7 females, and 17 controls consisting of 10 males and 7 females. All traumatic brain injury patients had sustained an impact injury to the brain on one occasion (from a road traffic accident, fall, sports injury or assault) causing a loss of consciousness. All patients had been treated conservatively after their traumatic brain injury and had not required any neurosurgical intervention. All healthy subjects had no history of significant medical or psychiatric illness. They had no known physical disability and were on no regular medication. These participants (patients and controls) had participated in the traumatic brain injury research as part of an MRI study conducted by Imperial College.

The study presented in this chapter particularly focusses on the physiological and behavioural measures obtained in the previous experiments presented in this thesis, pertaining to callosal transfer, as also used by the studies by Wahl and colleagues, and Fling and colleagues (Wahl et al., 2007, Fling et al., 2011a). The microstructural measure for all correlation analysis in this current study, is the region pertaining to the callosal motor fibres; this measure had been obtained in the previous study, using the coordinates obtained from the tractography work by Wahl and colleagues (Wahl et al., 2007). In addition, this region was also investigated in the work by Fling and colleagues, as part of their correlation analysis between behaviour and microstructure (Fling et al., 2011a).

With regards to determining a relationship between structure and physiology, I proposed that I would be able to reproduce the relationship between fractional anisotropy of the callosal motor fibres and amount of interhemispheric inhibition in the control group, as demonstrated by Wahl and colleagues. This demonstrated that the higher the fractional anisotropy value (i.e

the better the microstructure of the callosal motor fibres), the greater the amount of interhemispheric inhibition (Wahl et al., 2007). In the context of the reduced interhemispheric inhibition and lower fractional anisotropy in the traumatic brain injury patient group demonstrated in the earlier studies presented in this thesis, there were two possibilities; either there would be a stronger positive relationship between the two measures, proposing that the existing callosal fibres following injury were compensating physiologically (as demonstrated by the lack of group difference with transcallosal recruitment in Chapter 4). The alternative was that any relationship between the microstructure and physiology would not be evident in the traumatic brain injury group, due to the previously observed findings of compromised microstructure, and reduced interhemispheric inhibition in the same patient group in the previous study (Chapter 4) presented in this thesis.

With regards to determining a relationship between structure and behaviour, I proposed that the better the microstructure, the better the performance in the behavioural task. This would be evident by a higher fractional anisotropy value of the corpus callosal fibres being associated with a lower variability of the behavioural measure. This has already been demonstrated in the older adults in the study by Fling and colleagues (Fling et al., 2011a).

8.2.1 Data and Statistical analysis

Normality of all the data sets used for this analysis had been previously tested with Kolmogorov – Smirnov tests. Correlation analysis was therefore undertaken and calculated by Pearson linear correlation. For the analysis between interhemispheric inhibition and diffusion tensor imaging metrics, the method used by Wahl and colleagues was implemented (Wahl et al., 2007). Interhemispheric inhibition was expressed for each intensity of the conditioning pulse by (1 – mean conditioned MEP/mean unconditioned MEP) x 100%. Therefore interhemispheric inhibition = 0% indicates no inhibition, and interhemispheric inhibition = 100% indicates complete inhibition. The peak of inhibition was used as the constant interstimulus interval for each participant. To relate the microstructure to physiology, individual mean fractional anisotropy values of the hand callosal motor fibres were correlated to interhemispheric

inhibition in both the patient and control groups. This was then repeated for radial diffusivity for both groups.

For the analysis of behavioural measures and diffusion tensor imaging metrics, the method used by Fling and colleagues was implemented (Fling et al., 2011a). Firstly, a paired t test was undertaken in the control group to determine whether the variability (standard deviation of the intertap interval of a single hand) of the unimanual state was significantly different in the non dominant and dominant hand. As there was no significant difference, this was combined. A further paired t test was undertaken for the variability (standard deviation of the between hand lag) of the between hand lag variability, for both asynchronous conditions (non dominant to dominant hand, dominant hand to non dominant hand). As there was no statistically significant difference between the two measures, the values were combined. Correlation analysis was then undertaken for unimanual variability versus fractional anisotropy of the corpus callosum, and between hand lag variability (bimanual simultaneous condition) versus fractional anisotropy of the corpus callosum and between hand lag variability (bimanual asynchronous condition) versus fractional anisotropy of the corpus callosum. This was then repeated for the patient group. Furthermore, the same measures were correlated with the respective radial diffusivity metrics of the corpus callosum.

For all significant relationships, it was intended to use a Fisher r – to – z transformation to identify differences in the strengths of correlations between groups. However, no significant relationships were identified. For the report of the statistical differences, the significant threshold of $\alpha = 0.05$ was used. Statistical procedures were conducted using the statistical package SPSS version 19.0 for Windows; SPSS Inc. All data are given as mean ± standard error of the mean unless otherwise stated.

8.3 Results

8.3.1 Correlation analysis between physiology and structure

As there was no significant difference between the two groups in interhemispheric inhibition recruitment, an average was obtained of the amount of interhemispheric inhibition produced across the intensities (90% RMT, 100% RMT, 110% RMT, 120% RMT), for each group. For all graphs, the y axis corresponds to percentage of inhibition produced. Therefore interhemispheric inhibition = 0% indicates no inhibition, interhemispheric inhibition = 100% indicates complete inhibition.

Firstly, the relationship between fractional anisotropy and amount of inhibition was explored between the control group and traumatic brain injury group. This demonstrated a tendency for higher fractional anisotropy values to be associated with more inhibition in the patient group. However, this was not evident in the healthy control group. There was no significant correlation between the fractional anisotropy and interhemispheric inhibition in the control group ($r = 0.01$, $p = 0.98$) or traumatic brain injury group ($r = 0.41$, $p = 0.15$).

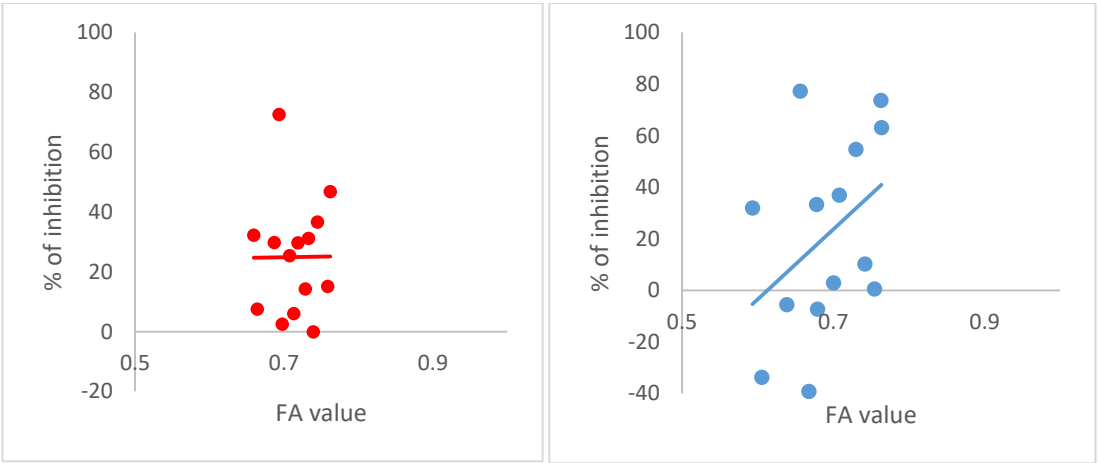


Figure 8.1 The relationship between FA versus average interhemispheric inhibition RMT. Left panel: Control group (red). Right panel: TBI group (blue)

Then the relationship between radial diffusivity and amount of inhibition was explored. This demonstrated a tendency for higher radial diffusivity values to be associated with less inhibition in the patient group, although this was less evident in the healthy controls. However, there was no significant correlation between the radial diffusivity and interhemispheric inhibition in the control group (r = - 0.03, p = 0.92) or traumatic brain injury group (r = -0.22, p = 0.45).

