Long-term outcomes of traumatic brain injury: an epidemiological, neuropsychological, and neuropathological study

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Abstract

In this thesis, I investigate the long-term outcomes of traumatic brain injury (TBI) using data from the Military Hospital for Head Injuries (MHHI) cohort, which was established during the Second World War to advance the care and study of individuals affected by TBI. While these materials have remained dormant for more than two decades, they offer a unique opportunity to examine key contemporary topics in clinical neuroscience, encompassing epidemiology, neuropsychology, and neuropathology. Through the study of this cohort, I address the long-term effects of brain injury, the contributions of specific brain regions to cognitive function, and the pathological mechanisms driving Alzheimer's disease.

In the first results chapter, I compare long-term neurological, cognitive, psychiatric, and functional outcomes after open traumatic brain injury (OTBI) and closed traumatic brain injury (CTBI) through a retrospective cohort analysis. Compared to CTBI, OTBI was associated with a higher incidence of seizures, visual impairment, and dysphasia, but unexpectedly lower rates of other cognitive and psychiatric symptoms. These findings support clinical impressions of CTBI as a more global cerebral insult than OTBI. Prolonged post-traumatic amnesia was identified as an early predictor of seizures up to five years after OTBI. Seizures, sensorimotor impairment, and visual deficits were associated with greater long-term disability after OTBI. I also perform exploratory analysis of long-term mortality outcomes, which suggest reduced life expectancy and a higher burden of neurological causes of death among survivors of OTBI compared to population controls.

In the second results chapter, I develop and validate a novel method for digital lesion-symptom mapping (LSM) using film-based CT radiographs from penetrating brain

injury cases to explore structure-function relationships in the human brain. Replicating previous research, damage to the left prefrontal cortex was associated with chronic insomnia, independent of mood symptoms. A further study investigating performance in the clock-drawing test found results consistent with time-setting errors being linked to left hemisphere damage and visuospatial errors to right hemisphere damage, despite limited statistical power. These findings affirm the value of TBI lesion studies, which offer more diverse lesion distributions than stroke-based LSM.

In the final results chapter, I investigate the effect of disrupted neuronal connectivity on the propagation of tau pathology in Alzheimer's disease. Post-mortem brains of individuals who survived for decades after unilateral penetrating brain injuries revealed reduced levels of hyperphosphorylated tau (p-tau) in association cortex that had been disconnected from the mesial temporal lobe, compared to association cortex that remained connected in the contralateral hemisphere. The same effect was not observed for amyloid-β (aβ). This finding supports the tau propagation hypothesis through comparative human post-mortem evidence for the first time.

Together, these studies provide valuable insights into the lifelong effects of OTBI, reveal structure-function relationships through a novel approach to digital lesion mapping, and offer unique neuropathological evidence of the mechanisms contributing to Alzheimer's disease. A key theme emerging from this work is that focal injuries in otherwise healthy individuals serve as robust models for exploring brain function and disease across the neuroscientific spectrum, from neuropsychology to neuropathology. By reviving a historical dataset with contemporary analytical tools, these findings illustrate the lasting scientific value of archival resources to clinical neuroscience.

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Acronyms and Abbreviations

Acronym	Definition
α-syn	Alpha-synuclein
AAL	Automated anatomical labelling
AC	Association cortices
AD	Alzheimer's disease
AICHA	Atlas of Intrinsic Connectivity of Homotopic Areas
AP	Antero-posterior
APP	Amyloid precursor protein
APPsα	Amyloid precursor protein soluble ectodomain alpha
APPsβ	Amyloid precursor protein soluble ectodomain beta
APTES	3-aminopropyltriethoxysilane
ARD	Attributable rate differences
ARTAG	Aging-related tau astrogliopathy
AT8-ir	AT8-immunoreactivity
Aβ	Amyloid beta
BBB	Blood-brain barrier
BF	Bone fragment
BIC	Brain Injuries Committee
BOLD	Blood-oxygenation-level dependent
CA	Cornu ammonis
CAA	Cerebral amyloid angiopathy
CI	Confidence interval
CNS	Central nervous system
CSF	Cerebrospinal fluid
CT	Computed tomography
CTA	Computed tomography angiography
CTBI	Closed traumatic brain injury
CTE	Chronic traumatic encephalopathy
DAB	3,3'-diaminobenzidine
dlPFC	Dorsolateral prefrontal cortex
dmPFC	Dorsomedial prefrontal cortex
DP	Dementia pugilistica
DTI	Diffusion tensor imaging
EC	Entorhinal cortex
EEG	Electroencephalography
fAD	Familial Alzheimer's disease
FFPE	Formalin-fixed paraffin-embedded
FFT	Formalin fixation time

fMRI	Functional MRI
FOV	Field of view
GCS	Glasgow Coma Scale
GEE	Generalised estimating equation
GFAP	Glial fibrillar acidic protein
GHQ-30	Goldberg's 30-point General Health Questionnaire
GP	General practitioner
H&E	Haematoxylin and eosin
HAM-A	Hamilton Anxiety Rating Scale
HHA	Head Hospital Archive
HRP	Horseradish peroxidase
ICC	Intraclass correlation coefficient
ICD	International Classification of Disease
ICH	Intracranial haematoma
ICP	Intracranial pressure
IDA	Industrial denatured alcohol
IF	Immunofluorescent
IHC	Immunohistochemistry
ILF	Inferior longitudinal fasciculus
IQR	Interquartile range
IRR	Incidence rate ratio
LBCV	Luxol fast blue and cresyl violet staining
LOC	Loss of consciousness
LP	Lumbar puncture
LSM	Lesion-symptom mapping
MFB	Metallic foreign body
MHHI	Military Hospital for Head Injuries
MND	Motor neuron disease
MNSU	Mobile neurosurgical unit
MRC	Medical Research Council
MRI	Magnetic resonance imaging
MS	Multiple sclerosis
MTL	Mesial temporal lobe
NFT	Neurofibrillary tangle
NHS	National Health Service
NHSCR	National Health Service Central Register
NINDS	National Institute of Neurological Disorders and Stroke
NP	Neuritic plaque
NREM	Non-rapid eye movement
NT	Neuropil thread
OBB	Oxford Brain Bank

ONS	Office for National Statistics
OPTIMA	Oxford Project to Investigate Memory and Ageing
OR	Odds ratio
OTBI	Open traumatic brain injury
P-tau	Hyperphosphorylated tau
PART	Primary age-related tauopathy
PBS	Phosphate buffered saline
PD	Parkinson's disease
PET	Positron emission tomography
PFC	Prefrontal cortex
PHF	Paired helical filament
PM	Post-mortem
PMI	Post-mortem interval
PTA	Post-traumatic amnesia
pTDP-43	Phosphorylated transactive response DNA-binding protein 43
PTE	Post-traumatic epilepsy
PTH	Post-traumatic headache
PTSD	Post-traumatic stress disorder
PVS	Persistent vegetative state
RA	Retrograde amnesia
RHI	Repetitive head impact
rTMS	Repetitive transcranial magnetic stimulation
SD	Standard deviation
SUB	Subiculum
SWI	Susceptibility weighted imaging
TAI	Traumatic axonal injury
TBI	Traumatic brain injury
TCVI	Traumatic cerebral vascular injury
TDP-43	Transactive response DNA binding protein-43
TEC	Transentorhinal cortex
TICA	Traumatic intracranial aneurysm
TNA	The National Archives
TP	Table position
TREM2	Triggering receptor expressed on myeloid cells 2
VHIS	Vietnam Head Injury Study
vmPFC	Ventromedial prefrontal cortex
WWI	World War 1, or the First World War
WWII	World War 2, or the Second World War

1 Introduction and Background

To begin this chapter, I provide an overview of traumatic brain injury (TBI), defining the terms used in this thesis and establishing key concepts. After this, I summarise the scientific literature relevant to each study reported in the following chapters, with **Section 1.2** considering epidemiology, **Section 1.3** discussing neuropsychology, and **Section 1.4** reviewing neuropathology. In **Section 1.5**, I introduce the Military Hospital for Head Injuries (MHHI) cohort and highlight the unique opportunities it offers to gain insights into the long-term outcomes of TBI. Finally, in **Section 1.6**, I summarise the aims and rationale of the research that underpins this thesis. The steps taken to prepare the research materials used in this thesis are summarised in **Appendix 1.1**.

The remainder of this thesis is structured as follows:

- **Chapter 2**: Epidemiological investigation of long-term morbidity and mortality outcomes following open traumatic brain injury (OTBI).
- **Chapter 3**: Neuropsychological study of penetrating brain injury cases to gain insights into structure-function relationships in the human brain.
- **Chapter 4**: Neuropathological study of penetrating brain injury cases to gain insights into Alzheimer's disease.
- **Chapter 5**: Synthesis and interpretation of findings across all three aims.

1.1 An overview of traumatic brain injury

1.1.1 Definition of traumatic brain injury

A TBI is defined as any alteration in brain function, or other evidence of brain pathology, caused by an external force [1,2]. For example, according to this definition, the following are all forms of TBI: an individual with no symptoms after a fall but whose computed tomography (CT) head scan shows microhaemorrhages, an individual who experiences temporary symptoms after a collision while playing sport, an individual

who survives a gunshot wound to the head and lives with permanent disability, and an individual who enters a minimally conscious state after a high-speed road traffic collision. This term therefore encompasses a wide range of conditions in which the brain is structurally or functionally altered by a mechanical insult.

In this thesis, the long-term outcomes of individuals affected by TBI are discussed. Features arising downstream from the initial event will be described as occurring *after* or *following* TBI, but this is not meant to imply resolution of symptoms or pathophysiological disturbances associated with the injury. TBI is conceptualised as a dynamic disease process triggered by an initial insult, from which adverse outcomes may arise [3,4].

1.1.2 Epidemiology of traumatic brain injury

The incidence of hospital admissions due to TBI across Europe is estimated to be between 262–287 cases per 100,000 people [5,6]. Each year in England and Wales, more than one million people attend emergency departments with a recent head injury, leading to approximately 200,000 hospital admissions [7]. An audit of 187 emergency departments across England and Wales identified 15,820 patients who presented to hospital with TBI over a 15-month period, which equates to one attendance every 41 minutes [8]. Globally, the incidence of TBI is thought to be rising due to the growing number of motorised vehicle users and the increasing incidence of falls among ageing populations [9,10].

The prevalence of TBI is difficult to estimate because mild TBIs, which account for the majority of cases, are often not diagnosed [11]. However, it is estimated that one in two people will suffer a TBI during their lifetime, making the condition extremely common [12]. In terms of demographics, TBI tends to be more common among men than women and shows a bi-modal age distribution, mostly affecting people aged under 25

years and over 75 years [5,13]. Certain groups are known to be at particularly high risk, including individuals who participate in contact and collision sports [14], individuals who experience intimate partner violence [15], and individuals who live in conflict zones or serve in the military [16,17].

1.1.3 Classification of traumatic brain injuries

Classifying TBI serves to define more homogenous groups for the purposes of both clinical management and research. Traditionally, TBI has been classified using clinical measures of severity and by their mechanism of injury.

1.1.3.1 Clinical measures of injury severity

For more than 50 years, the Glasgow Coma Scale (GCS) has been central to the clinical assessment of TBI severity. To use the GCS, eye opening, verbal, and motor responses are rapidly assessed to determine the conscious level of the patient, which is scored between 3–15 [18]. Using this scale, TBI severity is classified as either mild (13–15),[1] moderate (9–12), or severe (3–8) [3]. The GCS can be combined with other indicators of injury severity using the Mayo TBI severity classification system [20]. Other indicators of severity include the duration of post-traumatic amnesia (PTA), which is defined by an inability to form new memories after injury, often leading to confusion and disorientation [12]. The Mayo system also accounts for loss of consciousness and neuroimaging abnormalities, but the GCS alone remains the most widely used clinical assessment tool [20]. The GCS was initially developed and validated to predict the outcomes of more severe brain injuries, although using the current definition of TBI,

[1] A concussion is currently defined as a mild TBI with no structural neuroimaging abnormalities. Although the term is widely used, this definition is not universally accepted [19].

mild TBIs account for up to 90% of all cases [21]. Outcomes following mild TBI are extremely heterogenous, which highlights the need for a more biologically-driven approach to TBI classification [22]. Reflecting this, the US National Institute of Neurological Disorders and Stroke (NINDS) recently recommended combining clinical assessment with biomarkers, imaging, and outcome modifying factors in an updated 'CBI-M' model of TBI classification [23]. Approaches like this have the potential to transform how TBI is defined, classified, managed.

1.1.3.2 Classification by mechanism of injury

Alongside injury severity, TBI is also traditionally classified by its mechanism of injury to form groups with similar injury patterns. The most fundamental distinction relates to the damage sustained by the skull and underlying dura, which can be used to distinguish between open and closed brain injuries [24].

Closed TBI (CTBI) occurs when external forces are transmitted through the skull to the brain without perforating the dura. The most common causes of CTBI is falls and road traffic collisions, which together account for more than 50% of cases, followed by assault and other violent encounters [5]. CTBIs comprise impact injuries (i.e. when blunt trauma causes rapid acceleration/deceleration of the brain) and non-impact injuries (i.e. when forceful motions of the head or body cause linear/rotational acceleration/deceleration of the brain) [3]. Impact injuries can cause focal tissue damage in the form of scalp bruising or lacerations, skull fractures, and intracranial haematomas or contusions, while non-impact injuries are associated with more diffuse damage resulting from strain and shearing forces in the brain. More recently, the long-term effects of other forms of exposure have been characterised, including exposure to blast-waves and repetitive head impacts (RHIs). Both blast-waves and RHIs can cause the neuropathological features of chronic traumatic encephalopathy (CTE) [25,26], and

both also appear to be associated with a syndrome of cognitive, mood, and behavioural disturbance in later life, although this is an important area of ongoing investigation [27,28].

Open TBI (OTBI), by contrast, occurs when external forces are transmitted to the brain and the dura is perforated by skull fragments or foreign bodies. OTBI accounts for between 5–12% of all cases of TBI resulting in hospitalisation [29,30]. The causes of OTBI can be divided between missile injuries (i.e. gunshot wounds from direct fire), blast fragment injuries (i.e. shell/mortar/bomb explosion wounds from indirect fire), and low-velocity mechanisms (e.g. knife wounds) [24]. OTBIs comprise tangential injuries (i.e. a foreign body deflects off the skull causing a displaced skull fracture), penetrating injuries (i.e. a foreign body penetrates the skull and dura and remains within the skull), and perforating injuries (i.e. a foreign body penetrates the skull and dura, creating both entry and exit points) (**Figure 1.1**). In OTBI, therefore, brain dysfunction arises from the combination of forces transmitted to the brain through the skull and direct injury to the brain itself.

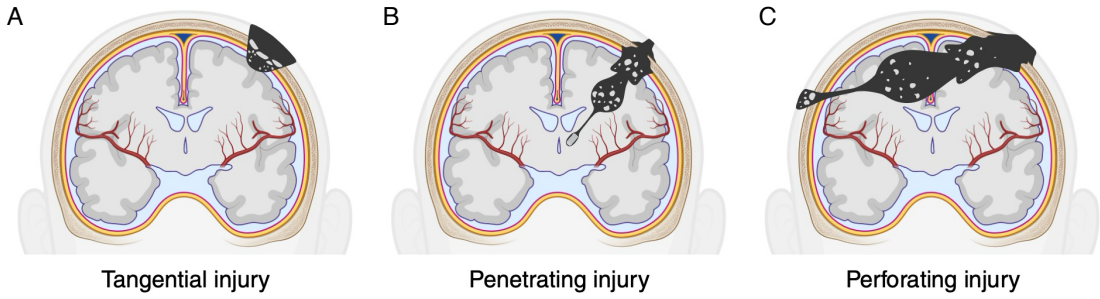

Figure 1.1 Classification of open traumatic brain injury based on mechanism of injury. A. Tangential injuries. **B.** Penetrating injuries. **C.** Perforating injuries. Adapted from Newcombe (1966) according to Hawryluk et al. (2022) [24,31]. Created with BioRender.com.

1.1.4 Pathophysiology of traumatic brain injuries

All forms of TBI involve the pathophysiological effects of external forces acting on the brain. In CTBI, these forces primarily cause differential acceleration, deceleration, and rotation of the brain, producing shearing forces that lead to traumatic axonal injury (TAI) [3]. These forces trigger a neurometabolic cascade of ionic flux, energy disturbance, cytoskeletal damage, altered neurotransmission, neuroinflammation, and neurovascular disruption, which can ultimately lead to cell death [32]. When this process reaches a certain threshold, it results in detectable brain dysfunction manifesting as the wide range of symptoms associated with TBIs.

In moderate-severe CTBI, the processes described above are often compounded by secondary brain injuries, which arise from local and systemic pathophysiological processes [9]. Locally, inflammation resulting from TAI and the neurometabolic cascade can lead to cerebral oedema, increasing intracranial pressure (ICP) and causing wider damage. At the same time, ICP may also rise due to intracranial haemorrhage, resulting from the exertion of shearing forces on the cerebrovasculature. Systemically, physiological disturbances that occur either as a result of TBI or associated injuries (i.e. hypotension, hypoxaemia, hypercapnia, hyponatraemia, hyperthermia, hyper-/hypoglycaemia) may further exacerbate secondary brain injury [33].

Compared to CTBI, relatively little is known about the pathophysiology of OTBI. By definition, skull fragments and/or foreign bodies produce lacerations of the brain parenchyma, its meningeal covering, and the cerebral blood vessels [34–36]. The extent to which these structures are destroyed will be determined by the size, shape, velocity, mass, and material of any object that perforates the skull [37]. The pathophysiology of OTBIs may therefore vary substantially according to the properties of the object responsible for the injury.

As an object moves through the brain, it crushes the tissue in its path to form a permanent cavity (**Figure 1.2**) [37]. The movement of the object also stretches the walls of the permanent cavity, causing damage to the surrounding region, which can be especially severe in softer organs such as the brain. The histopathological result is a permanent cavity surrounded by a peri-lesional region of damaged tissue. Post-mortem studies of fatal self-inflicted gunshot wounds caused by low-velocity firearms at close range show that the permanent cavity is surrounded by a zone of haemorrhage and necrosis [35,36,38]. Beyond this zone is a wider region of oedema, astrocyte destruction, haemorrhage, activated glial cells, and axonal injury that decrease in severity with increasing distance from the cavity.

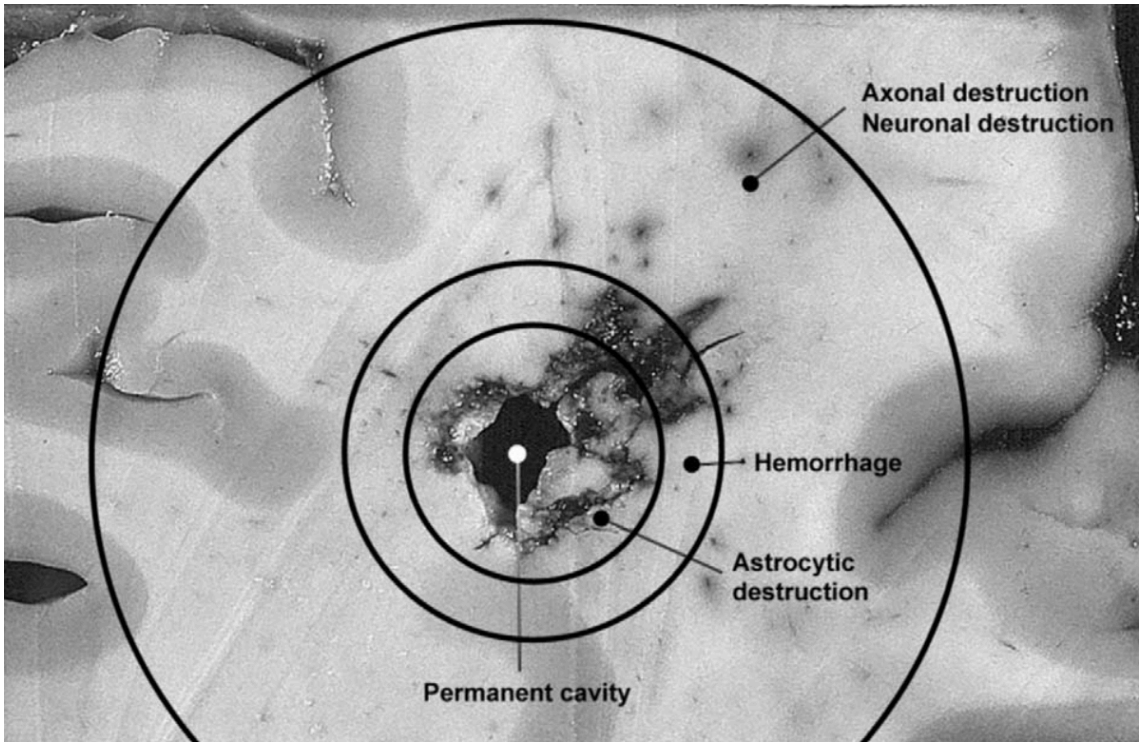

Figure 1.2 Neuropathological features of penetrating brain injury. The permanent cavity is surrounded by a peri-lesional region of damaged tissue. Adapted from Oehmichen et al. (2004) [38].

Post-mortem studies have also identified axonal injury remote from the permanent cavity, most severely involving the brainstem and corpus callosum [38,39]. This form of

axonal injury displays wave-like arrangements, suggesting it may be caused by the blast wave generated by the explosion that propels the bullet, as well as more irregular patterns which may be the result of oedema or ischaemia [40]. Involvement of the brainstem, rather than cavitation caused directly by the projectile, is thought to be responsible for respiratory arrest and death in close-range self-inflicted gunshot wounds [38–40]. The extent of axonal injury remote from the permanent cavity in survivors of OTBI, and how this compares to CTBI, is unclear. However, studies of injuries caused by bolt stunners in sheep suggest that axonal damage is more widespread after non-penetrating injuries compared to penetrating injuries [41].

Secondary brain injury is practically inevitable after OTBI. In the short-term, cerebral oedema and intracranial haemorrhage are common and the extent of blood loss can precipitate hypovolaemic hypotension leading to systemic hypoxia and ischaemia [42]. In the medium-term, an open wound and intrusion of foreign bodies act as potent sources of primary and secondary infection [43]. In the long-term, retained projectiles can have damaging effects, such as heavy metal toxicity after retention of projectiles composed of lead [44].

1.1.5 Management of traumatic brain injuries

Reflecting the range of pathophysiological disturbances associated with TBI, clinical management differs considerably between mild and moderate-severe TBI. Mild TBIs are associated with a wide spectrum of symptoms, including physical (e.g. headache, dizziness, photosensitivity), cognitive (e.g. concentration or memory impairment), and behavioural symptoms (e.g. irritability, emotional lability) [45]. The acute management of mild TBI involves pain management, if required, and a short period of relative rest followed by graduated return to activity. The aim of reducing activity during this period is to enable resolution of the pathophysiological changes described above without

unnecessary exertion, although there is evidence to suggest that early sub-symptom threshold exercise is beneficial to recovery [46,47]. In 80–90% of mild TBI cases, symptoms resolve completely within 1–2 weeks of the injury [48]. The remaining 10–20% may experience persistent symptoms [49]. There are currently limited therapeutic options to support recovery from mild TBI, but cervico-vestibular rehabilitation is recommended for persistent headache or dizziness [11].

By contrast, moderate-severe TBI is associated with a high degree of disability and mortality [50]. Strikingly, severe TBI carries a 60% risk of severe disability six months after injury and a 39% risk of death [33]. The acute management of people with moderate-severe TBI combines access to urgent neurosurgical intervention for cranial decompression when necessary, reversal of major haemorrhage, anti-seizure management, and stabilisation of physiological parameters in pre-hospital, emergency department, and neuro-intensive care settings [51]. Long-term management is centred upon neurorehabilitation, with evidence supporting early intensive rehabilitation to maximise functional gains and minimise disability [52].

1.2 Epidemiology background

1.2.1 Long-term morbidity outcomes following traumatic brain injury

Moderate-severe TBI is associated with an increased risk of developing a range of neurological and psychiatric conditions later in life, including epilepsy [53,54], depression [55–57], sleep disorders [58], dementia [59–62], and ischaemic stroke [63,64]. However, the extent to which these risks apply to individuals with OTBI is unclear, largely because opportunities to study cohorts who survive these injuries are rare [65]. The pathophysiological differences between OTBI and CTBI suggest that OTBI may confer distinct risks for specific long-term conditions. Individuals with OTBI are also at greater risk of complications following injury compared to those with CTBI

[66], which may further affect outcomes. Despite these concerns, current TBI guidelines do not incorporate targeted strategies for the prevention or mitigation of long-term risks specific to OTBI [65]. To address this knowledge gap, an epidemiological study comparing long-term morbidity and mortality outcomes after OTBI and CTBI is reported in **Chapter 2**. To inform this study, this section summarises what is known about long-term outcomes after CTBI and contrasts this with what little is known about outcomes following OTBI.

1.2.1.1 Neurological outcomes

1.2.1.1.1 Seizures

Up to 5% of individuals admitted to hospital with TBI experience seizures within seven days of injury [67]. These early post-traumatic seizures are thought to result from a temporary reduction in the seizure threshold after brain injury and are not associated with a significantly increased risk of seizures long-term. By contrast, seizures that occur more than seven days after injury are linked to long-lasting structural and physiological changes in the brain and are associated with an increased risk of seizures in the long-term [68,69]. As such, individuals who experience late post-traumatic seizures are said to have post-traumatic epilepsy (PTE).

PTE is the leading cause of epilepsy in young adults, accounting for an estimated 10–20% of symptomatic epilepsy cases and 5% of all epilepsy in the general population [67,70,71]. In terms of injury severity, approximately 25% of individuals with severe TBI develop PTE [53,72]. Moderate TBI increases the risk of epilepsy three-fold compared to the general population, while the relationship between mild TBI and PTE is less clear [53,72]. The risk of seizures is highest within the first year after injury [54], but remains elevated for more than a decade thereafter [54,73]. Approximately 80% of seizures in

PTE are generalised, or focal with secondary generalisation, while 20% are focal only [74].

The relationship between OTBI and seizures has been studied in several large veteran cohorts throughout the twentieth century. Over this time, PTE prevalence after OTBI appears to have stayed relatively constant. Across veterans who served in WWI, WWII, the Korean War, the Vietnam War, and the wars in Iraq and Afghanistan, the prevalence of PTE up to 5 years after injury varied between 31–43% [75,76]. The Vietnam Head Injury Study (VHIS) is the largest study of long-term outcomes following OTBI performed to date, involving 1,221 veterans of the Vietnam War and spanning more than 40 years [77]. In the VHIS cohort, the prevalence of PTE up to 15 years after OTBI rose to 50—53% [76]. This reflects a significant number of cases with latent onset PTE. 35 years after injury, 44% of veterans affected by OTBI continued to have seizures [78]. In terms of seizure type, approximately 60% were generalised or focal with secondary generalisation and 40% were focal only [79].

Several large population-based cohort studies have investigated risk factors for seizures after all forms of TBI [53,80,81]. A meta-analysis including these studies identified signs of greater injury severity (e.g. loss of consciousness, post-traumatic amnesia lasting more than 24 hours), focal neurological signs, focal radiological signs (e.g. contusion, intracranial haematoma, skull fracture) and excessive alcohol use as predictors of PTE [73]. In the VHIS, predictors of PTE after OTBI specifically were lesion volume, focal neurological signs (including motor weakness, visual impairment, and dysphasia), headache, retained metallic foreign bodies, and intracranial haematoma [82].

The relationship between PTE and cognitive and psychiatric outcomes is complex, as the effects of the initial injury are difficult to disentangle from the potentially additive effects of seizures, reciprocal relationships between impairments and epilepsy, and confounding effects of anti-seizure medications [83,84]. In terms of functional outcomes, among survivors of severe TBI admitted to a neurotrauma centre, PTE was associated with worse disability outcomes (measured using the Glasgow Outcome Scale) up to two years after injury [72]. Similarly, in survivors of moderate-severe TBI who underwent inpatient neurorehabilitation, those who developed PTE also experienced worse neurological and rehabilitation outcomes [85]. The VHIS also explored the relationships between PTE and other long-term outcomes, finding that PTE was associated with depression, impaired overall cognitive performance, and unemployment [86].

1.2.1.1.2 Headache

Headache affects approximately 40% of individuals with moderate-severe TBI [87,88]. Post-traumatic headache (PTH) is defined by headache that develops within seven days of TBI [89]. PTH often resembles migraine or tension-type headache and may be relieved by traditional treatments for migraine [90,91]. PTH seems to be more common following mild TBI than after moderate-severe TBI, although this may be partly due to sampling bias [90,92]. Risk factors for headache following moderate-severe TBI include female sex, a pre-injury history of headache (especially migraine), and OTBI [93]. A longitudinal study exploring predictors of headache severity after TBI found that OTBI increased the odds of more severe headache six months after injury, but not at three-month or twelve-month time-points [93]. Predictors of headache among those affected by OTBI were not explored in this study, but addressing this question could serve to identify those at higher risk of persistent and debilitating pain.

1.2.1.1.3 Other neurological outcomes

Dizziness is a prominent feature of mild TBI and is also reported following moderate-severe TBI [94]. The term itself is ambiguous and can be used to describe symptoms arising from dysfunction of vestibular, autonomic, or motor systems, among others. Specifically, following mild TBI, abnormalities of smooth pursuit, saccades, and the vestibulo-ocular reflex can be identified on examination [95], and are often associated with vertigo, nausea, and blurred vision [96,97]. Dysautonomia, leading to orthostatic intolerance and light-headedness, is increasingly recognised as a feature of mild TBI [98–100], and gross motor deficits including postural instability, impaired balance, and gait abnormalities, can also persist up to 12 months after injury [101,102]. Relatively little is known about the extent to which dizziness occurs after OTBI. Indeed, a recent systematic review of vestibular disturbance after TBI excluded the small number of studies on this subject [103].

Disrupted sleep is reported in 30–60% of individuals after TBI and significantly affects recovery and quality of life [104,105]. A large meta-analysis found that a quarter of individuals affected by TBI of all forms are later diagnosed with a sleep disorder, such as insomnia, hypersomnia, or sleep apnoea [106]. A meta-analysis of polysomnography studies reported that individuals with TBI have measurable deficits in sleep duration and sleep efficiency compared with controls [107]. Although relatively little is known about predictors of sleep disturbance after TBI, poor sleep is thought to exacerbate other TBI symptoms, including pain, cognitive impairment, and mood disorders [104]. The prevalence of sleep disruption after OTBI is unclear, but damage to the left prefrontal region, specifically, is associated with higher rates of insomnia [108]. The relationship between lesion location and insomnia after OTBI is explored further in **Chapter 3**.

1.2.1.2 Cognitive outcomes

1.2.1.2.1 Specific cognitive impairments

Cognitive domains, including memory, attention, executive functions, and processing speed, can be affected after TBI across the spectrum of injury severity [109,110]. A meta-analysis of epidemiological studies found that mild, moderate, and severe TBI can have lasting effects on both memory and intelligence, with injury severity predicting the extent of impairment to intellectual ability [111,112]. Adverse cognitive outcomes after CTBI relate to the extent of white matter damage and the degree to which brain connectivity is disrupted after injury [113,114]. Indeed, neuroradiological evidence of diffuse damage in the form of TAI is associated with cognitive impairments that persist for months after injury, particularly those involving memory and executive functions [115].

Meanwhile, the effect of OTBI on cognitive outcomes is typically characterised in terms of the impairments resulting from damage to specific brain regions. These focal deficits, such as dysphasia, often arise from lesions involving cortical structures. However, the relationship between OTBI and cognitive symptoms at the group level is less clear. Understanding the cognitive effects of penetrating brain injuries irrespective of lesion location dates back to the concept of mass action, developed by Karl Lashley in 1931 [116]. More recently, while exploring the effects of OTBI on memory impairment in the VHIS, Schooler et al. found that short-term memory impairment occurred regardless of lesion location, unlike semantic memory, verbal episodic memory, and visual episodic memory, which were contingent on damage to specific structures [117]. The neuropsychological study of lesions caused by penetrating brain injuries to gain insights into structure-function relationships in the brain will be discussed further in **Chapter 3**. Evidence for epidemiological associations between OTBI and cognitive

outcomes including memory, concentration, and intelligence will be assessed in **Chapter 2**.

1.2.1.2.2 Dementia and neurodegeneration

TBI is widely recognised as a modifiable risk factor for dementia [4,118,62]. Numerous large meta-analyses have confirmed that a history of TBI is associated with a greater risk of clinically diagnosed dementia [60,119–121]. National registry-based cohort studies have demonstrated a dose-response relationship, with both the severity and number of TBIs correlating with greater dementia risk, and have established that this risk is higher after TBI compared with non-brain trauma [61,122]. Former professional athletes with exposure to RHIs and multiple mild TBIs display higher rates of neurodegenerative diseases, including Alzheimer's disease (AD), Parkinson's disease (PD), and motor neuron disease (MND), compared with population controls [123,124]. RHI exposure is also associated with the neuropathological finding of CTE [27,125–127]. Meanwhile, studies investigating the association between TBI and the neuropathological diagnosis of AD, PD, and MND have produced inconsistent findings [118]. Indeed, the pathophysiological links between TBI and neurodegeneration are not well understood and will be discussed further in **Chapter 4**.

OTBI may be associated with accelerated cognitive decline, but the clinical impact of this is ambiguous. Studies investigating cognitive function decades after OTBI among WWII veterans reported greater decline in memory and reasoning compared to age-matched controls without TBI, although these longitudinal studies were at high risk of attrition bias [128,129]. In the VHIS, only 5% of veterans who survived OTBI showed evidence of dementia on the mini-mental state examination when tested at 60 years of age [130]. As such, the relationship between OTBI and dementia remains unclear, but

linking OTBI cohorts to national registry-based data may help to shed light on the lifelong cognitive effects of these injuries.

1.2.1.3 Psychiatric outcomes

Individuals who sustain TBIs of any severity show higher rates of mood and anxiety disorders compared with the general population, including depression, post-traumatic stress disorder (PTSD), and generalised anxiety disorder [131–133]. The estimated prevalence of both mood and anxiety disorders increases slightly in the first year after TBI and remain elevated for several years compared with pre-injury levels [134]. Older age, female sex, unemployment, and pre-existing psychiatric illness are all associated with a higher risk of developing these conditions after TBI of all forms [133,134]. Sustaining more than one TBI has not been shown to confer additional risk in adults [56,132]. However, a large registry study did demonstrate a dose-response relationship between both the number and severity of TBIs and rates of inpatient and outpatient psychiatric attendance among children and young adults [135]. Psychosis is more common after TBI than in the general population, but its relationship with brain injury is complex, as TBI is also more common among those with schizophrenia [133]. TBI also appears to be a risk factor for suicidal ideation, suicidal behaviour, and suicide completion [136].

Several functional neuroimaging studies have identified altered connectivity across emotion networks among individuals with depression after CTBI compared to those without depression [137–139]. The extent of alteration correlates with the severity of depressive symptoms, and this pattern of disrupted connectivity differs from that seen in individuals with depression unrelated to TBI [137–139]. The extent of white matter damage after CTBI also correlates with altered dopaminergic signalling among those with depression after CTBI [140]. Together, these findings support the notion that white

matter damage and altered connectivity after CTBI may contribute directly to depression, in a manner akin to cognitive outcomes described above [133]. At the same time, it is important to recognise the potential indirect effects of functional impairment on mood and anxiety after brain injury, as well as the influence of social and environmental factors on psychiatric outcomes [133].

The characterisation of adverse psychiatric outcomes after OTBI often focuses on personality changes associated with damage to the frontal lobe, including aggression, irritability, and disinhibition. In the VHIS, psychiatric and neuroimaging studies showed that damage to specific prefrontal regions was associated with anxiety and depression as well as irritability [77]. This approach will be discussed in more detail in **Chapter 3**. However, like cognitive outcomes, relatively little is known about the relationship between OTBI and psychiatric symptoms at the group level. Evidence for epidemiological associations between OTBI and psychiatric outcomes including anxiety, depression, and personality change will be assessed in **Chapter 2**.

1.2.1.4 Social and functional outcomes

Mild TBI is associated with transient reductions in academic performance [141,142]. but no long-term effect on educational attainment, employment, or standard of living [143]. By contrast, a meta-analysis of employment outcomes after moderate-severe TBI found that while employment rates increased over time, long-term unemployment remained over 50% and only a third of those affected were able to return to their pre-injury level of work [144]. A large registry cohort study showed a dose-response relationship between both TBI severity and frequency and negative social outcomes, including low educational attainment, receipt of means-tested welfare benefits, and disability pension [135]. The impact of TBI also extends to caregivers, who may experience challenges including missed work due to caring responsibilities [145]. A

wide range of injury-independent factors act to modify social and functional outcomes following TBI across the spectrum of injury severity [88,146]. These include: i) patient factors (e.g. age, sex, ethnicity, education, premorbid conditions) [147–149], ii) management factors (e.g. rehabilitation) [47,150,151], and iii) environmental factors (e.g. socioeconomic status, peer relationships, and other social determinants of health) [152–154]. Nonetheless, the injury itself remains an important determinant of social outcomes. For instance, a recent cohort study of military personnel found that elevated plasma levels of glial fibrillar acidic protein (GFAP) approximately eight years after TBI were associated with unemployment, suggesting a direct link between chronic glial activation, disability, and employment status [155].

OTBI often results in focal impairments such as limb weakness or sensory disturbance, visual impairment, dysphasia, and other cognitive impairments. In the VHIS, one in two veterans experienced persistent limb weakness or sensory disturbance, and one in four experienced dysphasia, although in a third of cases this resolved within ten years [156]. About a third of those with motor weakness were prevented from working by their impairment [86]. 15 years after injury, 56% were currently employed and up to 80% had worked since their injury [82]. Smaller lesion volume and higher pre-injury intelligence were both predictors of with return to work. Unemployment was associated with specific neurological outcomes (i.e. PTE, paresis, sensory loss, visual impairment), psychiatric outcomes (i.e. mood disturbance, anti-social or violent behaviour), and cognitive outcomes (visual and verbal memory loss, impaired sustained attention).

1.2.2 Long-term mortality outcomes following traumatic brain injury

Although an early case-control study found a greater risk of death up to 15 years after hospitalisation for mild TBI in Glasgow [157], subsequent cohort studies have not

confirmed this association [4]. By contrast, moderate-severe TBIs are associated with increased long-term mortality and reduced life expectancy [158]. Large retrospective studies have found that individuals with TBI remain at higher risk of death than age- and sex-matched members of the general population for up to 10 years after injury, equating to an average reduction in life expectancy of seven years [159–161].

People affected by TBI are significantly more likely to die from seizures, as well as non-neurological causes of death such as aspiration pneumonia, sepsis, accidental overdose, and falls [71,159]. External causes of death, including suicide, injury, and assault, are up to four times more common among individuals with a lifetime history of TBI compared with the general population [160]. Epidemiological studies have also indicated that TBI is associated with an increased risk of developing other neurological conditions that can be fatal, such as stroke and potentially also brain cancer [162,163].

TBI of all forms is associated with a mild increase in the risk of ischaemic stroke (1.3–1.6 times) and a greater increase in the risk of haemorrhagic stroke (4.8–6.2 times) [162,164]. In some studies, the additional risk that a history of TBI conferred on the risk of stroke from either cause was equivalent to that of hypertension [64]. The risk of stroke appears to become elevated within months of injury, and while moderate-severe TBIs carry the greatest risk, mild TBIs also appear to be associated with an increased risk compared to controls [164,165]. OTBI, specifically, is associated with traumatic intracranial aneurysms (TICAs), which form after laceration of cerebral blood vessels and can rupture to cause haemorrhagic stroke. A recent case series found TICAs in 8% of civilian gunshot wound cases using CT angiography (CTA) on admission to hospital and showed that TICAs were associated with intracranial haemorrhage [166]. Although a third of cases resolved spontaneously, a further third required endovascular

or open neurosurgical treatment, suggesting that using CTA to screen for TICAs may serve to mitigate the risk of haemorrhagic stroke after OTBI.

The relationship between TBI and brain cancer is less clear, largely due to the low incidence of primary brain tumours, which makes the epidemiological study of their associated risks challenging. A systematic review of epidemiological studies concluded that there is insufficient evidence of a causal relationship between TBI and brain tumours [167]. However, a more recent study involving nearly two million US veterans reported that moderate-severe TBI was associated with developing brain cancer, and that OTBI was an independent risk factor [163]. Given the poor prognosis of many brain cancers and the opportunity to deploy screening programs among those at higher risk, the relationship between OTBI and brain cancer warrants further investigation.

To address keys gaps in the scientific literature identified above, the epidemiological study of long-term morbidity and mortality outcomes after OTBI is reported in **Chapter 2**.

1.3 Neuropsychology background

1.3.1 Origins of human brain lesion studies

While the structure of the human brain was characterised and illustrated nearly 500 years ago by the anatomists Andreas Vesalius and Thomas Willis, our current understanding of how the nervous system functions is based on little over 150 years, or five generations, of observation and experimentation. Throughout this time, the study of individuals who survive injuries to the brain has remained a cornerstone of neuroscience, providing valuable insights into the functional consequences of damage to specific brain regions. In 1861, French surgeon Paul Broca adopted a systematic approach to the case of Louis Victor Leborgne. Broca characterised Leborgne's

difficulty with producing speech, and after his death, related this to a cavity in the left peri-sylvian region of his brain, producing the first clinico-pathological study of brain injury [168]. Inspired by Broca, Carl Wernicke applied the same approach to a series of cases with language difficulties [169]. In 1874, Wernicke observed that language production was associated with damage to the left frontal region of the brain while language reception was associated with left temporal damage. He combined these observations to develop a theoretical system of language processing. Importantly, Wernicke understood that while certain regions of the brain may have specialised functions, the connections between regions are equally, if not more, salient, and that damage to these connections can produce subtly different symptoms to destruction of the regions themselves. In so doing, Wernicke started the debate between 'localisationist' and 'connectionist' views of brain function, which continues today [170].

1.3.2 Brain lesion studies in the twentieth century

1.3.2.1 The First World War (1914–1918)

Large-scale conflicts accelerated the scientific progress made by brain lesion studies throughout the twentieth century. The high volume of penetrating brain injuries sustained by young and often otherwise healthy individuals led to unprecedented opportunities to study the effects of focal brain injuries. During the First World War, the neurologist Gordon Holmes investigated the visual impairments of British soldiers to establish the structural and functional organisation of the human visual system (**Figure 1.3**) [171,172]. Additionally, the neuropsychological study of First World War veterans advanced the understanding of dysphasia, led to the development of new cognitive tests, and generated early functional maps of the cerebral cortex [173–175]. At the same time, Kurt Goldstein's pioneering approach to the psychological aspects of brain injury highlighted the power of multi-disciplinary rehabilitation [176].

1.3.2.2 The Second World War (1939–1945)

In the aftermath of WWII, the psychologist Hans-Lukas Teuber oversaw a series of studies that were among the first to link damage to specific brain regions with impaired cognitive performance beyond dysphasia. Emulating Broca's approach, Teuber combined systematic neuropsychological evaluation with lesion localisation using skull x-rays and demonstrated that damage to distinct cortical areas was associated with focal deficits, challenging Lashley's 'mass action' view of brain function. For example, Teuber showed that temporal lobe lesions resulted in memory problems, while parieto-occipital lesions produced visuospatial and perceptual impairments [177,178]. Through a series of longitudinal assessments, Teuber also highlighted the long-term social impacts of brain injury, although these were captured even more vividly by the Russian neuropsychologist Alexander Luria [179–181].

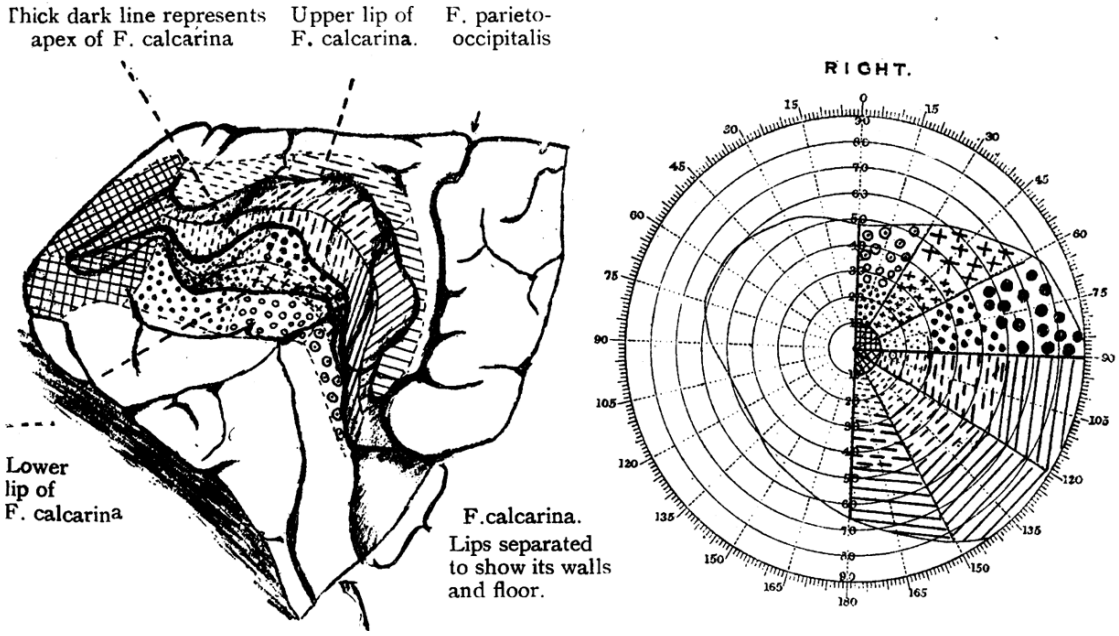

Figure 1.3 The visual cortex was mapped through studying the visual field impairments of soldiers injured during the First World War. Adapted from Holmes (1918) [172].