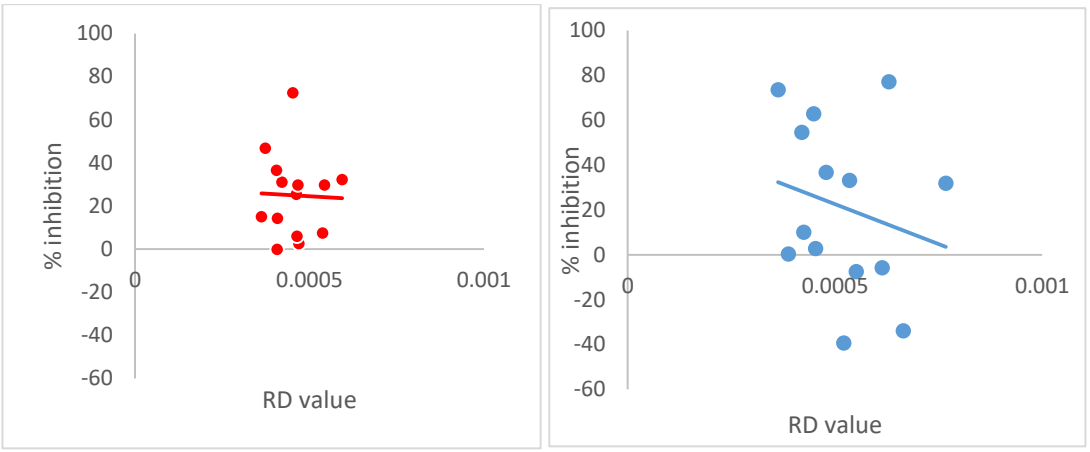


Figure 8.2 The relationship between RD versus average interhemispheric inhibition RMT. Left panel: Control group (red). Right panel: TBI group (blue)

However, because the analysis by Wahl and colleagues demonstrated differences in the strengths of the correlation at different intensities, the correlations were then separated. There was no significant correlation between the fractional anisotropy and amount of interhemispheric inhibition. There were no significant correlations at any intensity, although there was a tendency in patients for higher FA values to be associated with more inhibition, particularly at the higher intensities.

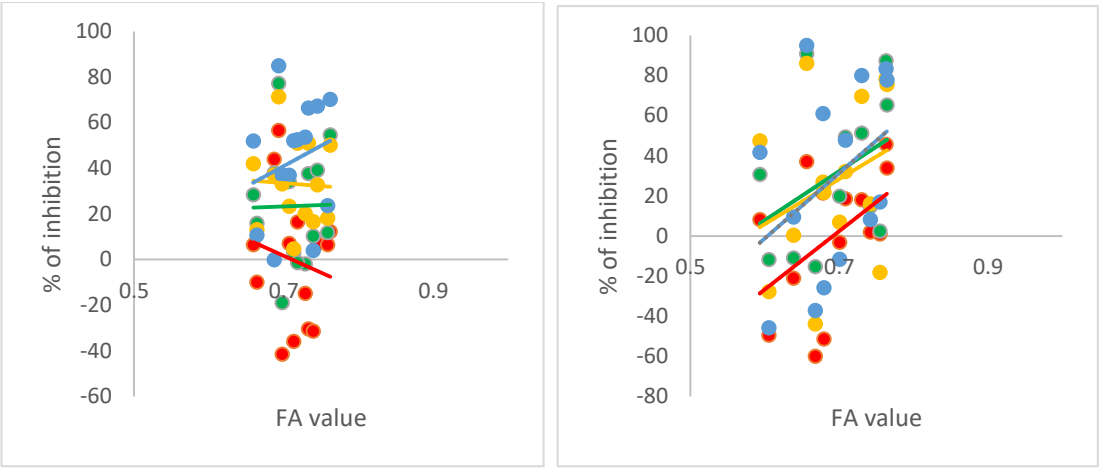


Figure 8.3 The relationship between FA versus Interhemispheric inhibition at each % RMT. Red IHI 90, green IHI 100, yellow IHI 110, blue IHI 120. Left panel: Control group. Right panel: TBI group

The values of the correlation coefficients and their respective p value are provided in Table 8.1.

% RMT	Control	TBI
IHI 90	r = -0.16 p = 0.58	r = 0.48 p = 0.08
IHI 100	r = 0.02 p = 0.96	r = 0.39 p = 0.16
IHI 110	r = -0.05 p = 0.88	r = 0.31 p = 0.29
IHI 120	r = 0.22 p = 0.45	r = 0.38 p = 0.18

Table 8.1 The relationship between FA versus Interhemispheric inhibition

This was then repeated for the radial diffusivity. There was no significant correlation between the radial diffusivity and amount of interhemispheric inhibition. There were no significant correlations at any intensity, although there was a tendency in patients for higher RD values to be associated with less inhibition, at all intensities.

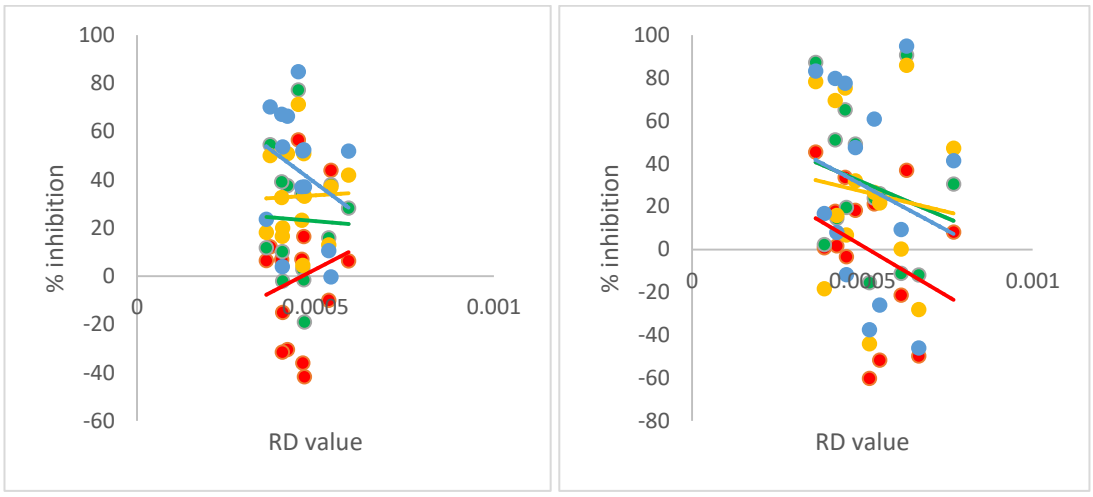


Figure 8.4 The relationship between RD versus Interhemispheric inhibition at each % RMT. Red IHI 90, green IHI 100, yellow IHI 110, blue IHI 120. Left panel: Control group. Right panel: TBI group

The values of the correlation coefficients and their respective p value are provided in Table 8.2.