Meanwhile in Britain, studies of the MHHI cohort explored the role of lesion location on limb weakness and sensory disturbance [182], visual field impairment [183], seizures

[184], dysphasia [185], and amnesia [186]. Early neuropsychological studies of this cohort were conducted by Oliver Zangwill, who described a case of impaired number perception after left parieto-occipital injury, an individual who stopped dreaming after right parietal injury, and a left-handed veteran with visuospatial neglect after injury to the right parietal lobe [187–189]. In 1963, Freda Newcombe was appointed to lead the systematic neuropsychological assessment of the MHHI cohort [129]. Newcombe described the array of impairments displayed by this group in her thesis, concluding that selective impairments persisted without evidence of generalised cognitive decline [31]. In her subsequent book, she contrasted the association between language impairments and damage to the left hemisphere with the visuospatial deficits of those with right hemisphere damage [190]. Specifically, in terms of language, Newcombe went on to describe the difference between deep and surface dyslexia [191–193]. Notably, she also observed how visual perception and visuo-spatial impairments could be dissociated after damage to the right hemisphere, informing the influential macaque lesion studies of Ungerleider and Mishkin, which would later establish the distinction between ventral and dorsal pathways of visual processing (**Figure 1.4**) [194–196]. Newcombe's studies involving the MHHI cohort continued into the 1990s. Results from the latter stages of this work established contributions of the left hemisphere to visuospatial processing [197–199] and generated novel findings regarding how the brain processes visual information to perform facial recognition [200], spatial orientation [201,202], object naming [203], imagery [204], semantic memory [205], and verbal reasoning [206].

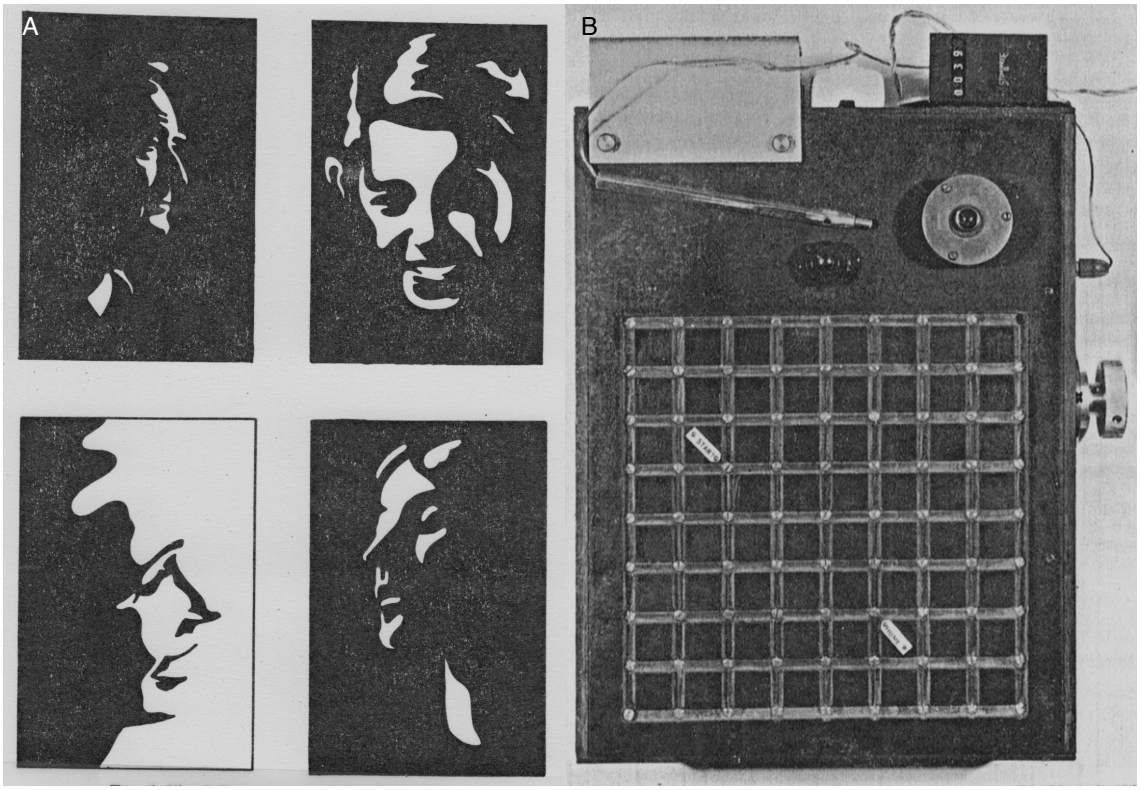

Figure 1.4 Newcombe established that impairments of visual perception and visuo-spatial performance could be dissociated after damage to the right hemisphere. A. The visual closure task was used to assess visual perception: subjects were asked whether they can perceive a face, partially delineated by white contours on a black ground or by black contours on a white ground and identify it as that of a man or woman, old man or old woman, boy or girl. **B.** The maze-learning task was used to assess visuo-spatial performance: subjects were shown a particular path between start and end points then asked to trace out the same path. Adapted from Newcombe (1969) [190].

1.3.2.3 The Vietnam War (1955–1975)

Neuropsychological studies in the VHIS were led by the neurologist Andres Salazar and neuropsychologists Jordan Grafman and Alex Martin. Building on Newcombe's conclusion that cognitive decline was not inevitable after penetrating injury, this group showed that pre-injury intelligence was the strongest predictor of overall post-injury cognitive performance, followed by lesion size, and that lesion location only affected performance in specific subtests [207]. The injuries sustained by veterans in the VHIS predominantly affected the frontal lobes [108,208,209], and Grafman used this to show

that damage to the ventromedial prefrontal cortex (vmPFC), in particular, was associated with aggression and violence [210].

More recently, the combination of neuropsychological and neuroimaging data collected by the VHIS has been used to perform lesion-symptom mapping (LSM). LSM is a form of statistical neuroimaging analysis that tests the association between damage to specific brain regions and cognitive impairments or symptoms. Using LSM to analyse the CT scans of 192 veterans with penetrating brain injuries, Grafman's group showed that damage to the left dorsomedial prefrontal cortex (dmPFC) was associated with insomnia approximately 35 years after injury (**Figure 1.5.A**) [108]. They went on to report an association between damage to cortical and limbic areas in the left hemisphere and higher levels of anxiety (**Figure 1.5.B**) [208] and associations between damage to the left dorsolateral prefrontal cortex (dlPFC) and higher levels of neuroticism and lower levels of conscientiousness (**Figure 1.5.C–D**) [209].

1.3.3 The contemporary role of human brain lesion studies

Today, human brain lesion studies remain an important tool for the discovery, development, and validation of neuroscientific theories [211]. While functional neuroimaging has become the mainstay for investigating structure-function relationships in the brain, lesion studies play an important complementary role. The impairments revealed by damage to particular brain regions serves to generate hypotheses that can then be tested through functional neuroimaging. For example, functional magnetic resonance imaging (fMRI) identifies regional blood-oxygenation-level dependent (BOLD) activity that correlates with performance of a certain task. In this way, lesion studies highlight which areas are strictly necessary for a task to be performed, while functional neuroimaging identifies a pattern of BOLD activity that is

sufficient to perform a certain task in healthy individuals [212]. Conversely, fMRI activation maps can be used as a spatial guide for areas to be investigated by lesion studies [170,213]. Additionally, underlying physiology of the BOLD signal is uncertain, and fMRI is limited to the investigation of tasks that can be performed (and states that can be experienced) within the confines of a scanner, while lesion studies can give insights into a wider range of human experience. For these reasons, human brain lesion studies continue to play an important role in neuroscientific research.

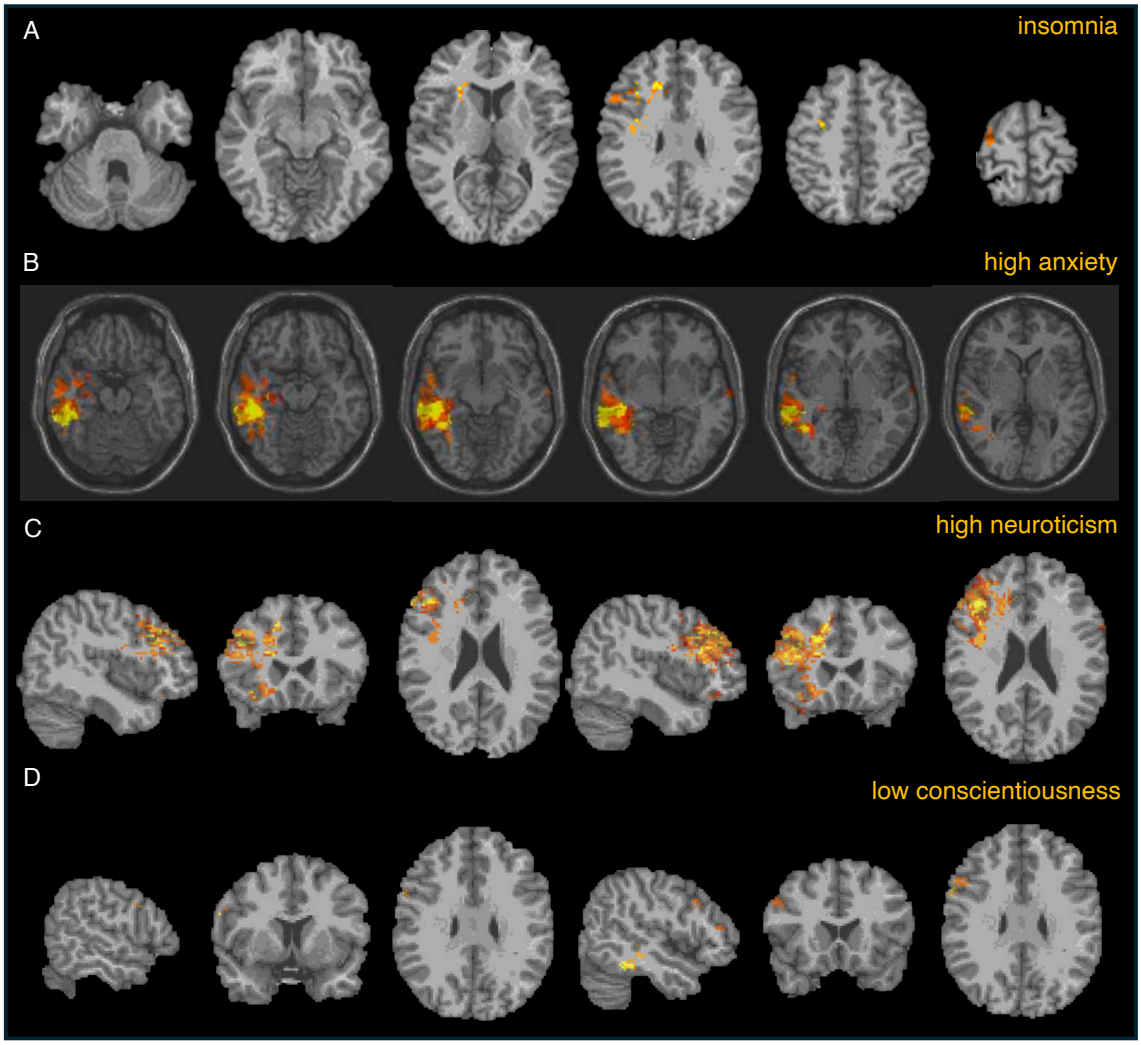

Figure 1.5 Key findings from lesion-symptom mapping studies performed in the Vietnam Head Injury Study. A. Damage to the left dorsomedial prefrontal cortex is associated with insomnia, adapted from Koenigs et al. (2010) [108]. **B.** Damage to cortical and limbic areas in the left hemisphere is associated with higher levels of anxiety, adapted from Knutson et al. (2013) [208]. **C–D.** Damage to the left dorsolateral prefrontal cortex is associated with higher

levels of neuroticism and lower levels of conscientiousness/self-discipline, adapted from Forbes et al. (2014) [209].

1.3.4 Lesion-symptom mapping

In the last 30 years, the field of lesion studies has widely adopted LSM to uncover insights into the functional architecture of the brain [214]. Building on the approach pioneered by Broca and Wernicke more than a century ago, LSM leverages the power of statistical comparisons between brain regions among large case series to distinguish between injured areas that are associated with impairment from areas that are simply vulnerable to injury [215]. For the most part, these studies have involved individuals with brain damage caused by ischaemic stroke, as the commonest form of acquired brain injury [213].

This approach is illustrated by Tranel et al. (2008), who used LSM in a stroke cohort to explore the neuroanatomical correlates of errors in the clock-drawing test [216]. In this study, the authors found that damage to the left hemisphere was associated with time-setting errors (**Figure 1.6.A**), and that damage to the right hemisphere was associated with visuospatial errors (**Figure 1.6.B**). The clock-drawing test remains widely used as part of the structured neurological examination of cognitive function and as a component of dementia screening tools like the Montreal Cognitive Assessment (MoCA) [217,218]. However, while time-setting and visuospatial components of the test are scored separately in the MoCA, the extent to which impaired performance in these aspects of the test localises to specific brain regions is unclear [219–221]. Functional neuroimaging studies have shown brain activation in bilateral frontal, parietal, and occipital lobes when performing the clock-drawing test [222], and both time-setting and visuospatial components of the test involve bilateral activation [223]. As such, there is

scope for further lesion studies to investigate which of these regions, if any, are necessary to perform discrete components of the clock-drawing test.

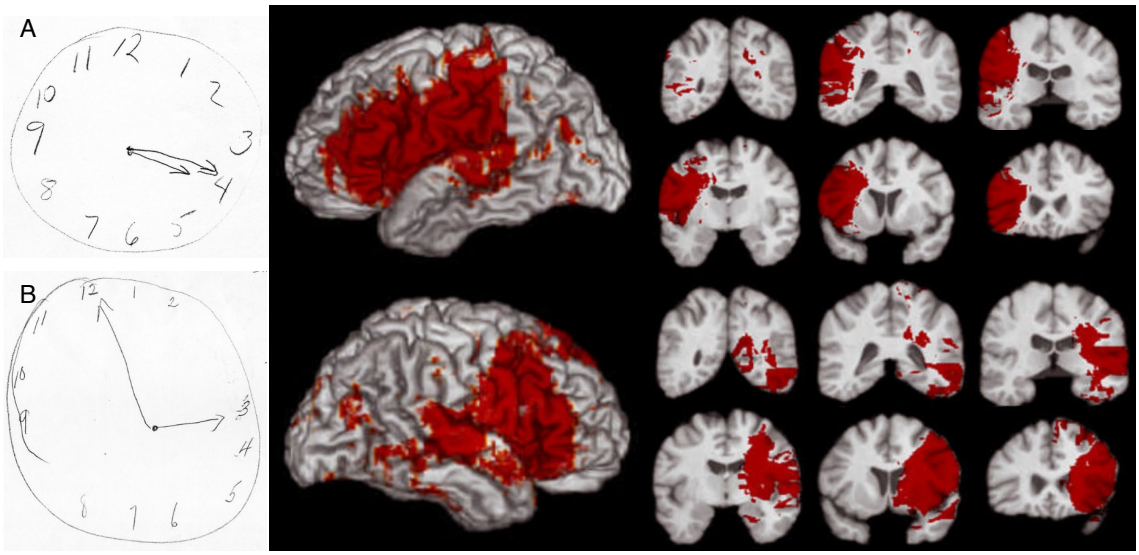

Figure 1.6 Lesion-symptom mapping using a stroke cohort to explore the neuroanatomical correlates of errors in the clock-drawing test. Adapted from Tranel et al. (2008) [216]. **A.** Time-setting errors are associated with left hemisphere damage. **B.** Spatial errors are associated with right hemisphere damage.

LSM has also been used to analyse the effects of lesions caused by TBI, multiple sclerosis (MS), brain tumours, and surgical resection for treatment of neoplasia or epilepsy [213,224]. In this context, studying veterans who have undergone surgical resection for treatment of penetrating brain injuries offers unique advantages. First, traumatic lesions do not conform to the vascular territories affected by ischaemic stroke [225], the regions most vulnerable to cerebral malignancy [226], or the areas most often associated with epilepsy [227]. Second, penetrating brain injuries caused by low-velocity projectiles produce focal deficits, and surgical resection of unhealthy tissue around these deficits produces lesional areas that are more discrete than those caused by MS or malignancy [228]. Third, military personnel tend to be healthy individuals who sustain injuries at a relatively young age, minimising the confounding effects of coincidental disease and ageing [75].

The timing of both neuroimaging and neuropsychological assessment have varied across the large number of LSM studies performed in stroke cohorts [213,229]. Acquiring data in the acute phase provides insights into the immediate impact of an injury and avoids both the structural distortion that can occur with time and the functional re-organisation that can be achieved through rehabilitation [213,229]. By contrast, scanning in the chronic phase provides a clearer delineation of lesional boundaries, once cerebral oedema and haemorrhage have resolved, and assessing performance years after injury highlights the impairments that persist despite rehabilitation, which may have the greatest bearing on long-term quality of life [229].

Consequently, the neuroimaging and neuropsychological data that were collected from the MHHI cohort provide a valuable opportunity to study the effects of clearly delineated lesions on persistent impairments in an otherwise healthy cohort. However, utilising these archival data for LSM presents a unique set of challenges. Most significantly, the CT head radiographs acquired from this cohort were printed on film. While early pioneers of lesion-symptom mapping developed techniques to transfer film-based CT radiographs into digital programs for statistical analysis, the tools used to perform LSM have advanced over the intervening 30 years [230,231]. To the best of our knowledge, there are no reports describing how film-based CT head radiographs can be used to perform LSM using modern neuroimaging programs.

To address this, in **Chapter 3**, a novel adaption of existing lesion reconstruction methods will be described and validated both structurally and functionally. Then, this method will be applied to conduct LSM using cases from the MHHI cohort, with the aim of replicating the findings described above in relation to insomnia and the clock-drawing test.

1.4 Neuropathology background

1.4.1 Neuropathology of traumatic brain injury

1.4.2 A brief history of traumatic brain injury neuropathology

The neuropathological study of TBI dates back to 1928, when Harrison Martland described the features of 'punch-drunk syndrome' among boxers [232]. Thirty years later, Sabina Strich with Peter Daniel characterised the acute neuropathological features of TBI [233]. Strich described the neuropathological features of individuals who entered a persistent vegetative state (PVS) after TBI caused by road traffic collisions [234–236]. These cases were not complicated by skull fracture, intracranial haematoma, or oedema, and yet all remained unresponsive for months after injury. Survival after injury ranged from four weeks to two years. Strich observed that the most striking abnormality was a 'diffuse and often severe degeneration of the white matter throughout the cerebral hemispheres' [234–236]. Grey matter changes were less common, but, when present, consisted of contusions in the crests of gyri, small infarcts at the bottom or sides of sulci, and small haemorrhages. Strich concluded that this diffuse white matter degeneration was most likely due to physical damage to nerve fibres at the time of injury, as opposed to anoxia, oedema, vascular disturbance, or fat embolism. Specifically, she reasoned that such widespread damage could be accounted for by the 'stretching and tearing of nerve fibres and blood vessels' under shear forces exerted on the brain during its rotation within the skull. Sparing of specific tracts in each case was explained by the directions of the shear strains relative to fibre direction, drawing on Holbourn and Cairns' work on the biomechanics of head injury at the MHHI [237].

Following Strich, Oppenheimer described microscopic lesions containing ‘tight clusters of active microglial cells’ scattered throughout the cerebral white matter, basal ganglia, and brainstem following TBI [238]. While these clusters were seen in cases of PVS following TBI, they were also seen in several cases in which concussion occurred shortly before death from non-neurological causes. Oppenheimer concluded that ‘permanent damage, in the form of microscopic destructive foci, can be inflicted on the brain by what are regarded as trivial head injuries’ [238]. These early observations have proven to be seminal [3]. Today, the diffuse white matter degeneration described by Strich is known as traumatic axonal injury (TAI), which can be detected after mild, moderate, and severe TBI using diffusion tension imaging (DTI) [239]. The extent of TAI is closely related to injury severity, recovery, and long-term outcomes following TBI [3]. Similarly, the microhaemorrhages described by Oppenheimer can be identified using susceptibility weighted imaging (SWI), and also serve as a measure of injury severity and recovery [240].

1.4.3 Acute neuropathological features of traumatic brain injury

The acute neuropathological features of TBI can be grouped into focal and diffuse features [241]. Focal features include lacerations of the brain parenchyma, contusions, and intracranial haemorrhage. Diffuse, or multifocal, features include TAI, microhaemorrhages, brain swelling, disruption of the blood-brain barrier (BBB), and neuroinflammation. Different injury mechanisms are associated with variable combinations of focal and diffuse neuropathological features [241]. For example, CTBI caused by acceleration forces or blast waves typically results in multifocal TAI, microhaemorrhages, and perivascular inflammation [25]. CTBI caused by blunt impact is more likely to exhibit diffuse TAI, contusions in coup and contrecoup regions, a greater inflammatory response, and intracranial haemorrhage, often in association with

depressed skull fracture without dural perforation. Penetrating brain injuries, by definition, are characterised by compound skull fracture, dural perforation, and brain laceration, which is usually associated with intracranial haemorrhage [241]. Brain laceration produces a permanent cavity surrounded by a proximal zone of haemorrhagic necrosis and a wider region of activated glial cells, oedema, astrocyte destruction, and axonal injury [35,36,38].

1.4.4 Chronic neuropathological features of traumatic brain injury

The chronic neuropathological features of TBI include inflammation [242], disruption of the BBB [243], astrogliosis [244], multifactorial white matter change [245], and neuronal loss [246]. Reactive microglia have been identified up to 18 years post-injury [247]. Their presence is associated with reduced corpus callosum thickness, suggesting that persistent inflammation may contribute to white matter degeneration and neuronal loss [242]. Fibrinogen and immunoglobulin G (IgG) are plasma proteins that do not normally cross the BBB, but both accumulate in multi-focal parenchymal and perivascular regions following TBI, distinct from areas of haemorrhage, persisting for years [243]. Deposition of fibrinogen and also iron are associated with inflammation and neuronal loss after TBI [248,249].

Abnormal protein aggregation is the hallmark of neurodegenerative conditions such as AD, and it is also a chronic neuropathological feature of TBI [250,118]. AD is defined by the build-up of neurofibrillary tangles (NFTs) composed of hyperphosphorylated tau proteins (p-tau) and extracellular plaques formed of amyloid-β (aβ) peptides [251–254]. Both features can appear in the brain shortly after TBI [255–259]. However, there are important differences between the distribution, extent, and form of these proteins when they arise after TBI and AD.

A link between TBI and NFTs was first established through the neuropathological description of dementia pugilistica (DP) in 1973 [260]. Twenty years later, the distribution of NFTs in the brains of boxers was shown to be distinct from that of AD [261]. In 2005, this pattern was also described in the brains of American football players, and it was re-named CTE [262,263]. CTE is defined by the presence of p-tau aggregates within neurons and astrocytes surrounding small blood vessels in the deep layers of cortical sulci, and in superficial layers elsewhere in the cortex [127]. Identification of multiple CTE lesions and NFTs in hippocampal and subcortical structures denotes more severe disease [264]. This is in contrast to AD, where p-tau aggregates confined to neurons develop in the entorhinal cortex and hippocampus before appearing throughout the neocortex, predominantly in deeper layers of association cortex, irrespective of gyri or sulci [265]. The morphologies of p-tau filaments in CTE also differ from AD, suggesting distinct aggregation processes in the two conditions [266].

Early descriptions of DP emphasised the presence of p-tau, but later studies showed that diffuse aβ plaques often coincide with NFTs in these cases [267–269]. Diffuse aβ plaques have subsequently been identified in a third of individuals who died shortly after TBI, regardless of age [255,270]. Diffuse plaques can develop within hours of TBI, but while they have been shown to persist beyond one year, they appear to regress in the longer term, and do not appear to develop into the dense plaques that are characteristic of AD pathology [271]. Nevertheless, while early diffuse aβ plaques may not provide a direct pathophysiological link between TBI and AD, the mechanisms underlying their appearance and removal may play an important role [255]. Briefly, amyloid precursor protein (APP) is a transmembrane protein which accumulates in damaged axons, and it is thought that abundant APP in areas of TAI may undergo aberrant cleavage by secretases to form diffuse aβ plaques [255]. TBI also appears to

remodel the perivascular matrix, including endothelial components involved in aβ clearance, which may slow the removal of aβ plaques [272]. In the longer term, aβ plaques are thought to be removed through microglial phagocytosis and degradation by the enzyme neprilysin, which is upregulated for months after TBI [255]. Intriguingly, variants of the neprilysin gene, and other microglia-associated proteins such as Triggering Receptor Expressed on Myeloid cells 2 (TREM2), are associated with the extent of aβ plaque formation and removal after TBI [273,274].

Therefore, while NFTs and aβ plaques are both chronic neuropathological features of TBI, p-tau in the form of CTE and transient diffuse aβ plaques stand in contrast to AD-related NFTs and neuritic aβ plaques. The mechanistic links underlying the epidemiological association between exposure to TBI and long-term risk of AD therefore remain unclear, in large part due to the incomplete understanding of AD pathogenesis. In **Chapter 4** of this thesis, penetrating brain injury will be used as a model to gain valuable insights into the pathological processes underlying AD.

1.4.5 Neuropathology of Alzheimer's disease

1.4.5.1 Origins and clinico-pathological characterisation

In 1907, Alois Alzheimer reported an 'unusual illness of the cerebral cortex' [275]. He described the case of a 51-year-old woman who rapidly developed severe impairments of recent memory and language, which progressed over four years to result in a profound dementia [275]. Post-mortem examination showed generalised atrophy of the brain and silver staining revealed 'striking changes of the neurofibrils' affecting up to one third of neurons in the cortex, often causing them to disintegrate, alongside 'minute miliary foci which are caused by the deposition of a special substance in the cortex' [275]. In the 1960s, electron microscopy and x-ray diffraction revealed that these

neurofibrils were made up of paired helical filaments (PHFs) and the extracellular substance, described as ‘amyloid’ (meaning starch-like), showed a β-pleated sheet structure [276–278]. Soon after, it was shown that most older individuals affected by dementia displayed the same neuropathological features as had been described by Alzheimer, and that the extent of these changes correlated with the severity of cognitive impairment [279,280]. Subsequently, the term ‘Alzheimer’s disease’ became widely used to describe a clinical syndrome of progressive memory impairment accompanied by other cognitive deficits among people aged over 65 [281]. A diagnosis of ‘probable Alzheimer’s disease’ began to be offered when other causes of dementia had been excluded, while the presence of Alzheimer’s pathology was only confirmed by post-mortem examination in a minority of cases [281]. Through this shift, what is now referred to as Alzheimer’s disease emerged as a clinico-pathological entity, defined conceptually by core pathological features thought to cause a relatively broad set of clinical symptoms which vary significantly between individuals [282].

The constituent proteins making up the two cardinal structures described by Alzheimer were identified and sequenced in the 1980s. Amyloid plaques were found to contain a small novel peptide, aβ, and the PHFs in neurofibrils were shown to contain p-tau [283–286]. By then, it was well established that these features were spatially distributed throughout the brain in patterns that were highly conserved between individuals, and which appeared to progress with both age and cognitive decline. In the following decade, neuropathological staging systems were developed to define the spatiotemporal patterns of both aβ and p-tau pathology.

1.4.5.1.1 Amyloid-beta

APP is produced in the neuronal cytoplasm and transported along axons to synaptic terminals [287]. APP appears to promote neuronal survival, growth, motility, and neurite

formation. Its effects are largely mediated by multi-step cleavage pathways involving α- and β-secretases, releasing soluble ectodomains (APPsα or APPsβ, respectively). APPsα appears to support neuronal survival and growth, while APPsβ is associated with neuronal apoptosis. Residual portions of APP may then be cleaved again by γ-secretase. When γ-secretase acts after α-secretase, the p3 peptide is produced, and when γ-secretase acts after β-secretase, soluble aβ peptides (aβ40 and aβ42) are produced. Both aβ isoforms have a propensity to polymerise from monomers into oligomers, fibrils, and ultimately aβ plaques [288]. When aβ40 coalesces it forms diffuse plaques, which are seen after TBI and in ageing. Beyond parenchymal deposition, aβ40 also accumulates within the walls of capillaries, arterioles, and arteries of the leptomeninges, in a condition known as cerebral amyloid angiopathy (CAA) [289]. CAA is seen in up to 90% of AD cases, and when severe can produce ischaemic and haemorrhagic insults [290]. Soluble aβ42 has beneficial neurotrophic effects and modulates synaptic plasticity, but when aβ42 aggregates it forms dense core plaques, which are often associated with tau-positive dystrophic neurites, and are therefore referred to as neuritic plaques (NPs) [291]. What governs the balance between soluble and aggregated aβ42 remains unclear, but catalysts of aggregation include metal ion dysregulation and oxidative stress, as well as inflammatory cytokines, such as S100A9, and microbes, such as herpes simplex virus 1, which both offer potential links between TBI and the development of AD [292–295].

The distribution of NPs in AD appears to progress in phases, beginning with widespread deposits throughout the neocortex (mainly layers II, III, and V) before affecting the allocortex, subcortical nuclei, brainstem, and lastly the cerebellum, as described by Braak and Braak and later by Thal (**Figure 1.7.A**) [291]. In areas with a high density of plaques, other forms of aβ deposition can be seen, including sub-pial

bands, fleecy amyloid, and lake-like amyloid [296]. However, quantitative studies show that neither the overall extent of aβ deposition nor the density of diffuse plaques consistently correlate with the severity of cognitive impairment in AD, while associations between cognition and NP density have been mixed [297,298]. To date, aβ burden alone cannot explain disease progression in AD, prompting investigation into its role in the broader context of neurodegeneration and its relationship with tau pathology.

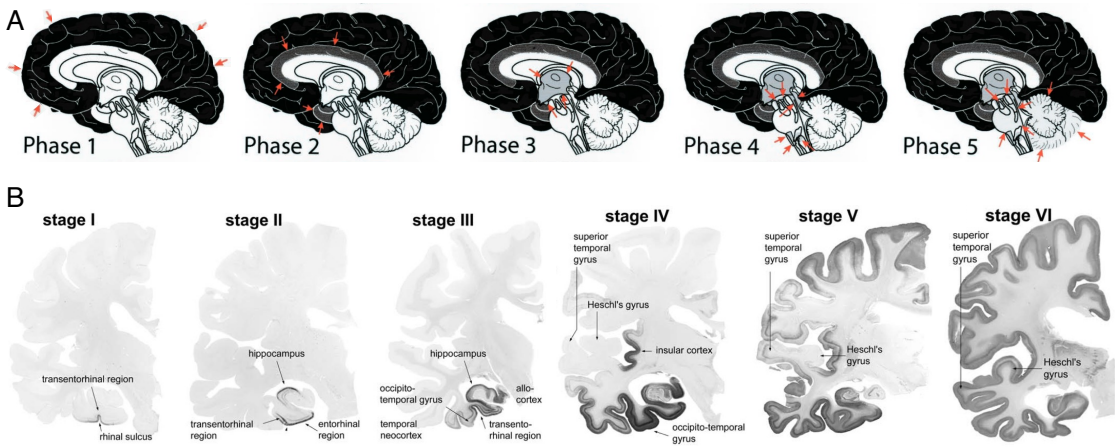

Figure 1.7 Stage-dependent pathology of Alzheimer's disease (AD). A. Thal phases of amyloid beta (aβ) deposition in AD: aβ deposits are widespread throughout the neocortex (phase 1), before sequentially affecting allocortex (phase 2), thalamus (phase 3), brainstem (phase 4), and cerebellum (phase 5). Adapted from Thal et al. 2002 [291]. **B.** Braak stages of hyperphosphorylated tau (p-tau) pathology in AD: NFTs develop in entorhinal (stage I) and hippocampal (stage II) regions before affecting temporal neocortex (stages III–IV) and association cortex (stages V–VI). Adapted from Braak et al. (2006) [265].

1.4.5.1.2 Hyperphosphorylated tau

Tau (tubulin associated unit) proteins are predominantly found in neuronal axons where they promote the assembly and stability of microtubules. Tau exists in six isoforms, each containing either three or four microtubule-binding domains (3- or 4-repeat, 3R/4R tau) and multiple phosphorylation sites on serine or threonine residues. In AD, hyperphosphorylation of tau (p-tau) reduces its propensity to bind microtubules and promotes its aggregation into insoluble complexes organised in PHFs [299]. PHFs

accumulate within the neuronal cytoplasm to form NFTs, as well as axonal and dendritic components, known as neuropil threads (NTs), which are pathological hallmarks of AD [265,300]. NFTs develop from pre-tangles into mature tangles, and can persist beyond the death of the neuron in which they formed, producing ghost tangles [301]. P-tau oligomers are particularly neurotoxic and the formation of mature NFTs is associated with microtubule disassembly, impaired axonal transport, and synaptic dysfunction [302]. The precise roles of p-tau and NFTs in AD pathogenesis remain unclear, but emerging evidence in mouse models has recently shown that the formation of NFTs may in fact protect against cell death, suggesting that non-fibrillar p-tau oligomers may be the main cause of deleterious effects [303]. This would be in-keeping with the early observation that neuronal loss correlates with, but greatly exceeds, NFT formation in AD [304].

Like aβ plaques, the distribution of NFTs in AD appears to progress in stages, appearing first in the entorhinal cortex (EC) of the mesial temporal lobe (MTL) before accumulating in the hippocampus, temporal neocortex, and AC (primarily layers III and V), defined by Braak and Braak (**Figure 1.7.B**) [300,305,265]. This staging appears to be largely symmetrical between the two cerebral hemispheres, although asymmetrical tau pathology can be seen in early-onset and atypical forms of AD, such as primary progressive aphasia [306–309]. Unlike aβ, the extent of p-tau pathology correlates closely with neuronal loss and the severity of cognitive impairment, and the regions affected align with the clinical features of atypical AD variants [310,298,297,304,311–313].

1.4.5.2 Re-defining Alzheimer's with biomarkers and the path to disease-modifying treatments

Today, the emergence of biomarkers detectable during life is transforming AD from a clinico-pathological entity to a biologically defined disease. Illustrating this transition, the most recent criteria for AD diagnosis and staging combine clinical assessment of cognition and function with biomarkers reflecting core neuropathological features (e.g. plasma markers of aβ and p-tau) and the degree of neurodegeneration and inflammation (e.g. plasma neurofilament light chain and glial fibrillary acid protein, respectively) [314]. This evolution is enabling diagnosis based on underlying biology to be made during life, often prior to the development of symptoms, which has in turn created a therapeutic window for the use of disease-modifying treatments. At the same time, biomarkers provide an opportunity to define new sub-types of AD based primarily on molecular abnormalities, which may then be amenable to specific therapies. The has led some to suggest that it will soon be more accurate to refer to Alzheimer's diseases, *plural* [315].

The need for such a radical approach is highlighted by the disappointing effects of the first wave of AD disease-modifying treatments, which target its core neuropathological features. More than 20 monoclonal antibodies designed to bind aβ monomers, oligomers, fibrils, and plaques (including bapineuzumab, solanuzemab, crenezumab) failed to slow cognitive impairment in phase 2 or 3 clinical trials, despite effectively clearing their targets from the extracellular space [316–319]. The latest versions of these treatments (donanemab and lecanemab) were associated with a moderate reduction in cognitive decline, although this amounted to a small absolute change and produced significant adverse effects [320–322]. As such, these challenges have highlighted our limited understanding of AD pathophysiology and its heterogeneity.

Consequently, attention is now focused on formulating a more complete model, or models, of AD pathogenesis, combining altered protein metabolism with neurodegeneration, neuroinflammation, neurovascular disruption, and neuronal network dysfunction.

1.4.5.3 Reconfiguring the 'amyloid cascade' hypothesis

The fact that the first genetic studies of familial AD (fAD) converged on the production of aβ lent support to the primacy of this molecule in the model AD pathogenesis, despite this form of AD accounting for less than 5% of all cases. In what became known as the 'amyloid cascade hypothesis', AD was formulated as a proteinopathy, wherein aβ plaques promote the formation of NFTs, neuronal loss, and vascular damage [323]. However, there are now a number of significant challenges to this model. As discussed above: i) the extent of aβ plaques in people with AD does not correlate with the degree of neuronal loss or the severity of cognitive decline, ii) a significant proportion of older people have aβ plaques without cognitive impairment, and iii) removing aβ plaques does not significantly slow cognitive decline [297,298,324,325,320]. In 2006, a widely-cited study claimed for the first time that administering a form of aβ (aβ*56) in mice was associated with memory impairment; however, this article was later retracted and the findings has not been replicated [326].

More recently, an updated hypothesis has been advanced, which emphasises the harm caused by the depletion of soluble aβ42, rather than the presence of aβ plaques [327,328]. This proteinopaenia hypothesis is supported by the findings that high levels of aβ42 in the cerebrospinal fluid (CSF) predict normal cognition in amyloid-positive individuals with fAD and were also associated with slower cognitive decline in a meta-analysis of anti-aβ drug trial data [329,328,330]. As such, it may be that the depletion

soluble aβ42 is deleterious, while the formation of aβ plaques merely coincides with this process. Further research into this hypothesis is warranted, but in the meantime, aberrations of aβ processing are best understood as being necessary, but not sufficient, early steps in the pathogenesis of AD, with a wide range of potential downstream effects which themselves mediate the extent and nature of cognitive impairment more directly [331,332].

Among these downstream mechanisms, neuroinflammation and the formation and spread of tau pathology attract attention. Aβ peptides can activate microglia, leading to chronic inflammation which may then contribute to neuronal death and synaptic loss [333–336]. Aβ also promotes the phosphorylation of tau proteins by activating kinases such as glycogen synthase kinase-3 beta and cyclin-dependent kinase 5, thereby inducing the accumulation of p-tau and the formation of NFTs. This relationship is thought to underlie the proximity of cored plaques, dystrophic neurites, and NFTs in layers III and V of the cortex. In the MTL, Aβ oligomers can mediate long-term depression of synaptic connections to drive tau hyperphosphorylation, but this pathway can also be activated by other means of chemical or electrophysiological stimulation [337]. Aβ oligomers may also promote tau phosphorylation through glutamate-mediated long-term depression in the MTL [337]. Importantly, NFTs can develop independently of aβ plaques in the MTL, as described in primary age-related tauopathy (PART), although this is extremely common among older individuals and associated with a slower rate of cognitive decline than in AD or mild cognitive impairment [338]. Outside of the MTL, a significant burden of aβ pathology appears to be necessary for NFTs to accumulate [339,340]. Recent studies show that aβ can trigger synaptic hyperactivity and promote neuronal hyperconnectivity, potentially facilitating the spread of p-tau according to the spatiotemporal pattern defined by Braak and Braak [341–

343]. This finding suggests a crucial pathophysiological link between aβ and the means by which tau pathology is thought to spread through the brain.

1.4.5.4 The ‘tau propagation’ hypothesis

Although the post-mortem studies underpinning Braak staging of p-tau pathology in AD were cross-sectional, the shifting pathological patterns and increasing clinical severity between cases implied a dynamic process. This prompted further investigation into the potential role of p-tau propagation in the spread of NFTs throughout the brain. Subsequently, *in vitro* and animal studies have shown that tau proteins can be transported along axons and across synapses [344–349]. In transgenic mouse models, p-tau in the EC gradually accumulates in anatomically connected regions [350–352]. Transection of these connections prevents the accumulation of p-tau in these regions [353]. Furthermore, intracerebral injection of p-tau leads to accumulation in regions that are connected to the site of injection [354,355]. Interestingly, the uptake and release of tau was shown to depend on neuronal activity [356,357]. Together, these studies indicate that the progression of NFTs in AD may occur by the propagation of p-tau between connected neurons in a manner that is dependent on their signalling activity [349,358]. This is known as the tau propagation hypothesis.

Alongside the cell and animal models discussed above, a small number of descriptive post-mortem studies have shown findings that support the tau propagation hypothesis [359]. Esiri et al. observed that in AD, NFTs affect the AC and the cingulate cortex, which both have strong anatomical connections to the MTL, while sparing the primary motor and sensory areas, which do not [360,361]. It was also noted that NFTs in the AC appear in register within layers III and V. These layers are the major source of cortico-cortical and subcortical projection neurons. At around the same time, other groups reported that NFTs in the MTL predominantly affect neurons that connect the

EC and hippocampus with the AC [362]. Esiri et al. also observed that NFTs were arranged in clusters, which is in-keeping with the involvement of specific neurons depending on their immediate microenvironment, including the presence of NPs. More recently, interrogation of the subcortical distribution and subcellular localisation of p-tau in human post-mortem brain tissue using electron microscopy has revealed appearance within pre-synaptic terminals of glutamatergic neurons connecting the mammillary bodies with the antero-dorsal nucleus of the thalamus, which is itself strongly connected to the EC [363]. Thus, collectively, these studies lend support to the tau propagation hypothesis. However, comparative studies using human post-mortem brain tissue from individuals with contrasting exposures to test this hypothesis are lacking. This gap is addressed in **Chapter 4**, using post-mortem brain tissue from the MHHI cohort to assess the effect of penetrating brain injuries that disconnect the MTL and AC on the extent of distribution of p-tau pathology in the human brain.

1.5 The Military Hospital for Head Injuries cohort

In this section, I introduce the MHHI cohort and highlight the unique opportunities it offers to address key knowledge gaps outlined in the previous sections, spanning long-term epidemiological, neuropsychological, and neuropathological outcomes of TBI.

1.5.1 Establishment and operation of the Military Hospital for Head Injuries

In the years leading up to the Second World War, or World War II (WWII), it was recognised that head injuries, including TBI, were likely to be a significant source of casualties [364]. Accordingly, prominent neurosurgeons and neurologists were tasked with delivering a national programme of advanced head injury assessment, investigation, treatment, rehabilitation, and follow-up [365,366]. In February 1940, the

MHHI opened in St Hugh's College, Oxford under the leadership of Hugh Cairns and Charles Symonds (**Figure 1.8**) [367]. Over the course of the war, it is estimated that more than 13,000 military personnel were admitted to the MHHI following TBIs sustained in the battlefield or during non-combat activities including training, transportation, and recreation.

Emergency treatment in the battlefield was delivered by a network of nine mobile neurosurgical units (MNSUs), working alongside military field hospitals (**Figure 1.8.A**) [368]. Military personnel who survived the immediate phase after injury were transferred by land, sea, and air from across Europe, Africa, and Asia to the MHHI for further assessment and treatment. Criteria for admission included persistent symptoms, signs, or other evidence of brain dysfunction after head injury (**Appendix 1.2**). Individuals with chronic symptoms attributed to TBI were also referred from other hospitals. The acuity of cases admitted to the MHHI therefore ranged from those transferred within days of injury to those referred months or even years later due to unsatisfactory progress [75,369].

1.5.2 Assessment and investigation at the Military Hospital for Head Injuries

Clinical assessments at the MHHI were performed in a standardised manner and undertaken repeatedly to guide management and identify the first signs of deterioration that might herald complications such as intracranial haemorrhage or meningitis.[2] Medical history taking, neurological examination, and neuropsychological assessment

[2] 'Form 2151', The National Archives (TNA) FD 1/5342.

were all performed according to a routine scheme (**Appendix 1.3**). Even the language used to describe psychiatric and cognitive sequelae of brain injury was standardised according to a glossary of terms (**Appendix 1.4**).

Figure 1.8 Mobile neurosurgical units (MNSUs) and staff at the Military Hospital for Head Injuries. A. Each MNSU contained the staff and equipment needed to run a neurosurgical operating theatre. Later units had space to operate inside the vehicle with a tent attached to serve as a ward. **B.** Cairns (centre) and Symonds ran the hospital with the support of Lord Nuffield (left). **C.** Patients and nurses shown outside the temporary buildings. **D.** Military, Red Cross, and civilian nurses worked together to run the hospital. Photographs reproduced by kind permission of the Principal and Fellows of St Hugh's College, Oxford.

Antero-posterior (AP) and lateral skull x-rays were acquired on admission to assess for fractures, deformities, bone fragments (BFs), and metallic foreign bodies (MFBs) (**Figure 1.9.A**).[3] Pneumoencephalograms, where CSF was replaced by air via cisternal

[3] 'Classification of x-ray cases – period 20/6/40–31/12/40', TNA WO 222/845.

puncture, were used to identify hydrocephalus and cerebral abscesses. Wound swabs and CSF were analysed by microscopy, biochemistry, and bacterial culture. White blood cell counts were reported per cubic millimetre (i.e. µL), with 0–2 cells/µL considered normal, and the presence of red cells and xanthochromia were also described [370]. Protein level was measured in milligrams per centilitre (i.e. mg/dL) with >50 mg/dL considered abnormally high. CSF pressure was recorded in millimetres of water with a normal range of 100–200 mmH_2O.

Electroencephalography (EEG) was used to investigate seizures and assess the severity of brain injury (**Figure 1.9.B–C**) [371,372]. Diagnosis of epilepsy was supported by EEG with periods of hyperpnoea used to lower the seizure threshold. Waveforms with a frequency of 8–12 Hz were considered normal, and those with lower frequences were considered indicative of seizures, although it was recognised that the EEG may be normal in epilepsy. Careful observation of seizure semiology was emphasised and recorded using structured forms to characterise the disorder and distinguish epilepsy from 'hysterical' fits (i.e. functional seizures) (**Appendix 1.3**). An EEG suggestive of cerebral trauma was defined by a) widespread abnormally slow waves with a frequency of 0.5–7 waves/second (i.e. delta or theta waves), b) suppression of the normal dominant frequencies of 8–12 waves/second (i.e. absence of normal alpha waves), or c) large amplitude discharges of 2–3 waves/second lasting up to 3 seconds (i.e. epileptiform discharges) [371,372].