% RMT	Control	TBI
IHI 90	r = 0.18 p = 0.55	r = -0.32 p = 0.26
IHI 100	r = -0.03 p = 0.91	r = -0.23 p = 0.44
IHI 110	r = 0.03 p = 0.91	r = -0.11 p = 0.71
IHI 120	r = -0.28 p = 0.33	r = -0.21 p = 0.47

Table 8.2 The relationship between RD versus Interhemispheric inhibition

8.3.2 Correlation analysis between behaviour and structure

There was no significant correlation between the fractional anisotropy and unimanual variability in the control group (r = 0.21, p = 0.41) or traumatic brain injury group (r = - 0.27, p = 0.29) (Figure 8.5). For all graphs, the lower the standard deviation (variability), the better the performance.

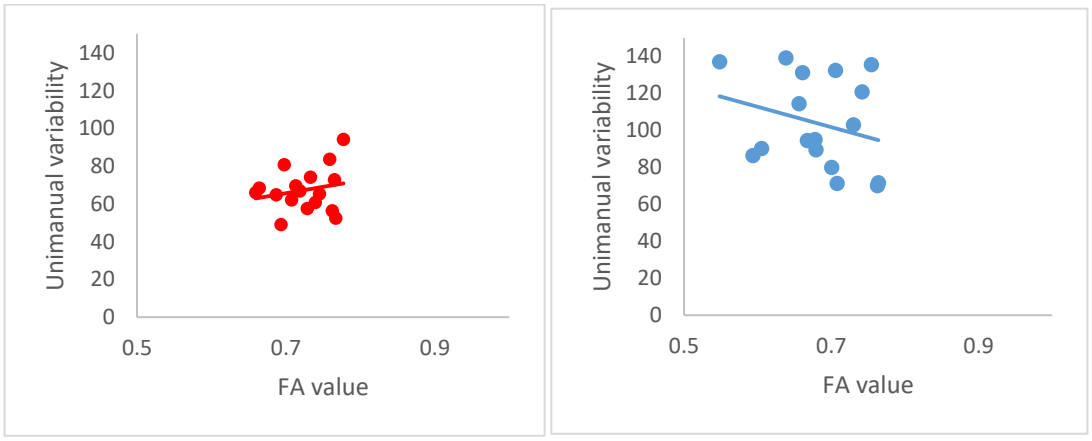


Figure 8.5 The relationship between FA versus unimanual variability. Left panel: Control group (red). Right panel: TBI group (blue)

There was no significant correlation between the fractional anisotropy and the between hand lag variability in the bimanual simultaneous condition in the control group (r = 0.02, p = 0.94) or traumatic brain injury group (r = - 0.33, p = 0.20) (Figure 8.6).

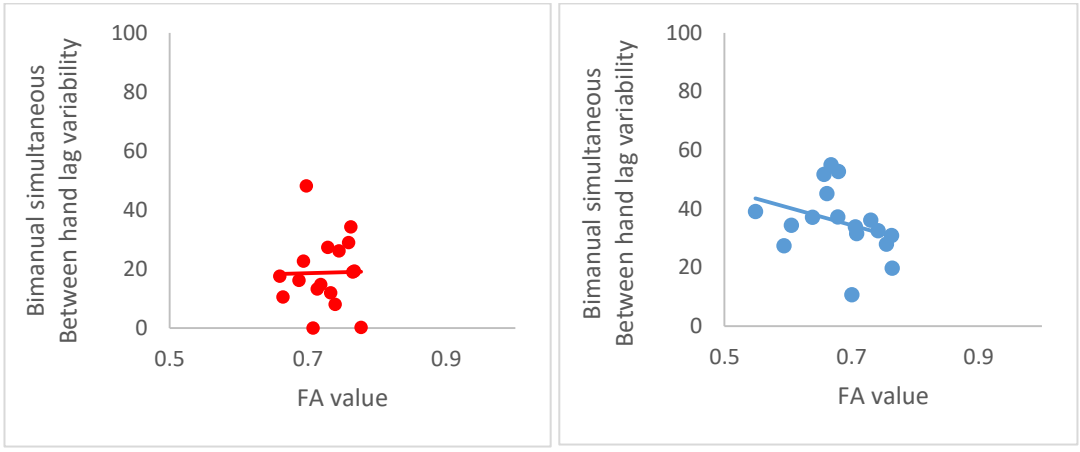


Figure 8.6 The relationship between FA versus between hand lag variability in the bimanual simultaneous condition. Left panel: Control group (red). Right panel: TBI group (blue)

There was no significant correlation between the fractional anisotropy and between hand lag variability in the bimanual asynchronous condition in the control group (r = -0.26, p = 0.31) or traumatic brain injury group (r = 0.17, p = 0.51) (Figure 8.7).

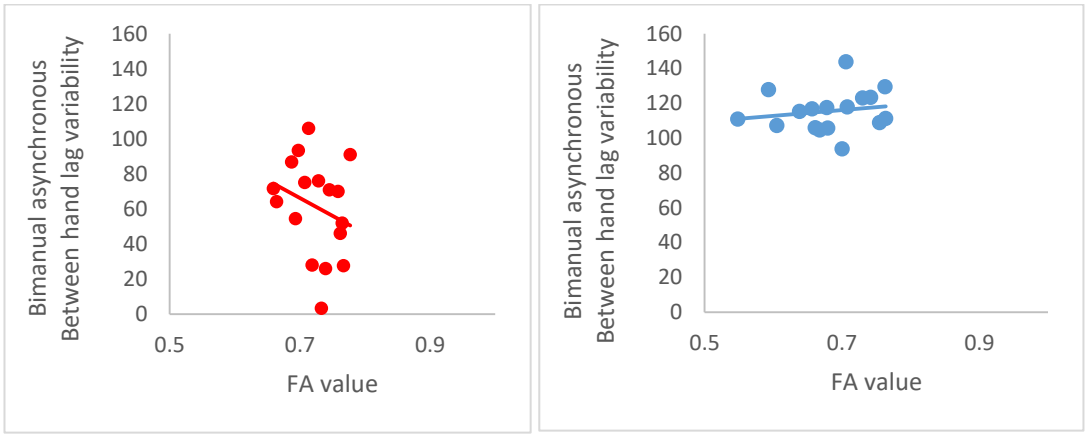


Figure 8.7 The relationship between FA versus between hand lag variability in the bimanual asynchronous condition. Left panel: Control group (red). Right panel: TBI group (blue)

There was no significant correlation between the radial diffusivity and unimanual variability in the control group (r = -0.06, p = 0.81) or traumatic brain injury group (r = 0.14, p = 0.60) (Figure 8.8).