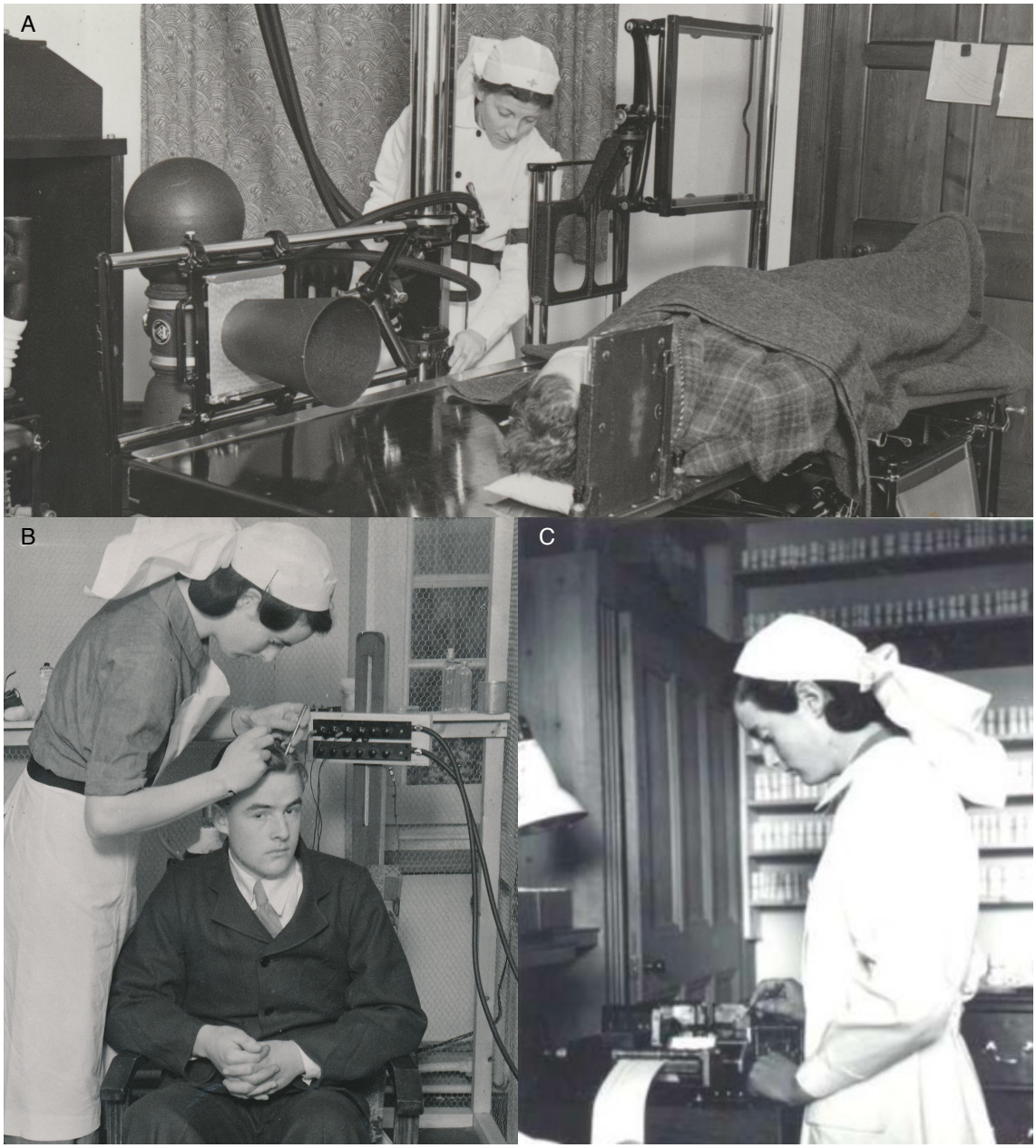

Figure 1.9 X-ray and electroencephalography (EEG) performed at the Military Hospital for Head Injuries. A. A lateral skull x-ray being acquired. **B.** EEG probes being applied to the scalp of a patient. **C.** An EEG tracing being printed. Photographs reproduced by kind permission of the Principal and Fellows of St Hugh's College, Oxford.

1.5.3 Management and rehabilitation at the Military Hospital for Head Injuries

Cases at the MHHI were classified as either closed or open head injuries according to whether the scalp was intact or broken.[4]

For closed head injuries, the aim of treatment was 'to provide for the damaged brain optimum conditions for spontaneous recovery'.[5] A detailed description of the approach to closed head injury management at the MHHI is provided in **Appendix 1.5**. Notably, temporary loss of consciousness, headache, dizziness, concentration difficulty, irritability, insomnia, and 'nervous instability' after closed head injury were attributed to a combination of 'general cerebral concussion', contusions, intracranial haemorrhage, and skull fracture [373]. It was understood that 'the brain may be severely damaged in the absence of fracture and sometimes without any external injury of importance'. At the outset of the war, symptoms not identified as 'organic' were considered to be the result of 'psychoneurosis', which included 'cases of stupor, amnesia, depression, and neurotic headache or fits'.[6] In this way, mood disorders were not distinguished from what are now considered functional neurological disorders. However, by 1942, the team at the MHHI recognised the ambiguity of this division:

> '…with increasing experience of all classes of injury from a variety of circumstances, more and more involved [sic] mixtures of organic and functional sequelae were defined by modern methods… The patient who has suffered an

[4] 'Memorandum on the Treatment of Head Injuries in War', TNA FD 1/5342.
[5] 'Memorandum on the Treatment of Head Injuries in War', TNA FD 1/5342.
[6] 'Comments on treatment and disposal', TNA WO 222/845.

apparently trivial head injury and later wanders, with loss of memory, is sometimes shown by the electroencephalogram or subsequent attacks to be suffering from epilepsy… The patient behaving in a clearly neurotic manner may be found to have a xanthochromic spinal fluid' [373].

For open head injuries, the aim of treatment was:

> 'to convert an open wound into a clean closed one at the earliest possible moment; when the brain itself is penetrated or exposed, to remove dead tissue or foreign bodies, so far as this may be possible without the affliction of further injury; in all open injuries it is advisable to operate as soon as possible, preferably within 10 hours of the injury'.[7]

The focus of neurosurgical care was early aggressive infection control achieved through meticulous wound debridement, antiseptic irrigation, and systemic antibacterial prophylaxis to prevent meningitis or abscess. More details about this approach are provided in **Appendix 1.5**. The risk of bacterial infection was substantially reduced by the use of penicillin, which was used at scale to prevent and treat meningitis for the first time at the MHHI, after being received by the first patient in Oxford in 1941 [374]. Penicillin was administered intrathecally as it had been shown not to cross the BBB, and levels in the CSF were monitored, along with protein levels, cell counts, and cultures [375].

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[7] 'Memorandum on the Treatment of Head Injuries in War', TNA FD 1/5342.

After treatment at the MHHI, individuals were either medically discharged from military service or returned to their unit. Medical discharge, or 'invalidation', was decided by a board and recorded using a structured form (**Appendix 1.3**). The invaliding board also assessed eligibility for a war pension to compensate veterans according to the extent of 'disablement' attributed to injuries or illness sustained through their service [376]. The extent of disability was quantified as a percentage, which corresponded to the degree of financial compensation, with a one-off payment provided to veterans with less than 20% disability and a monthly stipend for those exceeding this threshold. Compensation was not routinely provided for 'psychoneurosis' in the early years of the war, but this changed over the course of the conflict, and ultimately between 2–10% of pensions were awarded with this diagnostic label [377]. This included individuals with psychiatric conditions as they are recognised today, providing there was no evidence of an existing 'predisposition to mental disorder'.

Veterans with ongoing impairments were transferred to dedicated rehabilitation units. Weekly rounds of these units were performed by resident neurologists at the MHHI, including William Ritchie Russell and Derek Denny-Brown. Physiotherapy, speech therapy, and occupational therapy were the mainstays of rehabilitation, with a focus on enablement, adapting tools and machinery to reduce disability, and gaining new skills to maximise employability. The benefits of holistic care, which included dance, outdoor theatre, swimming, and boat trips to restore mental health were also recognised.[8]

[8] 'Comments on treatment and disposal', TNA WO 222/845.

1.5.4 Follow-up and longitudinal research

Of the 13,000 military personnel admitted to the MHHI, more than 3,250 cases were systematically indexed (**Figure 1.10)**. A coordinated program of follow-up was conducted to monitor and record the progress of all indexed cases, serving a dual purpose, as Russell described: 'I have here opened a Head Injury Bureau to act as both an advice bureau for those who have had head wounds, and also as a centre for research'.[9] This took place in five phases over a period spanning more than 70 years. The data collected at each phase, and how they have been utilised in this DPhil, are outlined in the remaining sections of this chapter.

[9] Russell's letter to the Chairman of the Nuffield Provincial Hospitals Trust, 17th December 1946, TNA FD 1/6137

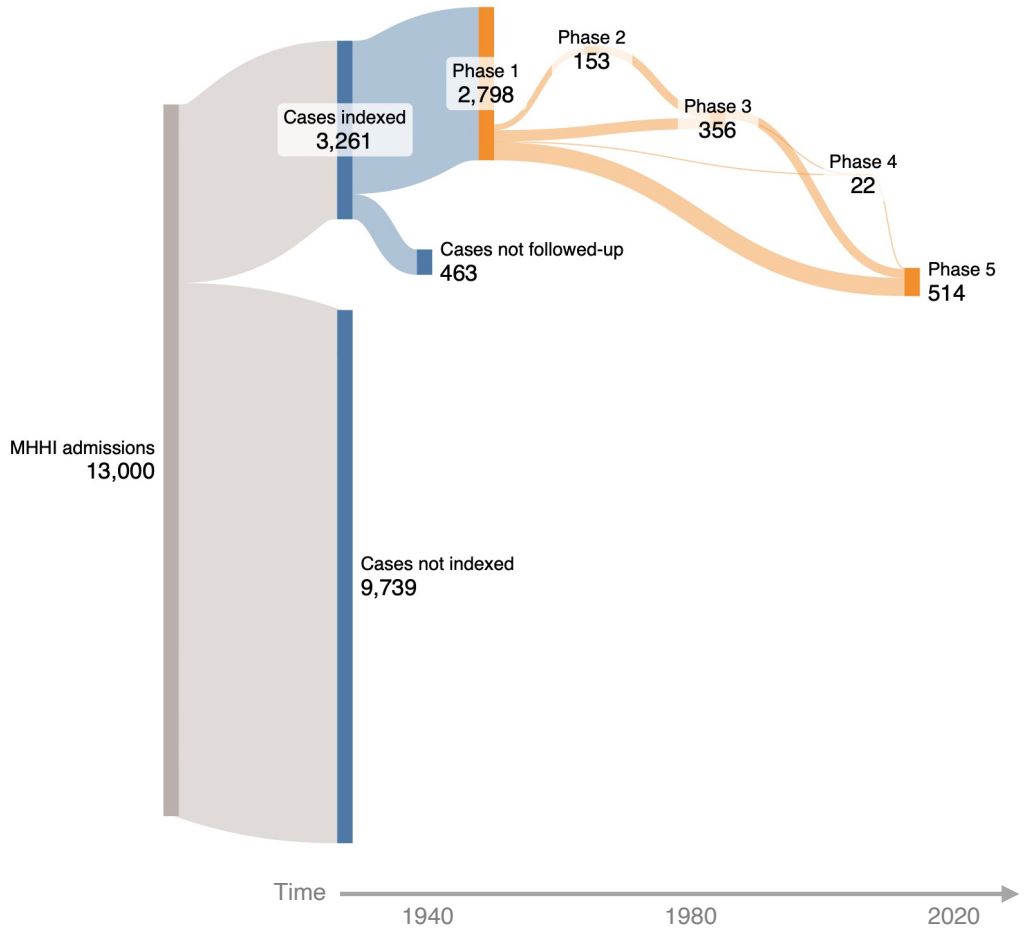

Figure 1.10 The five follow-up phases of the Military Hospitals for Head Injuries cohort.

Information describing the assessment, investigation, management, rehabilitation, and follow-up of every indexed case was recorded using custom-made punch cards (**Figure 1.11**) [378]. All cases admitted between February 1940–January 1941 were indexed, until the number of beds in the hospital expanded.[10] Subsequently (1941–45), only 'cases of special interest' were indexed; these comprised cases with: penetrating brain injury, post-traumatic epilepsy, air sinus fracture, fungal abscess, skull fracture

[10] 'Report from Oxford Military Hospital (Head Injuries), Appendix I: Headings under which material is indexed', TNA FD 1/5342

and post-traumatic amnesia lasting more than one week, extradural or subdural haematoma, and injury to the hypothalamus or brainstem.[11] In the last year of the war (1944–45), indexing prioritised the large number of cases admitted to the MHHI with penetrating brain injuries sustained during the D-Day landings of June 1944 [75]. To define the cohort of all indexed cases, from which study cohorts are drawn for the epidemiological studies reported in **Chapter 2**, the information recorded on these cards was entered into a digital database. Data processing steps are described fully in **Section 2.2**. A summary of baseline characteristics and injury details for the cohort of all indexed cases at the MHHI is presented in **Appendix 1.6**. For additional context, a review of the aetiology of TBIs during WWII is provided in **Appendix 1.7**.

1.5.4.1 Phase 1 (1940–70, *n* = 2,798): questionnaire follow-up

Follow-up was conducted by the Oxford Head Injury Bureau, which was staffed by a neurologist, psychologist, social worker, and secretary, with the support of the War Office, Air Ministry, and the Medical Research Council (MRC).[12] Structured questionnaires were posted between 1940–70 and responses were added to the punch card made for each case (**Appendix 1.8**). Details of military service, civilian employment, and pension receipts were ascertained directly from the Ministry of Pensions and added to the punch card for each case.

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[11] BIC 54, 'Report of the Sub-Committee on "Follow-Up", Category of Cases of Head Injury for "Follow-Up"', 9th November 1941, TNA FD/1 5343.
[12] Russell's letter to the Chairman of the Nuffield Provincial Hospitals Trust, 17th December 1946, TNA FD 1/6137.

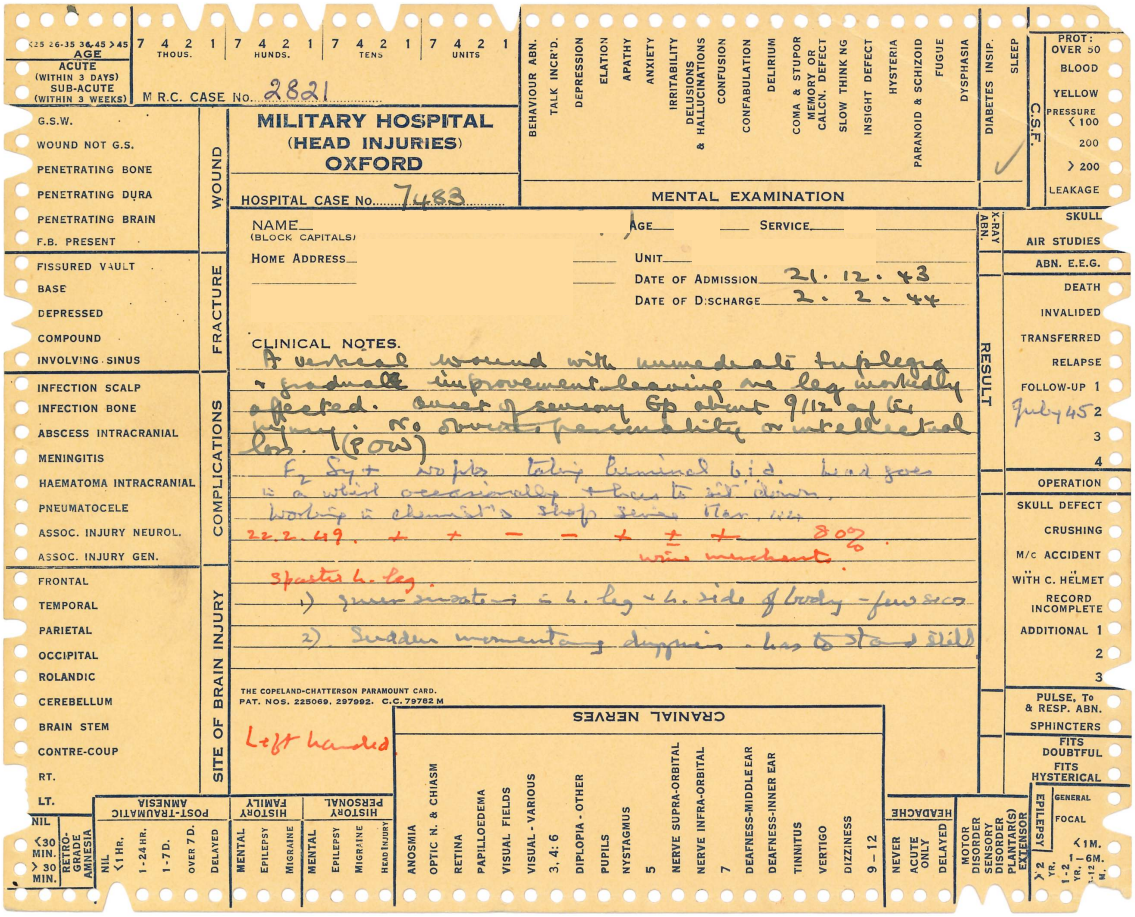

Figure 1.11 A punch card used to record details of an indexed case. This version was used to record details of all indexed cases admitted between 1940–44.

Phase 1 of follow-up proceeded in four stages that mirrored the indexing of cases at the MHHI. In the first stage (1940–41), all cases admitted consecutively to the MHHI were followed-up until the rate of cases overwhelmed the administrative capacities of the bureau [369]. The majority of these cases involved ‘closed head injuries’. These individuals constituted ‘the first thousand cases’ admitted to the MHHI and sustained their injuries during active duty, training exercises, or recreation. In the second stage

(1941–45), only ‘cases of special interest’ were followed-up.[13] In the last year of WWII (1944–45), follow-up prioritised the large number of cases admitted with ‘gunshot wounds’ sustained during the D-Day landings [75]. Finally, after WWII, the MHHI was relocated to Wheatley Military Hospital, just outside Oxford, where a small number of cases with gunshot wounds were admitted during the Korean War, up until 1953. Of these, approximately 50 were followed up.

In 1946 alone, 800 questionnaires were distributed and from the responses over 300 veterans received advice and support (e.g. information regarding entitlement to pension allowances and letters explaining their impairments to officers or employers) and more than 100 veterans were re-admitted for examination and treatment (e.g. diagnosis of latent onset post-traumatic epilepsy). This was performed in concert with the general practitioner (GP) of each veteran and often in collaboration with the British Red Cross, the British Legion, the Royal Air Force Benevolent Fund, and the National Council of Mental Health. As recalled by Daphne Martin-Hurst, who was the social worker at the bureau:

> ‘Our patients have presented many problems, and the attempt at satisfactory solution has been made possible by only the close co-operation with a medical staff which considers the social as well as the medical implications of each patient’.[14]

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[13] BIC 54, ‘Report of the Sub-Committee on “Follow-Up”, Category of Cases of Head Injury for “Follow-Up”’, 9th November 1941, TNA FD/1 5343.
[14] ‘Head Injury Bureau, Social Worker’s Report’, 18th December 1946, TNA FD 1/6137.

In this DPhil, I have used the records collected in this phase to conduct an epidemiological study of long-term morbidity outcomes after TBI, which is reported in **Chapter 2**.

1.5.4.2 Phase 2 (1963–70, *n* =153): neuropsychological assessment

In 1963, Russell appointed Freda Newcombe to lead the systematic neuropsychological assessment of the MHHI cohort and to sustain long-term follow-up [129]. Recruitment for this phase was overseen by Russell between 1963–1970. Veterans with penetrating brain injuries who participated in Phase 1 were approached via their GPs and invited by letter to attend the Churchill Hospital for residential neuropsychological assessments [190]. Veterans were selected from the cohort to reflect a range of injury severities (estimated by loss of brain tissue and depth of penetration) and to ensure a representative distribution of lesion locations (determined by operation notes and skull x-rays). However, as later described by Newcombe, this group was 'drawn from the more severe end of the continuum' of penetrating brain injuries:

> 'Russell's initial selection of cases in the early 1960s was biased towards men with a relatively high pension rate, thus reflecting more severe neurological (especially visual field) defects than those documented in the rest of the roster. This selection was expected to maximize the opportunity to study persisting, albeit selective, impairments' [129].

A total of 153 veterans completed the assessments, which covered 'a range of verbal and spatial functions' and took approximately 10 hours to administer [190]. The full list of assessments, including details of how they were administered, is provided in 'Missile Wounds of the Brain' [190].

1.5.4.3 Phase 3 (1983–87, *n* = 356): neuropsychological assessment and neuroimaging

Phase 3 was conducted in two stages. In the first stage, Newcombe invited all veterans who had participated in Phase 2 to return to Oxford for further neuropsychological assessment. This involved the repetition of certain tests from Phase 2, along with more detailed assessments of visual perception, facial recognition, and visuospatial abilities, conducted in the Neuropsychology Unit at the Radcliffe Infirmary [129]. Assessments were administered by a team of investigators, including Edward de Haan and Ziyah Mehta, with full details published in studies from that period [197,200,199]. Computed tomography (CT) head scans were also performed during these visits. Anatomical drawings were made by Hanna Damasio from the CT radiographs using standard templates to aid lesion localisation [231]. In total, 137 veterans completed the first stage of Phase 3.

In the second stage of Phase 3, a further series of postal questionnaires was completed by 356 veterans with penetrating brain injuries. This group included 153 veterans who also participated in the residential assessments, 203 who had not been assessed since discharge from the MHHI, and 36 age- and sex-matched controls with peripheral combat injuries [129]. The questionnaires collected details about general health and included self-reported measures of cognition (Broadbent's Cognitive Failures Questionnaire) [379], mental health (Goldberg's General Health Questionnaire–30) [380], and personality traits (Brooks' Personal Description Questionnaire) [381]. I have used the assessments performed during Phases 2 and 3 to perform neuropsychological studies of long-term outcomes after TBI, which are reported in **Chapter 3**.

1.5.4.4 Phase 4 (1978–2012, *n* = 22): post-mortem brain donation

In Phase 4, veterans who had survived for decades after penetrating brain injuries were invited to donate their brains for post-mortem research. This phase was coordinated by Freda Newcombe and Margaret Esiri, a neuropathologist at the Radcliffe Infirmary and later the John Radcliffe Hospital. Veterans with persistent, specific neuropsychological impairments were approached, resulting in 22 brain donations over approximately 30 years. Professor Esiri performed post-mortem examinations, produced diagnostic reports, took anatomical photographs, and prepared tissue specimens for research. The first case was described in a clinical and anatomical report published in 1987, which linked left hemisphere injury to dysphasia, dyslexia, dysgraphia, and verbal memory impairment [206]. Details of the remaining cases were not published. In this DPhil, I have used this tissue to conduct a neuropathological study of long-term outcomes after TBI in relation to AD, which is reported in **Chapter 4**.

1.5.4.5 Phase 5 (1995–2003, *n* = 514): death registration

In the final phase, death certificates were collected to record the age and cause of death of MHHI veterans with penetrating brain injuries. Study cards containing the personal identifiers of indexed cases were sent to the Office for National Statistics (ONS) and National Health Service Central Registry (NHSCR), who matched the case details with death registration records and returned death certificate summaries to the original investigators. The deaths of MHHI veterans were reported to Newcombe from 1995 until her death in 2001, and subsequently to her colleague, Andy Young. A total of 514 records were collected up to 2003, at which point it was clear that a large proportion of the cohort was still alive. Data collection did not continue, and analysis of

mortality data was not performed. In **Chapter 2** of this thesis, I have used these records to study long-term mortality outcomes after TBI.

1.5.5 Thesis aims and rationale

This thesis has three overarching aims that will be achieved through three separate studies using a combination of clinical records and longitudinal follow-up details, neuropsychological assessments and neuroimaging, and post-mortem brain tissue collected from the MHHI cohort.

1.5.6 Aim 1: Epidemiological study

TBI poses a significant global health challenge due to its high prevalence, wide range of potential long-term outcomes, and substantial contributions to morbidity, disability, and mortality [3,12]. Each year in England and Wales, more than 200,000 people are admitted to hospital with a recent head injury [7]. Between 5–12% of these cases involve open traumatic brain injury (OTBI), which accounts for an even higher proportion of military injuries [29,30]. Yet relatively little is known about the long-term outcomes of OTBI because opportunities to study large cohorts of survivors are limited [24]. To the best of our knowledge, the MHHI cohort constitutes the second largest OTBI cohort, after the VHIS, and therefore provides a valuable opportunity to address this gap [77]. During WWII, more than 3,000 cases were indexed at the MHHI, including approximately 1,000 cases of OTBI. The clinical and research records of these cases have been preserved in the Head Hospital Archive (HHA) at St Hugh's College, Oxford. Therefore, **Aim 1** of this thesis is to investigate long-term morbidity and mortality outcomes following OTBI using this resource.

To meet this aim, a retrospective cohort study using data from the MHHI cohort was performed to compare long-term neurological, psychiatric, and cognitive outcomes after OTBI and CTBI. This study is presented in **Chapter 2**.

1.5.7 Aim 2: Neuropsychological study

The study of individuals who survive focal brain damage has been a cornerstone of neuroscience for more than 150 years, providing valuable insights into the functional consequences of damage to specific brain regions. Today, while functional neuroimaging has become the mainstay for investigating structure-function relationships in the brain, lesion studies still play a vital complementary role [214,211]. Most lesion studies involve individuals with brain damage caused by ischaemic stroke. But the study of individuals with penetrating brain injuries retains unique advantages, including clearly delineated lesions sustained by an otherwise healthy cohort with a spatial distribution that is not restricted by vascular territories. Consequently, neuropsychological and neuroimaging data collected from the MHHI cohort are of great scientific interest. However, utilising these archival data presents a unique set of challenges. Most significantly, CT head radiographs acquired from the cohort were printed on film. This prohibited the digital processing required to perform LSM, which has been widely used to perform lesion studies for the last 30 years [230,231]. While early pioneers of LSM developed techniques to transfer film-based CT radiographs into digital programs, to the best of our knowledge, these methods have not been updated to enable the use of film-based CT head radiographs for modern neuroimaging analysis. Therefore, **Aim 2** of this thesis is to enable the use of these archival resources for the investigation of structure-function relationships in the human brain.

First, a novel digital lesion reconstruction method for film-based CT head radiographs was structurally and functionally validated. Then, this method was applied to perform two human brain lesion studies using cases from the MHHI. These studies are presented in **Chapter 3**.

1.5.8 Aim 3: Neuropathological study

Epidemiological links between TBI and dementia, particularly AD, have been consistently demonstrated, but the pathophysiological relationships between brain injury and neurodegeneration remain unclear. In part, this is because the understanding of AD pathogenesis remains incomplete. Post-mortem brain tissue donated by veterans of the MHHI provides a unique opportunity to address a question that is central to this issue. AD is characterised by aβ plaques and NFTs composed of p-tau [251–254]. The distribution of NFTs appears to progress in stages, appearing first in the MTL before accumulating in the AC [300,305,265]. Unlike aβ, the extent of p-tau pathology is closely associated with the severity of cognitive impairment and neuronal loss, and the regions affected appear to match specific cognitive impairments, suggesting that p-tau plays a direct role in AD pathogenesis [310,298,297,304,311–313]. A growing body of evidence from cell and animal models indicates that the progression of NFTs in AD reflects the propagation of p-tau between connected neurons in a manner that is dependent on their signalling activity [349,358]. A small number of descriptive human post-mortem studies also support the tau propagation hypothesis [359]. However, comparative studies using human post-mortem brain tissue to test this hypothesis are lacking. Therefore, **Aim 3** of this thesis is to investigate the pathogenesis of AD. To do this, post-mortem brain tissue from veterans who survived for decades after unilateral penetrating brain injuries was used to evaluate the effect of these injuries on the distribution of NFTs and aβ. This study is presented in **Chapter 4**.

2 Epidemiological study

In this chapter, I present a study in which I used epidemiological methods to investigate long-term morbidity and mortality outcomes following OTBI.

2.1 Introduction

In **Section 2.1.1**, I summarise the epidemiological relationships between OTBI and relevant long-term outcomes (**Figure 2.1**). In **Section 2.1.2**, I present the study rationale, followed by the aim, objectives, and hypotheses in **Section 2.1.3**.

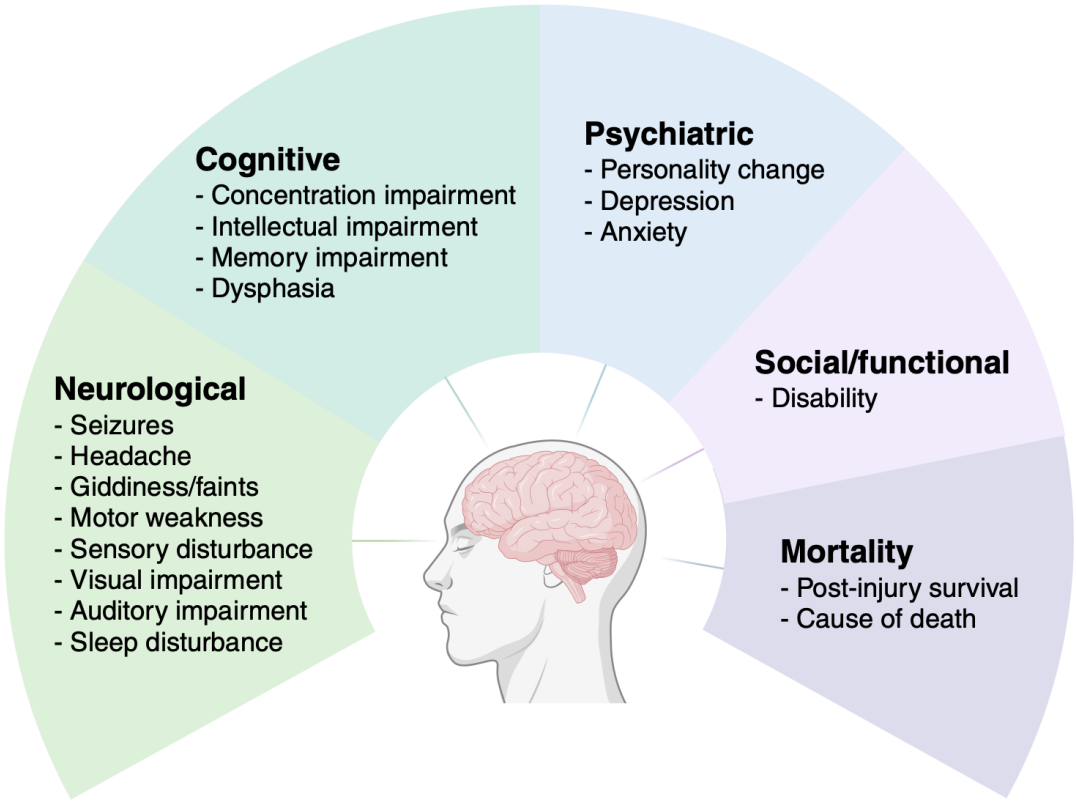


Figure 2.1 Long-term morbidity and mortality outcomes that will be explored in Chapter 2. Created with BioRender.com.

2.1.1 Background summary

Adverse neurological outcomes in the longer term after OTBI are often characterised by focal impairments, including motor weakness, sensory disturbance, and visual loss.

Notably, sensorimotor deficits were observed in nearly half of veterans in the VHIS, with substantial functional implications limiting employment and daily activities [86]. Additionally, OTBI significantly elevates the risk of seizures, with prevalence reaching approximately 50% within 15 years post-injury [382]. Post-traumatic headache is a common and debilitating complication of CTBI, but the relationship between OTBI and headache, by contrast, is relatively under-explored.

The study of cognitive outcomes after OTBI has tended to focus on either the focal impairments that result from damage to specific brain regions or the association between brain injury and accelerated cognitive decline. However, the extent to which OTBI is associated with more generalised impairments to concentration and memory at the group level is unclear [77,129]. Similarly, little is known about the impact of OTBI on psychiatric outcomes, beyond the effects of damage to specific brain regions and, in particular, the pre-frontal cortex [77]. Comparing the extent of these outcomes after OTBI and CTBI may shed light on the pathogenesis of cognitive and psychiatric conditions after brain injury.

These adverse neurological, cognitive, and psychiatric outcomes after OTBI can conspire to significantly impact social outcomes, frequently hindering daily functioning and employment through disability. In the VHIS, unemployment after OTBI was associated with seizures, sensorimotor and visual impairment, mood disturbance, anti-social or violent behaviour, visual and verbal memory loss, and impaired attention [82]. Further insights into early predictors of long-term disability can support the anticipation and mitigation of the most significant outcomes.

Mortality outcomes following OTBI are also poorly understood compared to CTBI, which is thought to reduce life expectancy by up to seven years and increase mortality

from neurological conditions, such as seizures [159–161]. OTBI may pose distinct risks, such as formation of TICAs, which form after cerebral vessel injury and increase the risk of haemorrhagic stroke [166].

2.1.2 Rationale for the proposed study

OTBIs account for a significant minority of all hospitalised TBI cases, but as these cases occur sporadically, opportunities to conduct longitudinal studies involving large groups who survive these injuries are limited. As a result, the long-term neurological, cognitive, psychiatric, and social outcomes of OTBI remain unclear [65]. Reflecting this lack of evidence, strategies for the prevention and management of adverse long-term outcomes after OTBI do not form part of current clinical guidelines [65]. In 2022, the Brain Trauma Foundation emphasised the urgent need for more research into the long-term effects of OTBI in order to update management guidelines [24]. For example, studies of small OTBI cohorts have shown that functional recovery continues for at least two years after injury and can be predicted by GCS at presentation, supporting an early and sustained role for neurorehabilitation [383].

To address this gap, I have utilised data collected from the MHHI cohort to compare long-term outcomes between two large and well-matched groups exposed to either OTBI or CTBI in a retrospective cohort study. Describing the prevalence of these outcomes will strengthen our understanding of the long-term impacts of these injuries. Identifying predictors of selected outcomes may help to formulate strategies to anticipate and mitigate these adverse effects. Moreover, comparing these outcomes after exposure to open and closed brain injuries will offer insights into the pathophysiological differences between OTBI and CTBI, which may, in turn, inform our approach to the management of both conditions. In light of the research gaps

described above, the aim, objective, and hypotheses for this study have been formulated as follows.

2.1.3 Aim, objectives, and hypotheses

Aim: Investigate long-term morbidity and mortality outcomes following OTBI.

Objectives:

1. Characterise the MHHI cohort.
2. Conduct an analytic cohort study to i) describe the prevalence of long-term neurological, psychiatric, and cognitive outcomes after OTBI, ii) compare the incidence of these outcomes after OTBI and CTBI, iii) identify early predictors of seizures, headache, and disability in the subgroup exposed to OTBI, and iv) determine which long-term outcomes were associated with disability after OTBI.
3. Perform a descriptive cohort study to explore mortality outcomes up to 60 years after OTBI.

Hypotheses:

A. The rates of outcomes that are traditionally associated with focal brain damage (i.e. seizures, sensorimotor impairment, visual impairment, and dysphasia) will be higher after OTBI compared to CTBI; while the rates of other outcomes (i.e. headache, giddiness/faints, auditory impairment, sleep disturbance, other cognitive outcomes, and psychiatric outcomes) will be equivalent.
B. Predictors of seizures after OTBI will include lesion depth, injury severity, focal neurological impairment, retained metallic foreign bodies, and intracranial haematoma, but not retained bone fragments.
C. Predictors of headache after OTBI will not include injury severity.

D. Predictors of disability after OTBI will include lesion depth and focal neurological impairment.

2.2 Materials and methods

2.2.1 Analytic cohort study or morbidity outcomes

2.2.1.1 Study design

A retrospective analytic cohort study of long-term neurological, psychiatric, cognitive, and disability outcomes was performed among veterans exposed to OTBI and CTBI during WWII.

2.2.1.2 Setting and participants

This study used archived records of indexed cases admitted to the MHHI between 01/01/1940–31/12/1945. The details of this setting are described in **Section 1.2**, including admissions criteria for the MHHI (**Appendix 1.2**). Eligible participants were male adults (≥18 years) admitted within three months of a TBI who completed at least one follow-up questionnaire after discharge. This threshold was applied to select acute TBI cases, excluding admissions for the evaluation of persistent symptoms, and was set at three months to align with current definitions of post-concussion syndrome and persistent post-traumatic headache [89,384]. Given that the criteria for indexing admissions to the MHHI changed throughout WWII (as described in **Section 1.5.3**), only consecutive admissions were included in this study in order to minimise selection bias. Exclusion criteria were therefore: female sex, age at admission <18 years or unknown, admission date after 31/12/1945 or unknown, time from injury to admission ≥3 months or unknown, no response to follow-up after discharge or date of response unknown, or indexing outside of stages 1 and 3 of follow-up phase 1. Baseline characteristics of eligible participants admitted more or less than three months after

TBI and with and without follow-up were compared to explore the effects of these criteria.

2.2.1.3 Variables

For comparative analyses the exposure variable was OTBI, and the comparator variable was CTBI. OTBI was defined by perforation of the dura. For comparative analysis, outcome variables were symptoms reported in at least one follow-up questionnaire after discharge, which were recorded using standard terms (**Table A.2.1**). Neurological outcome variables were seizures, headache, giddiness/faints, motor weakness, somatosensory disturbance, visual impairment, auditory impairment, and sleep disturbance. Cognitive outcomes were dysphasia, memory impairment, intellectual impairment, and concentration impairment. Psychiatric outcomes were anxiety, depression, and personality change. Given that dysphasia would limit the ability to report other cognitive or psychiatric symptoms, these were only assessed in cases without language impairment. For predictive analyses within the subgroup exposed to OTBI, outcomes were seizures and headache reported in follow-up questionnaires, and the extent of disability as determined by a military invaliding board who recommended the degree of financial compensation to be provided through war pensions.

Co-variates comprised baseline characteristics, military details, lesion depth, indicators of injury severity, early signs and symptoms, abnormal investigations, complications, discharge status, and relevant time intervals. Baseline characteristics, including age at admission and personal or family history of seizures or headache, were routinely collected on admission to the MHHI along with military details including rank and division. Lesion depth was measured at neurosurgical operation. Various measures of

clinical injury severity were available, including conscious level on admission, duration of both PTA and retrograde amnesia (RA), and presence of associated injuries. Of these, PTA duration was selected as the most objective and reliable indicator of injury severity. Details of the complete neurological examination were also available, from which dysphasia (expressive or receptive), sensorimotor impairment of the limbs, visual impairment (i.e. blindness, visual field impairment, diplopia, extraocular muscle palsy, or papilloedema), auditory impairment (deafness or tinnitus), and vestibular impairment (vertigo) were selected as the most salient early signs and symptoms.

EEG and lumbar puncture were routinely performed in the investigation of TBI at the MHHI. Abnormal EEG was defined by widespread delta or theta wave activity (0.5–7 Hz), absence of normal alpha waves (8–12 Hz), or paroxysmal epileptiform discharges (2–3 Hz for up to 3 seconds) [371,372]. CSF underwent microscopy and biochemistry to identify abnormally elevated white blood cell counts (>2 cells/μL) and protein levels (>50 mg/dL) [370]. Complications included infection of the brain or meninges (diagnosed by raised CSF white cell counts and positive bacterial cultures), retained metallic foreign bodies or bone fragments (identified using AP and lateral skull x-rays), intracranial haematoma (diagnosed by neurosurgical exploration or bloodstained CSF), and neuroendocrine dysfunction (defined by significant polyuria).[15] At discharge from the MHHI, individuals were either medically discharged from military service ('invalided') or returned to their unit. Dates of injury, admission, and discharge were routinely recorded, enabling the calculation of relevant time intervals. Due to a

[15] BIC 54, 'Report of the Sub-Committee on "Follow-Up", Category of Cases of Head Injury for "Follow-Up"', 9th November 1941, TNA FD/1 5343.

significant proportion of missing values for date of discharge (9% missing), follow-up duration was defined as the interval from date of injury to the date of the last questionnaire returned, which both had no missing values. From this range of co-variates, predictors and confounders of each outcome were pre-specified, and supplementary predictors and effect mediators were identified through exploration of statistical associations (see **Section 2.2.1.8**)

2.2.1.4 Data sources

Data describing all variables were collected from punch cards that were created during the follow-up period and accessed from the Archive of St Hugh's College, Oxford for the purpose of this study [75]. Originally, clinical information was ascertained from routine assessments performed by medical staff at the MHHI according to a systematic evaluation scheme (**Appendix 1.3**) and documented using agreed terminology (**Appendix 1.4**) to produce standardised case records (detailed in **Section 1.5.1**) [378]. These records were then transcribed, indexed, and checked by senior neurologists at the MHHI (Charles Symonds and Ritchie Russell), who then recorded key details in a punch card for each case.[16]

Data describing long-term outcomes were gathered from individuals after discharge from the MHHI using structured follow-up questionnaires sent by post between 1940–69 (described in **Section 1.5.3.1**).[17] Individuals who were discharged from military

[16] BIC 61a, 'Analysis of first thousand cases of head injury admitted to the Military Hospital for Head Injuries', 9th November 1941, TNA FD/1 5343.
[17] Russell's letter to the Chairman of the Nuffield Provincial Hospitals Trust, 17th December 1946, TNA FD 1/6137.

service by a medical board were contacted via the Ministry of Pensions and completed the questionnaire themselves with the help of their next-of-kin if required (**Appendix 1.8**). Those who returned to military service were contacted via their Unit Medical Officer who completed the questionnaire on their behalf (**Appendix 1.8**). Responses were added to the punch card for each case, along with the extent of disability, which was acquired directly from the Ministry of Pensions for both groups.

2.2.1.5 Confounders

Medical invalidation determined who completed the follow-up questionnaire and may therefore have influenced how conditions were reported. Consequently, discharge status was pre-specified as a confounder of both seizures and headache. A personal or family history of seizures or headache were also pre-specified confounders of these outcomes. Age at admission was pre-specified as a confounder of seizures, headache, and disability.

2.2.1.6 Data processing

Punch cards contained data in the form of labelled punch holes, dates, and handwritten text. Labelling and formatting varied between an early version of the punch card used between 1940–45 (**Figure A.2.1**) and a later version used for 'gunshot wound' cases admitted between 1944–45 (**Figure A.2.2**). To form a unified dataset, data processing therefore involved the following steps: loading, exploring, de-duplicating, transforming, and merging.

Data were inputted into MS Excel. Punch holes in the early cards were encoded to form dichotomous variables (e.g. 'penetrating dura': punched → 1=yes, not punched → 0=no) consistent with original analyses [369]. Punch holes in the later cards were initially encoded to form categorical variables reflecting their original labels (e.g.

‘infection’: ‘both’ punched → 1, ‘brain’ punched → 2, ‘meninges’ punched → 3, not punched → 0), before being transformed into dichotomous variables, where appropriate (e.g. infection of brain or meninges: ‘infection’ 1, 2, or 3 → 1=yes, 0 → 0=no). Dates were recorded in a standard format (dd/mm/yyyy) and used to calculate time intervals. Partially missing dates were imputed using the first possible date (i.e. ‘March 1943’ was recorded as ‘01/03/1945’). Text was encoded using numbers to form continuous variables (e.g. age in years, disability as a percentage) and standard terms to form dichotomous variables (e.g. ‘seizures’: 1=yes, 0=no) or ordinal variables (e.g. military rank: 1=enlisted, 2=non-commissioned officer, 3=commissioned officer). Sex was derived from military unit details.

Data errors were diagnosed by exploring distributions to identify values that were implausible (e.g. ‘06/22/1940’) or inconsistent (e.g. discharge date earlier than admission date). If the correct value could not be found in other records, these values were set as missing. Cases recorded on more than one card were identified as duplicates by their hospital number and values from the card with the latest data were loaded. Data fields present on both early and later versions of the cards were merged where possible (**Table A.2.2**). Selected data fields were transformed to enable merging when the same field was encoded differently between punch card versions.

2.2.1.7 Study size and statistical power

The number of cases indexed after admission to the MHHI determined the sample size (OTBI: *n* = 558, CTBI: *n* = 462). Using post-traumatic epilepsy as an example, assuming a prevalence of 50% in the exposure group and 10% in the comparator group [77], a sample size of 500 in each group provides more than 99% power to

detect a true difference at the 5% significance level. Sensitivity analyses indicate that power falls below 80% with a prevalence of 15% in the exposure group.

2.2.1.8 Statistical methods

2.2.1.8.1 Comparative analysis

Baseline characteristics and injury details were described using frequency and percentage for discontinuous variables and mean and standard deviation (SD) or median and interquartile range (IQR) for continuous variables after exploring distributions. Extreme outliers (i.e. more than 3 times the IQR above the upper quartile) were excluded. Follow-up duration was reported in person-years and follow-up density was calculated as the number of questionnaire responses per year. The number of outcomes was reported using period prevalence (i.e. the proportion of individuals who reported an outcome during the follow-up period) with 95% confidence intervals (CI). The frequency of outcomes was described using incidence rate (i.e. the rate of reported outcomes over the follow-up period). To compare the strength of association between OTBI and each outcome, relative risks were calculated as incidence rate ratios (IRRs). To assess the impact of OTBI on each outcome, absolute risks were calculated as attributable rate differences (ARDs).

2.2.1.8.2 Predictive analyses

Co-variates were explored using univariable regression analyses to calculate unadjusted odds ratios (ORs). Logistic regression was used to assess associations with seizures and headache, and linear regression was used to evaluate associations with disability. Multivariable regression analyses were then performed to calculate the adjusted OR of predictors and effect mediators, controlling for confounders. Potential predictors and confounders tested by multivariable regression were pre-specified according to the experimental hypotheses and included regardless of significance in

univariable regression (**Table 2.1**). However, predictors with extremely low frequencies (n<5) were excluded to ensure stable statistical estimation and to avoid quasi-complete separation in multivariable analyses. Other co-variates that were significantly associated with a given outcome in univariable regression ($p < 0.05$) were assessed for association with pre-specified predictors using the χ^2, Mann-Whitney U, and Kruskal-Wallis tests. Co-variates that were not associated with pre-specified predictors were included as supplementary predictors. Those that were associated with both predictors and outcomes were included as mediators if they were on the causal pathway between predictors and outcomes, or as supplementary confounders otherwise. To avoid overadjustment from redundant or overlapping variables, sensitivity analyses were performed to combine related co-variates that lost their association between univariable and multivariable analyses. Goodness of fit was assessed using pseudo-R^2 (Nagelkerke) for logistic regression and R^2 for linear regression models.

Table 2.1 Categorisation of co-variates into predictors, confounder, and mediators.

Outcome	Pre-specified predictors	Supplementary predictors	Pre-specified confounders	Supplementary effect mediators
Seizures	Depth >3cm PTA duration Dysphasia Sensorimotor Visual Retained MFB Retained BF ICH	None	Age at admission Personal history[a] Family history[a] Invalided	None
Headache	PTA duration	Military division Sensorimotor Visual	Age at admission Personal history[a] Family history[a] Invalided	Dysphasia
Disability	Depth >3cm Dysphasia Sensorimotor Visual	Military rank Infection	Age at admission	Retained MFB Retained BF Invalided Length of stay

[a] Excluded due to low frequency (n<5).

Epidemiological analyses were performed in R (v.4.4.1) using `epiR` to calculate prevalence and incidence rate and `fmsb` to calculate IRR and ARD [385,386]. Forest plots were produced in R using `readxl` for data import, `dplyr` and `tidyr` for data manipulation, and `forestplot` for visualisation. IRRs were log transformed for visualisation. Descriptive and predictive analyses were performed using SPSS (v.29.0.2.0). Missing values were excluded from analyses and the number of cases analysed are shown alongside the results. Two-tailed tests were performed and p-values less than 0.05 were considered significant.