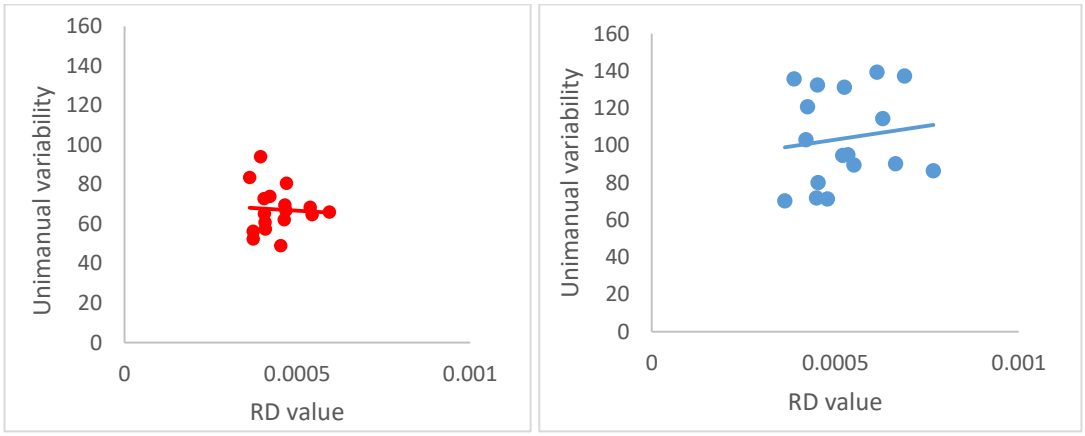


Figure 8.8 The relationship between RD versus unimanual variability. Left panel: Control group (red). Right panel: TBI group (blue)

There was no significant correlation between the radial diffusivity and between hand lag variability in the bimanual simultaneous condition in the control group (r = -0.17, p = 0.53) or traumatic brain injury group (r =0.29, p = 0.27) (Figure 8.9).

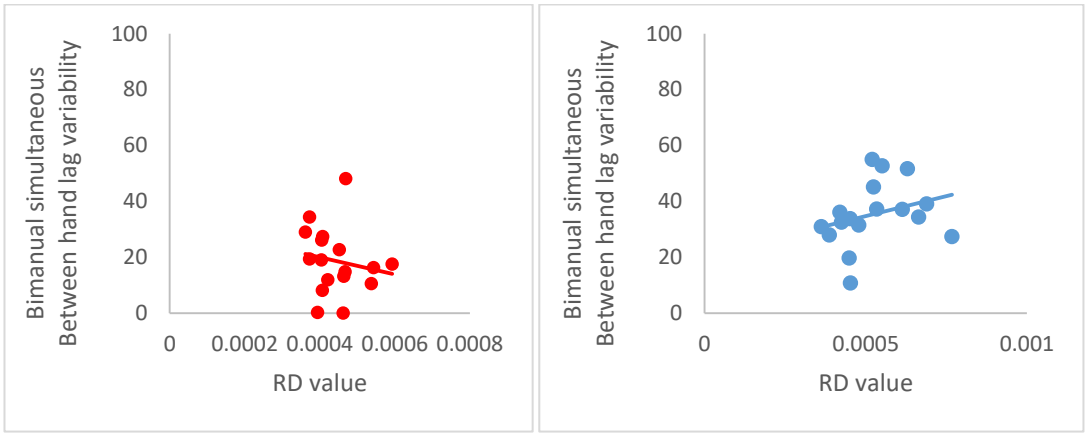


Figure 8.9 The relationship between FA versus in-phase variability. Left panel: Control group (red). Right panel: TBI group (blue)

There was no significant correlation between the radial diffusivity and between hand lag variability in the bimanual asynchronous condition in the control group (r = 0.30, p = 0.24) or traumatic brain injury group (r = - 0.10, p = 0.69) (Figure 8.10).

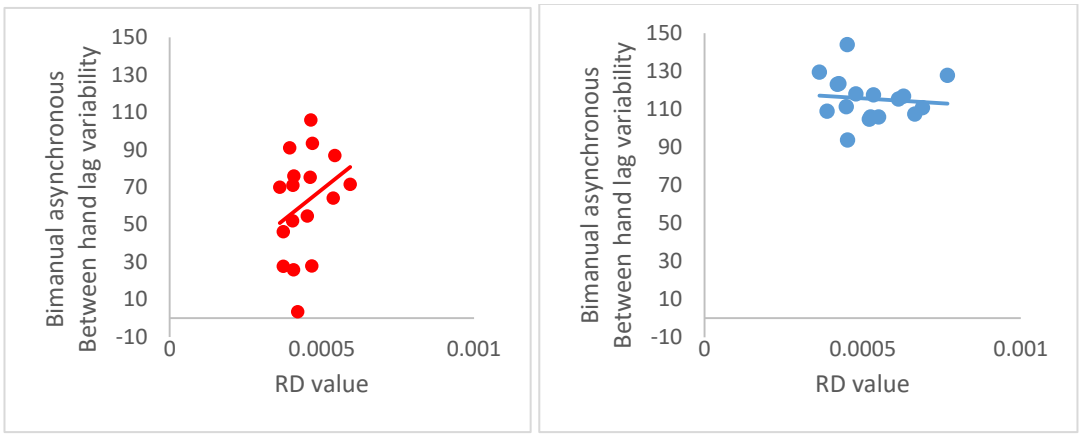


Figure 8.10 The relationship between FA versus out of phase variability. Left panel: Control group (red). Right panel: TBI group (blue)

8.4 Discussion

The main finding from this study was that there was no relationship identified between the physiological measures of callosal transfer and corpus callosum microstructure, or behavioural measures of callosal interaction and corpus callosum microstructure in the traumatic brain injury group, or controls.

8.4.1 Physiology and corpus callosum microstructure

There was no significant relationship identified between the corpus callosal microstructure and interhemispheric inhibition. This meant that the strength of the correlation could not be compared between the patient group and control. The corpus callosum is particularly vulnerable in traumatic brain injury (Gentry et al., 1988a). This is due to the perceived shearing of fibres in the context of the injury. Through the previous study of short and long tracts in traumatic brain injury patients using transcranial magnetic stimulation presented in this thesis, the main difference between the traumatic brain injury group and controls was reduced interhemispheric inhibition, with an interaction effect. This demonstrated that the relationship between the interstimulus interval and subsequent amount of inhibition was different between the two groups. Interhemispheric inhibition is a physiological measure of callosal transfer and is thought to be mediated by excitatory transcallosal fibres that originate from the hand area of the conditioning M1 and project onto the local inhibitory interneurons of the hand M1 in the opposite hemisphere (Ferbert et al., 1992, Chen et al., 2003). The microstructure of the corpus callosum in the current study demonstrated lower fractional anisotropy and higher radial diffusivity in the traumatic brain injury group, compared to the controls. This supported the hypothesis that vulnerability of the corpus callosum can be associated with abnormal physiological transfer across the corpus callosum after traumatic brain injury. The lack of a significant relationship between the microstructure and physiology in both the controls and patient group, was therefore surprising.

In this current study, the microstructure of the callosal motor fibres had been obtained using the co-ordinates that represented this region, by Wahl and colleagues (Wahl et al., 2007). The callosal motor fibres connect the primary motor cortices of the two hemispheres. The study by Wahl and colleagues had tracked the callosal motor fibres using fMRI. They had used interhemispheric inhibition as the physiological measure of connectivity (Ferbert et al., 1992). Their study had demonstrated positive correlation between the fractional anisotropy and interhemispheric inhibition in healthy participants; the higher the fractional anisotropy of the corpus callosum, the stronger the interhemispheric inhibition (Wahl et al, 2007). In the context of these findings, I expected there to be a replication of the positive correlation between fractional anisotropy and interhemispheric inhibition in the healthy controls in this current study. However, there was no significant relationship between corpus callosal microstructure and physiology in either the patients or controls.

However, it was apparent that there were different trends between the two groups, with the relationship having an opposite effect, between patients and controls. For example, at low stimulus intensities (90 RMT), there was a minimal relationship between the fractional anisotropy and the amount of inhibition elicited in the control group. This is not unexpected. However, in the patient group, a relationship between fractional anisotropy and amount of inhibition elicited was evident, even at the low intensities; the higher the fractional anisotropy of the callosal motor fibre microstructure, the more inhibition elicited. This is an important finding (even though not reaching statistical significance) as it suggests the physiological function of the remaining fibres is more dependent on the integrity of the remaining fibres. As expected, the relationship between microstructure and amount of inhibition elicited became more positive as the stimulus intensity increased in the control group. This was also evident in the study by Wahl and colleagues, with the more inhibition elicited at the higher intensities and correlations becoming significant. This implies recruitment of transcallosal fibres. However, the relationship between the microstructure and amount of inhibition appeared relatively maintained in the patient group. Although, not statistically significant, the respective relationships were similar when radial diffusivity was investigated, however, the trends were opposite; the lower the radial diffusivity, the better the inhibition.

The observed differences between the study by Wahl and colleagues and the controls in the current study may also be due to subtle differences in methodologies. The study by Wahl and colleagues involved a slighter larger sample size (n = 25) than our study (n = 17). Although Wahl and colleagues used the interhemispheric inhibition protocol devised by Ferbert and colleagues, the conditioning coil was orientated laterally on the left hemisphere, to induce a lateral to medial current in that M1 region, rather than the coil pointing postero-laterally at a 45^0 angle to induce a posterior to anterior current as used in the current study. The coil orientation used in this current study was chosen based on the findings that the lowest motor threshold is achieved when the induced current flows approximately perpendicular to the line of the central sulcus. However, it is understood that the lateral to medial current at high stimulus intensity evokes direct waves, and the posterior to anterior directed current preferentially elicits early I – waves (Werhahn et al., 1994, Sakai et al., 1997, Day et al., 1989, Di Lazzaro et al., 1998b). The current flow will inevitably have impact on the descending volleys and is an important difference in the two studies. In addition, although the study by Wahl and colleagues tested conditioning intensities from 100% to 150%, the positive correlations were reported at the higher intensities (130%, 140% and 150%). The conditioning intensities used in the current study were 90%, 100%, 110%, 120%. The study by Wahl and colleagues does not report the correlation values for the lower intensities used in their study, but this may be because no relationship was found in their study at 100%, 110%, 120%. It is therefore possible that the combination of the higher intensities and potentially the different evoked waves by the coil orientation, contribute to the lack of relationship between interhemispheric inhibition and microstructure in the current study, compared to the study by Wahl and colleagues.

The current study then explored the corpus callosal microstructure beyond the fractional anisotropy, which was evaluated by Wahl and colleagues. The relationship between radial diffusivity of the corpus callosum and interhemispheric inhibition was then evaluated. This was because radial diffusivity was shown to be higher for traumatic brain injury patients in the corpus callosum, than healthy controls. This has been reflected in previous studies in traumatic brain injury (Kinnunen et al., 2011, Fabota et al., 2012) and could reflect the nature of shearing

injury in traumatic brain injury resulting in residual demyelination in the chronic phase following injury. If this is the case, it is possible that dispersion of conduction velocity in this context would affect interhemispheric inhibition. However, there was also no relationship demonstrated between radial diffusivity and interhemispheric inhibition.

For exploratory purposes, the possibility for a relationship between the axial diffusivity of the corpus callosum and interhemispheric inhibition was also investigated. This also did not demonstrate a relationship at the intensities investigated. This was less surprising; although axial diffusivity was higher in the traumatic brain injury group than the controls, this was not found to be statistically different.

The interstimulus interval was set at 12ms in the study by Wahl and colleagues. This was because previous studies had demonstrated reliable interhemispheric inhibition at that level (Ferbert et al., 1992, Hanajima et al., 2001). The interstimulus interval used in the current study was individualised as the interval that achieved the peak of interhemispheric inhibition for each participant (control and traumatic brain injury patient). This was because most previous studies on interhemispheric inhibition demonstrate interhemispheric inhibition from 6 to 12ms (Ferbert et al., 1992, Netz et al., 1995, Di Lazzaro et al., 1999, Daskalakis et al., 2002). Therefore this is a strength of this current study, and reinforces the relevance of the findings.

There are additional factors that could contribute to the lack of relationship between the microstructure and physiology. Firstly, the microstructure values used in this study were based on the callosal motor fibres mask established in the study by Wahl and colleagues (Wahl et al., 2007), and not individualised to each participant in the current study. It is possible that a more individualised method may have increased yield of a relationship. However, in the context of the differences between the two groups, it remains possible that the described relationship between microstructure and physiology in healthy participants, as demonstrated by Wahl and colleagues, is weak and not reproducible. Although callosal transfer was

assessed with the ipsilateral silent period, a study by Wegrzyn and colleagues also failed to demonstrate a relationship between TMS and structural integrity at the corpus callosum (Wegrzyn et al., 2013). Although, outside of the focus of this study of the corpus callosum, existing studies have attempted to determine the relationship between the corticospinal tract and microstructure. The study by Kloppel and colleagues actually reported a negative correlation between resting motor threshold and fractional anisotropy, and concluded that motor threshold was an indicator of white matter microstructural integrity (Kloppel et al., 2008). However, the relationships reported did not include the structural areas (such as the internal capsule) that would be anatomically associated with the corticospinal tract. In fact, a further study of healthy individuals by Hubers and colleagues also reported significant correlations between the active motor threshold of both hemispheres and fractional anisotropy. However, no correlations were demonstrated for 1mV MEP, input-output curve slope of central motor conduction time. In addition, none of the respective regions had any proximity to the corticospinal tract, raising the possibility that existing reported correlations may not have a plausible biological basis (Hubers et al., 2012).