2.2.2 Descriptive cohort study of mortality outcomes

2.2.2.1 Study design

A descriptive cohort study was performed to explore long-term mortality outcomes among veterans exposed to OTBI during WWII.

2.2.2.2 Setting and participants

This study used archived records of indexed cases admitted to the MHHI between 01/01/1940–31/12/1953. Eligible participants were male adults (≥18 years) admitted with OTBI whose archived records included a death certificate. Exclusion criteria were therefore: female sex, age at admission <18 years or unknown, CTBI, or no archived death certificate. To identify an age-matched population cohort for exploratory comparisons, the range of birth years was extrapolated using the IQR of age and the range of MHHI admission dates (i.e. lower limit: a 22-year-old admitted in 1945, upper limit: a 30-year-old admitted in 1940). This corresponded to men born between 1910–1923.

2.2.2.3 Variables

Age at death and time from injury to death were collected to describe survival after OTBI. Neurological and psychiatric conditions recorded on death certificates were also explored. Underlying and multiple causes of death were combined for descriptive analysis. Only underlying causes of death were compared with the population cohort.

2.2.2.4 Data sources

Study cards containing the personal identifiers of indexed cases (e.g. name, date of birth, National Health Service [NHS] number, address) were sent to the ONS / NHSCR by the original investigators and a death certificate summary card was returned for matched cases (**Figure 2.2**). Approximately 500 summaries were collected between 1995–2003, after which data collected stopped as a large proportion of the cohort were still alive. Details from these cards were entered into a digital dataset, in which individuals were listed by MRC number and Hospital number alongside date of injury, date of death, and causes of death. For this study, data describing all variables were collected from this dataset, which was stored in a .csv file on a floppy disk in the Archive of St Hugh's College. Average population life expectancy was estimated using life-tables for men in England and Wales aged between 28–41 years in 1951 [387]. Causes of death for men over 70 years old who died between 1994–2000 in England and Wales were collected from national mortality data [388].

Figure 2.2 Mortality data collected from the Military Hospital for Head Injuries. A. A study card containing personal identifiers used for matching. **B.** A death certificate summary was returned for matched cases, containing ICD-9 codes for multiple causes (M/C) and underlying causes (U/C) of death.

2.2.2.5 Data processing

Data processing involved transforming, harmonising, and categorising. Post-injury survival was calculated from date of injury and date of death. Underlying and multiple causes of death were recorded using the 9th revision of the International Classification of Disease (ICD-9) codes until 2000 and the 10th revision (ICD-10) codes from 2001

onwards [389]. ICD-10 codes were therefore converted to ICD-9 codes for harmonisation. A sub-group of ICD-9 codes was then defined to categorise neurological or psychiatric conditions [390]. The full list of codes included is given in **Table A.2.3**. In summary, the following conditions were included: Chapter II Neoplasms – benign and malignant neoplasms of the brain, meninges, spinal cord, pituitary and pineal glands; Chapter V: Mental Disorders – psychiatric conditions except personality disorders, those relating primarily to substance use, or those associated with childhood onset; Chapter VI: Diseases of the Nervous System and Sense Organs – all conditions except those involving the peripheral nervous system, eyes, and ears, or those associated with childhood onset; Chapter VII: Diseases of the Circulatory System – all forms of cerebrovascular disease.

2.2.2.6 Study size and statistical power

The number of death certificates matched historically from the MHHI cohort determined the sample size (n = 400). Using cerebrovascular disease as an example, with a prevalence of 10% in the comparator group based on population mortality data [388], a sample size of 400 in each group provides 83% power to detect a 70% increase in prevalence at the 5% significance level, but only 71% power to detect a 60% increase and 57% power to detect a 50% increase. Performing comparative analyses with this sample size therefore carries a high risk of missing differences that may be clinically relevant (i.e. type II errors). As such, only descriptive analyses will be performed in this study.

2.2.2.7 Statistical methods

Age at death and survival will be described using medians and IQRs. Associations between age at admission and these outcomes were explored using Spearman rank

correlation after exploring distributions. Causes of death were described using frequencies and percentages.

2.2.3 Research ethics

Ethical approval for these studies was waived by the Research Governance, Ethics & Assurance Team at the University of Oxford, who advised that 'because it involves only anonymised retrospective data obtained from an existing archive, it requires neither sponsorship nor research ethics review'.[18]

2.3 Results

2.3.1 Long-term morbidity

2.3.1.1 Participants

Of the 3,216 indexed admissions to the MHHI, there were 1,572 eligible participants after applying the exclusion criteria (**Figure 2.3**). From this group, consecutive admissions were selected, and extreme outliers were excluded to form the study cohort (n = 1,020), which comprised 558 OTBI cases and 462 CTBI cases. The main reasons for non-participation were being admitted more than three months after TBI (n = 879, 27.0%) and not participating in postal follow-up (n = 307, 9.4%). As anticipated, individuals admitted more than three months after TBI (or after an unknown time interval) differed to those admitted within three months of injury, being slightly older (26.0 vs 25.0 years, $U = 9.328\text{x}10^5$, $p < 0.001$) and more likely to have a personal history of epilepsy (3.3% vs 1.2%, $\chi^2 = 15.576$, $p < 0.001$) and a family history of both

[18] Letter from Dr Karen Melham, Sponsorship and Ethics Lead, University of Oxford, 8th August 2022.

epilepsy (7.2% vs 2.1%, $\chi^2 = 46.500$, $p < 0.001$) and headache/migraine (4.1% vs 1.5%, $\chi^2 = 19.272$, $p < 0.001$, **Table A.2.4**). After applying this criterion, individuals who did not participate in follow-up were not significantly different to those who did participate in terms of age, personal or family history, or military details (**Table A.2.5**), suggesting a low risk of responder bias resulting from the use of follow-up questionnaires.

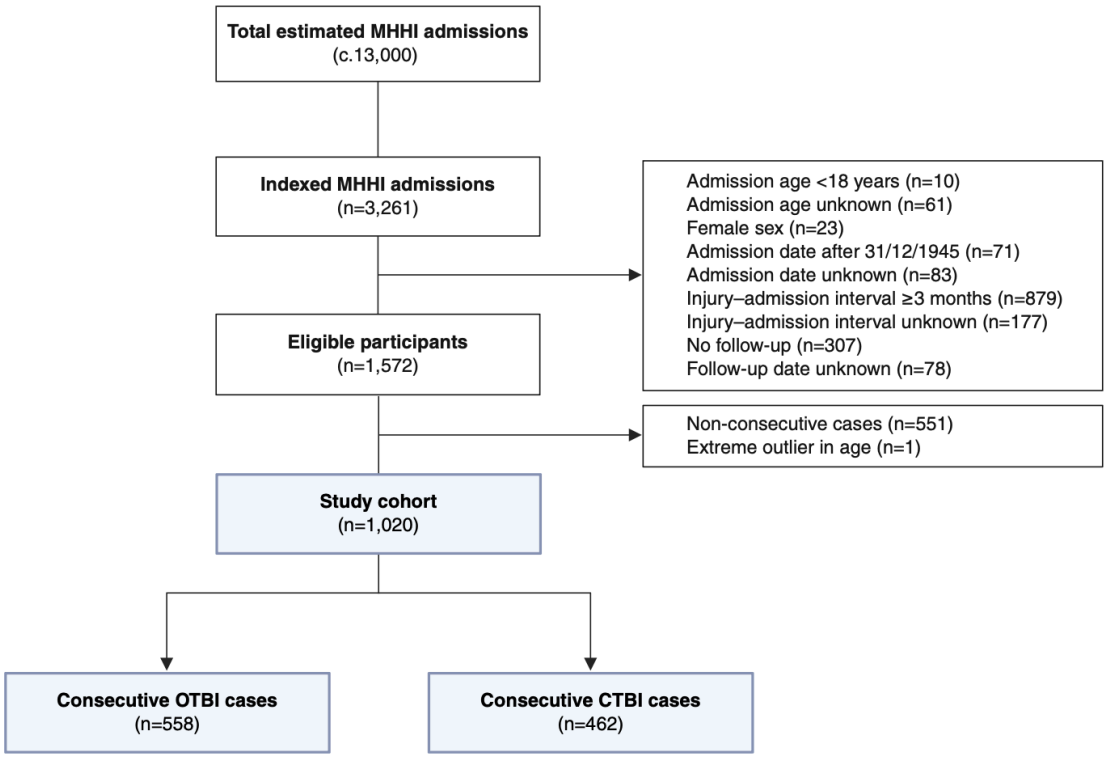


Figure 2.3 Analytic study cohort. Eligible participants were assembled from indexed cases admitted to the Military Hospital for Head Injuries (MHHI) within three months of traumatic brain injury (TBI) who participated in follow-up. The study cohort was selected from consecutive admissions with either open TBI (OTBI) or closed TBI (CTBI). Created with BioRender.com.

2.3.1.2 Baseline characteristics and injury details

The exposure and comparator groups were matched in terms of demographics (age, sex) and the average age at admission was 25 years in both groups (**Table 2.2**). A personal or family history of epilepsy and headache/migraine was rare among those admitted with OTBI but more common among CTBI cases. The proportion of military

ranks was similar between groups, but the representation of military divisions differed substantially. More than 90% of those who sustained OTBIs served in the army, compared to two thirds of CTBI cases, with air force personnel making up most of the remaining third. This is consistent with the fact that aircraft crash was among the most common cause of CTBIs among those admitted to the MHHI (**Appendix 1.6**).

Table 2.2 Baseline characteristics of the exposure and comparator groups.

Characteristic	OTBI	CTBI
Age – median (IQR)	n=558	n=462
Age at admission	25.0 (21.0 to 30.0)	25.0 (21.0 to 30.0)
Sex – n (%)	n=558	n=462
Male	558 (100.0)	462 (100.0)
Personal history – n (%)	n=558	n=462
Epilepsy	1 (0.2)	10 (2.2)
Headache/migraine	2 (0.4)	13 (2.8)
Family history – n (%)	n=558	n=462
Epilepsy	3 (0.5)	22 (4.8)
Headache/migraine	2 (0.4)	15 (3.2)
Military rank – n (%)	n=552	n=452
Enlisted	347 (62.9)	268 (59.3)
Non-commissioned officer	152 (27.5)	121 (26.8)
Commissioned officer	53 (9.6)	63 (13.9)
Military division – n (%)	n=547	n=461
Army	518 (92.8)	313 (67.7)
Air force	21 (3.8)	143 (31.0)
Navy	8 (1.4)	5 (1.1)

Comparing the injury details between groups illustrates important differences between the nature of OTBI and CTBI (**Table 2.3**). On average, individuals who sustained OTBIs were admitted to the MHHI four days after injury, and, on admission, nearly one in five cases were comatose (17.6%) and a third had additional injuries (32.4%). Almost a third of cases experienced no PTA after OTBI, and while another third experienced less than one day of amnesia, it was not uncommon to experience more than a day (16.7%) or more than seven days (13.8%) of amnesia. The majority had no

RA (62.4%). Early signs and symptoms revealed a high burden of focal neurological impairments, including visual impairment (45.7%), sensorimotor impairment (33.7%), and dysphasia (32.4%). Auditory and vestibular impairments were less common. A small number of injuries were complicated by infection of the brain or meninges (7.2%) and intracranial haematoma (3.0%), while neuroendocrine dysfunction was rare (0.5%). The average length of stay at the MHHI was 88 days after OTBI, and more than three quarters of case were invalided at discharge (78.0%).

By contrast, individuals who sustained CTBIs were admitted to the MHHI about four weeks after injury. Fewer than one in fifty cases were comatose on admission (1.9%) and associated injuries were half as common as in the OTBI group (14.7%). PTA was more common, affecting nearly 90% of CTBI cases, with the proportions experiencing a greater extent of amnesia following a similar pattern. Only 15% has no RA. The burden of focal neurological impairments was substantially lower, affecting between 5–10% of cases after CTBI, and infection was much less common (0.6%). Interestingly, the proportion of cases with an abnormal EEG was similar between groups (OTBI: 37.3%, CTBI: 34.2%) and elevated CSF protein was more than twice as common after CTBI (OTBI: 4.3%, CTBI: 10.2%). The average length of stay after CTBI, 43 days, was less than half that of OTBI, and just over a third of cases were invalided (36.8%). After discharge, the average number of questionnaires returned was higher after OTBI (2.8 vs 2.0). The time from discharge to last questionnaire response was also longer after OTBI (5.6 vs 2.4 years), but the density of responses was greater after CTBI (0.51 vs 0.86 questionnaires/year).

Table 2.3 Injury and follow-up details of the exposure and comparator groups.

Injury and follow-up details	OTBI	CTBI
Admission latency – median (IQR)	n=558	n=462
Time from injury to MHHI admission (days)	4.0 (2.0 to 8.0)	29.5 (10.0 to 49.0)
Conscious level – n (%)	n=558	n=462
Coma/stupor	98 (17.6)	9 (1.9)
Associated injuries – n (%)	n=558	n=462
Present	181 (32.4)	68 (14.7)
Post-traumatic amnesia – n (%)	n=276	n=456
Nil	88 (31.9)	50 (11.0)
Less than 1 day	104 (37.7)	238 (52.2)
1–7 days	46 (16.7)	96 (21.1)
More than 7 days	38 (13.8)	72 (15.8)
Retrograde amnesia – n (%)	n=399	n=442
Nil	249 (62.4)	68 (15.4)
Momentary to 30 minutes	127 (31.8)	314 (71.0)
More than 30 minutes	23 (5.8)	60 (13.6)
Early signs and symptoms – n (%)	n=558	n=462
Dysphasia	181 (32.4)	26 (5.6)
Sensorimotor impairment	188 (33.7)	47 (10.2)
Visual impairment	255 (45.7)	35 (7.6)
Auditory impairment	36 (6.5)	35 (7.6)
Vestibular impairment	4 (0.7)	38 (8.2)
Complications – n (%)	n=558	n=462
Infection	40 (7.2)	3 (0.6)
Intracranial haematoma	17 (3.0)	11 (2.4)
Neuroendocrine dysfunction	3 (0.5)	2 (0.4)
Investigations – n (%)	n=558	n=462
Abnormal EEG	208 (37.3)	158 (34.2)
CSF protein >50g/dL	24 (4.3)	47 (10.2)
Discharge status – n (%)	n=558	n=462
Invalided	435 (78.0)	170 (36.8)
Length of stay – median (IQR)	n=558	n=462
Time from admission to discharge (days)	88.0 (45.0–135.5)	43.0 (27.0–64.0)
Follow-up – mean (SD)	n=558	n=462
Number of questionnaires returned	2.8 (0.5)	2.0 (0.7)
Follow-up – median (IQR)	n=558	n=462
Follow-up duration (person-years)	5.62 (5.12–8.46)	2.39 (2.09–2.89)
Follow-up density (questionnaires/year)	0.51 (0.34–0.58)	0.86 (0.65–1.05)

2.3.1.3 Prevalence of morbidity after OTBI and CTBI

During the first five years after injury, three quarters of those affected by OTBI reported an adverse neurological, cognitive, or psychiatric outcome (76.2%, 95% CI:72.5–79.5). Adverse neurological outcomes affected the majority (62%, 95% CI: 57.9–65.9) but cognitive impairments (28.3%, 95% CI: 24.7–32.2) and psychiatric symptoms (19.1%, 95% CI: 15.9–22.8) also contributed significantly to morbidity. Overall, the most common outcome after OTBI was seizures, affecting nearly one in three within five years of injury (32.6%, 95% CI: 28.9–36.6, **Table 2.4**). Conversely, the most common outcome after CTBI was headache, which also affected approximately a third of cases (38.5%, 95% CI: 34.2–43.0). About one in eight experienced headache after OTBI (13.6%, 95% CI: 11.0–16.7), and one in ten experienced giddiness or faints (10.0%, 95% CI: 7.8–12.8). The prevalence of focal neurological and cognitive impairments after OTBI was substantial, including sensorimotor impairment (14.5%, 95% CI: 11.8–17.7), visual impairment (11.8%, 95% CI: 9.4–14.8), and dysphasia (12.7%, 95% CI: 10.2–15.7). Compared to visual impairment, auditory impairment was much less common (2.0%, 95% CI: 1.1–3.5). The prevalence of sleep impairment was also relatively low (3.0%, 95% CI: 1.9–4.8). More than one in nine experienced concentration impairment after OTBI (11.5%, 95% CI: 9.0–14.6). Memory impairment affected slightly fewer (9.0%, 95% CI: 6.8–11.9), with the prevalence of intellectual impairment being much lower (1.9%, 95% CI: 1.0–3.5). The most common psychiatric outcome after OTBI was personality change (11.3%, 95% CI: 8.8–14.4), while smaller proportions reported depression (5.8%, 95% CI: 4.0–8.2) and anxiety (4.3%, 95% CI: 2.8–6.5).

Table 2.4 Period prevalence of neurological, cognitive, and psychiatric outcomes after open and closed traumatic brain injury.

	OTBI (n=558)		CTBI (n=462)	
Outcome	**n**	**Prevalence % (95% CI)**	**n**	**Prevalence % (95% CI)**
Neurological	346	62.0 (57.9–65.9)	209	45.2 (40.8–49.8)
Seizures	182	32.6 (28.9–36.6)	31	6.7 (4.8–9.4)
Headache	76	13.6 (11.0–16.7)	178	38.5 (34.2–43.0)
Giddiness/faints	56	10.0 (7.8–12.8)	90	19.5 (16.1–23.3)
Sensorimotor impairment	81	14.5 (11.8–17.7)	14	3.0 (1.8–5.0)
Visual impairment	66	11.8 (9.4–14.8)	4	0.9 (0.3–2.2)
Auditory impairment	11	2.0 (1.1–3.5)	4	0.9 (0.3–2.2)
Sleep disturbance	17	3.0 (1.9–4.8)	13	2.8 (1.7–4.8)
Cognitive	158	28.3 (24.7–32.2)	72	15.6 (12.6–19.2)
Dysphasia	71	12.7 (10.2–15.7)	4	0.9 (0.3–2.2)
Memory impairment[a]	44	9.0 (6.8–11.9)	25	5.5 (3.7–7.9)
Intellectual impairment[a]	9	1.9 (1.0–3.5)	5	1.1 (0.5–2.5)
Concentration impairment[a]	56	11.5 (9.0–14.6)	50	10.9 (8.4–14.1)
Psychiatric	93	19.1 (15.9–22.8)	96	21.0 (17.5–24.9)
Anxiety[a]	21	4.3 (2.8–6.5)	45	9.8 (7.4–12.9)
Depression[a]	28	5.8 (4.0–8.2)	30	6.6 (4.6–9.2)
Personality change[a]	55	11.3 (8.8–14.4)	44	9.6 (7.2–12.7)
Any	425	76.2 (72.5–79.5)	239	51.7 (47.2–56.3)

[a] After excluding cases with dysphasia (OTBI n=487, CTBI n=458).

2.3.1.4 Incidence of morbidity after OTBI and CTBI

In terms of neurological outcomes, the incidence rate of experiencing seizures was nearly two times higher after OTBI compared to CTBI (IRR 1.74, 95% CI 1.20 to 2.54, **Table 2.5**, **Figure 2.4**). This corresponded to two more individuals experiencing seizures per 100 person-years (ARD 20.59, 95% CI 8.60 to 32.74, **Figure 2.5**). The rate of visual impairment was more than four times higher after OTBI (IRR 4.64, 95% CI 1.70 to 12.73), corresponding to one more individual experiencing visual impairment per 100 person-years (ARD 12.95, 95% CI 7.67 to 18.24). The rates of headache (IRR 0.12, 95% CI 0.09 to 0.15), giddiness or faints (IRR 0.17, 95% CI 0.12 to 0.23), and sleep disturbance (IRR 0.35, 95% CI 0.17 to 0.71) were all significantly lower after OTBI. This corresponded to 14 fewer cases of headache (ARD -141.54, 95% CI: -164.94 to -117.20), six fewer cases of giddiness or faints (ARD -67.85, 95% CI: -84.79

to 50.43), and under one fewer cases of sleep disturbance per 100 person-years compared to CTBI (ARD -7.60, 95% CI: -14.16 to -0.97). The incidence of sensorimotor and auditory impairment did not differ between groups.

Table 2.5 Incidence rate of neurological, cognitive, and psychiatric outcomes after open and closed traumatic brain injury.

	OTBI (n=558)	CTBI (n=462)
Outcome	**Incidence /1,000 py (95% CI)**	**Incidence /1,000 py (95% CI)**
Neurological		
Seizures	48.5 (41.7–56.1)	27.9 (19–39.6)
Headache	18.8 (14.8–23.6)	160.4 (137.7–185.7)
Giddiness/faints	13.7 (10.4–17.8)	81.5 (65.6–100.2)
Sensorimotor impairment	19.0 (15.1–23.6)	12.4 (6.8–20.9)
Visual impairment	16.5 (12.8–21)	3.6 (1.0–9.1)
Auditory impairment	2.6 (1.3–4.6)	3.6 (1.0–9.1)
Sleep disturbance	4.0 (2.4–6.5)	11.6 (6.2–19.9)
Cognitive		
Dysphasia	17.6 (13.7–22.2)	3.6 (1.0–9.1)
Memory impairment[a]	12.2 (8.8–16.3)	22.6 (14.7–33.4)
Intellectual impairment[a]	2.4 (1.1–4.6)	4.5 (1.5–10.5)
Concentration impairment[a]	15.7 (11.9–20.4)	45.2 (33.5–59.6)
Psychiatric		
Anxiety[a]	5.7 (3.5–8.8)	40.8 (29.8–54.6)
Depression[a]	7.6 (5.1–11)	27.2 (18.3–38.8)
Personality change[a]	15.4 (11.6–20.1)	40.2 (29.2–54)

[a] After excluding cases with dysphasia (OTBI n=487, CTBI n=458).

Among cognitive outcomes, OTBI was associated with a higher rate of dysphasia (IRR 4.94, 95% CI 1.81 to 13.54), but rates of memory impairment (IRR 0.54, 95% CI 0.33 to 0.88) and concentration impairment (IRR 0.35, 95% CI 0.24 to 0.51) were significantly lower compared to CTBI (**Figure 2.4**). This corresponded to three fewer cases with impaired concentration per 100 person-years after OTBI compared to CTBI (ARD -29.46, 95% CI: -42.65 to -16.28, **Figure 2.5**). The incidence of intellectual impairment did not differ significantly. All psychiatric outcomes occurred less often after OTBI compared to CTBI, including anxiety (IRR 0.14, 95% CI 0.08 to 0.24), depression (IRR 0.28, 95% CI 0.17 to 0.47), and personality change (IRR 0.38, 95% CI 0.26 to

0.57, **Figure 2.4**). This corresponded to three fewer cases of depression (ARD -35.06, 95% CI: -47.23 to -22.89) and two fewer cases of anxiety (ARD -19.53, 95% CI: -29.66 to -9.41) and personality change (ARD -24.78, 95% CI: -37.34 to -12.22) per 100 person-years (**Figure 2.5**).

Outcome	OTBI Events	OTBI Person-yrs	CTBI Events	CTBI Person-yrs	IRR (95% CI)
Neurological					
Seizures	182	3,751.49	31	1,110.10	1.74 (1.20 to 2.54)
Headache	76	4,036.93	178	1,109.96	0.12 (0.09 to 0.15)
Giddiness/faints	56	4,086.58	90	1,103.62	0.17 (0.12 to 0.23)
Sensorimotor impairment	81	4,264.55	14	1,124.63	1.53 (0.86 to 2.69)
Visual impairment	66	3,998.58	4	1,123.56	4.64 (1.70 to 12.73)
Auditory impairment	11	4,237.12	4	1,124.63	0.73 (0.23 to 2.29)
Sleep disturbance	17	4,214.00	13	1,117.40	0.35 (0.17 to 0.71)
Cognitive					
Dysphasia	71	4,035.37	4	1,123.63	4.94 (1.81 to 13.54)
Memory impairment[a]	44	3,615.67	25	1,103.85	0.54 (0.33 to 0.88)
Intellectual impairment[a]	9	3,703.51	5	1,109.80	0.54 (0.18 to 1.61)
Concentration impairment[a]	56	3,560.94	50	1,106.44	0.35 (0.24 to 0.51)
Psychiatric					
Anxiety[a]	21	3,663.75	45	1,103.07	0.14 (0.08 to 0.24)
Depression[a]	28	3,665.13	30	1,104.04	0.28 (0.17 to 0.47)
Personality change[a]	55	3,564.44	44	1,094.34	0.38 (0.26 to 0.57)

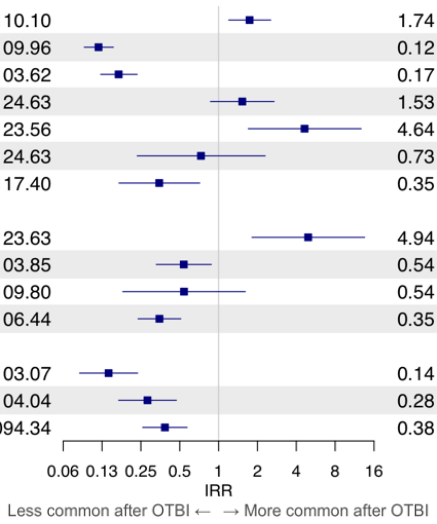


Figure 2.4 Association of open traumatic brain injuries with rates of neurological, cognitive, and psychiatric outcomes, compared to closed traumatic brain injury.

Outcome	OTBI Events	OTBI Person-yrs	CTBI Events	CTBI Person-yrs	ARD (95% CI)
Neurological					
Seizures	182	3,751.49	31	1,110.10	20.59 (8.60 to 32.74)
Headache	76	4,036.93	178	1,109.96	-141.54 (-164.94 to -117.20)
Giddiness/faints	56	4,086.58	90	1,103.62	-67.85 (-84.79 to -50.43)
Sensorimotor impairment	81	4,264.55	14	1,124.63	6.55 (-1.18 to 14.27)
Visual impairment	66	3,998.58	4	1,123.56	12.95 (7.67 to 18.24)
Auditory impairment	11	4,237.12	4	1,124.63	-0.96 (-4.75 to 2.85)
Sleep disturbance	17	4,214.00	13	1,117.40	-7.60 (-14.16 to -0.97)
Cognitive					
Dysphasia	71	4,035.37	4	1,123.63	14.03 (8.67 to 19.42)
Memory impairment[a]	44	3,615.67	25	1,103.85	-10.48 (-20.06 to 0.90)
Intellectual impairment[a]	9	3,703.51	5	1,109.80	-2.08 (-6.33 to 2.18)
Concentration impairment[a]	56	3,560.94	50	1,106.44	-29.46 (-42.65 to -16.28)
Psychiatric					
Anxiety[a]	21	3,663.75	45	1,103.07	-35.06 (-47.23 to -22.89)
Depression[a]	28	3,665.13	30	1,104.04	-19.53 (-29.66 to -9.41)
Personality change[a]	55	3,564.44	44	1,094.34	-24.78 (-37.34 to -12.22)

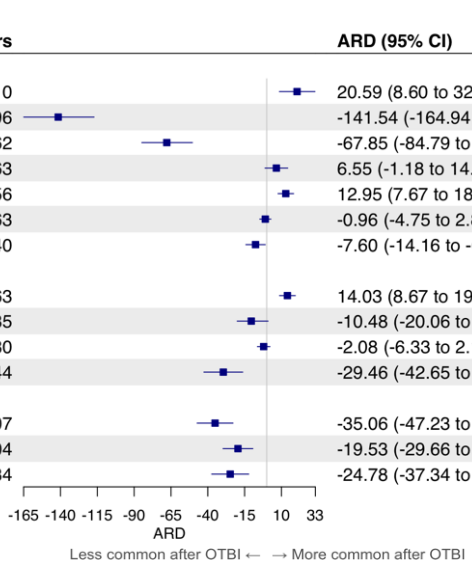


Figure 2.5 Impact of open traumatic brain injury on rates of neurological, cognitive, and psychiatric outcomes compared to closed traumatic brain injury.

2.3.1.5 Early predictors of long-term seizures after OTBI

Age at admission and discharge status were pre-specified confounders of seizures after OTBI. Personal and family history of epilepsy were excluded as confounders due to extremely low frequencies ($n = 1$ and $n = 3$, respectively). Pre-specified predictors were lesion depth, PTA duration, dysphasia, sensorimotor impairment, visual impairment, retained metallic foreign bodies (MFBs), retained bone fragments (BFs), and intracranial haematoma (ICH). Of these, unadjusted estimates suggested that PTA lasting more than seven days (OR 4.04, 95% CI: 1.79–9.11), dysphasia (OR 1.85, 95% CI: 1.28–2.68), and sensorimotor impairment (OR 1.94, 95% CI: 1.34–2.79) were associated with higher odds of developing seizures (**Table 2.6**, **Table A.2.6**). Univariable regression analyses identified no supplementary predictors, mediators, or confounders (**Table A.2.6**).

Table 2.6 Predictors of seizures after open traumatic brain injury.

Covariate	x/n	Unadjusted OR (95% CI)	x/n	Adjusted OR (95% CI)
Lesion				
Depth >3cm	471/540	0.69 (0.41–1.16)	230/258	0.59 (0.24–1.46)
Post-traumatic amnesia				
Nil	88/276	ref	87/258	ref
<1 day	104/276	1.54 (0.80–2.98)	95/258	1.68 (0.84–3.38)
1–7 days	46/276	1.59 (0.71–3.56)	40/258	1.67 (0.69–4.04)
>7 days	38/276	4.04 (1.79–9.11)	36/258	2.75 (1.11–6.82)
Early signs and symptoms				
Dysphasia	181/558	1.85 (1.28–2.68)	77/258	1.03 (0.55–1.94)
Sensorimotor impairment	308/558	1.94 (1.34–2.79)	145/228	1.36 (0.76–2.42)
Visual impairment	255/558	1.06 (0.75–1.51)	109/258	1.06 (0.59–1.90)
Complications				
Retained MFB	142/546	1.35 (0.91–2.02)	67/258	1.20 (0.64–2.24)
Retained BF	69/546	1.10 (0.65–1.87)	35/258	1.25 (0.57–2.72)
Intracranial haematoma	16/546	0.92 (0.32–2.70)	8/258	2.03 (0.43–9.61)

Adjusted for age at admission and discharge status.

After adjusting for age and discharge status, only the association between PTA and seizures remained significant (**Table A.2.7**). Individuals with PTA lasting more than

seven days were nearly three times more likely to develop seizures than those without PTA (adjusted OR 2.75, 95% CI: 1.11–6.82). However, sensitivity analysis showed that focal neurological impairment (i.e. dysphasia, sensorimotor impairment, or visual impairment) was also a predictor of long-term seizures (adjusted OR 2.52, 95% CI:1.05–6.06, **Table A.2.8**). Psuedo-R^2 of the predictive model was 0.13, consistent with modest explanatory power. Adjusted estimates confirmed that lesion depth, retained MFBs, retained BFs, and ICH were not predictors of seizures (**Table 2.6**).

2.3.1.6 Early predictors of long-term headache after OTBI

Age at admission and discharge status were pre-specified confounders of headache after OTBI. Personal and family history of headache or migraine were excluded as confounders due to extremely low frequencies ($n = 2$, both). The only pre-specified predictor was PTA duration. Unadjusted estimates suggested that this was not associated with higher odds of headache (**Table 2.7**, **Table A.2.9**). After univariable regression analyses, military division, sensorimotor impairment, and visual impairment were included as supplementary predictors, and dysphasia was included as a mediator (**Table A.2.9,10**).

After adjusting for age and discharge status, only the associations of military division and dysphasia with headache remained significant (**Table A.2.11**). Individuals in the air force were nearly five times as likely to report headache as those in the army (adjusted OR 4.71, 95% CI: 1.12–18.32). This effect was mediated by dysphasia, with affected and individuals being slightly less likely to report headache than those without dysphasia (adjusted OR 0.38, 95% CI 0.15–0.99). Psuedo-R^2 of the predictive model was 0.11, consistent with modest explanatory power. Adjusted estimates confirmed

that PTA duration was not a predictor of headache (adjusted OR 1.56, 95% CI: 0.49–5.01 for PTA lasting more than seven days, **Table 2.7**).

Table 2.7 Predictors of headache after open traumatic brain injury.

Covariate	x/n	Unadjusted OR (95% CI)	x/n	Adjusted OR (95% CI)
Military division				
Army	518/546	ref	259/273	ref
Air force	20/546	3.42 (1.33–8.80)	11/273	4.71 (1.12–18.32)
Navy	8/546	2.28 (0.45–11.55)	3/273	1.74 (0.14–21.69)
Post-traumatic amnesia				
Nil	88/276	ref	88/273	ref
<24 hours	104/276	0.70 (0.31–1.55)	102/273	0.79 (0.34–1.83)
>24 hours	46/276	1.18 (0.47–2.96)	45/273	1.32 (0.49–3.55)
>7 days	38/276	1.10 (0.41–2.96)	38/273	1.56 (0.49–5.01)
Early signs and symptoms				
Dysphasia	181/558	0.56 (0.32–0.99)	79/273	0.38 (0.15–0.99)
Sensorimotor impairment	308/558	0.58 (0.36–0.94)	152/273	0.68 (0.34–1.36)
Visual impairment	255/558	0.47 (0.28–0.79)	111/273	0.62 (0.29–1.30)

Adjusted for age at admission and discharge status.

2.3.1.7 Early predictors of long-term disability after OTBI

Age at admission was a pre-specified confounder of disability. Pre-specified predictors were lesion depth, dysphasia, sensorimotor impairment, and visual impairment. Unadjusted estimates suggested that all these variables were associated with higher odds of disability (**Table 2.8**, **Table A.2.12**). After univariable regression analyses, military rank and infection were included as supplementary predictors, and retained MFB, retained BF, invalidation, and length of stay were included as mediators (**Table A.2.12,13**).

After adjusting for age, only the associations between sensorimotor impairment and visual impairment remained significantly associated with disability (**Table A.2.14**). Sensorimotor impairment was associated with 7% more disability (adjusted B 0.07, 95% CI: 0.01–0.14) and visual impairment was associated with 10% more disability

(adjusted B 0.10, 95% CI: 0.03–0.16). This effect was mediated by discharge status and length of stay. Invalidation at discharge was associated with 26% more disability than being invalided later (adjusted B 0.26, 95% CI: 0.18–0.34), and every additional ten days spent in hospital associated was associated with 0.05% additional disability (adjusted B 4.99×10^{-4}, 95% CI: 1.45×10^{-4}–8.54×10^{-4}). R^2 of the predictive model was 0.35, consistent with moderate explanatory power. Adjusted estimates confirmed that lesion depth (adjusted B -0.02, 95% CI: -0.13–0.09) and dysphasia (adjusted B 0.01, 95% CI: -0.06–0.08) were not predictors of disability (**Table 2.8**).

Table 2.8 Predictors of disability after open traumatic brain injury.

Covariate	x/n	Unadjusted B (95% CI)	x/n	Adjusted B (95% CI)
Military rank				
Enlisted	334/525	ref	137/213	ref
Non-commissioned officer	143/525	0.01 (-0.05 to 0.06)	58/213	-0.01 (-0.08 to 0.06)
Commissioned officer	48/525	-0.09 (-0.18 to 0.00)	18/213	-0.11 (-0.23 to 0.01)
Lesion depth				
>3cm	457/526	-0.14 (-0.21 to -0.07)	190/213	-0.02 (-0.13 to 0.09)
Post-traumatic amnesia				
Nil	82/254	ref	75/213	ref
<1 day	97/254	0.04 (-0.04 to 0.13)	82/213	0.05 (-0.02 to 0.12)
1–7 days	41/254	0.08 (-0.03 to 0.18)	34/213	0.01 (-0.08 to 0.10)
>7 days	34/254	0.26 (0.15 to 0.37)	22/213	0.06 (-0.06 to 0.18)
Early signs and symptoms				
Dysphasia	173/531	0.12 (0.07 to 0.17)	56/213	0.01 (-0.06 to 0.08)
Sensorimotor impairment	300/531	0.12 (0.07 to 0.16)	120/213	0.07 (0.01 to 0.14)
Visual impairment	250/531	0.15 (0.10 to 0.20)	89/213	0.10 (0.03 to 0.16)
Complications				
Infection	40/531	0.11 (0.02 to 0.20)	12/213	0.12 (-0.01 to 0.26)
Retained MFB	141/531	0.12 (0.07 to 0.18)	56/213	0.02 (-0.05 to 0.09)
Retained BF	68/531	0.08 (0.00 to 0.15)	25/213	0.09 (-0.01 to 0.19)
Discharge status				
Invalided	422/531	0.33 (0.28 to 0.38)	164/213	0.26 (0.18 to 0.34)
Time intervals				
Length of stay	n=440	8.71×10^{-4} (5.98×10^{-4} to 1.14×10^{-3})	n=213	4.99×10^{-4} (1.45×10^{-4} to 8.54×10^{-4})

Adjusted for age at admission.

2.3.1.8 Long-term impairments associated with disability after OTBI

Age at admission was considered a confounder of disability. Among neurological outcomes, unadjusted estimates suggested that seizures, headache, sensorimotor impairment, and visual impairment were all associated with disability, while giddiness or faints, auditory impairment, and sleep disturbance were not (**Table A.2.15**). Dysphasia also showed an association with disability, but other cognitive and psychiatric outcomes appeared to not be associated. Seizures, headache, visual impairment, and dysphasia remained significantly associated with disability, after adjusting for age (**Table A.2.16**). Seizures were associated with 16% higher disability (adjusted B 0.16, 95% CI: 0.12 to 0.21) and both visual impairment (adjusted B 0.11, 95% CI: 0.04 to 0.18) and dysphasia (adjusted B 0.11, 95% CI: 0.04 to 0.18) were associated with 11% higher levels (**Table 2.9**). Conversely, headache was associated with 10% lower levels of disability (adjusted B -0.10, 95% CI: -0.18 to -0.03).

Table 2.9 Impairments associated with disability after open traumatic brain injury.

Covariate	x/n	Unadjusted B (95% CI)	x/n	Adjusted B (95% CI)
Neurological				
Seizures	175/531	0.19 (0.14 to 0.24)	175/531	0.16 (0.12 to 0.21)
Headache	65/531	-0.16 (-0.23 to -0.09)	65/531	-0.10 (-0.18 to -0.03)
Giddiness/faints	50/531	-0.07 (-0.15 to 0.02)	50/531	-0.02 (-0.10 to 0.28)
Sensorimotor impairment	79/531	0.09 (0.02 to 0.16)	79/531	0.05 (-0.01 to 0.12)
Visual impairment	66/531	0.12 (0.05 to 0.20)	66/531	0.11 (0.04 to 0.18)
Auditory impairment	10/531	-0.02 (-0.20 to 0.06)	10/531	0.00 (-0.16 to 0.16)
Sleep disturbance	16/531	-0.04 (-0.18 to 0.11)	16/531	0.02 (-0.11 to 0.16)
Cognitive				
Dysphasia	70/531	0.14 (0.07 to 0.21)	70/531	0.11 (0.04 to 0.18)
Memory impairment	59/531	0.02 (-0.06 to 0.09)	59/531	-0.01 (-0.08 to 0.07)
Intellectual impairment	12/531	0.13 (-0.03 to 0.30)	12/531	-0.05 (-0.12 to 0.03)
Concentration impairment	60/531	-0.07 (-0.14 to 0.01)	60/531	0.15 (-0.00 to 0.30)
Psychiatric				
Anxiety	21/531	-0.03 (-0.16 to 0.10)	21/531	-0.03 (-0.13 to 0.07)
Depression	30/531	-0.02 (-0.13 to 0.08)	30/531	0.03 (-0.09 to 0.14)
Personality change	59/531	0.01 (-0.07 to 0.09)	59/531	0.04 (-0.04 to 0.12)

Adjusted for age at admission.

2.3.2 Long-term mortality

2.3.2.1 Participants

Of the 3,216 indexed admissions to the MHHI, there were 903 eligible participants after applying the exclusion criteria (**Figure 2.6**). From this group, cases with archived death certificates were selected (n = 400). The main reasons for non-participation were being exposed to CTBI rather than OTBI (n = 2,264, 69.4%) and not having an archived death certificate (n = 503, 15.4%). On average, individuals with no death certificate were one year younger at admission than those with a death certificate (25.0 vs 26.0 years, $U = 9.112 \times 10^4$, p = 0.015) but did not differ in terms of personal and family history or military details (**Table A.2.17**).

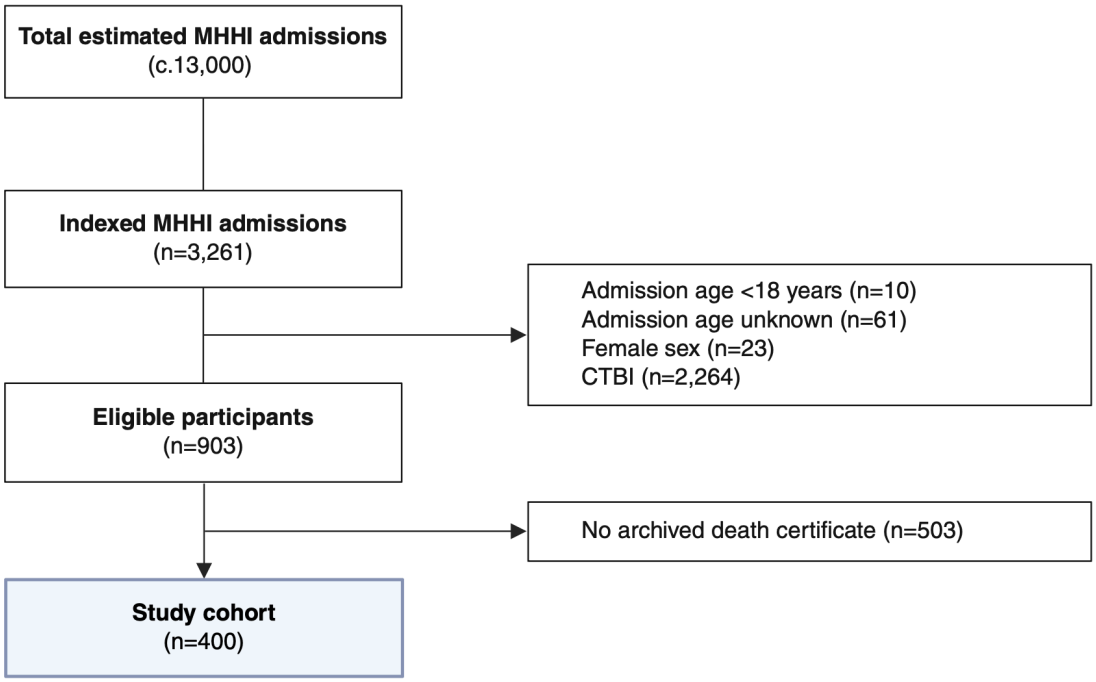


Figure 2.6 Descriptive study cohort. Eligible participants were assembled from indexed cases admitted to the Military Hospital for Head Injuries (MHHI) with open traumatic brain injury (OTBI). The study cohort was selected from individuals with archived death certificates. CTBI = closed traumatic brain injury. Created with BioRender.com.

2.3.2.2 Baseline characteristics and injury details

The average age at admission was 26 years and there was practically no personal or family history of epilepsy or headache/migraine among the descriptive study cohort (**Table 2.10**). The majority served in junior military ranks and more than 90% served in the army.

Table 2.10 Baseline characteristics of the descriptive study cohort.

Characteristic	
Age – median (IQR)	n=400
Age at admission	26.0 (22.0–30.0)
Sex – n (%)	n=400
Male	400 (100.0)
Personal history – n (%)	n=400
Epilepsy	0 (0.0)
Headache/migraine	0 (0.0)
Family history – n (%)	n=400
Epilepsy	1 (0.3)
Headache/migraine	0 (0.0)
Military rank – n (%)	n=386
Enlisted	237 (61.4)
Non-commissioned officer	107 (27.7)
Commissioned officer	42 (10.9)
Military division – n (%)	n=387
Army	367 (94.8)
Air force	11 (2.8)
Navy	9 (2.3)

On average, individuals in the descriptive study cohort were admitted to the MHHI six days after OTBI, and, on admission, nearly one in five were comatose (17.0%) and a third had additional injuries (30.5%, **Table 2.11**). Most experienced PTA and this lasted more than seven days for nearly one in five cases (19.7%). The majority had no RA (54.8%). Early signs and symptoms revealed a high burden of focal neurological impairments, including visual impairment (48.5%), sensorimotor impairment (56.5%), and dysphasia (35.8%). Auditory and vestibular impairments were less common. One

in ten injuries were complicated by infection of the brain or meninges (10.0%) and fewer were diagnosed with intracranial haematoma (2.5%), while neuroendocrine dysfunction was rare (0.3%). The average length of stay was 85 days, and four out of five cases were invalided at discharge (80.3%).

Table 2.11 Injury details of the descriptive study cohort.

Injury details	
Admission latency – median (IQR)	n=400
Time from injury to MHHI admission (days)	6.0 (2.0–47.0)
Conscious level – n (%)	n=400
Coma/stupor	68 (17.0)
Associated injuries – n (%)	n=400
Present	122 (30.5)
Post-traumatic amnesia – n (%)	n=173
Nil	46 (26.6)
Less than 1 day	56 (32.4)
1–7 days	37 (21.4)
More than 7 days	34 (19.7)
Retrograde amnesia – n (%)	n=119
Nil	154 (54.8)
Momentary to 30 minutes	101 (35.9)
More than 30 minutes	26 (9.3)
Early signs and symptoms – n (%)	n=400
Dysphasia	143 (35.8)
Sensorimotor impairment	226 (56.5)
Visual impairment	194 (48.5)
Auditory impairment	23 (5.8)
Vestibular impairment	4 (1.0)
Complications – n (%)	n=400
Infection	40 (10.0)
Intracranial haematoma	10 (2.5)
Neuroendocrine dysfunction	1 (0.3)
Investigations – n (%)	n=400
Abnormal EEG	176 (44.0)
CSF protein >50g/dL	13 (3.3)
Discharge status – n (%)	n=400
Invalided	321 (80.3)
Length of stay – median (IQR)	
Time from admission to discharge (days)	84.5 (46.8–137.0)

2.3.2.3 Survival

Average age at death was 67 years (IQR: 58.0–74.0) and the average time from injury to death was 41 years (IQR: 31.0–48.0, **Table 2.12**). The estimated average life expectancy of an age- and sex-matched population cohort was between 73.5–75.2 years. There was a modest positive correlation between age at admission and age at death ($\rho = 0.297$, $p < 0.001$, $n = 332$) and a negative correlation between age at admission and post-injury survival time ($\rho = -0.151$, $p = 0.006$, $n = 332$).