The lack of relationship demonstrated in this current study between the microstructure and physiology of the corpus callosum in both the control group as well as the patient group, is therefore a relevant one. Despite group differences in microstructure and physiology in the cohort of patients who participated in the experiments presented in this thesis, the fact that relationships were not found, even in the healthy controls, could propose additional factors are involved when trying to determine relationships between microstructure and physiology (such as the variability of TMS). One consistent factor through the studies that have demonstrated relationships between microstructure and physiology, is that they have only been demonstrated at high intensities in healthy adults (Wahl et al., 2007, Chiou et al., 2014). Patient studies using transcranial magnetic stimulation at higher intensities may be possible; however, it is possible patient groups would not tolerate transcranial magnetic stimulation at higher intensities. The findings from this current study also raises the possibility that where relationships have been demonstrated in the existing literature, they are not reproducible, as they are weak when they occur.

8.4.2 Behaviour and corpus callosum microstructure

There was no significant relationship identified between the corpus callosal microstructure and behavioural measures that investigated interhemispheric interaction. As previously mentioned, existing literature has demonstrated that the corpus callosum is anatomically vulnerable in traumatic brain injury (Gentry et al., 1988a). Interhemispheric interactions are required in bimanual movements to prevent interference from the opposite hemisphere (Bangert et al., 2010). Tasks where collaboration and coordination between the hands is required, such as opening a bottle, driving a car or playing a motor instrument, are difficult in patients after traumatic brain injury (Caeyenberghs et al., 2011a). Therefore the lack of relationship between the callosal microstructure and behavioural measure was also surprising.

However, it was apparent that there were subtle differences in the trends in the control group compared to the patients. In the healthy control group, for the unimanual variability and bimanual simultaneous variability, there was no trend between the fractional anisotropy (i.e. callosal motor fibre microstructure) and performance. However, in the out of phase/asynchronous bimanual variability, there was a trend; the greater the integrity, the lower the variability (i.e. the better the performance). This is in contrast to the study by Fling and colleagues, who demonstrated the opposite relationship in their control group. However, in the patient group, there appeared to be more of a relationship between the microstructure and behaviour in the unimanual and bimanual simultaneous task, where the better the microstructure, the better the performance. However, all relationship seems to be lost in the out of phase bimanual task. This could suggest that the level of damage is sufficient to destroy that relationship between microstructure and behaviour in the task that challenged callosal connections the most, requiring the highest levels of interaction.

The observed differences between the study by Fling and colleagues and the controls in the current study may also be due to subtle differences in methodologies. The study by Fling and colleagues involved 14 young participants (mean age 23.1 years) and 16 older participants (mean age 71.9 years). The control group and patient in the current study had a much wider

age range (controls between 22 and 52 years, and patient group 22 and 60 years) and were on average older than the “control” (young group) and younger than the “patient” (older group) investigated in the study by Fling and colleagues (Fling et al., 2011a). This raises the possibility of behavioural and structural relationships being less evident in the current study, due to age-related effects on structure and function present in the Fling study groups. The aging brain is also vulnerable to executive dysfunction, poor processing speed and impaired inhibitory control, which will undoubtedly have impact on the ability to execute a behavioural task (Hasher et al., 1988, Salthouse, 1996, Bangert et al., 2010). In fact, the study by Bangert and colleagues demonstrated a relationship between the asymmetrical bimanual movement and a self-reported executive dysfunction questionnaire (Bangert et al., 2010). Deficits in attention and executive function are well described as contributory factors in the persistent functional disability following traumatic brain injury (Centers for Disease Control and Prevention 2003, Castellanos et al., 2010, Kinnunen et al., 2011). However, there is the possibility that the significantly older age group in the study of healthy older adults by Fling and colleagues had impact on the relationship between structure and behaviour observed there.

The instruction to the participants was also different in the study by Fling and colleagues, compared to the current study; their participants were instructed to tap in synchrony with the cue. However, in the current study, the participants were instructed to tap in response to the cue. This subtle difference was due to clinical experience that patients with traumatic brain injury are slower in response time, compared to healthy counter-parts. In addition, tapping in synchrony with a cue is likely to require more complex cognitive processing in addition to the visual, perceptual, callosal and corticospinal processes that would be needed to execute the required tap. Although this instruction to tap “in response to the cue” was consistent through the current study, it is difficult to know whether this subtle difference was contributory to the lack of relationship.

There were also some findings with the Fling paper that were difficult to explain, and were not replicated in the current study. In the young adults involved in the study, poorer performance

on the unimanual and asynchronous (requiring higher levels of interhemispheric inhibition) bimanual finger tapping tasks was related to a larger corpus callosum and higher fractional anisotropy (Fling et al., 2011a). This seemed counter-intuitive; the expectation would be that better microstructure across the corpus callosum would be related to better performance in the behavioural tasks that require the most interhemispheric inhibition. Nevertheless, Fling and colleagues have concluded that a larger corpus callosum and better white matter integrity may be detrimental. However, they have acknowledged that they would need a physiological measure to understand this.

A more significant difference between the study by Fling and colleagues and the current study was the use of fewer diffusion tensor imaging diffusion gradient directions in the Fling study (six versus sixty four used in the current study). The greater the number of diffusion gradient directions, the more reliable the white matter microstructure measurement (Giannelli et al., 2009). The study by Giannelli and colleagues also demonstrates this is relevant to fractional anisotropy (i.e. the measure used in the current study), but not mean diffusivity. This raises the possibility that the microstructural measures were more reliable in the current study, which would be a strength of this study.

It is also relevant to note that there were no relationships identified between behaviour and the callosal microstructure in the area pertaining to the callosal motor fibres used by the Wahl mask, in the study by Fling and colleagues (Wahl et al., 2007, Fling et al., 2011a). As mentioned, the study by Fling and colleagues used a region of analysis approach, and the coordinates provided by the tractography work by Wahl and colleagues corresponded to corpus callosum region 5 in the study by Fling and colleagues. Although relationships between corpus callosal microstructure and behaviour were reported, they actually corresponded to the region that connected to the primary sensory cortex of the corpus callosum (referred to as corpus callosum region 6), and not the other regions investigated in the study. It is therefore important that the study by Fling and colleagues, despite using the same callosal motor fibre

coordinates used in the current study, was also not able to demonstrate a significant relationship between microstructure of the callosal motor fibres and behavioural measure.

In the existing traumatic brain injury literature, a strong relationship was observed between the microstructure and an assessment of bimanual coordination (Caeyenberghs et al., 2011a,b). However, in the initial study involving adult patients with traumatic brain injury, the task involved switching direction with a circular motion, so is not comparable to the tapping task used in the current study. In addition, the strong correlations that were observed in the study by Caeyenberghs and colleagues were identified in the prefrontal, primary sensory and parietal region of the corpus callosum, and not the primary motor region which was assessed in the current study (Caeyenberghs et al., 2011a). In the latter study by the same team involving adolescents with traumatic brain injury, despite the presence of reduced fractional anisotropy in the corticospinal tract, corpus callosum, cerebellum and anterior corona radiata, the reported correlations involved ball skills and cerebellum, manual dexterity and cerebellum, balance and corticospinal tract (Caeyenberghs et al., 2011b). However, interestingly these relationships were not replicated in their control group. This alludes to the possibility that performance in functional tasks may be more dependent on microstructure after injury, but this could only be tested with longitudinal studies of the same measures in the same patients. Again, the task is not comparable to the task from Chapter 6. However, it is relevant that the relationship between poorer white matter integrity and poorer performance in the behavioural measures is only described in areas independent to the corpus callosum; the correlations presented were not evident with the corpus callosum itself. This demonstrates the complexity of bimanual coordination in traumatic brain injury, and the involvement of connections with other brain regions, and not just the callosal motor fibres. This has already been demonstrated in brain activation patterns in bimanual movement, shown to involve the primary sensorimotor cortex, supplementary motor area, cingulate motor cortex, lateral premotor cortex, parietal cortex and subcortical structures (Sadato et al., 1997, Jancke et al., 2000a, Kermadi et al., 2000, Deiber et al., 2001, Immisch et al., 2001, Debaere et al., 2004, Witt et al., 2008).

Correlations between microstructure and behaviour have been demonstrated in studies to date outside of the traumatic brain injury literature. In multiple sclerosis, a relationship between fractional anisotropy of the anterior corpus callosum and bimanual finger opposition movements has been made (Bonzano et al., 2008). In the literature comparing older and younger adults, a strong correlation was identified between fractional anisotropy and bimanual scores in the body of the corpus callosum in a smaller sample (n = 10) of young adults (Johansen-berg et al., 2007). However, the task was different, and involved asynchronous thumb opposition at varying frequencies. Although this current study has attempted the findings by Fling and colleagues, a subsequent study of young and old adults by the same group has failed to demonstrate a relationship between microstructure (fractional anisotropy, radial diffusivity, mean diffusivity) and motor performance in a unimanual tapping task in their young and old participants. However, a strong relationship was evident between functional connectivity using fMRI and structural connectivity in the older but not younger adults; the stronger the functional connectivity, the poorer the structural connectivity. However, this adds to the complexity of the situation, as their study suggests that the better functional connectivity between the primary motor cortices, the poorer the performance in the older adults (Fling et al., 2012a). Findings of this nature in the existing literature have been attributed to potential over-recruitment of motor cortices in older adults relative to young adults, due to inefficient recruitment of additional areas in the brain to undertake tasks (Fling et al., 2012a, Riecker et al., 2006). However, this proposal counters previous work which demonstrates unimanual motor performance is strongly associated with callosal microstructural integrity in older adults (Fling et al., 2011a). Both studies by Fling and colleagues instructed the participant to tap in synchrony with the cue onset (rather than react to the cue), so the tasks themselves are comparable. Therefore, the lack of correlation in the latter study is probably due to methological differences in imaging, where the earlier study used a region of interest approach, whereas the latter study utilises fibre tractography (Fling et al., 2011a, Fling et al., 2012a).

8.4.3 Future work

When the study was being designed, one of the aims was to determine whether there were any significant relationships between the physiological and behavioural measures of callosal transfer with the microstructure of the callosal motor fibres, in both the traumatic brain injury and control groups. The corpus callosum is of particular importance to the theme of this thesis, as it is understood to be particularly vulnerable following TBI.

Wahl and colleagues had already explored such a relationship in healthy individuals, where the callosal motor fibres were identified in the posterior body of the corpus callosum in twelve healthy participants, and a significant correlation between microstructure and the physiological measure of interhemispheric inhibition was also demonstrated (Wahl et al., 2007). The method by Wahl and colleagues was also referred to in a behavioural study by the Fling group, which had explored a relationship between microstructure and bimanual coordination as part of their work (Fling et al., 2011a). Other studies had also supported this particular location of the callosal motor fibres (Hofer et al., 2006, Zarei et al., 2006). Therefore, at the time of designing this study I thought a similar approach had the potential to increase the possibility of demonstrating a relationship between the measures of callosal transfer investigated in this thesis, and microstructure in this current group of traumatic brain injury patients and controls.

Future work could look at alternative methods of analysis, such as a mass univariate approach. This would enable the analysis of simultaneously measured variables (as in Kloppel et al., 2008). This method would have the potential advantage of demonstrating relationships involving microstructure outside of the area of interest. This would theoretically increase the likelihood of demonstrating a relationship in this study. However, it also has the potential to demonstrate relationships with structural areas that are not anatomically related to the pathway.

8.5 Conclusions

Although there were differences physiologically, behaviourally and structurally between the traumatic brain injury and control groups when assessed separately in the experiments presented through this thesis, no obvious relationship was able to be demonstrated between them.

Nevertheless, other factors besides callosal transfer are likely to contribute to the measures. Interhemispheric inhibition also depends on the connections within each hemisphere, to include the inhibitory neurons on the receiving hemisphere that translate the callosal activity into local inhibition, and the corresponding corticospinal tract. Clearly behaviour across the corpus callosum is even more complex, with a widespread activation pattern on functional studies which is also dependent on the type of behavioural task (Sadato et al., 1997, Jancke et al., 2000a, Kermadi et al., 2000, Deiber et al., 2001, Immisch et al., 2001, Debaere et al., 2004, Witt et al., 2008). The pathology underlying traumatic brain injury may also involve damage to the cortex from the evidence available in post-mortem work and more recent longitudinal imaging studies (McKee et al., 2013, Cole et al., 2018). In fact, greater atrophy is seen in the sulcal regions of the cortex, and this has also been attributed to the vulnerability of the sulci with the biomechanical forces involved with traumatic brain injury (Cole et al., 2018). This therefore suggests that many other factors may be involved.

The findings from this analysis suggest that physiological and behavioural relationships with anatomical measures are difficult to reproduce. Although this may purely reflect subtle differences in study methods in the experiments presented in this thesis compared to those in the existing literature, it does raise the possibility that they are difficult to demonstrate, and weak (due to studies with small numbers) when they have been reported.

However, in the context of this unique dataset where all three measures of physiology, behaviour and microstructure have been made in the same group of healthy and traumatic brain injury patients, it was worth the exploratory analysis.

Chapter 9

Conclusions

9.1 Summary

This thesis has presented six studies incorporating three areas of investigation i) the physiological changes following traumatic brain injury, ii) the behavioural changes following traumatic brain injury and iii) microstructural differences in patients who have suffered a traumatic brain injury. Through this series of investigations, simple studies have been developed in healthy participants, which have then been used to assess a group of chronic traumatic brain injury patients. Although the imaging and cognitive consequences following traumatic brain injury are well described, physiology and behaviour are less well studied in the traumatic brain injury literature. However, their evaluation is important in understanding the functional consequences encountered by sufferers following traumatic brain injury.