Table 2.12 Long-term survival after open traumatic brain injury.

Survival	
	n=400
Age at death (years) – median (IQR)	67.0 (57.0–74.0)
Post-injury survival time (years) – median (IQR)	41.0 (31.0–48.0)

2.3.2.4 Causes of death

Nearly one in four individuals who were exposed to OTBI died of a neurological or psychiatric condition ($n = 93$, 23.3%, **Table 2.13**). Cerebrovascular disease was the most common neurological cause of death ($n = 66$, 16.5%), followed by epilepsy ($n = 12$, 3.0%) and neurodegenerative disease ($n = 9$, 2.3%). Central nervous system (CNS) infection affected four cases (1.0%) and there were three cases of malignant CNS neoplasia (0.8%). The frequency of neurological or psychiatric underlying causes of death was 13% after OTBI and 12% in an age-/sex-matched population cohort (**Table A.2.18**).

Table 2.13 Causes of death after open traumatic brain injury.

Neurological or psychiatric conditions recorded at death	n (%)
	n=400
Cerebrovascular disease	66 (16.5)
Unspecified	52 (13.0)
Haemorrhagic	9 (2.3)
Ischaemic	5 (1.3)
Neurodegenerative disease	9 (2.3)
Dementia, unspecified	2 (0.5)
Alzheimer's disease	2 (0.5)
Neurodegeneration, unspecified	2 (0.5)
Presenile dementia	1 (0.3)
Parkinson's disease	1 (0.3)
Motor neuron disease	1 (0.3)
Epilepsy	12 (3.0)
Generalised convulsive	1 (0.3)
Grand mal status	1 (0.3)
Petit mal status	1 (0.3)
Epilepsy, unspecified	9 (2.3)
CNS infection	4 (1.0)
CNS neoplasia	3 (0.8)
Malignant neoplasm of brain	3 (0.8)
Psychiatric disease	2 (0.5)
Demyelinating disease	1 (0.3)
Autonomic disease	1 (0.3)
Other CNS disease	6 (1.5)
Total number of conditions	104 (26.0)
Total number of individuals with a neurological or psychiatric condition	93 (23.3)

2.4 Discussion

2.4.1 Long-term morbidity

The objectives of this study were to a) describe the prevalence of long-term neurological, psychiatric, and cognitive outcomes after OTBI, b) compare the incidence of these outcomes after OTBI and CTBI, c) identify early predictors of seizures, headache, and disability in the subgroup exposed to OTBI, and d) determine which long-term outcomes were associated with disability after OTBI.

2.4.1.1 Key results

2.4.1.1.1 Prevalence of morbidity after OTBI

Within the first five years following injury, the burden of morbidity among individuals with OTBI was substantial, with more than three quarters experiencing adverse neurological, cognitive, or psychiatric outcomes. Neurological complications were the most prevalent, affecting over 60% of individuals, while approximately 30% experienced cognitive impairments, and 20% reported psychiatric symptoms. On average, a third of individuals experienced seizures, one in eight experienced headache, and one in ten experienced giddiness or faints. Focal neurological and cognitive impairments also contributed significantly to morbidity, with 15% experiencing limb weakness or sensory disturbance, 13% experiencing dysphasia, and 12% experiencing visual impairment. One in nine individuals experienced concentration impairment and one in eleven experienced memory impairment after OTBI, while intellectual impairment affected only one in fifty. In terms of psychiatric outcomes, 11% experienced personality change, while 6% were affected by depression, and 4% experienced anxiety.

2.4.1.1.2 Incidence of morbidity after OTBI and CTBI

It was hypothesised that the rates of outcomes traditionally associated with focal brain damage (i.e. seizures, sensorimotor impairment, visual impairment, and dysphasia) would be higher after OTBI compared to CTBI; while the rates of other outcomes (i.e. headache, giddiness/faints, auditory impairment, sleep disturbance, other cognitive outcomes, and psychiatric outcomes) would be equivalent. This study found that OTBI was associated with a nearly two-fold increase in the rate of seizures, a four-fold increase in the rate of visual impairment, and a five-fold increase in the rate of dysphasia compared to CTBI. The rate of sensorimotor impairment did not differ

between exposures. Contrary to the hypothesis, OTBI was also associated with lower rates of headache, giddiness or faints, sleep disturbance, memory impairment, concentration impairment, personality change, depression, and anxiety relative to CTBI. The rates of auditory impairment and intellectual impairment were equivalent between groups.

2.4.1.1.3 Early predictors of long-term seizures and headache after OTBI

It was hypothesised that predictors of seizures after OTBI would include lesion depth, injury severity, focal neurological impairment, retained MFB, and intracranial haematoma, but not retained BF. In this study, after adjusting for age and discharge status, injury severity (PTA lasting more than seven days) and focal neurological impairment predicted seizures after OTBI. The duration of PTA did not predict headache after OTBI, after adjusting for age and discharge status, supporting the hypothesis that headache would not be predicted by injury severity. Serving in the air force was associated with a nearly five-fold increase in the odds of headache compared to serving in the army. This association was mediated by dysphasia which itself slightly reduced the odds of reporting headache.

2.4.1.1.4 Early predictors and long-term impairments associated with disability after OTBI

It was hypothesised that long-term disability after OTBI would be predicted by lesion depth and focal neurological impairment. In this study, after adjusting for age, disability was predicted by both sensorimotor and visual impairment, which were associated with 7% and 10% higher disability levels, respectively, but not lesion depth or dysphasia. These effects were mediated by discharge status and length of stay. Throughout the first five years after injury, seizures were associated with 16% higher disability and both

visual impairment and dysphasia were associated with 11% higher disability, while headache was associated with 10% lower disability levels, after adjusting for age.

2.4.1.2 Limitations

The MHHI cohort provides a rare opportunity to characterise the long-term effects of OTBI among a large and relatively homogenous group of survivors, and to contrast these effects with those experienced by a comparable group who were exposed to CTBI. However, the use of archival materials posed significant challenges that were only partly mitigated by the study design, resulting in several noteworthy limitations.

2.4.1.2.1 Imprecision

Studying paper-based clinical records generated more than eighty years ago inherently carries a high risk of inaccuracy, but the systematic approach to evaluation, standardised use of terminology, and meticulous systems of documentation upheld by the MHHI collectively lend a remarkably high degree of precision to these resources, despite their age. In particular, the use of punch cards to encode and condense a large volume of clinical information was critical to the feasibility of this study. Moreover, the MHHI pioneered methods that were extremely advanced for its time, most notably the early use of penicillin to treat meningitis, and which in many ways shaped the modern approach to TBI in combat settings, including the deployment of mobile neurosurgical units and an aggressive approach to surgical debridement [391]. Nevertheless, practical limitations of how medicine was practised in the 1940s are evident. Most obviously, the nature of clinical investigations was substantially restricted. Neuroimaging was limited to AP and lateral skull x-rays, making the identification of MFBs and BFs and the diagnosis of ICH challenging. The resolution and interpretation of EEGs at the time was also limited. The fact that these investigations were not predictors of seizures or headache after OTBI is therefore of limited significance.

While the use of questionnaires in this study enabled the collection of patient-centred outcomes, it is also a further source of imprecision. Participants may have under-reported certain symptoms, particularly those relating to their cognition and mental health, due to stigma or limited insight. The true impact of cognitive and psychiatric outcomes may therefore be under-estimated. However, if participants were aware that their responses would be used to provide relevant advice and support (as they were), those with more severe symptoms may have been more inclined to respond, potentially leading to over-estimation of outcome severity. Additionally, the fact that questionnaires could be completed with the support of another individual reduces the chance that those with more severe impairments would not respond. It is therefore difficult to determine the effect of imprecision on estimates of disease prevalence. Assuming that these factors affected both groups equally, they are unlikely to introduce systematic bias.

2.4.1.2.2 Information bias

The use of data collected from questionnaires carries an additional layer of imprecision. Participants may have had incomplete recollections of their symptoms, leading to recall errors. Given that TBI can have cognitive effects, including memory impairment, these errors may have been systematic, introducing the risk of recall bias. This risk was partly mitigated by other individuals being involved in the completion of the questionnaire (i.e. Unit Medical Officer or next-of-kin). However, this also introduces the risk of observer bias, whereby the expectations or beliefs of the individual completing the questionnaire may have influenced how certain outcomes were reported. This risk was mitigated to some extent by standardised questionnaires and the fact that observers were unaware of exposure status or research hypotheses.

2.4.1.2.3 Selection bias

The risk of selection bias was reduced by only including consecutive admissions to the MHHI. This avoided the inclusion of CTBI cases indexed because of additional exposures or outcomes that would have enriched this group. Limiting analyses to only cases with at least one follow-up questionnaire also served to reduce the risk of attrition bias. However, to assess outcomes occurring several years after injury, up to three questionnaires were included in the analyses. On average, members of the OTBI group returned more questionnaires over a longer follow-up period, while members of the CTBI group responded more frequently within a shorter window. The groups therefore exhibited different patterns of follow-up, which should be considered when interpreting the results of this study. In particular, while it is tempting to compare the prevalence of outcomes between groups, given the longer duration of follow-up in the OTBI group, these estimates are at risk of attrition bias in favour of increased prevalence after OTBI, and so comparisons between groups must be interpreted with caution. By accounting for follow-up duration, incidence rates mitigate the risk of attrition bias and enable comparison between groups.

2.4.1.3 Confounding

When comparing the incidence rates of outcomes after OTBI and CTBI, it is important to bear in mind the range of differences that related to injury details between groups which may have acted as effect mediators. For example, the average time from injury to MHHI admission was four days after OTBI and 30 days after CTBI. However, it is important to remember that the MHHI would rarely be the first hospital attended after injury, with initial assessment being performed at a field hospital before transfer to the MHHI for specialist assessment and treatment. The rate of complications such as infection were also higher after OTBI compared to CTBI. However, as these details lie

on the causal pathway between exposures and outcomes, they are not considered confounders.

In the prediction of outcomes after OTBI, multivariable regression analyses were performed to control for potential confounders. Pre-specified confounders included age at admission, a personal or family history of epilepsy or headache, and discharge status. However, due to extremely low frequencies, personal and family history variables were excluded to preserve estimate stability. Because the person completing the questionnaire differed according to whether an individual was discharged from military service or returned to their unit, discharge status was also pre-specified as a potential confounder. Supplementary confounders and effect modifiers were identified by assessing all covariates for associations with exposures and outcomes. Nevertheless, the choice of variables that were assessed historically means that important characteristics such as socioeconomic status could not be controlled. This, along with the imprecision of the categories applied, means that residual confounding may still exert significant effects.

2.4.1.4 Interpretation

While the prevalence of long-term morbidity after OTBI has been estimated in this study, applying these estimates beyond the specific setting of this study is challenging, given the highly selected nature of the cohort and imprecision of data collection methods. Importantly, these findings serve to demonstrate that morbidity after OTBI can arise from not only seizures and persistent neurological impairments, but also from episodic neurological phenomena such as headaches and giddiness, cognitive impairments affecting concentration and memory, and mental health symptoms including anxiety and depression.

Comparing the incidence of neurological, cognitive, and psychiatric outcomes between groups exposed to OTBI and CTBI offers potential insights into the pathophysiology of these conditions. Simplistically, the increased rate of seizures after OTBI can be attributed to the presence of a cicatrix (or scar) in the cortex, which acts as a potent epileptogenic focus [392], and higher rates of visual impairment and dysphasia are consistent with focal damage to the regions specialised to perform these processes. However, the fact that rates of sensorimotor impairment were matched between OTBI and CTBI suggests the structures involved in these processes may be particularly vulnerable to CTBI. Indeed, the length of the ascending and descending tracts responsible for sensory and motor function in the limbs may make them more susceptible to TAI. The effects of TAI could also conceivably underlie the finding that other outcomes were more common after CTBI compared to OTBI, especially those involving cognitive, emotional, and regulatory processes arising from large brain networks that involve association, commissural, and projection fibres. This is consistent with reports that white matter damage and altered connectivity after CTBI contribute directly to adverse cognitive and psychiatric outcomes [115,113,114,133].

Of course, these epidemiological patterns are inevitably extreme simplifications of complex relationships that emerge from dynamic interactions between a wide range of factors. To give just one example, the most widely used anti-seizure medication in this period was phenobarbital, which is also an effective anxiolytic, therefore attenuating any association that may exist between OTBI and anxiety. However, the team at the MHHI, who became uniquely familiar with the natural history of both OTBI and CTBI through their work, described how certain symptoms were especially common after closed head injury, namely ‘headache, usually associated with vertigo, difficulty in concentration and insomnia, with or without depression’ [373]. This collection of

symptoms was variably referred to as ‘post-concussion syndrome’, ‘post-traumatic general cerebral syndrome’, and ‘minor contusion syndrome’, but all authors agreed that it was more common after closed head injury than after penetrating brain injury [393–395]. This observation is now supported by the findings of this study, which indicate that headache, giddiness or faints, sleep disturbance, concentration and memory impairment, depression, anxiety, and personality change are all more common after CTBI than OTBI.

Consistent with the VHIS, this study found that focal neurological impairment was an early predictor of long-term seizures after OTBI. In contrast, lesion depth, retained MFB, and ICH were not confirmed as predictors, most likely due to imprecision, as described above. Interestingly, PTA lasting more than seven days was also shown to predict long-term seizures after adjusting for confounders. Injury severity is a known risk factor for PTE after CTBI. Specifically, PTA lasting more than one day was associated with seizures in one of the largest population-based studies of PTE [53]. In the VHIS, PTA of more than one hour was not associated with seizures after OTBI, but subdivisions of this group were not analysed. Conscious level at first examination, an alternative measure of injury severity, was associated with PTE [82]. Extended PTA and reduced conscious level may both reflect a greater extent of neurometabolic derangement after OTBI, promoting excitotoxicity and heightening seizure risk.

PTA was, however, shown not to predict headache after OTBI, in keeping with previous research [93]. This suggests that any mechanism by which PTA might confer an increased risk of seizures does not influence the development of headache. No other covariates were associated with headache after OTBI, except for serving in the air force, which increased the odds more than four-fold compared to serving in the

army. Reasons for this association could conceivably include physiological stressors associated with altitude, such as low pressure and low atmospheric oxygen levels, increased vestibular and sensory stimulation leading to migraine, and the cognitive demands of flying. Equally, greater surveillance or a lower threshold for reporting headache due to concerns about fitness to fly may have contributed to the association. However, this would seem to contradict Russell's impression that those serving in the air force generally recovered from closed head injuries more quickly than others [369].

Sensorimotor and visual impairments were both predictors of long-term disability after OTBI. Dysphasia was not associated with greater disability, which, given that disability was interpreted in the context of pension compensation, may reflect language difficulties posing less of a barrier to employment than limb weakness or blindness, for example. It may also be the case that individuals with dysphasia were able to recover and adapt to a greater extent than those with other impairments. In the long-term, those who experienced seizures, visual impairment, and dysphasia were assessed to have higher levels of disability, indicating the functional impact of these impairments. Those with headaches were assessed to have lower disability, which could reflect less reporting of headache alongside other impairments or higher rates of headache among those engaged in employment.

2.4.1.5 Generalisability

The generalisability of these findings is limited by the demographics of the study cohort, which were shaped by the study setting and historical context in which the data were originally collected. The study cohort was exclusively male, and the majority were injured during early adulthood. While TBI is more common among men than women, the lack of female cases in this cohort is a significant limitation [3]. The study cohort

also sustained injuries through military service. However, in the context of WWII, the majority of these men were civilians who enlisted to serve, rather than professional military personnel. More specifically, only individuals who returned a follow-up questionnaire after discharge from hospital were included in the analyses, although the baseline characteristics of this group were not different to non-responders. Caution should therefore be exercised before applying any of these findings directly to an individual who sustains an OTBI today. However, the strength of this study lies in the study of two large and comparable groups who survived OTBI and CTBI to yield insights into the nature and extent of long-term impacts following these injuries. It is hoped that the findings of this study will motivate further research that seeks to replicate and extend the results reported here in more representative populations.

2.4.1.6 Future work

Building on this work, I plan to explore predictors of seizures, headache, disability, and other outcomes up to 35 years after OTBI. The relationship between lesion location and relevant outcomes up to 35 years after OTBI can also be explored by combining the data collected to date with the methods described in **Chapter 3**.

2.4.2 Long-term mortality

The objective of this study was to explore mortality outcomes up to 60 years after OTBI.

2.4.2.1 Key results

2.4.2.1.1 Survival

The average age at death was 67 years, which was approximately seven years less than the estimated average life expectancy of an age- and sex-matched population cohort.

2.4.2.1.2 Causes of death

Nearly one in four individuals who were exposed to OTBI died of a neurological or psychiatric condition. Considering only underlying causes of death, the proportion of neurological or psychiatric conditions recorded at death was 13% in the study cohort and 12% population cohort.

2.4.2.2 Limitations

Death certificate data carry several intrinsic limitations. Causes of death are prone to imprecision due to variable diagnostic certainty and the limited availability of information at the time of death. The use of ICD codes serves to mitigate this to some extent, but classification errors can still occur, along with a tendency towards specific conditions being classified under broader labels, such as ‘presenile dementia’, and the use of non-specific terms, such ‘cerebrovascular disease unspecified’. Also, conditions recorded on the death certificate only reflect those considered to have contributed to death and are therefore an incomplete record of the conditions affecting an individual. Neurodegenerative disease, epilepsy, and psychiatric conditions, in particular, are likely to not be captured as a result.

Mortality data were acquired by data linkage performed by the ONS and NHSCR. Data linkage errors can occur due to non-unique combinations of identifiers, errors in identifiers and matching variables, and missing data. This leads to missed links and false links which can reduce the sample size and introduce misclassification errors, measurement errors, and, if systematic, selection bias. Given that statistical comparisons were not undertaken in this exploratory study, the potential impact of these issues was not assessed.

2.4.2.3 Interpretation

The results of this study could be interpreted to suggest that OTBI is associated with reduced long-term term life expectancy compared to controls. However, when the collection of mortality data stopped in 2003, it can be assumed that a proportion of the study cohort were still alive, meaning that the true average age at death is likely to be higher than estimated here. The finding that nearly one in four individuals who were exposed to OTBI died of a neurological or psychiatric condition is potentially more revealing. Statistical comparison with a population cohort was not undertaken due to limited power. But numerical comparison highlights a further issue arising from the fact that only underlying causes of death are available from publicly accessible datasets. Using this measure, the proportions of neurological or psychiatric conditions recorded at death were similar. However, excluding multiple causes removed half of the conditions that were recorded on death certificates in the study cohort, preferentially affecting those that were not direct causes of death. Indeed, this excluded 11/12 cases with epilepsy, 8/9 cases with neurodegenerative disease, and 2/2 cases with psychiatric conditions. Therefore, to interrogate the long-term effects of OTBI on mortality, record-level data reporting multiple causes of death will be required.

2.4.2.4 Generalisability

The results of this exploratory study provide preliminary insights into the long-term effects of OTBI on mortality which should not be generalised at this stage. They will, however, act as a foundation for further research to address this important issue.

2.4.2.5 Future work

Access to record-level mortality data has been gained through data linkage applications to NHS England and NHS Scotland which were approved in 2025. The proposed study aims to complete the collection of mortality data from the group

exposed to OTBI by including deaths registered between 2003–2024. This will enable the average age at death to be calculated accurately and significantly increase the sample size to boost statistical power. Mortality data will also be collected from those exposed to CTBI and a control group who sustained peripheral nerve injuries, supporting comparisons between groups to evaluate the long-term effects of OTBI on survival, age at death, and cause of death. Data linkage will require the transfer of personal identifiers from the Archive at St Hugh's College to NHS England and National Records of Scotland without the informed consent of those in the cohort. Research ethics applications to the NHS Health Research Authority were initiated in 2022 and received final approval in 2024 (REC24/SC/0201). Additional approval to process confidential information without consent under Regulation 5 of the Health Service (Control of Patient Information) Regulations 2002 (Section 251 support) was gained from the Confidentiality Advisory Group (24/CAG/0094). This study will enable exploration of the association between OTBI and neurological causes of death, including the potential link between OTBI and haemorrhagic stroke.

An increased risk of stroke following CTBI has been confirmed by several meta-analyses [162,396,164,165]. CTBI is associated with a mild increase in the risk of ischaemic stroke and a larger increase in the risk of haemorrhagic stroke [162,164]. In some studies, the additional risk of stroke conferred by TBI was equivalent to that of hypertension [64]. It appears that the risk of stroke becomes elevated within months of injury, and while moderate-severe TBIs carry the greatest risk, even mild TBIs seem to be associated with an increased risk compared to controls [164,165].

CTBI results in multiple pathophysiological disturbances that affect the cerebrovascular system, including damage to components of the neurovascular unit (leading to

disruption of the BBB) [243], loss of cerebrovascular autoregulation [397], and altered coagulation [398]. These disturbances are collectively termed traumatic cerebral vascular injury (TCVI) [399]. Recovery from TCVI involves vascular regeneration, which may entail longer term disturbances to blood flow and platelet-endothelial interactions. These changes are thought to affect the balance of coagulation after CTBI and may predispose an individual to either haemorrhage or ischaemic stroke. A recent study also showed that CTBIs of any severity are associated with a higher number of cerebral microbleeds by middle-age compared with no history of TBI, but not with other measures of cerebral small vessel disease, suggesting that CTBI may be specifically associated with microvascular fragility [400]. Beyond the direct effects of CTBI on the cerebrovascular system, individuals with lasting impairments after CTBI may have reduced levels of physical activity and, as a result, may accumulate systemic vascular risk factors at a higher rate than the general population [162]. For this reason, people affected by TBI may benefit from lifestyle modifications and medications aimed at primary prevention of cerebrovascular disease.

OTBI is associated with TICAs, which form after laceration of cerebral blood vessels and can rupture to cause haemorrhagic stroke. A recent case series found TICAs in 8% of civilian gunshot wound cases using CTA on admission to hospital and showed that TICAs were associated with intracranial haemorrhage [166]. Although a third of cases resolved spontaneously, a further third required endovascular or open neurosurgical treatment, suggesting that using CTA to screen for TICAs may serve to mitigate the risk of haemorrhagic stroke after OTBI.

3 Neuropsychological study

In this chapter, I present a study in which I explored the neuropsychological sequelae of penetrating brain injuries to gain insights into structure-function relationships in the human brain.

3.1 Introduction

In **Section 3.1.1**, I provide a brief history of human lesion studies. In **Section 3.1.2**, I present the study rationale, followed by the aim, objectives, and hypotheses in **Section 3.1.3**.

3.1.1 Background summary

The study of focal brain injuries has underpinned our understanding of structure-function relationships in the human brain for more than 150 years. Throughout the 20th century, progress in this field was accelerated by opportunities to learn from large numbers of cases injured through conflict. In the First World War, Gordon Holmes mapped visual impairments to specific cortical areas, while research performed in the aftermath of WWII by Hans Teuber and Freda Newcombe, among others, significantly advanced the understanding of localised brain functions [129,172,178]. The VHIS refined this approach by combining systematic neuropsychological assessment with neuroimaging data to perform LSM [108,209].

Today, while functional neuroimaging methods such as fMRI dominate neuroscientific research, lesion studies remain a valuable methodological adjunct. Unlike functional neuroimaging, lesion studies uniquely establish causal relationships between specific brain regions and functional impairments, thus identifying brain regions that are not merely involved but are strictly necessary for cognitive performance. Moreover, lesion

studies can overcome some constraints of neuroimaging methods, such as the limited scope of tasks performable within a scanner environment, by enabling the investigation of a broader range of cognitive, emotional, and behavioural outcomes in naturalistic settings.

3.1.2 Rationale for the proposed study

Data from the MHHI cohort hold valuable potential for performing contemporary lesion-symptom investigations. Unlike stroke-induced lesions, penetrating brain injuries are not restricted by vascular territories, and in contrast to other aetiologies such as malignancy or epilepsy, produce clearly delineated regions of damage. Furthermore, the young age and relative lack of co-morbidities among military cohorts minimise the potential confounding effects of age-related cognitive decline and co-morbidities.

However, leveraging archival data from the MHHI presents significant practical challenges. Most fundamentally, the available CT radiographs are film-based rather than digital, limiting their compatibility with modern digital LSM techniques. Early approaches to digitising film-based radiographs have not been updated to align with contemporary neuroimaging programs. Addressing this gap is essential to leveraging the valuable data held in these archival materials.

Consequently, in the first part of this chapter, a novel digital lesion reconstruction method adapted for use with film-based CT head radiographs will be validated. Then, this method will be applied to perform two LSM investigations. The first of these seeks to replicate prior findings from the VHIS concerning the neural substrates of insomnia, which can be a debilitating consequence of TBI. The second aims to replicate findings from a stroke cohort regarding the neural correlates of performance in the clock-drawing test, a widely used clinical tool for cognitive assessment and dementia

screening. Collectively, these studies will advance methodologies for lesion-based neuropsychological research and strengthen our understanding of structure-function relationships in the brain.

3.1.3 Aim, objectives, and hypotheses

Aim: Investigate structure-function relationships in the human brain.

Objectives:

1. Validate a novel digital lesion reconstruction method for film-based CT head radiographs i) structurally, by comparing damage to anatomical regions between reconstructions made using film-based CT head radiographs and reconstructions made using post-mortem photographs, and ii) functionally, by assessing the performance of film-based CT reconstructions to identify brain regions required for motor control of the limbs and visual perception using overlap analysis and lesion-symptom mapping.

2. Applying this method, conduct human brain lesion studies using archival cases of veterans with penetrating brain injuries to attempt to replicate the findings of i) a LSM study using cases from the VHIS cohort to investigate insomnia, and ii) a LSM study using cases with ischaemic stroke to investigate performance in the clock-drawing test.

Validation hypotheses:

A) Unilateral limb weakness is associated with damage to the contralateral motor cortex and corticospinal tract.

B) Unilateral hemianopia is associated with damage to the contralateral visual cortex, and unilateral quadrantanopia is associated with damage to the contralateral optic radiation.

Experimental hypotheses:

A) Damage to the left dorsomedial prefrontal cortex is associated with insomnia [108].

B) When performing the clock-drawing test, damage to the right hemisphere is associated with visuospatial construction errors, and damage to the left hemisphere is associated with time-setting errors [216].

3.2 Materials and methods

3.2.1 Materials

3.2.1.1 Study cohort

Cases screened for inclusion in these studies were individuals admitted to the MHHI with penetrating brain injuries who later underwent CT head scans and participated in neuropsychological assessments or postal questionnaires during Phase 3 of follow-up (*n* = 73; **Figure 3.1**). As described in **Section 1.5.4.2**, recruitment for neuropsychological assessment was originally performed by Russell between 1963–1970. Veterans involved in Phase 1 of follow-up were approached via their general practitioner and invited by letter to complete residential neuropsychological assessments at the Department of Neurology in the Churchill Hospital, Oxford [190]. 153 veterans were selected from the larger group with OTBIs to provide a representative distribution of lesion locations, as determined by operation notes and skull x-rays, and a range of injury severities, estimated by the loss of brain tissue and depth of penetration (*n* = 153). From this group, 137 returned to Oxford between 1983–

87 for Phase 3 follow-up, which involved further neuropsychological assessments, including the clock-drawing test, and a CT head scan. The majority also completed postal questionnaires.

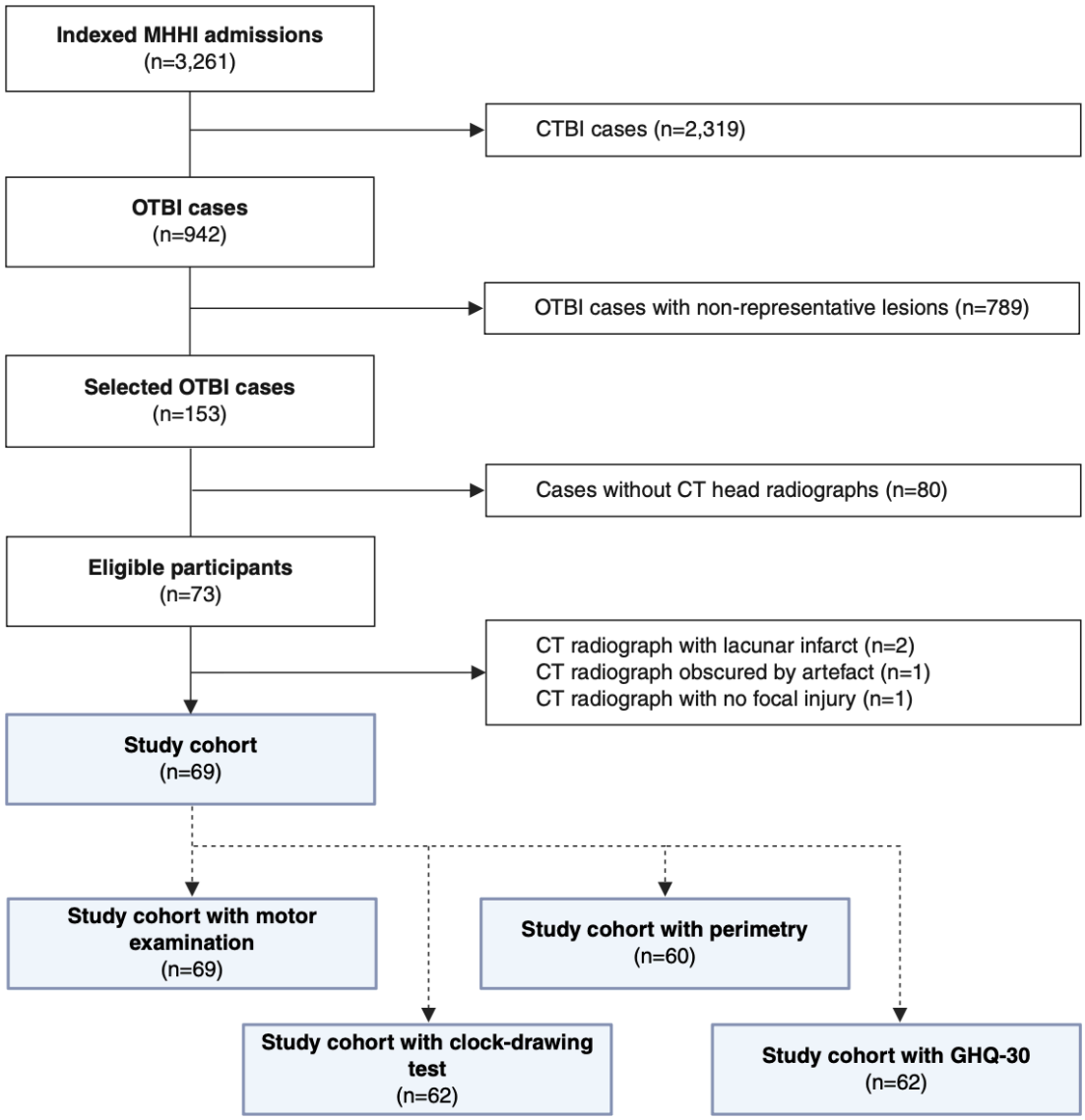


Figure 3.1 Neuropsychology study cohorts. The study cohorts were formed by screening the group of veterans who participated in neuropsychological studies between the 1960s and 1980s, who were a sub-group of the original Military Hospital for Head Injuries (MHHI) cohort. CTBI = closed traumatic brain injury. OTBI = open traumatic brain injury. Created with BioRender.com.

Inclusion criteria relating to the availability of relevant archival records (i.e. motor examination, perimetry, clock-drawing test, and questionnaire) were applied to form

study cohorts for each experimental hypothesis. Exclusion criteria for this study were: i) lacunar infarct visible on CT head, ii) CT head obscured by artefact, and iii) no evidence of focal brain injury on CT head. Cases with lacunar infarcts were excluded to avoid confounding from the potential effects of these lesions on effort and motivation [401,402]. Four cases were excluded in total (n = 2 with lacunar infarcts, n = 1 with artefact, n = 1 with no evidence of focal brain injury on CT).

3.2.1.2 Neuropsychological assessments and questionnaires

The neuropsychological assessment records and postal questionnaires of all participants in Phases 2 and 3 are preserved in the Archive, along with the film-based CT head radiographs of 73 participants.[19]

3.2.1.3 CT head radiographs and anatomical localisation drawings

CT head radiographs were available in the form of acetate films. These displayed sequential axial slices tiled onto A4-sized films (**Figure 3.2.A**). For each slide, a legend shows relevant technical details: kernel (TI), x-ray energy in kilovolts (125 KV), current in amperes (0.45 A), slice thickness (4–8 mm), gantry tilt (0, 6, 8, or 15˚), and table position (TP). Slice thickness was 4 mm in the brainstem and 8 mm in the cerebrum. Gantry tilt varied between scans, producing a variable degree of pitch (i.e. rotation about the x-axis). This resulted in a range of axial planes across the series of scans, corresponding approximately to the range used in current neuroimaging protocols [403]. Head position within the scanner also varied between scans, producing a variable degree of roll (i.e. rotation around the y-axis). Digital images were acquired by

[19] Kindly donated by Professor Andy Young, University of York.

photographing the original film with a digital camera (Canon EOS 500D) in front of a light box.

Anatomical localisation drawings were also available for each case. These were made by Hanna Damasio with reference to the CT head radiographs at the request of Freda Newcombe (**Figure 3.2.B**). In these drawings, manual delineations of hypodense regions are superimposed onto standardised anatomical templates, which can be found in the appendix of 'Lesion Analysis in Neuropsychology', Damasio & Damasio (1989) [231]. Four templates were used to accommodate the range of axial planes used across the series of scans (A1–A4).

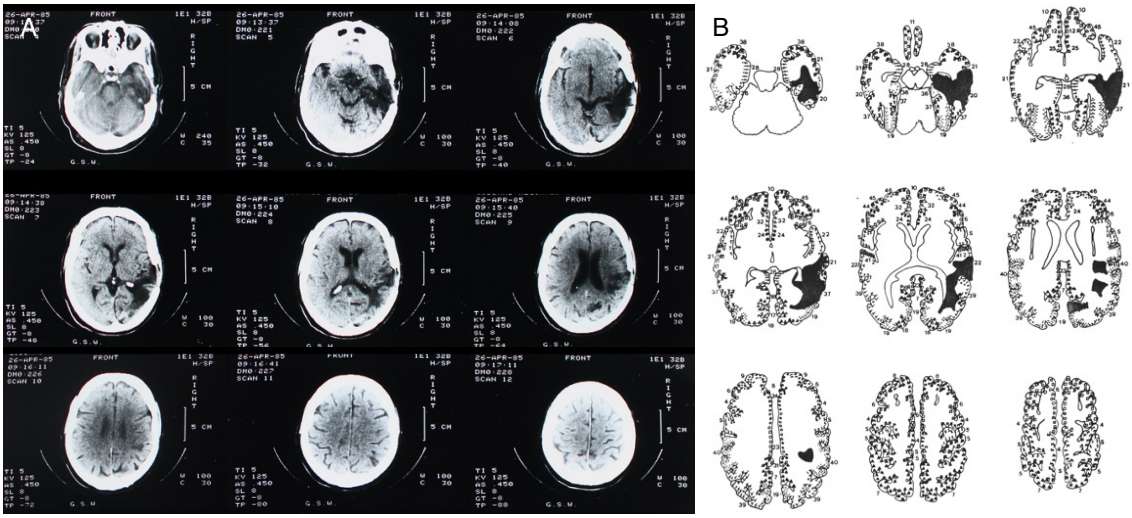


Figure 3.2 CT head scans acquired from the Military Hospital for Head Injuries cohort. **A.** A CT head radiograph showing an area of hypodensity in the right temporo-parietal region. **B.** The corresponding anatomical drawing produced by Hanna Damasio illustrates the location of the lesion.

3.2.1.4 Post-mortem reports and photographs

Finally, diagnostic post-mortem (PM) reports and photographs were available from the Oxford Brain Bank (OBB) for cases who completed neuropsychological assessments and CT head scans in Phase 3 and who after death donated their brains for research in Phase 4 of follow-up. Post-mortem reports described the extent of the lesional deficit

and the anatomical structures affected. Anatomical photographs showed the external appearance of the brain after removal from the skull and the gross appearance of internal structures in serial coronal sections. In total, PM reports and photographs were available for 22 cases, of which CT head radiographs were available for four. This larger PM group will form the focus of **Chapter 4**.

3.2.2 Methods

A combination of established neuropsychological assessment methods and a novel series of anatomical methods were applied to these materials to perform this study (**Figure 3.3**).

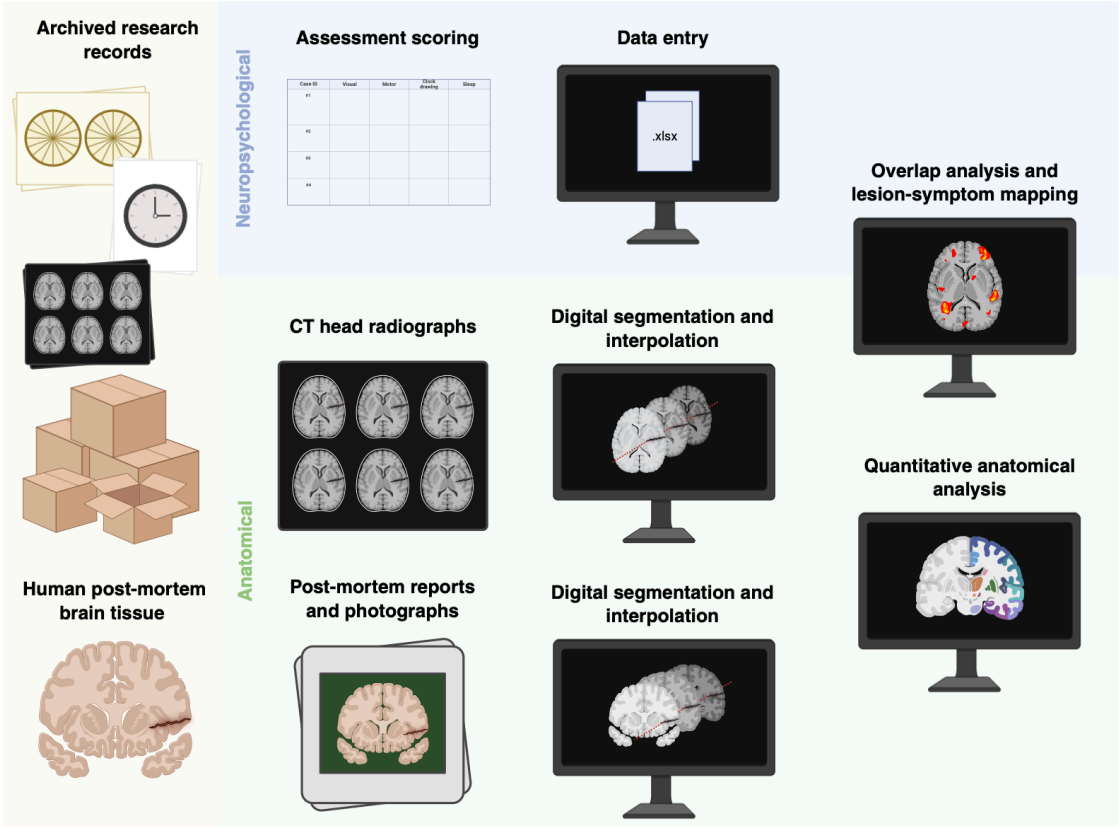


Figure 3.3 Overview of the materials and methods used in this chapter. Created with BioRender.com.

3.2.2.1 Neuropsychological methods

3.2.2.1.1 Limb weakness classification

The findings of neurological examination at discharge from the MHHI were recorded in the MRC study punch cards. Motor weakness of the arms, legs, or face was coded as none, slight, moderate, or severe. For this study, cases without motor weakness were classified as *unimpaired* and cases with slight, moderate, or severe weakness of the arms or legs were classified as *impaired*. Cases with facial weakness, or weakness not otherwise specified, were classified as *borderline*.

3.2.2.1.2 Visual field impairment classification

Visual field perimetry was performed during admission to the MHHI and at follow-up visits up to the 1980s. Visual field impairment was defined by inability to detect static white light at 3/330 resolution (i.e. a 3mm object 33cm away). For this study, cases with full visual fields were classified as unimpaired and cases with quadrantanopia or hemianopia were classified as impaired (**Figure 3.4**). Cases with scotoma or altitudinal deficits were classified as borderline.

3.2.2.1.3 Insomnia classification

Goldberg's 30-point General Health Questionnaire (GHQ-30) of self-reported mental health symptoms was completed by MHHI veterans throughout the 1960s and 1980s. Two questions relate to disturbed sleep (Q2: 'Have you lost much sleep over worry?' and Q3: 'Have you been having restless, disturbed nights?') [380]. Of these, only responses to Q3 were included, as Q2 was thought to be more closely related to anxiety than insomnia. Possible responses to Q3 were: 'not at all', 'no more than usual', 'rather more than usual', or 'much more than usual'. To identify all cases with evidence of longstanding sleep disturbance, 'not at all' responses were classified as unimpaired, and all other responses were classified as impaired (i.e. 0-1-1-1)

[404,405]. This approach is consistent with that of Koenigs et al., who analysed responses to a question in the Hamilton Anxiety Rating Scale (HAM-A) rating the difficulty falling or staying asleep from 0–4, and classified impaired as scores ≥2 (i.e. moderate or severe insomnia) [108]. The GHQ-30 was also used to quantify the prevalence of anxiety/depression in the study cohort using the recommended scoring system (i.e. 0-0-1-1) and a threshold of >4 to define 'caseness' [380,405].

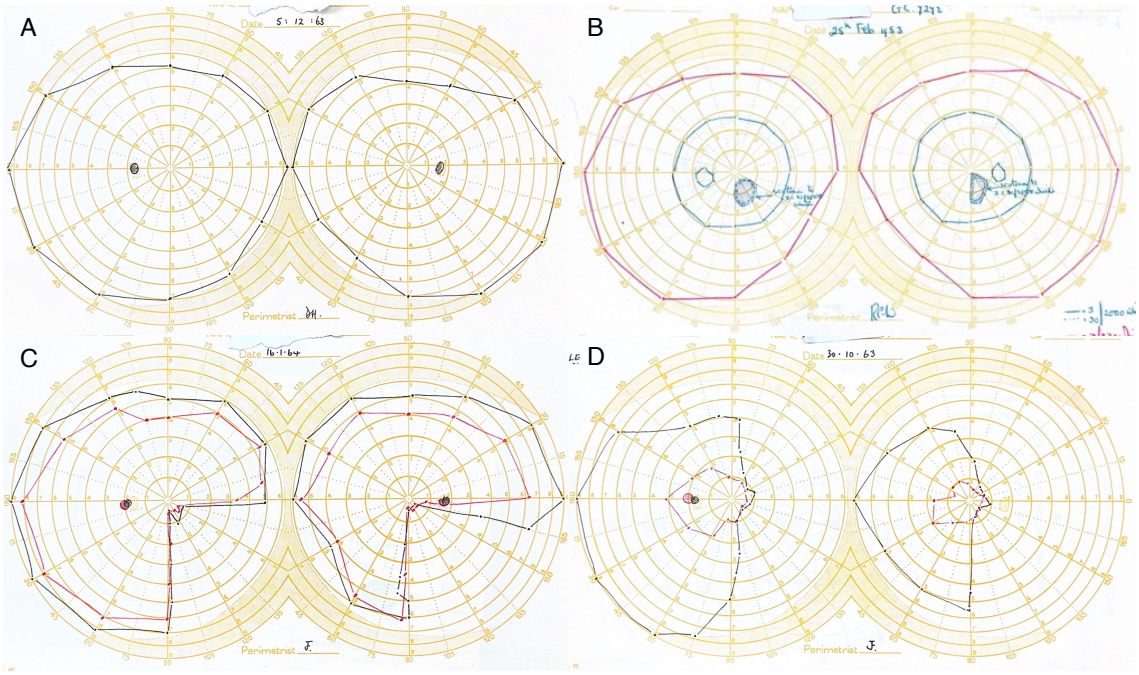


Figure 3.4 Classification of visual field impairments. A. Full visual fields. **B.** Scotoma. **C.** Right lower quadrantanopia. **D.** Right homonymous hemianopia.

3.2.2.1.4 Clock-drawing test classification

To perform the clock-drawing test, participants were provided with a blank sheet of paper and instructed to 'Draw a clock showing the time at twenty minutes to one'. To enable comparison with the findings of Tranel et al., the same two-step classification system was applied, combing the approaches described by Shulman et al. and Freedman [406,219,216]. Accordingly, in step one, responses were classified as borderline if they showed minor visuospatial errors (e.g. mildly impaired spacing,

numbers outside the contour, some numbers upside down, spokes used to orient spacing) and as *impaired* if they showed moderate-severe visuospatial disorganisation (e.g. moderately poor spacing, omission of numbers), inaccurate time-setting, or no reasonable representation of a clock (**Figure 3.5**) [406].

In step two, *impaired* responses were further classified according to specific error patterns (**Figure 3.6**) [219]. *Impaired visuospatial construction* was defined by incorrect number placement (i.e. numbers placed where other numbers should be) and/or omission of numbers. *Impaired time-setting* was defined by incorrect hand placement (i.e. more than 15˚ degrees away from the target number) and/or incorrect hand proportion (i.e. minute hand shorter than hour hand, or both hands equal). *Other errors* included mixed visuospatial and time-setting errors and other error types (e.g. missing hands, severely distorted contour).

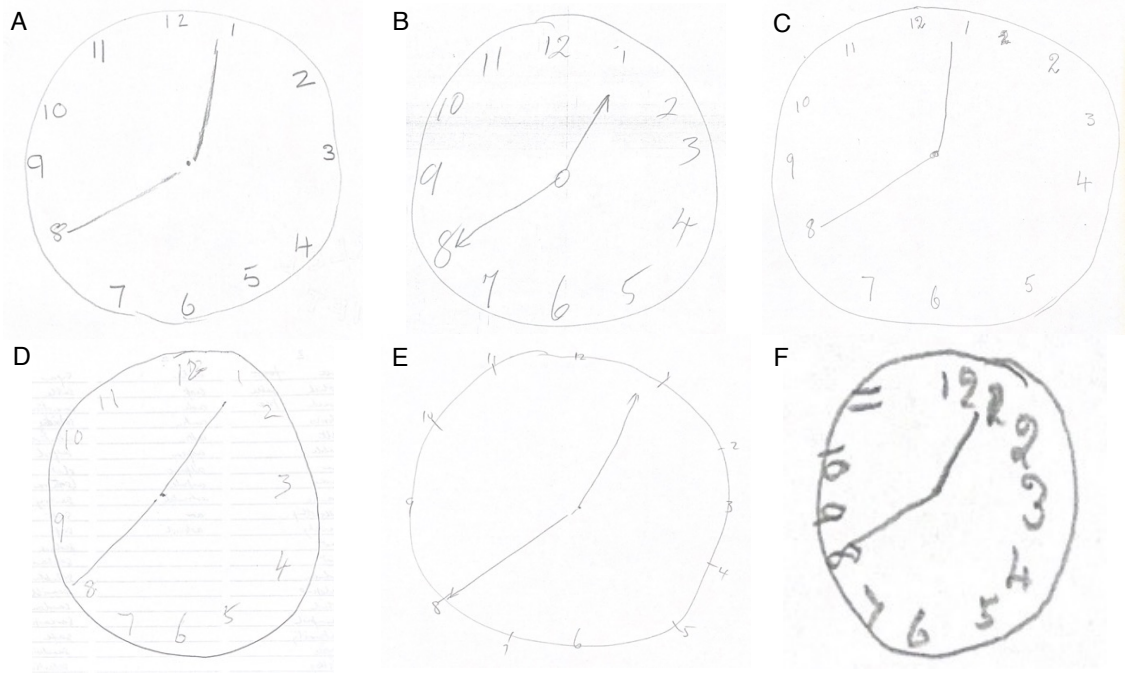


Figure 3.5 Classification of clock-drawing test impairments. A–C. Examples of unimpaired clock drawing. **C.** Greatest spatial inconsistency classified as unimpaired. **D–F.** Examples of borderline spatial impairment. **D.** Borderline spatial inconsistency. **E.** Numbers placed outside contour. **F.** Inconsistent number rotation.

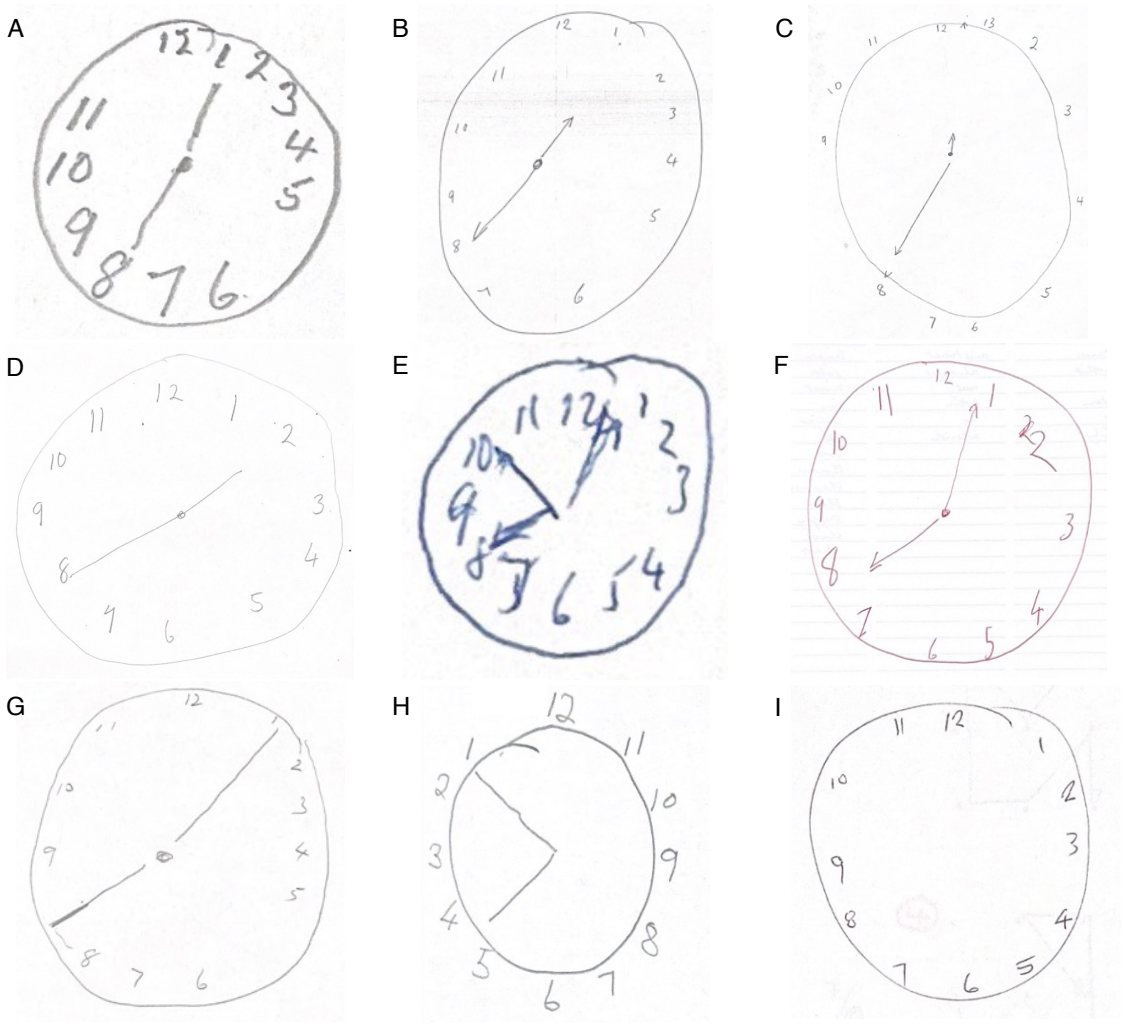


Figure 3.6 Classifications of clock-drawing test error patterns. A–C. Examples of impaired visuospatial construction with preserved time-setting. **D–F.** Examples of impaired time-setting with preserved visuospatial construction. **G–H.** Examples of mixed impaired time-setting and visuospatial construction. **I.** Example of other error patterns.

3.2.2.2 Anatomical methods

Regions of brain damage were digitally reconstructed in 3D from both CT head radiographs and PM photographs using a novel adaptation of existing lesion analysis methods.

3.2.2.2.1 3D reconstruction from CT head radiographs

In ITK-SNAP, a generic T1-weighted MRI brain volume (ch2better) was manually registered to the axial plane of each scan (**Figure 3.7**) [407,408]. To do this, the brain volume was rotated to match the plane of the corresponding anatomical drawing (A1: *x*

= +15, $y = 0$, $z = 0$; A2: $x = +8$, $y = 0$, $z = 0$; A3: $x = +4$, $y = 0$, $z = 0$; A4: $x = -6$, $y = 0$, $z = 0$), and further adjustments were made for each scan to match major anatomical landmarks (e.g. optic chiasm, ventricles) (**Figure 3.7.A**). The occiput was chosen as the centre of rotation ($x = 151$, $y = 1$, $z = 159$) on the basis that variation to gantry tilt would be around the point at which the head rests on the scanning bed (**Figure 3.7.B**). The rotated brain volume was then re-sliced using linear interpolation at a standard thickness (8 mm slice thickness = 18 voxels, 4mm slice thickness = 9 voxels) (**Figure 3.7.C**).

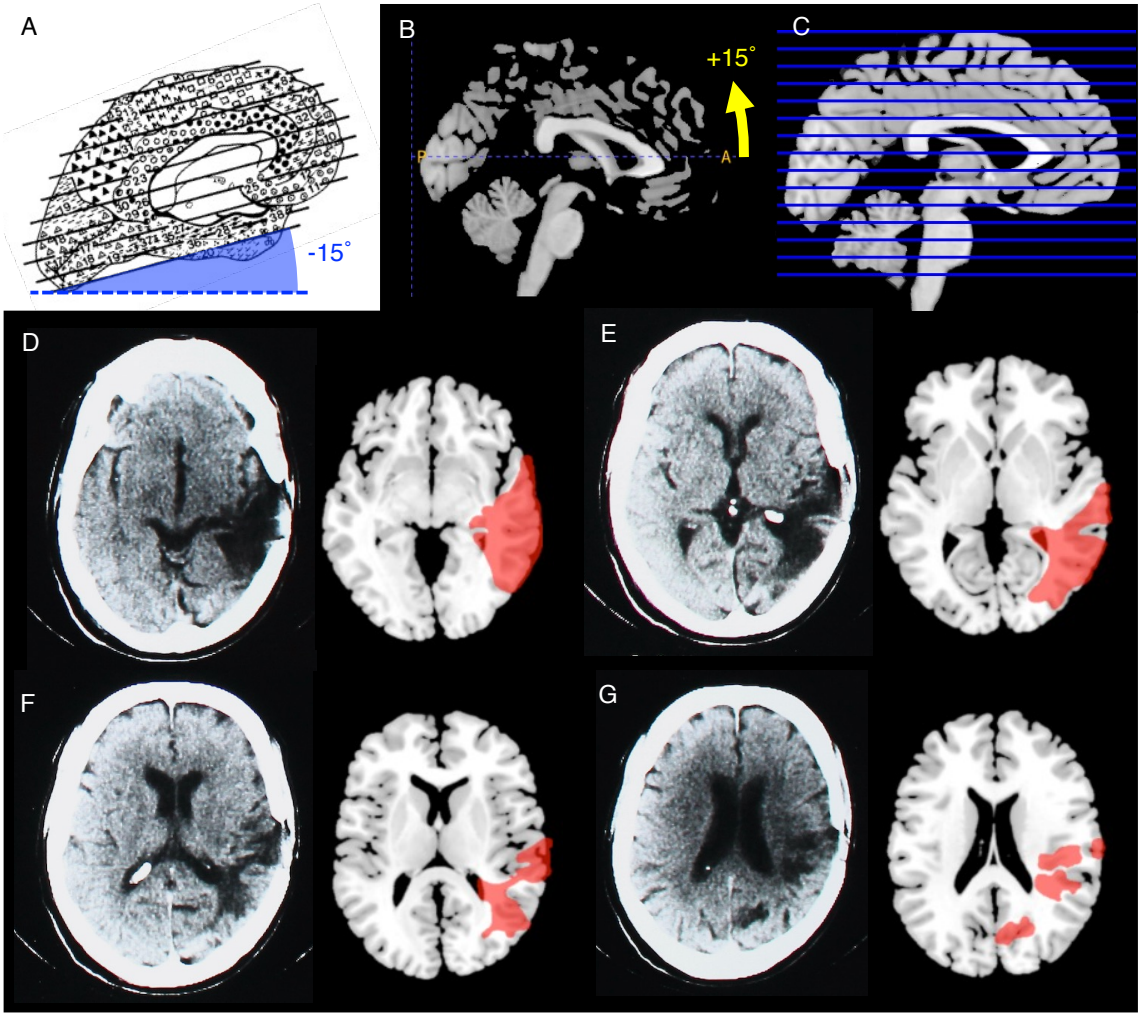


Figure 3.7 Registration and lesion segmentation using CT radiographs. A. In this case, the axial plane of the neuroanatomical template used (A1) was 15 degrees from the plane of a standard brain volume in MNI space. Adapted from Damasio & Damasio (1989) **[231]**. **B.** In ITK-SNAP, the brain volume was rotated 15 degrees around the occiput to match the template,

and small adjustments were made to match major anatomical landmarks on the axial radiographs for the individual case. **C.** The rotated volume was re-sliced in the axial plane. **D–G.** Using the re-sliced volume, corresponding axial slices were used to perform manual segmentation of the damaged region (red), with reference to the radiograph and anatomical localisation drawing.

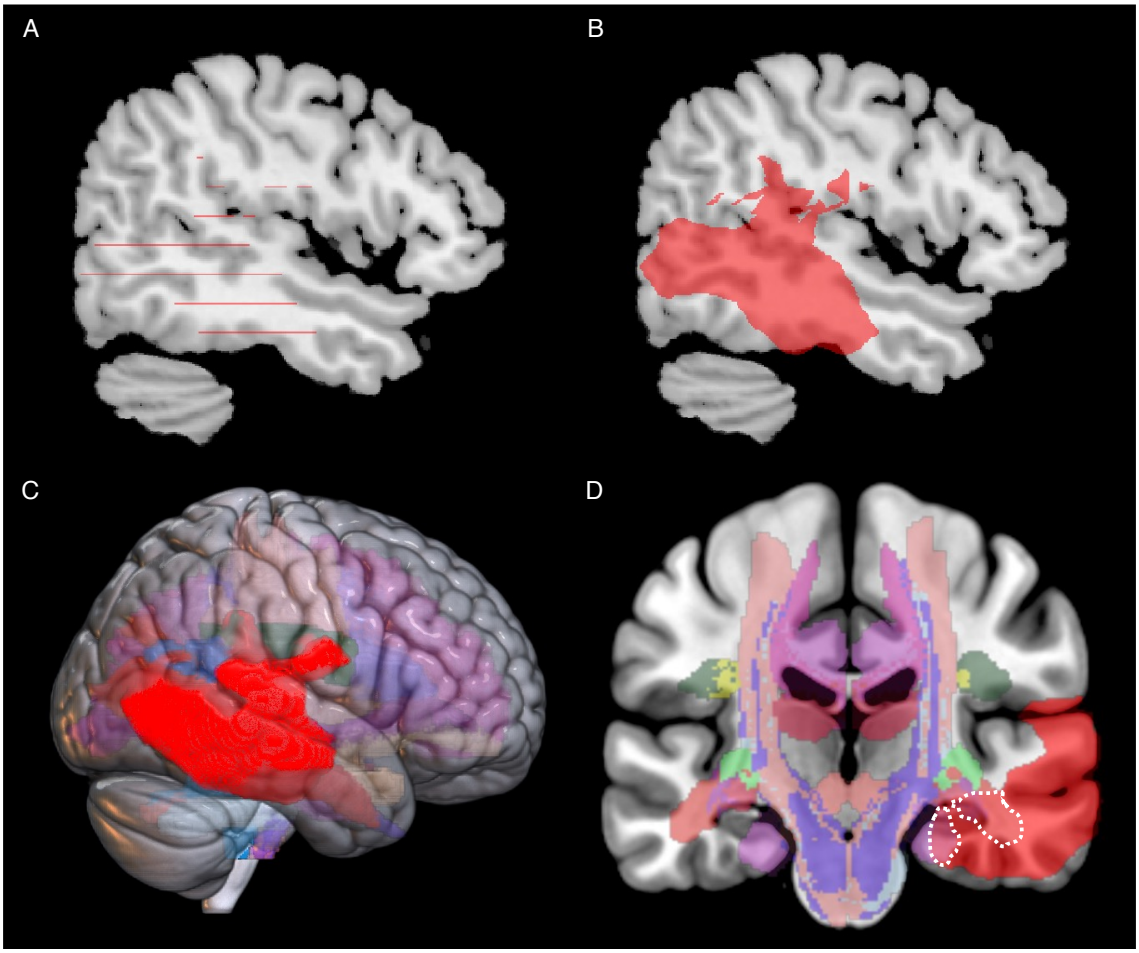


Figure 3.8 Interpolation and overlay onto a brain atlas to generate anatomical data from CT radiographs. A. Sagittal view of segmentations (red) in serial axial slices. **B.** Interpolation of segmentations to render a 3D volume. **C.** External view of the 3D volume. **D.** Overlay of the 3D volume onto a white matter atlas (natbrainlab) to extract anatomical data describing %damage to each region.

Radiographic lesions (i.e. regions of brain damage) were defined as areas of hypodensity. These regions were manually segmented in each axial slice with reference to the radiographs and anatomical drawings (**Figure 3.7.D–G**). For each case, morphological interpolation using optimal slice alignment was applied to render a 3D volume from serial 2D axial segmentations (**Figure 3.8.A–C**). Superior and inferior

ends of the volume were rounded using a 3D paintbrush. To enable overlay onto brain atlases for anatomical analysis, the volume was registered to another generic T1-weighted MRI brain volume (mni152) by reversing the initial rotation and re-slicing using nearest neighbour interpolation. In MRIcroGL, any areas of the volume extending outside of the brain volume were removed using an intensity filter and the volume was smoothed using a smooth and refine tool [409]. This method was applied to generate a lesional volume for each case. These volumes were then overlaid onto brain atlases to quantify the extent of damage to anatomical regions and used to perform overlap analyses and lesion-symptom mapping (**Figure 3.8.D**).

3.2.2.2.2 3D reconstruction from post-mortem photographs

In ITK-SNAP, a generic T1-weighted MRI brain volume (ch2better) was manually registered to the plane of PM sectioning (**Figure 3.9**) [407,408]. To do this, the brain volume was rotated ($x = +5$, $y = 0$, $z = 0$) to match the coronal plane of a neuroanatomical template from 'Oppenheimer's Diagnostic Neuropathology' (**Figure 3.9.A**) [410]. The inferior limit of the brainstem was chosen as the centre of rotation ($x = 151$, $y = 186$, $z = 1$) on the basis that the underside of the brain would be placed on a flat surface to be cut into coronal sections at PM (**Figure 3.9.B**). The rotated brain volume was then re-sliced using linear interpolation at a standard thickness (one coronal section = 16 voxels) (**Figure 3.9.C**).

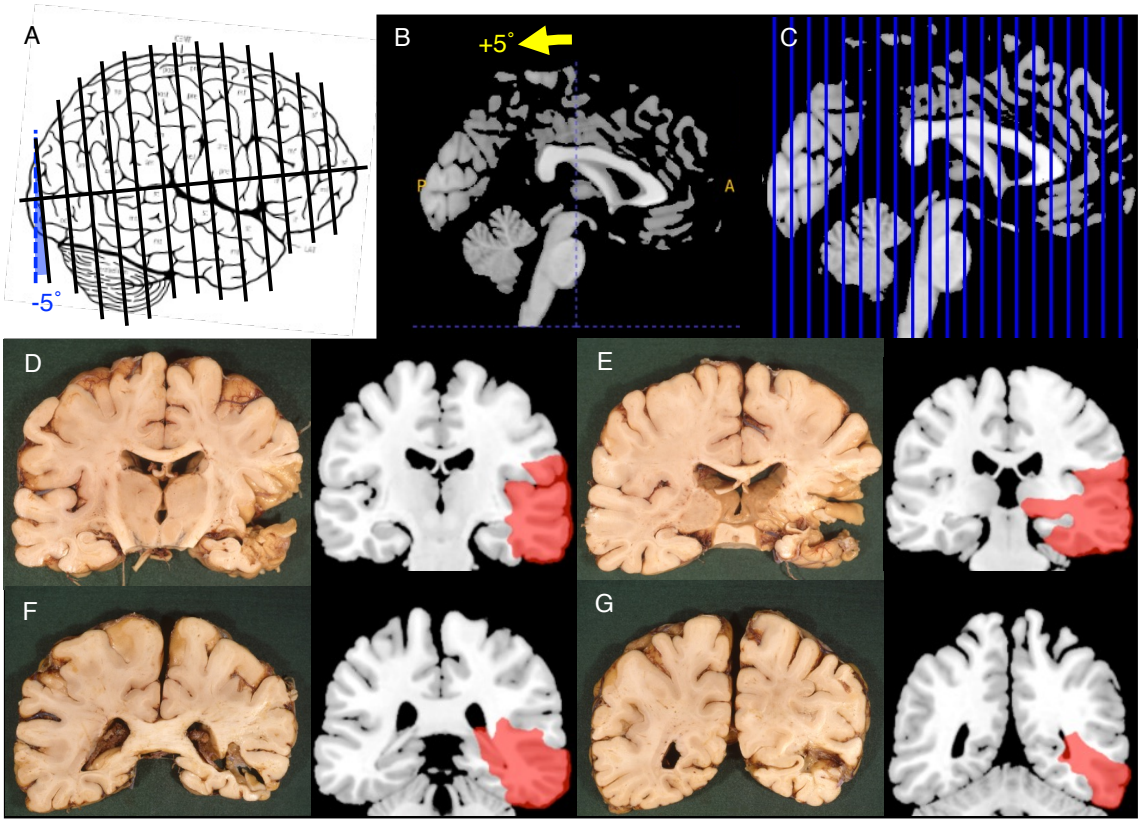


Figure 3.9 Registration and lesion segmentation using post-mortem photographs. A. In all cases, the coronal plane of post-mortem sections was 5 degrees from the plane of a standard brain volume in MNI space. Adapted from Esiri et al. (2012) **[410]**. **B.** In ITK-SNAP, the brain volume was rotated 5 degrees around the inferior brainstem to match the template. **C.** The rotated volume was re-sliced in the coronal plane. **D–G.** Using the re-sliced volume, corresponding coronal slices were used to perform manual segmentation of the damaged region (red), with reference to the post-mortem photograph, annotated template, and report.

Post-mortem lesions were defined as tissue deficits, resulting either from trauma or surgical debridement, and regions of encephalomalacia. In each coronal section, the extent of the damaged region was identified independently by two neuropathologists and traced onto template sections from 'Oppenheimer's Diagnostic Neuropathology' [410]. Lesions were manually segmented in each coronal slice with reference to the annotated templates, PM photographs, and PM reports (**Figure 3.9.D–G**). For each case, morphological interpolation using optimal slice alignment was applied to render a 3D volume from serial 2D coronal segmentations (**Figure 3.10.A–C**). As above, anterior and posterior ends of the volume were rounded using a 3D paintbrush. To

enable overlay onto brain atlases for anatomical analysis, the volume was registered to another generic T1-weighted MRI brain volume (mni152) by reversing the initial rotation ($x = -5$, $y = 0$, $z = 0$) and re-slicing using nearest neighbour interpolation. In MRIcroGL, any areas of the volume extending outside of the brain volume were removed using an intensity filter and the volume was smoothed using a smooth and refine tool [409]. This method was applied to generate a lesional volume for each case. These volumes were then overlaid onto brain atlases to quantify the extent of damage to anatomical regions (**Figure 3.10.D**).

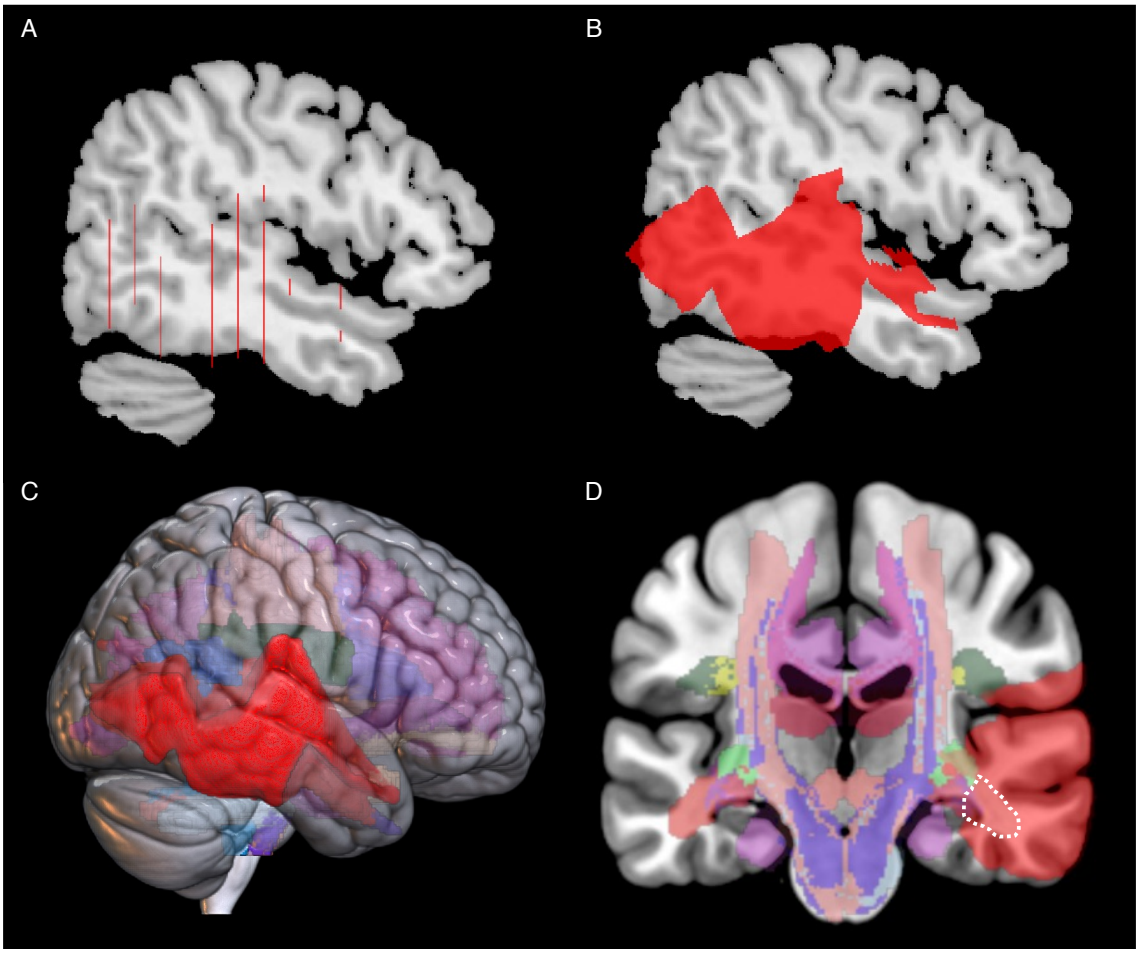


Figure 3.10 Interpolation and overlay onto a brain atlas to generate anatomical data from post-mortem photographs. A. Sagittal view of segmentations (red) in serial coronal slices. **B.** Interpolation of segmentations to render a 3D volume. **C.** External view of the 3D volume. **D.** Overlay of the 3D volume onto a white matter atlas (natbrainlab) to extract anatomical data describing %damage to each region.

3.2.2.2.3 Quantification of anatomical damage

To quantify the extent of damage to anatomical regions, lesional volumes were overlaid onto a grey matter atlas (automated anatomical labelling, AAL) and a white matter atlas derived from DTI tractography (natbrainlab) in MRIcroGL (**Figure 3.8.C–D, Figure 3.10.C–D**) [411–413]. Descriptive volumetric data were extracted to quantify the proportion of voxels in an anatomical region included within the volume (%damage).

3.2.2.2.4 Overlap analysis

Cases were classified into impaired and unimpaired groups for each assessment. In MRIcroGL, an overlap image was generated using all volumes from the cases in each group. The spatial distribution of areas with maximal overlap was reviewed to inform the approach to LSM. For example, because areas of maximal overlap for cases with arm and leg weakness were similar, these groups were combined into a single group with limb weakness for LSM. Conversely, because areas of maximal overlap for cases with hemianopia and quadrantanopia were distinct, these groups were analysed separately.

3.2.2.3 Statistical methods

Statistical analyses were performed using SPSS (v.29.0.2.0) and graphs were produced using GraphPad Prism (v.10.3.1), except where described below for specific LSM analyses.

3.2.2.3.1 Structural validation

Inter-rater reliability of %damage and reliability between reconstructions from CT and PM materials were assessed using an intraclass correlation coefficient (ICC) from a two-way, mixed effects model using absolute agreement of single measures, which is appropriate for continuous data. Coefficients were interpreted as follows: <0.5: poor

reliability, 0.5–0.75: acceptable reliability, 0.75–0.9: good reliability, >0.9: very good reliability [414].

3.2.2.3.2 Lesion-symptom mapping for functional validation and lesion studies

Atlas-based, voxel-wise, and region-specific LSM were performed to identify regions of brain damage associated with impaired performance or worse symptoms in each assessment. To perform LSM, cases were first classified into two groups according to whether the lesion affected a given region/voxel [415]. Assessment scores (i.e. 0=impaired, 1=unimpaired) were then compared between groups using general linear models to produce a z-statistic. This was repeated for every region/voxel. In order to minimise the chance of false positives, permutation testing (2,000 permutations) was used to correct for multiple comparisons [416]. A test statistic threshold equivalent to a corrected p-value <0.05 was used to highlight areas of damage that were associated with impaired performance or more severe symptoms (i.e. $z <$ z-statistic threshold for corrected $p < 0.05$). Thresholds were one-tailed following the assumption that brain damage is always associated with impairment [213]. To enable direct comparison between impaired and unimpaired groups cases with borderline impairment were excluded from LSM.

LSM was performed using SPM12 (https://www.fil.ion.ucl.ac.uk/spm/software/spm12/) and NiiStat (https://www.nitrc.org/projects/niistat) in MATLAB (R2023b Update 9). Atlas-based LSM was performed using both grey matter (AAL and Atlas of Intrinsic Connectivity of Homotopic Areas [AICHA]) and white matter atlases (natbrainlab) [412,413]. To test specific hypotheses regarding structure-function relationships, region-specific LSM was performed using restricted regions from the same atlases. Analyses were restricted to voxels affected in at least 5% of cases to minimise the

influence of rarely affected regions [214]. Given that lesion volume is often a significant predictor of impairment severity, this was regressed out from assessment scores [214]. Statistical maps produced by LSM were visualised using MRIcroGL [409,417].

3.2.2.3.3 Group comparisons

Associations between groups were tested for using the χ^2 test to compare frequencies and the t-test to compare means, as appropriate.

3.2.3 Research ethics

Ethical approval for this study was waived by the Research Governance, Ethics & Assurance Team at the University of Oxford, who advised that 'because it involves only anonymised retrospective data obtained from an existing archive, it requires neither sponsorship nor research ethics review'.[20]

3.3 Results

3.3.1 Baseline characteristics

Members of the neuropsychology study cohort were all male and the majority (87%) were right-handed (**Table 3.1**). Prior to injury, there was virtually no personal or family history of headache or migraine, mental health conditions, or epilepsy. The majority (83%) served in non-commissioned ranks within the Army. Median age at injury was 24 years (**Table 3.2**). At presentation to the MHHI, coma was rare (9%) but focal neurological impairments were common, including dysphasia (33%), cranial nerve deficit (64%), and limb weakness or sensory disturbance (61%). During their admission

[20] Letter from Dr Karen Melham, Sponsorship and Ethics Lead, University of Oxford, 8th August 2022.

to the MHHI, more than half experienced post-traumatic seizures, and at discharge, nearly three quarters were medically invalided.

Table 3.1 Baseline characteristics of the neuropsychology study cohort.

Baseline characteristics	
Sex – n (%)	
Male	69 (100.0)
Female	0 (0.0)
Handedness – n (%)	
Right	60 (87.0)
Left	4 (5.8)
Unknown	5 (7.2)
Personal history – n (%)	
Epilepsy	0 (0.0)
Headache/migraine	1 (1.4)
Family history – n (%)	
Epilepsy	0 (0.0)
Headache/migraine	0 (0.0)
Military force – n (%)	
Army	61 (88.4)
Air force	1 (1.4)
Navy	1 (1.4)
Unknown	6 (8.7)
Military rank – n (%)	
Enlisted	38 (55.1)
Non-commissioned officer	19 (27.5)
Commissioned officer	10 (14.5)
Unknown	2 (2.9)

Table 3.2 Injury details of the neuropsychology study cohort.

Injury details	
Age at injury – median (IQR)	
Age	24.0 (21.0–27.0)
Hemisphere – n (%)	
Left	29 (42.0)
Right	28 (40.6)
Bilateral	12 (17.4)
Conscious level on admission – n (%)	
Comatose	6 (8.7)
Retrograde amnesia – n (%)	
More than 30 mins	2 (2.9)
Up to 30 mins	26 (37.7)
None	23 (33.3)

Injury details	
Unknown	18 (26.1)
Neurological signs – n (%)	
Dysphasia	23 (33.3)
Cranial nerve deficit	44 (63.8)
Limb weakness or sensory disturbance	42 (60.9)
Post-traumatic seizures – n (%)	
Focal only	5 (7.2)
Generalised	31 (44.9)
None	33 (47.8)
Military status at discharge – n (%)	
Invalided	51 (73.9)

A neurological examination of the motor system recorded at discharge from the MHHI was available for all members of the study cohort (**Table 3.3**). Perimetry was available for 87% and was performed approximately 20 years after injury. When multiple perimetry records were available, the assessment performed closest to the date of the CT head scan was used. Responses to the clock-drawing test were available for 90% of the cohort. These were usually performed in the same visit as the CT scan, at an average age of 65 years. The GHQ-30 was completed by 90% of the cohort, on average one year after their CT scan. From this group, four veterans donated their brains to the OBB, with an average age at death of 85 years.

According to responses in the follow-up health questionnaire completed alongside the GHQ-30 at an average age of 66 years, most veterans in this cohort described their current health as fair, good, or excellent, and the majority (68%) saw their doctor every three months or less often (**Table 3.4**). Approximately a third experienced either hearing or visual problems, while one in four reported epilepsy, one in six reported migraine, and one in eight reported depression. Nearly three quarters were current or previous smokers, but less than one quarter consumed alcohol more than three days a

week. Only 6% were never employed, and while most retired for health reasons, the median age at retirement was 61 years.

Table 3.3 Assessment details of the neuropsychology study cohort.

Assessment details	
Assessment available – n (%)	
Motor examination	69 (100.0)
Perimetry	60 (87.0)
Clock-drawing test	62 (89.9)
CT head	69 (100.0)
GHQ-30	62 (89.9)
Post-mortem	4 (5.8)
Age at assessment – median (IQR), years	
Neurological examination	24.0 (21.0–27.0)
Perimetry	47.0 (40.0–54.0)
Clock-drawing test	65.0 (61.0–68.0)
CT head	65.0 (61.0–68.3)
GHQ-30	66.0 (63.0–70.0)
Post-mortem	84.5 (80.0–85.3)

Table 3.4 Health and social details of the neuropsychology study cohort.

Health and social details	
Frequency of healthcare use – n (%)	
Weekly	2 (2.9)
Monthly	13 (18.8)
Every 3 months	16 (23.2)
Every 6 months	8 (11.6)
Less than every 6 months	23 (33.3)
Unknown	7 (10.1)
Self-reported health status – n (%)	
Excellent	5 (7.2)
Good	22 (31.9)
Fair	28 (40.6)
Poor	8 (11.6)
Very poor	1 (1.4)
Unknown	5 (7.2)
Co-morbidities – n (%)	
Visual problems	19 (27.5)
Hearing problems	25 (36.2)
Migraine	11 (15.9)
Epilepsy	16 (23.2)

Health and social details	
Depression	8 (11.6)
Hypertension	9 (13.0)
Smoking history – n (%)	
Current smoker	18 (26.1)
Previous smoker	33 (47.8)
Non-smoker	14 (20.3)
Unknown	4 (5.8)
Alcohol intake – n (%)	
Daily	10 (14.5)
4–6 days/week	6 (8.7)
1–3 days/week	15 (21.7)
Less than weekly	10 (14.5)
Less than monthly	8 (11.6)
Never	11 (15.9)
Unknown	9 (13.0)
Employment – n (%)	
Self-employed	9 (13.0)
Manager	9 (13.0)
Foreman	14 (20.3)
Large firm	48 (69.6)
Small firm	11 (15.9)
Own	4 (5.8)
None	4 (5.8)
Unknown	4 (5.8)
Retirement age – median (IQR)	
Age	61.0 (54.0–64.0)
Retirement reason – n (%)	
Health	22 (31.9)
Age	16 (23.2)
Voluntary	11 (15.9)
Redundancy	4 (5.8)
Unknown	16 (23.2)

3.3.2 Structural validation

3.3.2.1 CT vs PM reconstructions

To perform structural validation of the novel methods described above, %damage to regions of the AAL atlas were compared between reconstructions from CT head radiographs and PM photographs in the four cases for whom both were available (collectively involving damage to 76 anatomical regions, **Table A.3.1**). This comparison

showed good reliability in determining damage to specific anatomical regions (ICC 0.824, 95% CI 0.736–0.885). Overlap analysis confirmed a high level of morphological congruence between the reconstructions for each case, irrespective of the location of the damage (**Figure 3.11**). The average overlap between CT and PM reconstructions was 69.1% (range: 59.4–88.5%, **Table A.3.2**). PM reconstructions were slightly larger than CT reconstructions, but this was not statistically significant (mean PM lesional volume: 57.5 cm^3, mean CT lesional volume: 51.3 cm^3; $p = 0.505$, paired t-test).

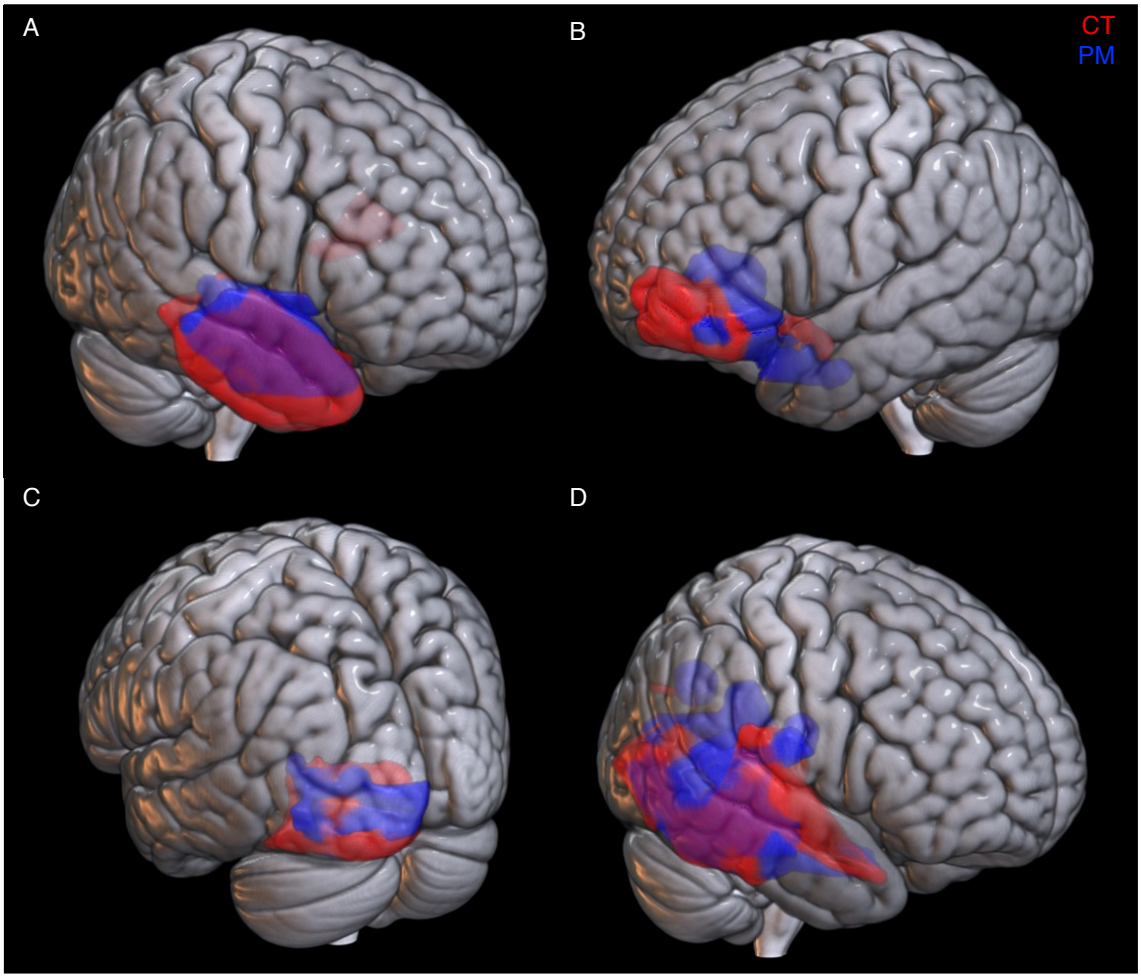


Figure 3.11 Structural validation of the novel lesion reconstruction method. Lesion reconstructions generated from CT head radiographs (red) and post-mortem photographs (blue) in four cases with both resources available.

3.3.2.2 Inter-rater reliability

To assess inter-rater reliability, CT reconstructions were generated by two independent assessors using the methods above for the same four cases, and %damage to regions of the AAL atlas were compared. This also showed good reliability (ICC 0.873, 95% CI 0.809–0.917).

3.3.3 Lesion characteristics

3.3.3.1 Lesion volume

Mean lesion volume was 58.7cm^3 with substantial variability across the cohort (SD: 41.3cm^3, range: 3.4–160.9cm^3). This equates to an average lesion occupying 3.3% of the generic mni152 brain volume (range: 0.2–9.2%). The average lesion volume in the MHHI cohort was slightly larger than that of the VHIS (**Table 3.5**) [108].

Table 3.5 Lesion volume in the Military Hospital for Head Injuries cohort compared with cohorts comprising other lesion aetiologies. ICA = internal carotid artery. MCA = middle cerebral artery.

Aetiology	Lesion volume – cm^3
Penetrating brain injury	
MHHI cohort – mean (SD)	58.7 (41.3)
VHIS cohort – mean (SD) [108]	40.4 (43.4)
Ischaemic stroke	
ICA or proximal MCA occlusion – mean (SD) [419]	91.3 (79.9)
Stroke leading to aphasia – mean (SD) [418]	101.0 (82.8)
Small volume stroke cohort – median (SD) [224]	5.8 (27.1)
Malignancy	
Brain tumour cohort – median (SD) [224]	66.5 (71.9)

Compared to other aetiologies used for LSM, lesion volume in the MHHI cohort is significantly smaller than that of strokes resulting in aphasia [418] and strokes caused by occlusions of the internal carotid artery or proximal middle cerebral artery [419],

although lesion volume can be much smaller in some stroke cohorts [224]. Lesion volume in this cohort was also slightly smaller than a typical brain tumour cohort used for LSM [224].

3.3.3.2 Lesion location

Overlapping lesions from all cases reveals the distribution of penetrating brain injuries in this cohort (**Figure 3.12**). The medulla, pons, and midbrain were spared, and relatively few lesions involved the cerebellum. Areas of maximal overlap are concentrated around the anterior and posterior horns of the lateral ventricles. This pattern contrasts with the regions damaged in veterans of the Vietnam war, whose injuries predominantly affected prefrontal areas.

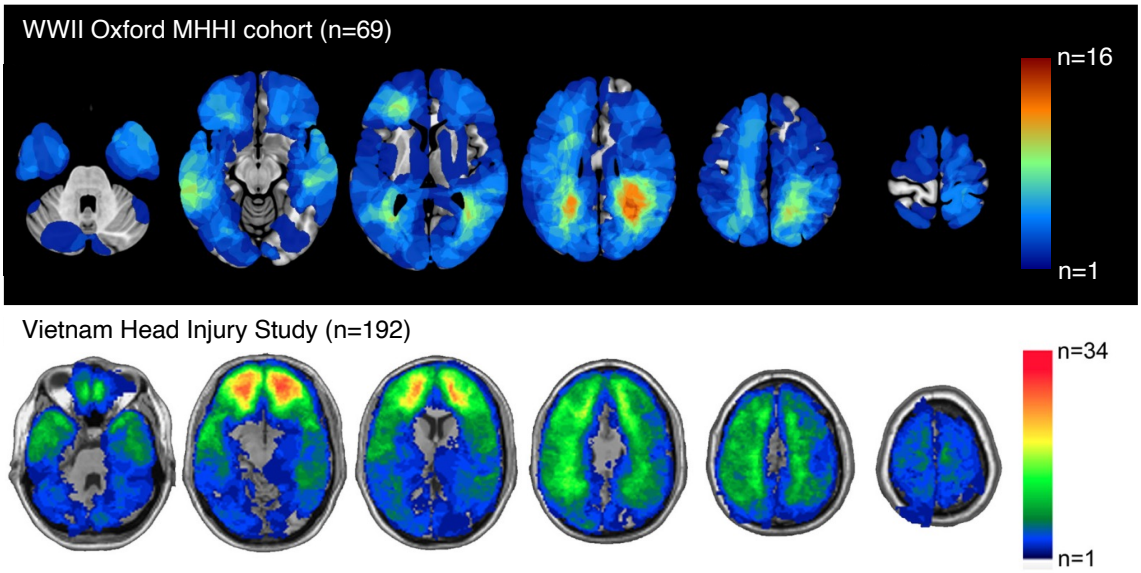


Figure 3.12 Lesion overlap for all cases in the Military Hospital for Head Injuries (MHHI) neuropsychology study cohort contrasts with the lesion overlap for all cases in the Vietnam Head Injury Study. Adapted from Koenigs et al. (2010) [108]. Colour bars indicate the number of overlapping lesions at each voxel.

3.3.4 Functional validation

To test the validation hypotheses and explore the limitations of the method described above, overlap analyses and LSM were used to assess the performance of film-based

CT reconstructions at identifying brain regions required for limb power and visual perception.

3.3.4.1 Limb weakness

Limb weakness was present in more than a third of the study cohort (**Table 3.6**), with slight weakness being more common than moderate or severe weakness. Of the 25 cases with impaired limb power, 13 experienced left-sided weakness, 11 experienced right-sided weakness, and one experience weakness bilaterally. Arm weakness was more common than leg weakness, although most experienced arm and leg weakness together.

Table 3.6 Limb weakness.