In Chapter 3, a selection of white matter tracts were assessed using transcranial magnetic stimulation in 34 healthy participants ("the normal brain"). The study explored the physiology across the corpus callosum, corticospinal excitability and the localised intracortical pathways. The results in the healthy cohort were comparable to that reported in the literature. This study included a larger number of participants than usual transcranial magnetic stimulation studies, and revealed a large variation in physiological measures between individuals. This finding emphasises the variation of standard TMS measures in healthy individuals, and the importance in acknowledging this when making inferences regarding differences in neurological disorders. In Chapter 4, the physiological study developed in the previous chapter was used to assess seventeen patients who have suffered a traumatic brain injury, and compared to healthy controls. The main finding was difference in callosal transfer between the two groups. Interhemispheric inhibition was the measure of callosal transfer. The findings suggested the relationship between the interstimulus interval and subsequent inhibition was different in the patient group, compared to the controls. There was no difference in the other measures of corticospinal excitability and the intracortical pathway time course. Therefore, this supports the fact that the physiological abnormalities in interhemispheric inhibition relate to the corpus callosum. The nature of the acceleration and deceleration shearing injury

preferentially affecting the fibres across the corpus callosum therefore seems a plausible explanation. The corpus callosum may be more vulnerable than the corticospinal tract due to the orientation of the respective fibres in these tracts.

Using the findings from the physiological work, Chapter 5 developed a behavioural reaction time tapping task to assess the callosal transfer in twenty nine healthy individuals. The intention was to develop a simple task which could then be used in the patient group. The behavioural measure examined a range of interhemispheric interactions that had been previously used to assess callosal interactions in two separate groups of older adults. It provided a baseline set of data of expected behavioural responses in a range of interhemispheric interactions in a larger healthy cohort. Chapter 6 used the behavioural measure to understand the callosal interactions in traumatic brain injury patients. Although performance by the patient group was, on average, comparable to their healthy counterparts, the variability of that performance was significantly different. The findings of this behavioural study added support to the hypothesis that the physiological findings presented in Chapter 4 are due to a pathological process, rather than an adaptive physiological process. Importantly, this study was conducted in the same group of patients, which undoubtedly strengthens this conclusion.

As the patient studies presented in this thesis (Chapter 4 and Chapter 6) demonstrated abnormalities in the physiology and behaviour involved with the corpus callosum, this was evaluated further in Chapter 7. The corpus callosum is a structure commonly affected by traumatic brain injury in post mortem studies. It is already recognised that visible areas of structural damage on conventional neuroimaging do not necessarily reflect the clinical deficit. Chapter 7 assessed the microstructure following traumatic brain injury, using the diffusion tensor imaging metrics for the traumatic brain injury and control groups who participated in the physiological and behavioural studies presented in this thesis. The main finding was that there were widespread differences in fractional anisotropy, and that this did include the corpus callosum. Chapter 8 explored whether there was a relationship between microstructure and

physiology, and microstructure and behaviour. The findings from this analysis were that a physiological relationship with microstructure was not present, despite using a DTI mask produced using the concept in a previous study in healthy individuals (Wahl et al., 2007). It is possible that the relationship between physiology and microstructure is only demonstrable at higher stimulus intensities. However, it is also possible that the lack of relationship is because interhemispheric inhibition also depends on the interneurons in each hemisphere. Thus, a failure to find a relationship in the study presented in Chapter 8 may also indicate that the contribution of these networks is greater than the microstructure of the corpus callosum. The absence of a relationship between behaviour and microstructure may also reflect the additional factors involved in performance, so the relative contribution of the microstructure is small. In addition, the lack of relationship between physiology, behaviour and anatomical measures may also reflect subtle differences in study methods in the experiments presented in this thesis, compared to those in the existing literature. This raises the possibility that they are difficult to demonstrate, and weak (due to pre-existing studies with small numbers) when they have been reported.

9.2 Implications of this research in understanding traumatic brain injury

These studies have demonstrated breakdown in the physiology and behaviour of measures that are thought to involve the corpus callosum. Thus, they support the hypothesis that there are abnormal interactions across the corpus callosum following traumatic brain injury. However, this also needs to take into account the fact that the corpus callosum is only part of an extensive brain network required to execute a bimanual movement, and that bimanual movement is also likely to be influenced by other factors. Nevertheless, this is likely to result in implications in every day functional tasks following traumatic brain injury, which may have previously been solely attributed to cognitive dysfunction following injury. This therefore demonstrates a further area that can be explored in neurorehabilitation. Bimanual coordination is essential in motor tasks, and will have implications in many aspects of daily life, including typing, tasks in the kitchen and playing a musical instrument. The comparable reaction time

performance in the behavioural task in the earlier study is therefore an important finding, as it is the consistent performance that is not being maintained in the patient group. This could be a product of poor sustained attention, or the effort to maintain consistent performance could require more attention to complete a task. Although outside the scope of this thesis, this may also contribute to the fatigue commonly experienced by patients who have suffered a traumatic brain injury. Inability to perform consistently may pose additional problems in day to day life, further impacting on the morbidity associated with traumatic brain injury.

9.3 Future work

This thesis has presented work detailing physiological and behavioural changes following traumatic brain injury, proposing possible mechanisms and clinical consequences for these changes. Future work will benefit from increasing numbers of individuals participating in traumatic brain injury research. Studies that incorporate transcranial magnetic stimulation will be limited by safety criteria, but a larger patient database would enable patients to be stratified according to their brain injury type, so more detailed inferences can be made with respect to mild and moderate-severe injury. Although these studies suggest that abnormalities in physiology and behaviour are present at least a year after the original injury, formal longitudinal studies would help evaluate this in more detail, and investigate whether this correlates with functional outcome.

Now these abnormalities have been identified, this opens an exciting line of enquiry in furthering traumatic brain injury research. The main issue is whether these identified abnormalities can be targeted with dedicated therapy input, or modulated to improve functional recovery accounting for the additional factors detailed above. Interhemispheric interactions have been found to be different in this patient group. Therefore future work could target improving bimanual coordination recovery with known methods to increase interhemispheric inhibition, such as GABA- agonist medication (for example, Baclofen) or methods to modulate the pathways such as repetitive transcranial magnetic stimulation.

9.4 Concluding remarks

The studies presented in this thesis represent findings in chronic traumatic brain injury patients. This is a unique dataset where all three measures of physiology, behaviour and microstructure have been made in the same group of healthy and traumatic brain injury patients. Although the abnormal findings between the two groups are not extensive, they do demonstrate abnormalities in an area under-researched in traumatic brain injury. Therefore the presence of these physiological and behavioural differences highlights the importance in not restricting research to the cognitive deficits encountered after traumatic brain injury.

The findings reported in this thesis demonstrate the complexity of the functional problems encountered after traumatic brain injury. The differences observed between the patient and healthy control group in this collection of studies therefore provides valuable insight into the difficulty returning to normal physical function following injury. Importantly, the findings from this thesis identify an additional area to target in neurological rehabilitation following traumatic brain injury. This area is likely to form part of the “hidden disability” experienced by traumatic brain injury suffers, having functional impact on their ability to return to normal daily function. This knowledge can therefore be used to develop strategies to address neurorehabilitation needs and enhance functional recovery following traumatic brain injury.

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