Limb weakness	
Limb power – n (%)	
Unimpaired	44 (63.8)
Impaired	25 (36.2)
Slight weakness	15 (60.0)
Moderate weakness	5 (20.0)
Severe weakness	5 (20.0)
Left-sided weakness – n (%)	13
Left arm and leg	6 (46.2)
Left arm only	5 (38.5)
Left leg only	2 (15.4)
Right-sided weakness – n (%)	11
Right arm and leg	7 (63.6)
Right arm only	3 (27.3)
Right leg only	1 (9.1)
Bilateral weakness – n (%)	1
Both arms	0 (0.0)
Both legs	1 (100.0)

3.3.4.1.1 Left limb weakness

Lesion overlap in cases with left limb weakness showed that areas of maximal overlap were concentrated around the right central sulcus (**Figure 3.13.A**). Atlas-based LSM showed that left limb weakness was associated with damage to the right pre-central

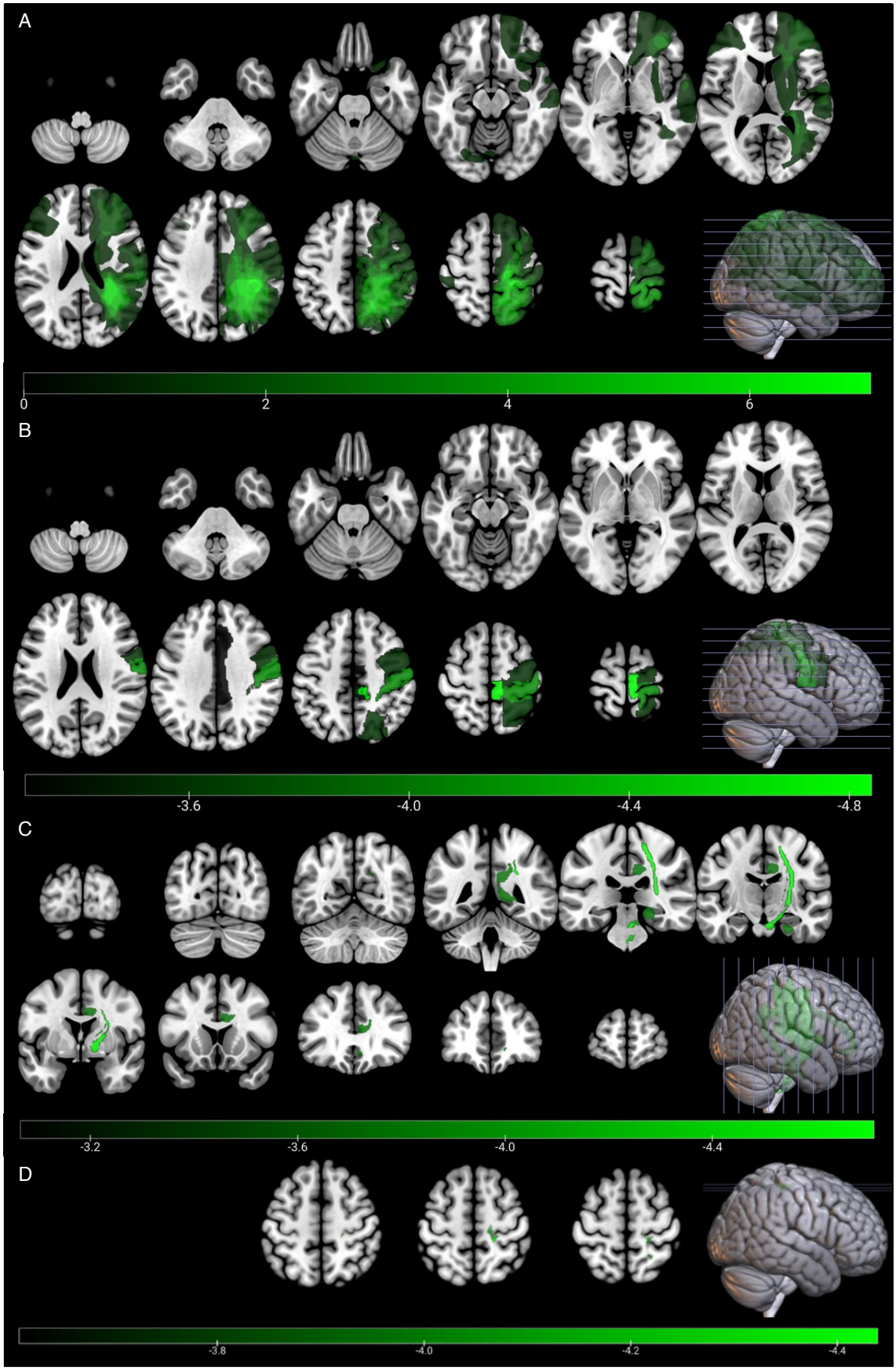
gyrus, right mid cingulum, right post-central gyrus, right superior parietal gyrus, and right paracentral lobule grey matter regions ($z < -3.303$, **Figure 3.13.B**), and the right cortico-spinal tract and right cingulum white matter regions ($z < -3.065$, **Figure 3.13.C**). Voxel-wise LSM identified a small volume involving the right pre-central gyrus, right post-central gyrus, and right cortico-spinal tract that was associated with left limb weakness (812 voxels, $z < -3.608$ **Figure 3.13.D**). Region-specific LSM that was restricted to the right pre-central gyrus ($z = -3.561$, $z < -1.791$) and right cortico-spinal tract ($z = -4.711$, $z < -1.779$) confirmed that damage to these regions was associated with left limb weakness.

3.3.4.1.2 Right limb weakness

Lesion overlap in cases with right limb weakness showed that areas of maximal overlap were concentrated in the left central sulcus (**Figure 3.14.A**). Atlas-based LSM showed that right limb weakness was associated with damage to the left post-central gyrus and left supramarginal gyrus grey matter regions ($z < -3.628$, **Figure 3.14.B**), and the left cortico-spinal tract, left arcuate, and left long segment white matter regions ($z < -3.155$, **Figure 3.14.C**). Voxel-wise LSM identified a small volume involving the left cortico-spinal tract, left arcuate, and left long segment that was associated with right limb weakness (68 voxels, $z < -3.962$, **Figure 3.14.D**). Region-specific LSM that was restricted to the left pre-central gyrus ($z = -3.494$, $z < -1.815$) and left cortico-spinal tract ($z = -3.522$, $z < -1.772$) confirmed that damage to these regions was associated with right limb weakness.

3.3.4.2 Visual field impairment

Visual field impairment was present in more than a third of the study cohort (**Table 3.7**), with quadrantanopia more common than hemianopia, and a small number of cases with scotoma.


A
0
2
4
6
B
-3.6
-4.0
-4.4
-4.8
C
-3.2
-3.6
-4.0
-4.4
D
-3.8
-4.0
-4.2
-4.4

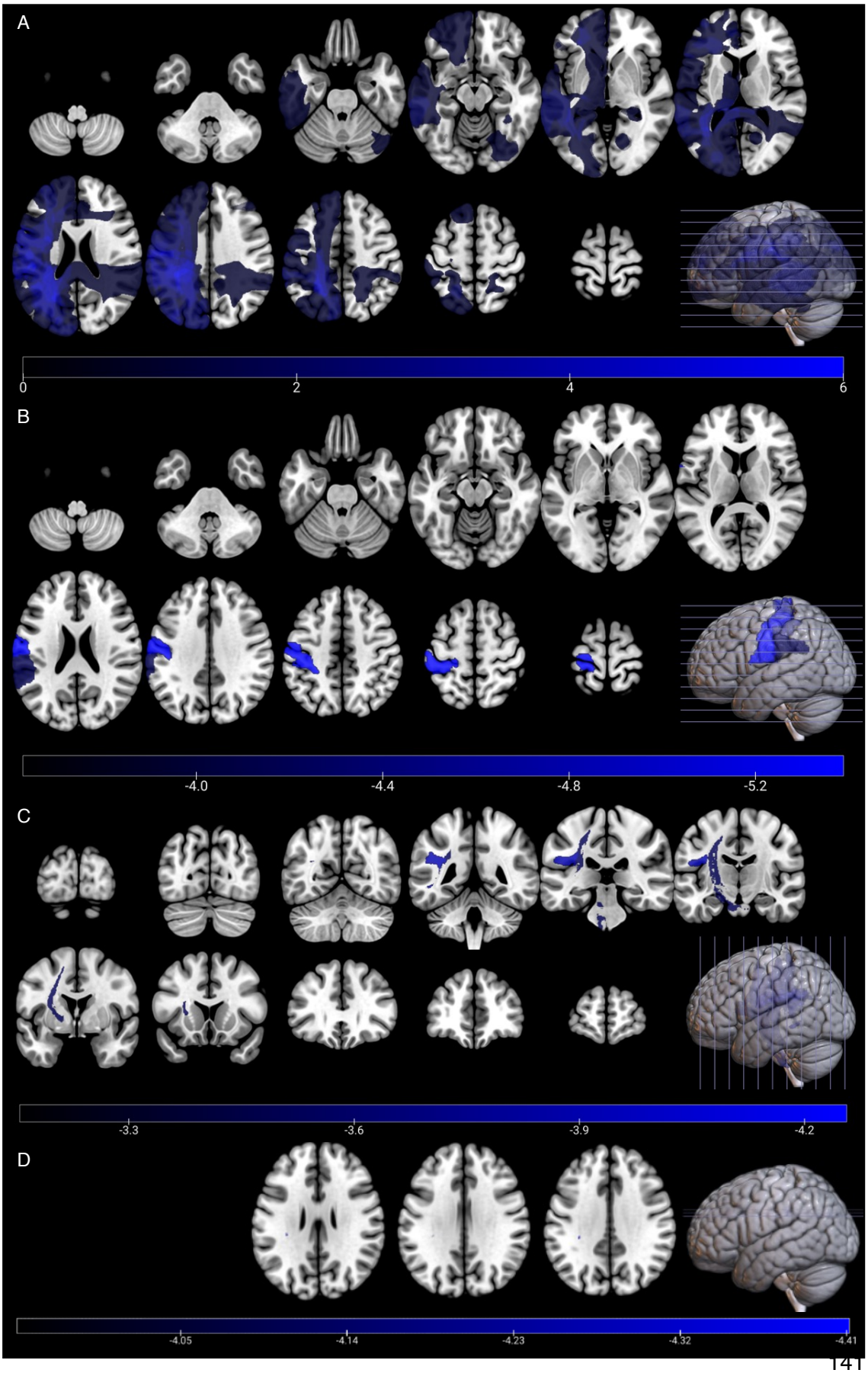

A
0
2
4
6
B
-4.0
-4.4
-4.8
-5.2
C
-3.3
-3.6
-3.9
-4.2
D
-4.05
-4.14
-4.23
-4.32
-4.41

Figure 3.13 Brain regions damaged in cases with left limb weakness (*n* = 14). A. Lesion overlap. **B.** Grey matter atlas-based lesion-symptom mapping (LSM). **C.** White matter atlas-based LSM. **D.** Voxel-wise LSM. Colour bars indicate (**A**) the number of overlapping lesions and (**B–D**) z-values exceeding the threshold for a corrected p-value <0.05 at each voxel.

Figure 3.14 Brain regions damaged in cases with right limb weakness (*n* = 11). A. Lesion overlap. **B.** Grey matter atlas-based lesion-symptom mapping (LSM). **C.** White matter atlas-based LSM. **D.** Voxel-wise LSM. Colour bars indicate (**A**) the number of overlapping lesions and (**B–D**) z-values exceeding the threshold for a corrected p-value <0.05 at each voxel.

Table 3.7 Visual field impairment.

Visual field impairment	
Visual fields – n (%)	
Unimpaired	37 (61.7)
Impaired	23 (38.3)
Hemianopia – n (%)	8
Left	3 (37.5)
Right	4 (50.0)
Inferior	1 (12.5)
Superior	0 (0.0)
Quadrantanopia – n (%)	12
Left	5 (41.7)
Right	7 (58.3)
Scotoma	3

3.3.4.2.1 Left hemianopia

Lesion overlap in cases with left hemianopia showed that areas of maximal overlap were concentrated in the right occipital region (**Figure 3.15.A**). Atlas-based LSM showed that left hemianopia was associated with damage to the right calcarine, right cuneus, right superior occipital gyrus, right middle occipital gyrus, and right inferior occipital gyrus ($z < -5.764$, **Figure 3.15.B**). Voxel-wise LSM identified a large volume involving the right calcarine, right cuneus, right superior occipital gyrus, and right middle occipital gyrus that was associated with left hemianopia (53,889 voxels, $z < -3.420$, **Figure 3.15.C**). Region-specific LSM that was restricted to the right calcarine cortex ($z = -5.977$, $z < -3.082$) confirmed that damage to this region was associated with left hemianopia.

3.3.4.2.2 Left quadrantanopia

Lesion overlap in cases with left quadrantanopia showed that areas of maximal overlap were concentrated in the right optic radiation (**Figure 3.16.A**). Atlas-based LSM showed that left quadrantanopia was associated with damage to the right optic radiation, right inferior longitudinal fasciculus, and right inferior occipito-frontal fasciculus ($z < -3.699$, **Figure 3.16.B**). Voxel-wise LSM did not identify any voxels associated with left quadrantanopia (0 voxels, $z = -3.694$, $z < -3.994$). Region-specific LSM that was restricted to the right optic tract ($z = -3{,}731$, $z < -1.778$) confirmed that damage to this region was associated with left quadrantanopia.

3.3.4.2.3 Right hemianopia

Lesion overlap in cases with right hemianopia showed that areas of maximal overlap were concentrated in the left occipital region (**Figure 3.17.A**). Atlas-based LSM showed that right hemianopia was associated with damage to the left superior occipital gyrus ($z < -4.882$, **Figure 3.17.B**). Voxel-wise LSM identified a small volume involving the posterior part of the left optic radiation that was associated with right hemianopia (125 voxels, $z < -4.087$, **Figure 3.17.C**). Region-specific LSM that was restricted to the left calcarine cortex ($z = -3.129$, $z < -3.076$) confirmed that damage to this region was associated with right hemianopia.

3.3.4.2.4 Right quadrantanopia

Lesion overlap in cases with right quadrantanopia showed that areas of maximal overlap were concentrated in the left optic radiation (**Figure 3.18.A**). Atlas-based LSM showed that right quadrantanopia was associated with damage to the left optic radiation, left inferior longitudinal fasciculus, left inferior occipito-frontal fasciculus, and left posterior segment ($z < -3.419$, **Figure 3.18.B**). Voxel-wise LSM identified a small volume in the anterior part of the left optic radiation that was associated with right

quadrantanopia (1,920 voxels, $z < -3.515$, **Figure 3.18.C**). Region-specific LSM that was restricted to the left optic radiation ($z = -3.801$, $z < -1.884$) confirmed that damage to this region was associated with right quadrantanopia.

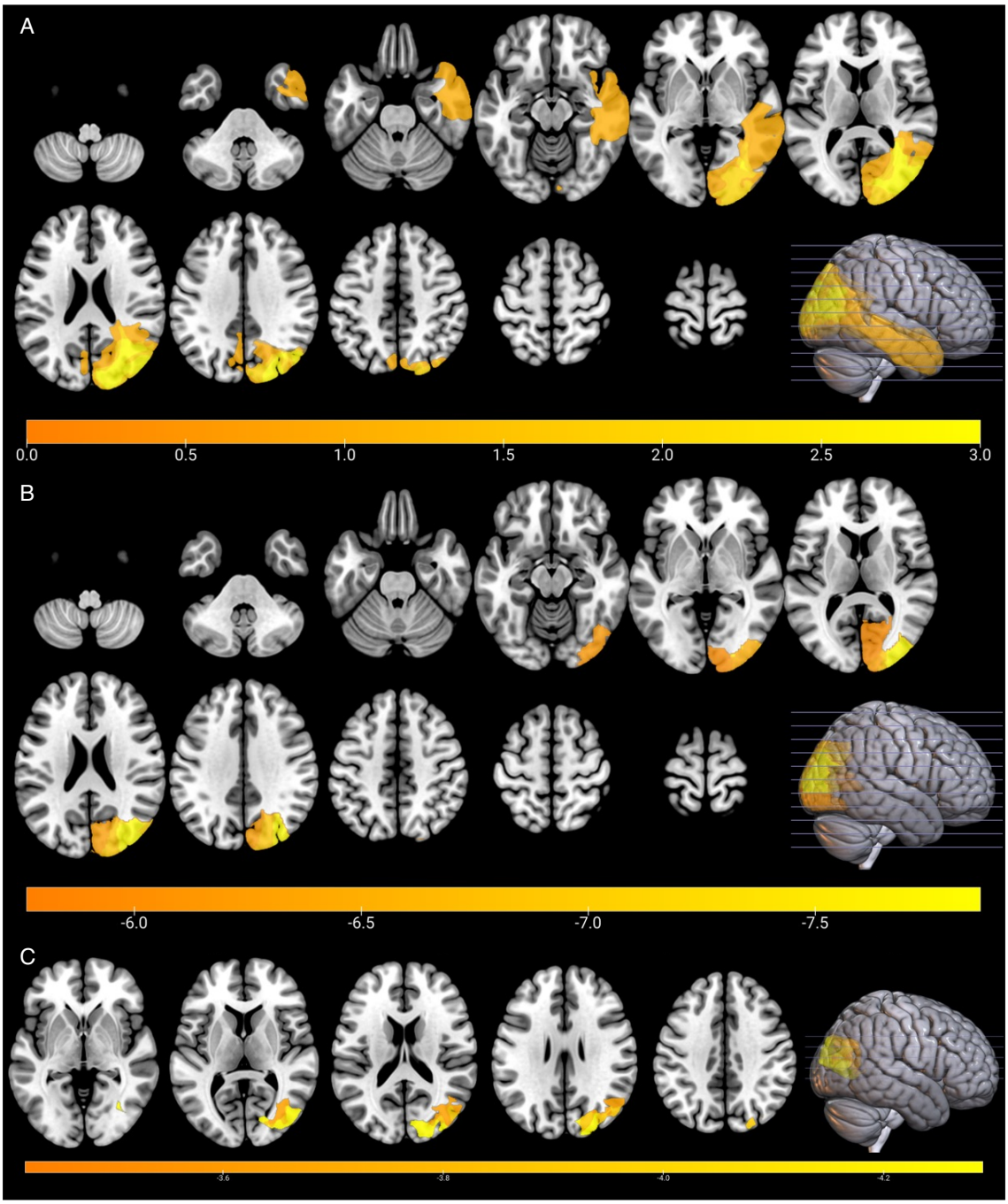


Figure 3.15 Brain regions damaged in cases with left hemianopia (*n* = 3). A. Lesion overlap. **B.** Grey matter atlas-based lesion-symptom mapping (LSM). **C.** Voxel-wise LSM. Colour bars indicate (**A**) the number of overlapping lesions and (**B–C**) z-values exceeding the threshold for a corrected p-value <0.05 at each voxel.

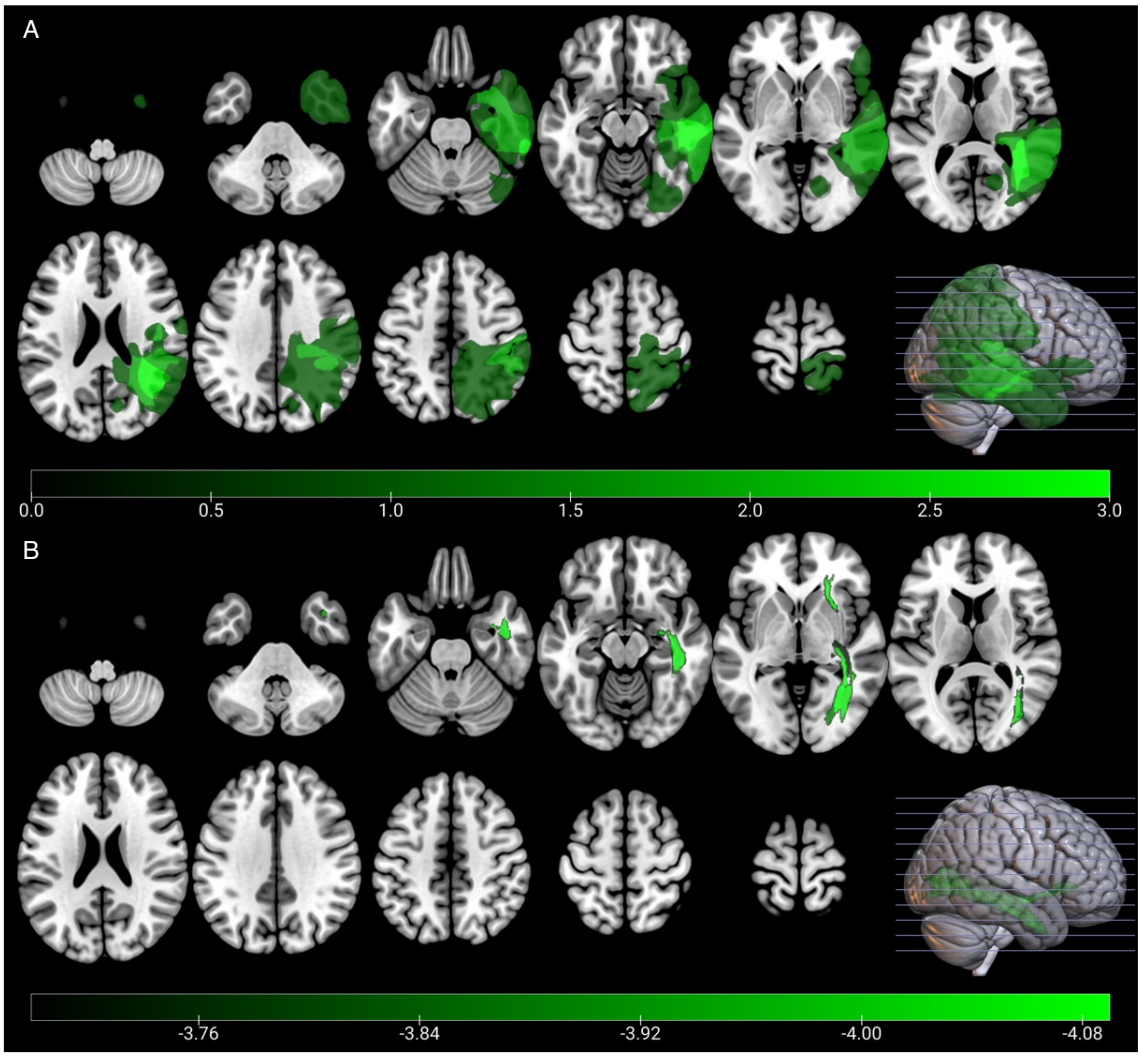


Figure 3.16 Brain regions damaged in cases with left quadrantanopia (*n* = 5). A. Lesion overlap. **B.** White matter atlas-based lesion-symptom mapping (LSM). Colour bars indicate (**A**) the number of overlapping lesions and (**B**) z-values exceeding the threshold for a corrected p-value <0.05 at each voxel.

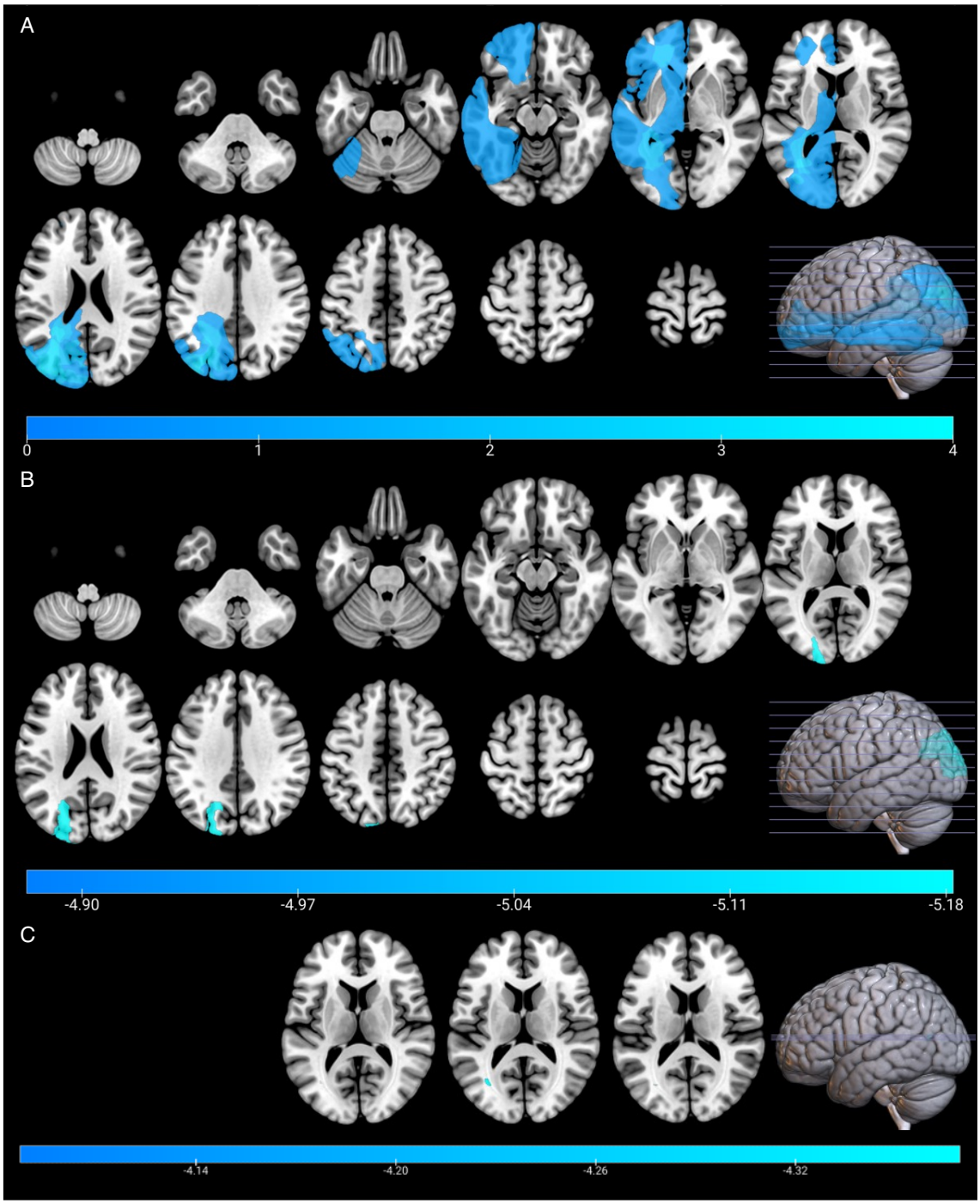


Figure 3.17 Brain regions damaged in cases with right hemianopia (*n* = 4). A. Lesion overlap. **B.** Grey matter atlas-based lesion-symptom mapping (LSM). **C.** Voxel-wise LSM. Colour bars indicate (**A**) the number of overlapping lesions and (**B–C**) z-values exceeding the threshold for a corrected p-value <0.05 at each voxel.

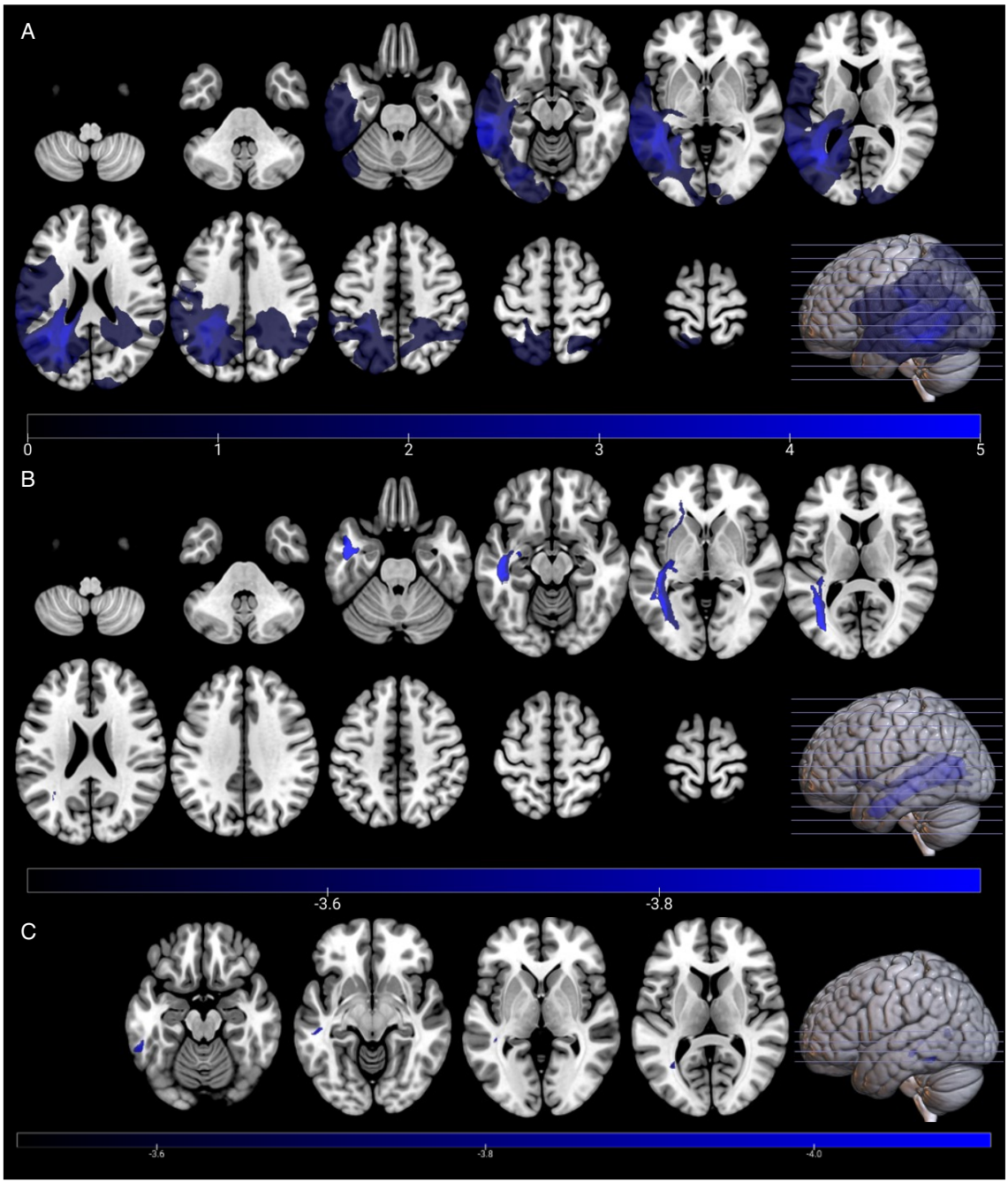


Figure 3.18 Brain regions damaged in cases with right quadrantanopia (*n* = 7). A. Lesion overlap. **B.** White matter atlas-based lesion-symptom mapping (LSM). **C.** Voxel-wise LSM. Colour bars indicate (**A**) the number of overlapping lesions and (**B–C**) z-values exceeding the threshold for a corrected p-value <0.05 at each voxel.

3.3.5 Lesion-symptom mapping in the Military Hospital for Head Injuries cohort

3.3.5.1 Insomnia

3.3.5.1.1 Sleep disturbance after penetrating brain injury

Sleep disturbance was reported by more than three quarters of the study cohort (**Table 3.8**). Of the 49 cases with insomnia, approximately two thirds reported no recent change (63.3%), while a third reported that their sleep had recently been worse (26.5%) or much worse (10.2%). Only three cases reported regular use of medications with a primary sedating effect (i.e. lorazepam, diazepam, and nitrazepam). There were no significant differences in age, self-reported symptoms of depression/anxiety, sedative medication use, or lesion volume between those with and without insomnia. Left hemisphere damage was not significantly more common among cases with insomnia (insomnia: 44.9%, no insomnia: 38.5%, $\chi^2 = 0.385$, $p = 0.825$).

Table 3.8 Baseline characteristics, lesion location and volume in the insomnia cohort.

Characteristic	Insomnia (*n* = 49)	No insomnia (*n* = 13)	p-value
Age at assessment – mean (SD)	65.9 (6.2)	67.9 (5.2)	0.278
Depression/anxiety – n (%)	22 (44.9)	5 (38.5)	0.677
Sedative medication use – n (%)	3 (6.1)	0 (0.0)	0.355
Hemisphere damaged – n (%)			0.825
Left	22 (44.9)	5 (38.5)	
Right	18 (36.7)	6 (46.2)	
Bilateral	9 (18.4)	2 (15.4)	
Lesion volume (cm^3) – mean (SD)	59.5 (40.3)	58.3 (45.5)	0.933

3.3.5.1.2 Damage to the prefrontal region is associated with insomnia

Lesion overlap in cases with insomnia showed that areas of maximal overlap were similar to the areas affected by penetrating brain injuries in the cohort generally (**Figure 3.12, Figure 3.19.A**). However, lesion overlap of cases without insomnia revealed a striking absence of damage to the left prefrontal region (**Figure 3.19.B**).

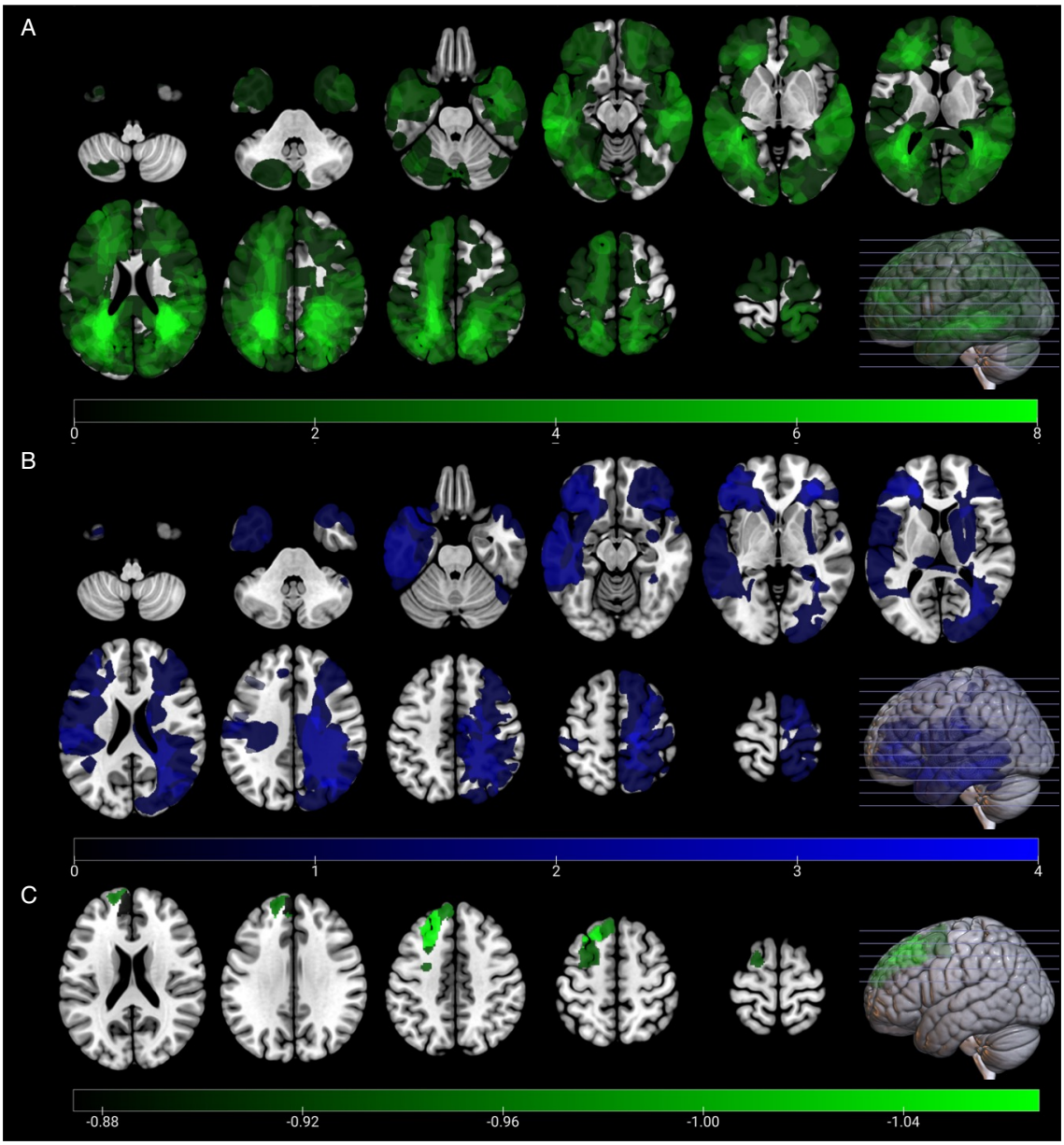


Figure 3.19 Lesion-symptom mapping (LSM) in the Military Hospitals for Head Injuries cohort supports an association between damage to the left prefrontal region and insomnia. A. Lesion overlap for cases with insomnia. **B.** Lesion overlap for cases without insomnia. **C.** Region-specific LSM restricted to the left prefrontal region confirmed that damage to the left superior frontal gyrus, left superior frontal sulcus, and left medial superior frontal gyrus is associated with insomnia.

Region-specific LSM that was restricted to left prefrontal regions of the AICHA atlas confirms that damage to the left superior frontal gyrus (part 2), left superior frontal sulcus (parts 4–6), and left medial superior frontal gyrus (part 2) was associated with insomnia (**Table A.3.3**). Region-specific LSM that was restricted to the left prefrontal

regions of the AAL atlas did not reach statistical significance (left medial superior frontal gyrus: $z = -0.939$, $z < -1.006$; left superior frontal gyrus: $z = -1.164$, $z < -1.235$). Atlas-based LSM found that no regions survived the threshold of significance (AAL: 0 regions, $z = -1.564$, $z < -1.710$; natbrainlab: AAL: 0 regions, $z = -1.696$, $z < -1.890$). Voxel-wise LSM also found that no voxels survived the threshold of significance (0 voxels, $z = -2.062$, $z < -2.194$, corrected $p < 0.05$).

3.3.5.2 Clock-drawing test

3.3.5.2.1 Clock-drawing test performance after penetrating brain injury

Performance in the clock-drawing test was impaired in more than a third of the study cohort (**Table 3.9**). Of the cases with impaired performance, six made visuospatial errors, ten made time-setting errors, and seven made mixed/other errors.

Table 3.9 Clock-drawing test performance and error pattern analysis.

Clock-drawing test	
Clock-drawing test performance – n (%)	
Unimpaired	30 (48.4)
Borderline	9 (14.5)
Impaired	23 (37.1)
Visuospatial error – n (%)	6
Left hemisphere damage	3 (50.0)
Right hemisphere damage	2 (33.3)
Bilateral damage	1 (16.7)
Time-setting error – n (%)	10
Left hemisphere damage	7 (70.0)
Right hemisphere damage	3 (30.0)
Bilateral damage	0 (0.0)
Mixed/other error – n (%)	7
Left hemisphere damage	2 (28.6)
Right hemisphere damage	2 (28.6)
Bilateral damage	3 (42.9)

3.3.5.2.2 Hemispheric laterality of clock-drawing test error patterns

Among cases making visuospatial construction errors there was no association with right hemisphere damage (left hemisphere: 3, right hemisphere: 2, $p = 0.733$, $\chi^2 = 0.117$, **Figure 3.20**). Among cases making time-setting errors, left hemisphere damage was more than twice as common as right hemisphere damage, but this was not statistically significant (left hemisphere: 7, right hemisphere: 3, $p = 0.226$, $\chi^2 = 1.463$, **Figure 3.20**). Three out of four cases with bilateral damage showed mixed/other error patterns.

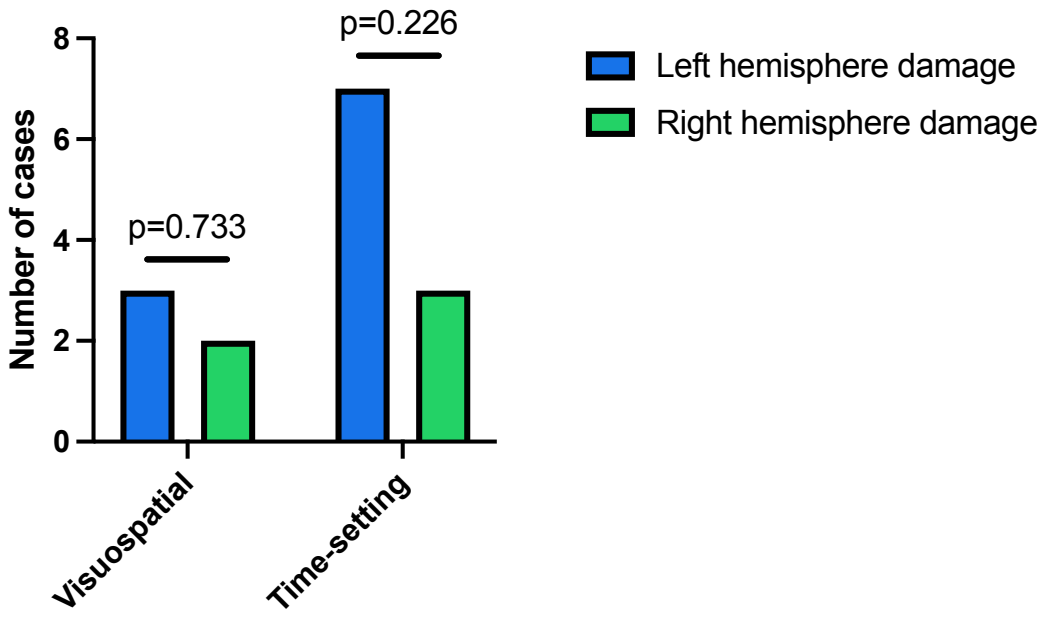


Figure 3.20 Clock-drawing test error patterns and laterality of hemispheric damage. While left-hemisphere damage was more common among cases with time-setting impairment, there was no association between laterality and clock-drawing test error patterns (χ^2 test, $n = 55$, cases with bilateral damage excluded).

Lesion overlap in cases making visuospatial construction errors showed that areas of maximal overlap were concentrated in the right hemisphere, although these regions involved only two cases (**Figure 3.21.A**). Lesion overlap in cases making time-setting errors showed that areas of maximal overlap were concentrated in the left hemisphere,

involving four cases (**Figure 3.21.B**). No regions or voxels were associated with visuospatial construction or time-setting errors by atlas-based or voxel-wise LSM.

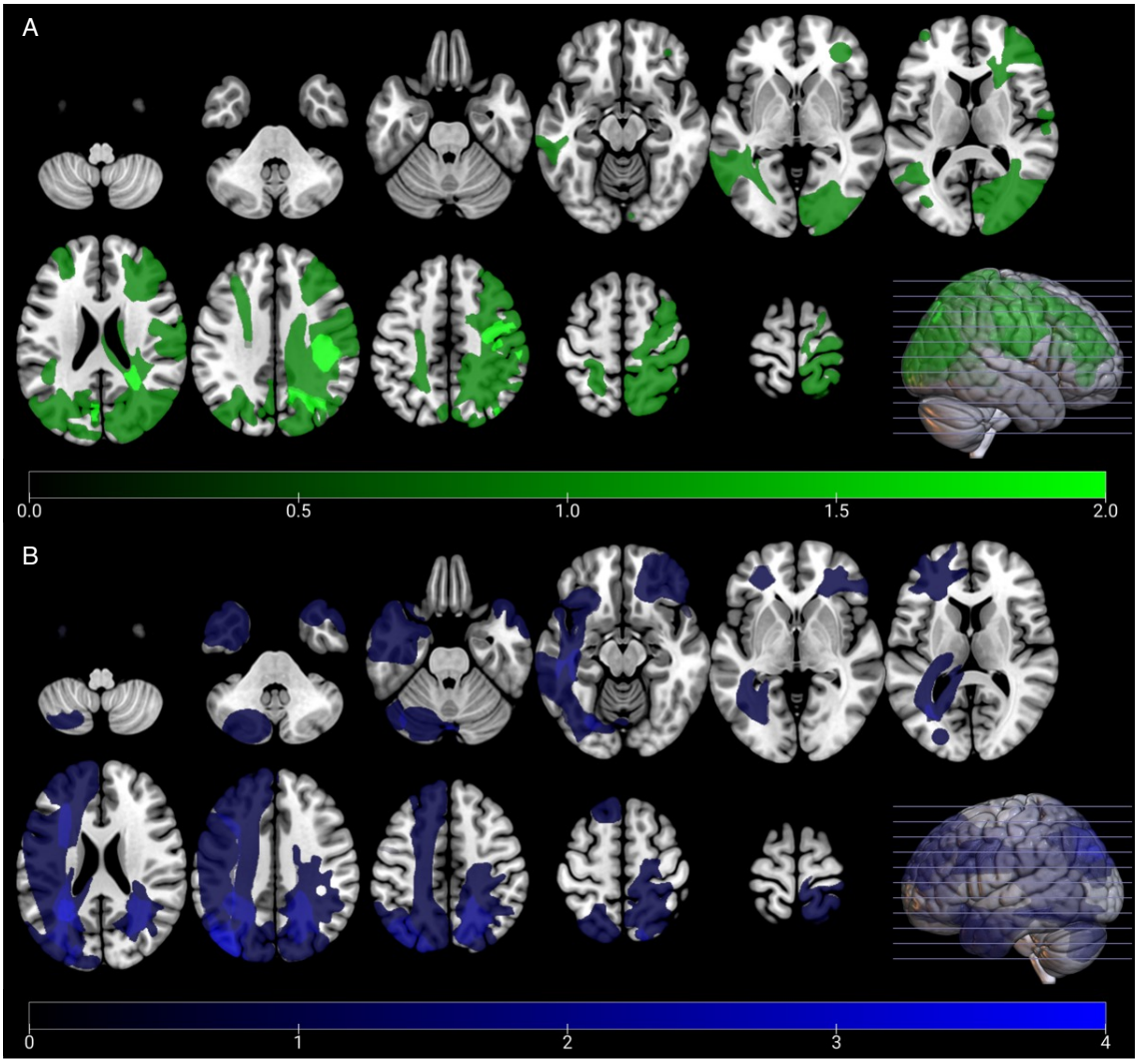


Figure 3.21 Brain regions damaged in cases with impaired clock-drawing. A. Lesion overlap for cases with impaired visuospatial construction. **B.** Lesion overlap for cases with impaired time-setting.

Atlas-based LSM found that no regions survived the threshold of significance for impaired visuospatial construction (AAL: 0 regions, $z = -2.761$, $z < -4.404$; natbrainlab: 0 regions, $z = -1.151$, $z < -3.745$) or impaired time-setting (AAL: 0 regions, $z = -2.428$, $z < -3.684$; natbrainlab: 0 regions, $z = -1.647$, $z < -3.375$). Voxel-wise LSM also found that no voxels survived the threshold of significance for visuospatial construction errors

(0 voxels, $z = -2.574$, $z < -3.976$) or time-setting errors (0 voxels, $z = -3.100$, $z < -3.715$).

3.4 Discussion

The objectives of this study were to structurally and functionally validate a novel digital lesion reconstruction method for film-based CT head radiographs. Then, applying this method, conduct human brain lesion studies using archival cases of veterans with penetrating brain injuries to attempt to replicate the findings of i) a LSM study using cases from the VHIS cohort to investigate insomnia, and ii) a LSM study using cases with ischaemic stroke to investigate performance in the clock-drawing test.

3.4.1 Baseline characteristics

Baseline characteristics confirm that the neuropsychology study cohort were a group of healthy young men serving in junior military ranks. The majority experienced focal neurological impairments after their injuries, leading to discharge from military service. Despite this, the vast majority maintained employment throughout their working years and, perhaps surprisingly, most enjoyed good physical and mental health into later life. This is consistent with the findings and impressions of Newcombe, who met nearly every member of the cohort [129]. A comparison of responses between the group of veterans who participated in residential assessments and those who only completed postal questionnaires suggested that the former were largely representative of the group who sustained penetrating brain injuries as a whole [129].

These characteristics are, for the most part, similar to those of the VHIS. Both groups comprised mostly right-handed men injured in early adulthood (MHHI: 24 years old, VHIS: 21 years old) [207]. Prolonged loss of consciousness was uncommon in both groups (MHHI: 9%, VHIS: 15%) and rates of PTE were nearly identical (MHHI: 52%,

VHIS: 51%) [82]. Rates of employment were also high in both cohorts (MHHI: up to 90%, VHIS: up to 80%) [82]. These similarities support comparison between findings from neuropsychological studies of the MHHI and VHIS cohorts.

3.4.2 Structural validation

Comparing lesions reconstructed from film-based CT radiographs and PM photographs showed high spatial overlap, reasonable morphological congruence between modalities irrespective of lesion location, and good reliability for determining damage to specific anatomical regions (**Figure 3.11**). Some variation between the reconstructions generated from these two modalities is to be expected, not least because of differences between the shape of the brain when imaged *in vivo* (i.e. encased by the skull, wrapped in the dura, and suspended in CSF) and *ex vivo* sections of brain displayed on a flat surface. Age-related atrophy during the twenty-year interval between CT scanning and PM examination will also be a significant source of variation. Considering these factors, the extent of agreement between reconstructions lends support to the structural validity of the reconstruction method used in this study.

3.4.2.1.1 Challenges and limitations

Nevertheless, the resolution of reconstructions made using this method is likely to be lower than those made from modern digital CT or MRI scans, due to the limited resolution of early CT radiographs. While manual delineation of lesional boundaries, semi-automated lesion interpolation, and the use of standardised brain volumes and anatomical templates also limits the accuracy of this approach, these steps are also taken when using modern digital scans for LSM. Therefore, the only significant difference between reconstructions generated from archival CT radiographs to those

made from digital scans is likely to be lower resolution. For this reason, atlas-based LSM may be more appropriate than voxel-wise LSM.

3.4.3 Lesion characteristics

3.4.3.1 Lesion volume

The volume of lesions caused by penetrating brain injuries in the MHHI cohort was slightly larger than that of the VHIS, but significantly smaller than typical ischaemic stroke cohorts (**Table 3.5**). Smaller lesions tend to produce less overlap between cases, potentially limiting the power of voxel-wise LSM analyses [416]. However, smaller lesions may be more appropriate than larger lesions for lesion-network mapping [420]. This will be discussed further below.

3.4.3.2 Lesion location

The relatively even distribution of lesions between cerebral hemispheres and across lobes reflects Russell's intention to recruit participants with a range of lesion locations to Phases 2–3 of follow-up (**Figure 3.12.A**). Sparing of the brainstem is to be expected given that injuries to this region are more likely to have been fatal. It is less clear why there are relatively fewer lesions involving the cerebellum, but this may simply be because veterans with cerebellar damage were not prioritised for neuropsychological assessment.

In this cohort, areas of maximal lesion overlap are more posterior than anterior and more deep than superficial. Up to 16 cases share lesions that affect these regions. However, the majority of the brain is covered by lesions shared by fewer than eight cases, confirming that in general there is relatively little overlap between lesions in this cohort. The largest areas of lesion overlap surround the posterior and anterior horns of the lateral ventricles. Around the posterior horn, this area is larger in the right

hemisphere than the left, while the reverse is true around the anterior horn. There is sparse coverage of the right inferior occipital, fusiform, and lingual regions, the right insula, the right superior frontal gyrus, and the left post-central gyrus. Sparing of these regions is most likely due to chance, but as it will limit the statistical power of voxel-wise LSM in these regions, this must be considered when planning and interpreting analyses from this cohort [416].

The pattern of lesion locations in the MHHI cohort contrasts with that of the VHIS, where prefrontal damage predominates (**Figure 3.12.B**). Comparing this pattern to the distribution of lesions caused by ischaemic strokes, infarcts of the middle cerebral artery account for approximately 60% of cases, while posterior cerebral artery strokes affect just 12%, and anterior cerebral artery strokes represent only 6% and are often bilateral [225]. Therefore, the MHHI cohort can provide a valuable opportunity to investigate processes which are thought to involve posterior and unilateral anterior brain regions, such as higher order visual processing and executive functions.

The relatively small size and lack of overlap between lesions in this cohort may offer a further advantage over stroke cohorts for LSM. The stereotyped patterns of damage that result from infarcts involving the anterior, middle, or cerebral arteries have been shown to distort lesion-symptom maps towards regions of maximal overlap at the centre of these vascular territories [421]. Multivariate voxel-wise LSM has become a popular way to overcome this challenge [422], but it has also been suggested that studying cohorts with small lesions that overlap minimally can also mitigate this problem, without recourse to the large sample sizes needed for multivariate analyses [421,423]. Cohorts with these properties are rare, but the group of veterans who were treated for penetrating brain injuries at the MHHI would appear to be one such

example. In this way, the relatively small size and even distribution of lesions in this cohort compensate for its small sample size.

3.4.4 Functional validation

Using the novel lesion reconstruction method described above to perform LSM of limb weakness and visual field impairment successfully identified all regions of brain damage specified in our validation hypotheses (**Figures 4.13–18**). This serves to functionally validate the use of this method to perform LSM in this cohort, noting that a similar approach was recently used to validate LSM in a large stroke cohort [424]. However, these examples also serve to illustrate the impact of limited resolution from film-based CT reconstructions and the restrictions imposed by the sample size of this cohort.

3.4.4.1.1 Challenges and limitations

While voxel-wise LSM successfully identified regions of brain damage that were hypothesised to be associated with limb weakness and visual field impairments, the volumes that exceeded the threshold for significance after correcting for multiple comparisons were small, and no voxels reached significance in the analysis of left quadrantanopia. This demonstrates the possibility of false negatives when applying this method within this cohort, due to the relatively small number of cases with lesions that overlap in regions relevant to certain hypotheses. More encouragingly, atlas-based LSM identified all hypothesised regions and would therefore appear to be a more appropriate form of LSM in this cohort. However, it is worth noting that this also identified other nearby regions, suggesting a tendency towards false positives. Region-specific LSM that was restricted only to areas that were hypothesised to be involved in a lesion-deficit relationship appeared to strike the best balance between sensitivity and

specificity, confirming the involvement of all hypothesised regions while avoiding false positives. This is therefore the most appropriate form of LSM to use in this cohort when conducting lesion studies to test an existing hypothesis.

3.4.4.2 A pathway towards clinical impact

The novel adaption of lesion reconstruction methods described above can be applied to utilise archival CT radiographs for digital LSM. While using film-based CT scans provides limited resolution, this can be mitigated by using atlas-based LSM to analyse the entire brain and region-specific LSM to test specific hypotheses. Where archival cohorts provide the opportunity to study unique lesion patterns, this limitation is likely to be outweighed. In the case of the MHHI cohort, this approach offers a valuable opportunity to study processes thought to involve more posterior brain regions, in contrast to the VHIS, and may also be less susceptible to the distortion that affects stroke lesion studies. This method can now be applied to other archival cohorts as well as more recent cohorts from lower income settings where digital CT scanning has more recently become available.

3.4.5 Lesion-symptom mapping in the Military Hospital for Head Injuries cohort

3.4.5.1 Insomnia

As discussed in the previous chapter, disrupted sleep is reported by between 30–60% of individuals after CTBI, and can have a significant impact on recovery and quality of life [104,105]. Up to a quarter of patients affected by CTBI go on to be diagnosed with a sleep disorder, including insomnia (i.e. difficulty falling or staying asleep), hypersomnia, or sleep apnoea [106]. What is more, poor sleep is thought to exacerbate

other symptoms of TBI, including pain, cognitive impairment, and mood disorders [104].

3.4.5.1.1 Sleep disturbance after penetrating brain injury

A large proportion of the study cohort reported long-standing sleep disturbance using the GHQ-30 approximately 30 years after injury (79%). This is significantly more than in the VHIS, where just 14% reported sleep disturbance using the HAM-A [108]. This may be because only moderate or severe insomnia was included by Koenigs et al. It was not possible to replicate this in our study as the GHQ-30 does not ask about the severity of sleep disturbance, although only three cases with insomnia reported regular use of a sedative medication. The groups with and without insomnia were of a similar age and had similar rates of depression/anxiety symptoms, suggesting that neither of these factors contributed significantly to sleep disruption (**Table 3.8**). Lesion volume was also equal between the two groups.

3.4.5.1.2 Damage to the left prefrontal region is associated with insomnia

As a result of the high prevalence of sleep disturbance, areas of maximal overlap among those with insomnia appeared similar to the areas affected by penetrating brain injuries in the study cohort generally. However, the overlap of lesions damaged among cases without insomnia notably spared the left prefrontal cortex, suggesting that damage to this region may be associated with insomnia (**Figure 3.19**). Region-based LSM confirmed that subregions of the left prefrontal region (including the left superior frontal gyrus and sulcus, which contribute to the dlPFC and dmPFC) were associated with insomnia. This result is consistent with the finding of Koenigs et al. from the VHIS and adds to a growing body of evidence supporting a role for the left prefrontal region in establishing and maintaining sleep [108].

3.4.5.1.3 Challenges and limitations

Many functional neuroimaging studies have found a correlation between altered prefrontal activity and insomnia, including a recent large UK Biobank study which identified increased connectivity between regions of the default mode network in people with insomnia [425]. In people whose sleep is improved by pharmacotherapy for insomnia, treatment is associated with higher prefrontal cortex activity and connectivity as measured by functional neuroimaging compared to placebo [426]. However, the causal links between sleep and the prefrontal region are not clear, and it has been suggested that prefrontal activity can be affected by sleep quality, leading to the effects of sleep deprivation on attention, decision-making, and executive function [427]. Equally, there is also evidence to suggest that altered prefrontal activity can affect sleep. For example, repetitive transcranial magnetic stimulation (rTMS), which modulates connectivity between brain regions, can improve sleep symptoms compared to a sham procedure when applied to the prefrontal region alongside pharmacological treatments for insomnia [428–430].

Human brain lesion studies, including the report from Koenigs et al. and now the findings of this study, support the conclusion that disrupting left dmPFC activity can lead to insomnia. More than 20 years ago, it was shown that slow wave activity during non-rapid eye movement (NREM) sleep originates from prefrontal and orbitofrontal regions and propagates to posterior regions of the brain [431]. Intriguingly, there is also evidence that this occurs preferentially in the left hemisphere [432]. Damage to the left prefrontal region may therefore disrupt the propagation of slow waves during NREM sleep to cause insomnia. Of course, it is also plausible that damage to the prefrontal region leads to sleep disturbance by indirect causation. For example, hypoactivity in the left prefrontal region is also linked to depressive symptoms, and depression is

strongly associated with sleep disruption. However, in this study, there was no difference in the rates of depression/anxiety between the groups with and without insomnia, reducing the likelihood of this explanation.

3.4.5.1.4 A pathway towards clinical impact

In the context of the literature described above, this finding supports further development of interventions that target the left dmPFC to improve sleep disorders, such as rTMS. A further implication of this study is that individuals who acquire damage to the left prefrontal region of the brain, whether this is caused by stroke, trauma, malignancy, or inflammation, may be more likely to experience poor sleep in the long-term, and strategies to identify and mitigate this should be considered in their management.

3.4.5.1.5 Future studies to confirm and extend this finding

Clinical trials to assess the effectiveness of interventions targeting the left dmPFC for treatment of insomnia will be vital to determine whether this result can be translated into clinical benefit. Regarding further LSM analyses using the MHHI cohort, first, we plan to test the remaining hypotheses that were generated from the VHIS, namely: i) that damage to cortical and limbic areas in the left hemisphere is associated with higher levels of anxiety [208], ii) that bilateral damage to the dorsal prefrontal cortex (PFC) is associated with higher levels of depression, while bilateral damage to the ventral PFC is associated with lower levels of depression [433], and iii) that damage to the left dlPFC is associated with lower levels of conscientiousness and self-discipline and higher levels of neuroticism [209]. Testing these hypotheses will also build on Freda Newcombe's finding that greater levels of anxiety, depression, and social dysfunction were reported by veterans in the MHHI cohort with damage to the left hemisphere [129]. We then intend to investigate the contribution of lesion volume and

location to the incidence of PTE, in an update of the report compiled by Russell using skull x-rays in this cohort [184]. Beyond this, the method described and validated here can also be applied more widely to test structure-function hypotheses advanced by functional neuroimaging studies, in a fashion that complements stroke lesion studies.

3.4.5.2 Clock-drawing test

More than a third of the study cohort displayed impaired performance in the clock-drawing test. However, the number of cases who made isolated visuospatial or time-setting errors was relatively small. This significantly limits the strength of any conclusions that can be drawn from this study. While the results indicate that impaired time-setting may be more common after damage to the left hemisphere, as was shown by Tranel et al. (2008), there is insufficient evidence to draw this conclusion from this study alone (**Figure 3.20**). The wide range of lesion locations among those with impaired performance is consistent with the clock-drawing test requiring multiple cognitive functions. Given the relative lack of overlap between lesions in groups who made time-setting and visuospatial errors, and the small lesion volume in this cohort generally, a network-based analysis may be an appropriate final step to fully address this question using the resources available in this cohort.

Notwithstanding the limited sample size in this study, there is also a substantial difference between the time from injury to assessment in this study and in that performed by Tranel et al., which may also have contributed to differences between findings. Tranel et al. assessed clock-drawing test performance in the acute phase, months after stroke, while this study assessed performance decades after injury, by which time significant functional reorganisation and cognitive recovery may have obscured the effects of the original deficit.

While the results of this study do not substantially add to our understanding of the neural correlates of the clock-drawing test, they do serve to illustrate the limitations of applying this method within the MHHI cohort, which will guide how this approach is used in future studies.

4 Neuropathological study

In this chapter, I present a study in which I examined the neuropathological effects of penetrating brain injuries to gain insights into the pathogenesis of AD.

4.1 Introduction

In **Section 4.1.1**, I provide a brief overview of AD. In **Section 4.1.2**, I present the rationale for the study, followed by the aim, objectives, and hypotheses in **Section 4.1.3**.

4.1.1 Background summary

Chronic features of TBI pathology extend beyond the immediate injury to encompass persistent inflammation, BBB disruption, neuronal loss, and accumulation of abnormal protein aggregates [241]. Central among these is the accumulation of p-tau forming NFTs, as observed in CTE [26]. CTE exhibits a unique pattern of tau deposition predominantly around small blood vessels within cortical sulci and superficial cortical layers, distinct from that seen in AD. Diffuse aβ plaques also appear rapidly after TBI but differ significantly from the dense plaques associated with AD [255]. How these features relate to the epidemiological links between TBI and AD remain unclear, in large part due to the incomplete understanding of AD pathogenesis.

AD is the most common form of dementia, accounting for between 60–90% of all cases [281]. The incidence of all-cause dementia in individuals aged over 65 is 1% per year, rising to 4% above the age of 80 [434]. In the UK, median life expectancy is now over 80 years, and one in six people aged over 80 have dementia (over 90 years, this rises to one in three) [435]. Globally, the prevalence of dementia is expected to triple from 50

million people in 2020 to more than 150 million by 2050 [436]. The estimated cost of dementia worldwide currently stands at US$ 1 trillion [281].

In AD, the characteristic distribution of NFTs progressively involves the MTL and AC, closely correlating with clinical symptom severity [254]. Increasing experimental evidence supports the tau propagation hypothesis, suggesting NFTs spread across synaptic connections in an activity-dependent manner [350,359,345]. Animal models have demonstrated p-tau transmission along neuronal connections, and limited descriptive studies in humans support the hypothesis, although definitive human comparative data remain scarce. By evaluating unilateral penetrating brain injuries in veterans with prolonged post-injury survival, this study aims to investigate how severed connections between the MTL and AC influence NFT distribution. Such an approach leverages the unique circumstances of these cases to address critical mechanistic questions about p-tau propagation in humans.

4.1.2 Rationale for the proposed study

If neuronal transport is the primary mechanism by which p-tau spreads in AD, then disruption of neuronal pathways connecting regions of early and advanced p-tau pathology would be expected to reduce NFT formation in the latter regions. Specifically, because p-tau pathology in AD typically progresses symmetrically through both cerebral hemispheres, and major cortico-cortical connection pathways are predominantly ipsilateral, unilateral disruption of connections between the MTL and AC should reduce AD-related p-tau pathology in the disconnected AC compared to the contralateral AC in the unaffected hemisphere of the same individual. In contrast, given that there is limited evidence of aβ propagation via neuronal pathways, such disruption is not expected to significantly alter the distribution of aβ pathology. To test this

hypothesis, I analysed post-mortem brain tissue from veterans who survived for decades following unilateral penetrating brain injuries (**Figure 4.1**).

Understanding whether disruption of neuronal connectivity directly affects tau pathology could yield substantial implications. Confirmation of the tau propagation hypothesis would support the identification of potential therapeutic targets, such as interception of extracellular tau oligomers or modulation of synaptic activity, to halt or slow AD progression. Moreover, insights derived from this work may extend beyond AD, offering new perspectives on treatment strategies for other tauopathies and related neurodegenerative conditions, which collectively represent a significant burden to aging populations.

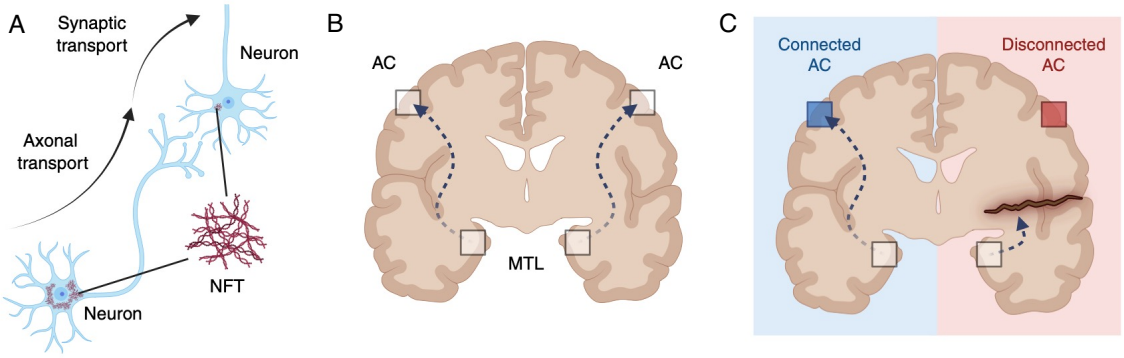


Figure 4.1 Neuropathology study rationale. A. The tau propagation hypothesis: tau proteins spread between neurons by axonal and synaptic transport mechanisms, leading to the formation of neurofibrillary tangles (NFTs). **B.** According to this hypothesis, the progression of p-tau pathology from mesial temporal lobe (MTL) to the association cortices (AC) depends on the anatomical connections between these regions. **C.** In this study, it is predicted that disrupting these connections is associated with reduced Alzheimer's-related p-tau pathology in the disconnected AC, compared to the unaffected hemisphere. Created with BioRender.com.

4.1.3 Aim, objectives, and hypotheses

Aim: Investigate the pathogenesis of Alzheimer's disease.

Objectives:

1. Compare the extent and distribution of AD-related p-tau and aβ pathology between the cerebral hemispheres of individuals who sustained unilateral penetrating brain injuries in early adulthood.

Hypothesis:

A. Disruption of the connections between the MTL and AC is associated with reduced AD-related p-tau, but not aβ, pathology in the disconnected AC, compared to the unaffected hemisphere.

4.2 Materials and methods

4.2.1 Materials

4.2.1.1 Study cohort

Cases screened for inclusion in this study were individuals admitted to the MHHI with penetrating brain injuries who donated brain tissue to the OBB in follow-up Phase 4 (*n* = 22). Inclusion criteria were: i) unilateral penetrating brain injury, ii) availability of tissue from MTL and AC in both hemispheres, and iii) availability of tissue from both hemispheres with similar formalin fixation time (FFT). The only exclusion criterion was coincidental (non-penetrating) damage in relevant areas due to other causes (e.g. a large vessel ischaemic lesion).

Controls without a history of TBI were drawn from participants of the Oxford Project to Investigate Memory and Ageing (OPTIMA) (*n* = 9). Specifically, non-demented controls comprised age-/sex-matched individuals with no history of cognitive impairment and no post-mortem AD pathology (*n* = 3). AD-positive controls comprised age- and sex-matched individuals with a history of cognitive impairment and post-mortem AD pathology (*n* = 3 mild pathology, *n* = 3 severe pathology).

4.2.1.2 Post-mortem reports and photographs

Diagnostic PM reports and photographs were available for all cases. Post-mortem reports described the lesional deficit and commented on the extent and distribution of other pathology. Photographs showed the external appearance of the brain after removal from the skull and the gross appearance of internal structures in serial coronal sections (**Figure 4.2**). These photographs, captured between 1978–2012, were available in the form of 35mm projector slides. Digital scanning and dust removal was performed for 253 slides with support from Oxford Medical Illustration to generate high quality digital images for onward analysis.

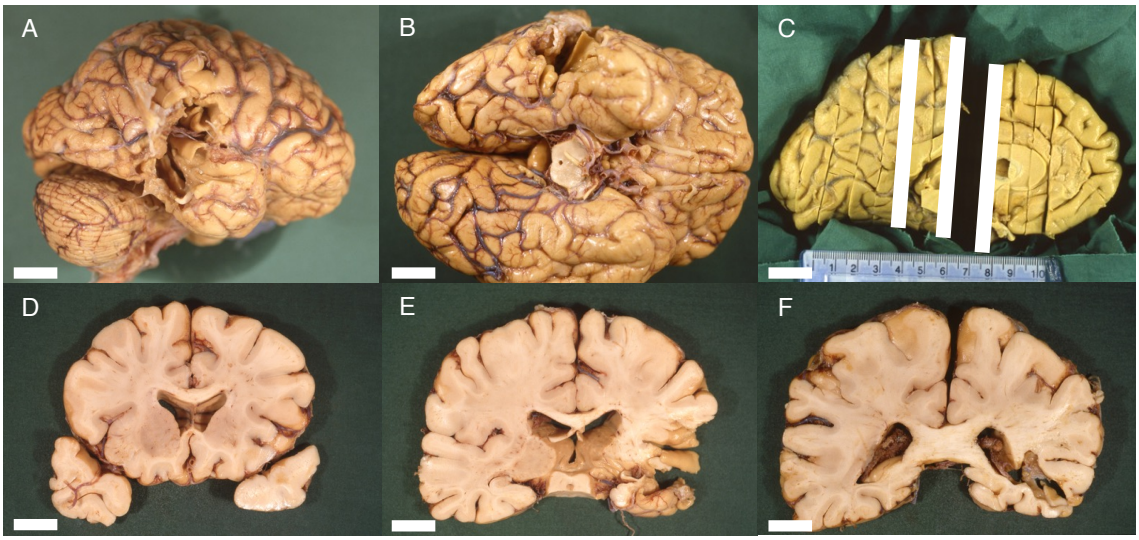


Figure 4.2 Brain tissue donated for post-mortem research. A–F. Post-mortem photographs of the same case shown in Figure 3.2. **A.** Right lateral view. **B.** Inferior view with cerebellum removed. **C.** Sagittal view of the left hemisphere cut into coronal sections approximately 1cm thick, with lines denoting the location of the coronal slices shown in **D–F. D–F.** Coronal sections reveal the anterior and posterior extent of the lesion (posterior side of each section shown). Scale bars: 2cm.

4.2.2 Methods

A combination of traditional neuropathological methods and a novel series of anatomical methods were applied to these materials to perform this study (**Figure 4.3**).

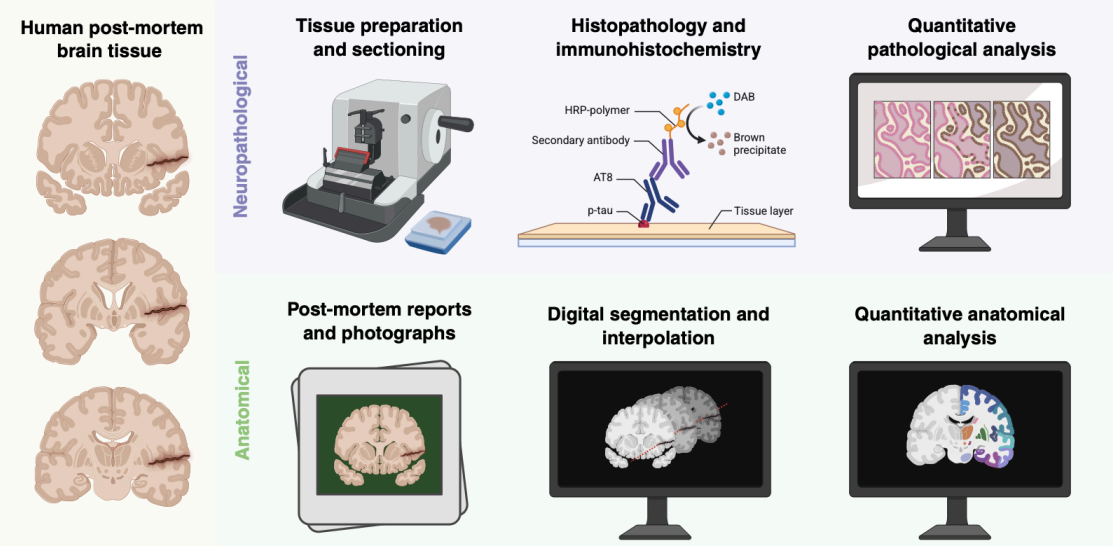


Figure 4.3 Overview of the materials and methods used in Chapter 4. Created with BioRender.com.

4.2.2.1 Neuropathological methods

4.2.2.1.1 Tissue sampling and preparation

Formalin-fixed paraffin-embedded (FFPE) blocks of post-mortem brain tissue from the MTL and AC in both hemispheres (n = 7 frontal cortex, n = 8 parietal cortex) were sampled from each case (**Figure 4.4**). In total, 75 blocks were studied: 58 blocks from cases and 17 blocks from controls. Four blocks were sampled from each case, except for one case with two hemispheric blocks. Two blocks were sampled from each control (MTL and occipital blocks), except for one negative control without an occipital block. Blocks were sectioned at 6µm thickness using a rotary microtome (Leica RM2135) and mounted onto small- or medium-sized pre-coated slides (small: 75x25mm, medium: 75x50mm). Hemispheric blocks were sectioned using a sledge microtome (Reichert-Jung Hn-40) and mounted onto large slides (76x102mm) that were manually coated with 3-aminopropyltriethoxysilane (APTES). Diagnostic PM reports and photographs were available for all cases.

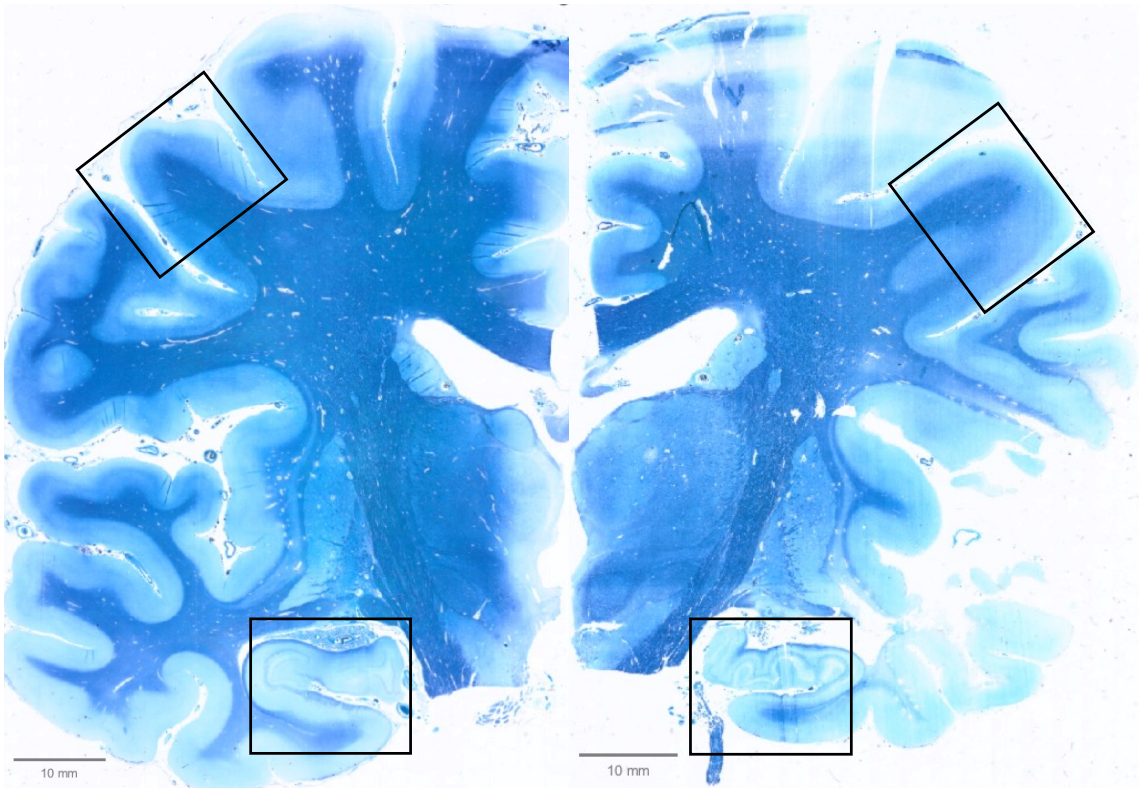


Figure 4.4 Post-mortem brain tissue sampling strategy. Luxol fast blue and cresyl violet staining. Hemispheric sections from the same case shown in Figure 4.2 with a right temporo-parietal deficit showing anatomical disconnection of the mesial temporal lobe (MTL) and association cortex (AC) in the affected hemisphere.

4.2.2.1.2 Histopathology

Histological staining was performed using standard techniques. Slides were baked at 60˚C for 30 minutes, cooled, and placed in serial xylene baths to clear paraffin, then rehydrated through serial dilutions of industrial denatured alcohol (IDA). To visualise cytoarchitecture, haematoxylin and eosin staining (H&E) was performed using filtered Harris haematoxylin and 0.25% aqueous acidified eosin, with 1% acid alcohol for differentiation. To examine white matter integrity, luxol fast blue and cresyl violet staining (LBCV) was performed using filtered and acidified luxol fast blue and cresyl violet, with 0.05% lithium carbonate for differentiation.

To visualise AD pathology, silver staining was performed using a simplified version of the Gallyas method [437,438]. After clearance and rehydration, slides were oxidised

using 5% periodic acid, impregnated with alkaline silver iodide, reduced using a developer solution of ammoniacal silver nitrate and sodium carbonate, toned with 0.2% gold chloride, fixed in 5% sodium thiosulphate, and counterstained with kernectrot.

4.2.2.1.3 Immunohistochemistry

To label features of AD pathology, immunohistochemistry (IHC) was performed using the Dako EnVision horseradish peroxidase/ 3,3'-diaminobenzidine (HRP/DAB+) system. Following clearance and rehydration, slides were incubated in 10% H_2O_2 in phosphate buffered saline (PBS) to block endogenous peroxidase activity. After antigen retrieval, primary antibody incubations were performed (**Appendix 4.1**). P-tau was labelled using AT8 (Thermo Fisher, MN1020, 1:300), which recognises the Ser202/Thr205 tau phosphorylation residue [439,265]. Aβ was labelled using 4G8 (Biolegend, SIG-39220, 1:24,000), which binds to amino acids 17–24 of the aβ peptide [291].

Primary antibody dilutions were optimised to enable visualisation of the full spectrum of AD pathology across the range of control tissue (**Figure 4.5**). Tissue from positive controls was used to confirm the absence of AT8 or 4G8 immunoreactivity in certain cases. Chromogenic visualisation was achieved by incubating slides with a secondary antibody conjugated to an HRP-labelled polymer before incubation with 0.02% DAB+ chromogen solution. Slides were counterstained with Harris haematoxylin, dehydrated through serial dilutions of IDA, fixed with xylene, and mounted using dibutylphthalate polystyrene xylene. Stained slides were viewed using a light microscope and digitally scanned to ascertain whole tiled images at 40x magnification. Small- and medium-sized slides were scanned using an Aperio ScanScope AT2 Turbo and large slides were scanned using an Evident VS200 scanner.

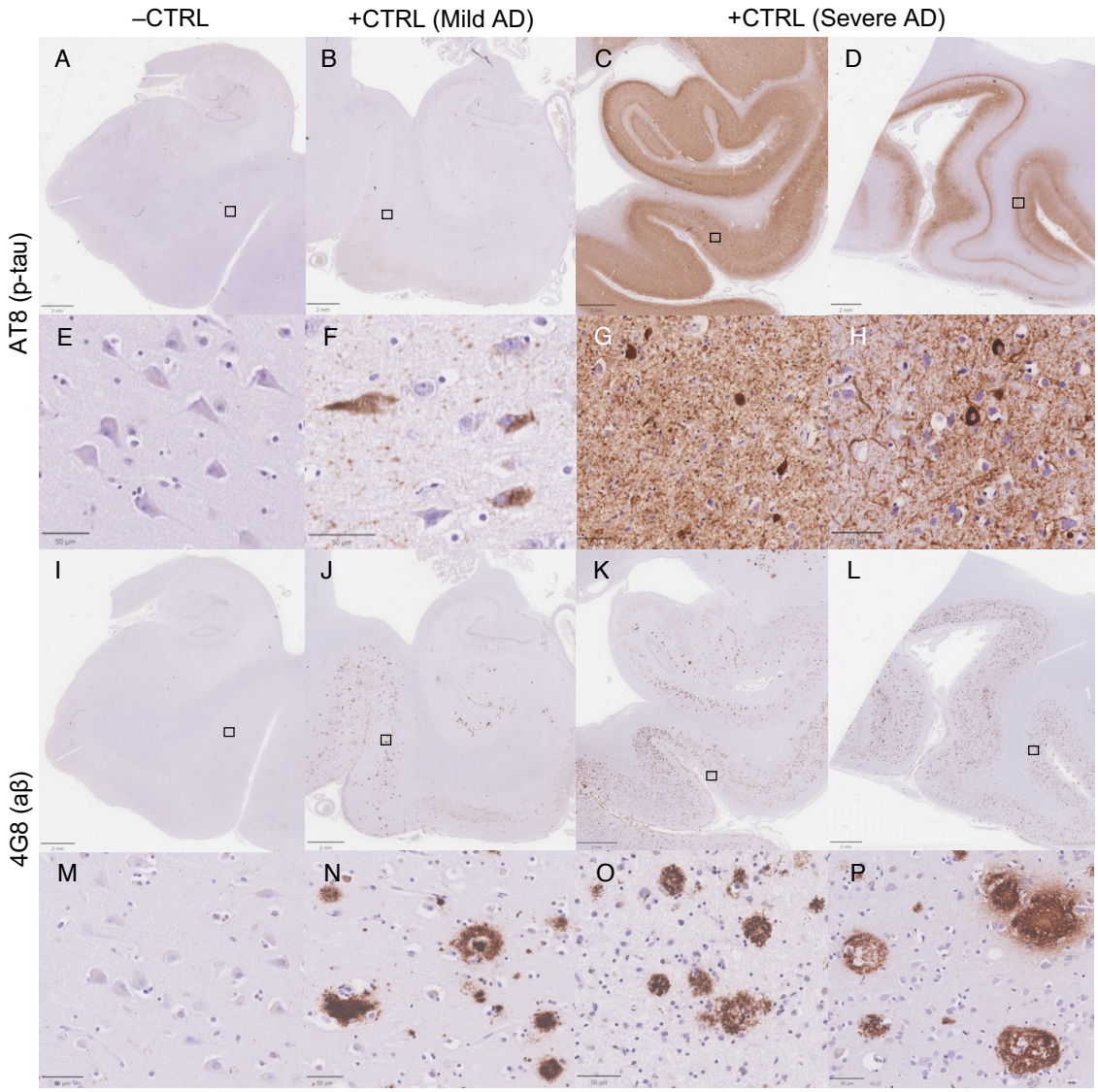


Figure 4.5 The spectrum of Alzheimer's disease (AD) pathology in controls. A–H. AT8 immunolabelling. **I–P.** 4G8 immunolabelling. **A.** MTL from a negative control showing no AT8-immunoreactivity (AT8-ir) or NFTs (**E** inset). **B.** MTL from a control with mild AD showing mild AT8-ir with sparse NFTs (**F** inset) in the TEC and EC. **C.** MTL from a control with severe AD showing widespread AT8-ir with NFTs and NTs (G inset). **D.** Occipital cortex from a control with severe AD showing widespread AT8-ir with NFTs and NTs (**H** inset) and prominent pathology in layer V of the striate cortex. **I.** MTL from a negative control showing no 4G8-ir or aβ plaques (**M** inset). **J.** MTL from a control with mild AD showing occasional NPs (**N** inset). **K.** MTL from a control with severe AD showing widespread NPs (**O** inset). **L.** Occipital cortex from a control with severe AD showing widespread NPs (**P** inset) throughout all cortical layers.

4.2.2.1.4 Accounting for prolonged formalin fixation time

Tissue fixation involves the formation of cross-links between proteins to preserve tissue morphology and prevent autolysis. Formalin, a 3.7% formaldehyde solution, is the most widely used fixative in diagnostic neuropathology because it is effective at preserving

morphology; it is also economical and easy to prepare and store [440]. However, the extent of cross-linking with formalin fixation is also more likely to alter the structure of protein epitopes and restrict the permeation of antibodies through tissue sections. Human postmortem brain tissue is often preserved in formalin for extended periods of time, but prolonged FFT can reduce the efficacy of histopathological and immunohistochemical techniques [441]. In forming the study cohort, the final inclusion criterion was applied to account for the fact that variation in FFT between hemispheres could confound the comparison of pathological markers between regions.

While this ensured that FFT was consistent between blocks from the same case, FFT still varied widely between cases, ranging from several days to 22 years. While silver staining efficacy declines beyond six years of FFT [442], IHC reliably detects AD pathology up to 14 years post-fixation [440]. Notably, aβ plaques and NFTs have been demonstrated using IHC after 50 years in formalin and 30 years in paraffin [443]. Cross-laboratory comparisons have confirmed that IHC labelling of p-tau and aβ is substantially more robust than silver staining and recommend their use in AD diagnostic protocols [444]. As such, given that FFT varied between cases and was often prolonged, data from IHC labelling was used for quantitative analysis, and statistical comparisons were clustered within cases *a priori*.

4.2.2.1.5 Analysis of Alzheimer's pathology

Qualitative descriptions of cytoarchitecture and AT8 and 4G8 labelling were made with reference to classical descriptions of AD and CTE pathology [291,265,289,127,26]. The extent and distribution of the features observed were applied to formulate the sampling and quantitation strategies for both p-tau and aβ pathology.

Using QuPath (v0.5.1), MTL and AC subregions involved in AD pathology were sampled using rectangular fields of view (FOVs) adapting a method developed by the De Luca group to sample the motor cortex in the study of multiple sclerosis (**Figure 4.6**) [445]. Subregions were identified using canonical descriptions of relevant microstructural anatomy [446–449]. The following subregions were sampled in the MTL: cornu ammonis zones 1–3 (CA1, CA2, CA3), subiculum (SUB), entorhinal cortex (EC, layers pre-α, -β, -γ and pri-α, -β, -γ), and transentorhinal cortex (TEC, layers pre-α and pri-α, -β, -γ). In the AC, FOVs were placed in cortical layers I–VI and in the subcortical white matter to help accurately identify each layer. Layers II, III, and V were sampled as they are most affected by p-tau pathology in AD. A systematic approach was used to minimise sampling bias. Trajectories running perpendicular to the pial surface were evenly spaced throughout each subregion avoiding artefacts of tissue processing (e.g. tears, dust). In the AC, trajectories were placed along the banks and crests of gyri, but not the sulci, to avoid sampling regions vulnerable to CTE pathology. FOV dimensions were set according to the typical thickness of cortical layers (layer I: 135x680µm, layers II–VI: 270x340µm). The number of FOVs placed within a subregion was determined by the relative area of each subregion (i.e. two FOVs in CA3, six FOVs in CA1). Both the dimensions and the number of FOVs (and therefore the overall area sampled from a subregion) was standardised between hemispheres and cases.

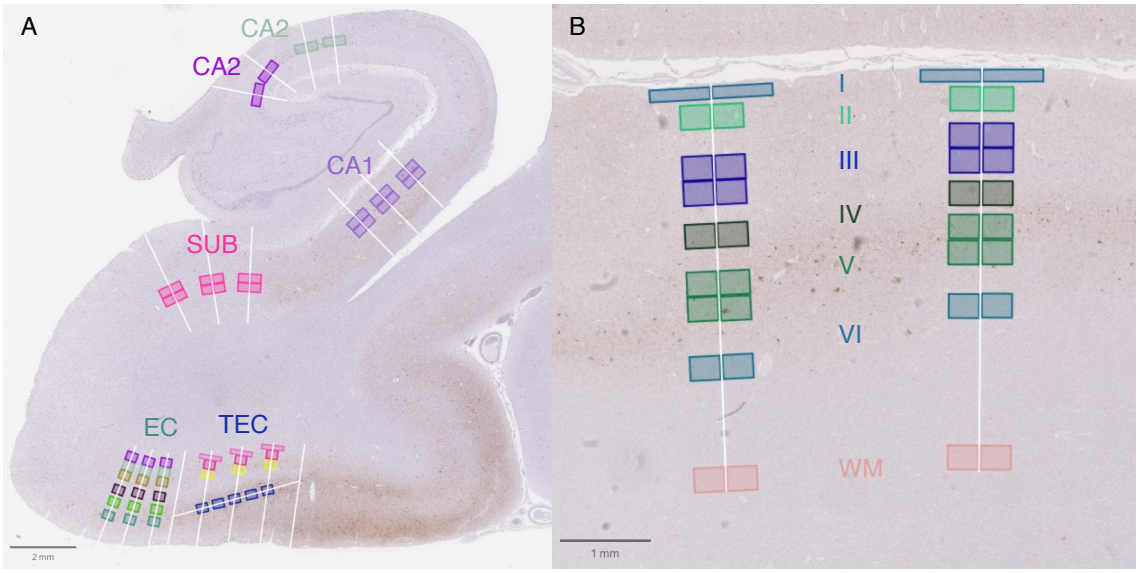


Figure 4.6 Sampling of the mesial temporal lobe (MTL, A) and association cortex (AC, B). AT8 immunolabelling. Fields of views (FOVs) were systematically placed in subregions involved in AD pathology. The following subregions were sampled in the MTL: cornu ammonis zones 1–3 (CA1, CA2, CA3), subiculum (SUB), entorhinal cortex (EC) layers pre-α, -β, -γ and pri-α, -β, -γ, and transentorhinal cortex (TEC) layers pre-α and pri-α, -β, -γ. In the AC, FOVs were placed in layers I–VI and in the subcortical white matter (WM) to help accurately identify each layer, but only layers II, III, and V were sampled as they are most affected by p-tau pathology in AD.

To quantify the extent of AD pathology within the sampled regions of the MTL and AC, the proportion of neurons affected by NFTs (%NFT) was calculated and the area coverage of aβ plaques (%Aβ) was measured. %NFT was used instead of the total count of NFTs to account for neuronal loss. To calculate %NFT, using slides labelled with AT8, the number of neurons was divided by the number of NFTs after manually counting both within each FOV (**Figure 4.7**). A neuron was counted if all the following criteria were satisfied: i) cell with a pyramidal shape (or stellate shape in EC and TEC), ii) cell diameter ≥10µm, and iii) visible nucleus and nucleolus within the FOV (**Figure 4.7.A–B**). NFTs were counted if fibrillar AT8-immunoreactivity (AT8-ir) could be identified in the cytoplasm of a neuron as defined above (**Figure 4.7.E–F**) [301,450]. If only granular AT8-ir was identifiable in the neuronal cytoplasm, this was marked as a pre-tangle and not counted as a NFT (**Figure 4.7.C–D**) [301,450]. This method

therefore excluded ghost tangles. NPs and NTs were also identifiable but were not quantified in this study (**Figure 4.7.G–H**).

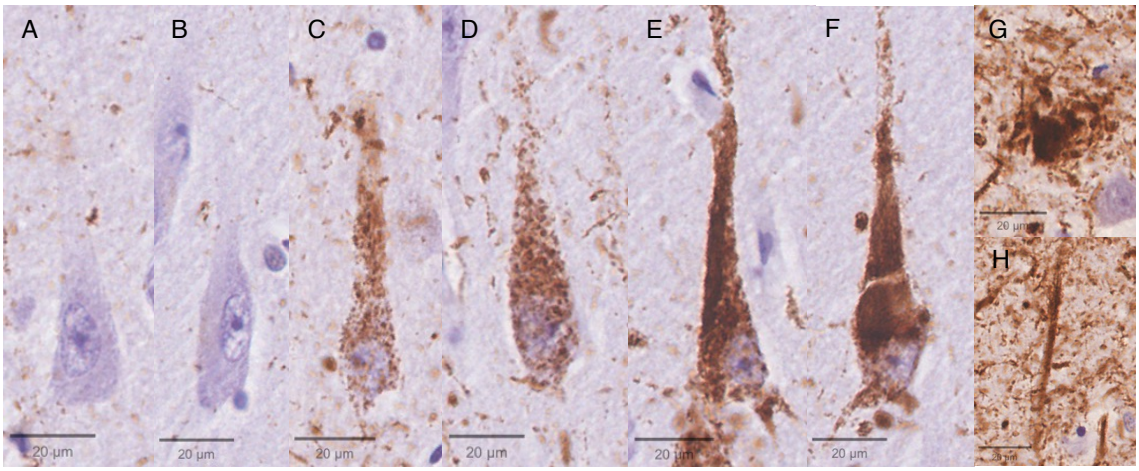


Figure 4.7 Features of Alzheimer's-related tau pathology. A–B. Neurons without hyperphosphorylated tau (p-tau) pathology. **C–D.** Neurons containing pre-tangles. **E–F.** Neurons containing neurofibrillary tangles (NFTs). **G.** Neuritic plaque. **H.** Neuropil thread.

To measure %Aβ, a semi-automated pixel classifier was used to detect areas of 4G8-immunoreactivity (4G8-ir) representing aβ plaques (**Figure 4.8**). In QuPath (v0.5.1), FOVs were transferred from slides labelled with AT8 to corresponding regions of slides labelled with 4G8. An artificial neural network pixel classifier was trained to detect the colour and shape of 4G8-ir plaques using representative images from multiple cases, in which plaques were manually defined as 'positive' and all other tissue areas were defined as 'negative' (**Figure 4.8.G,K**). Resolution (µm/pixel) was optimised to identify aβ plaques without detecting other amyloid deposits, such as fleecy amyloid or lake-like amyloid as defined by Thal et al. [296], and the trained classifier was applied within all FOVs (**Figure 4.8.H–J,L–N**).

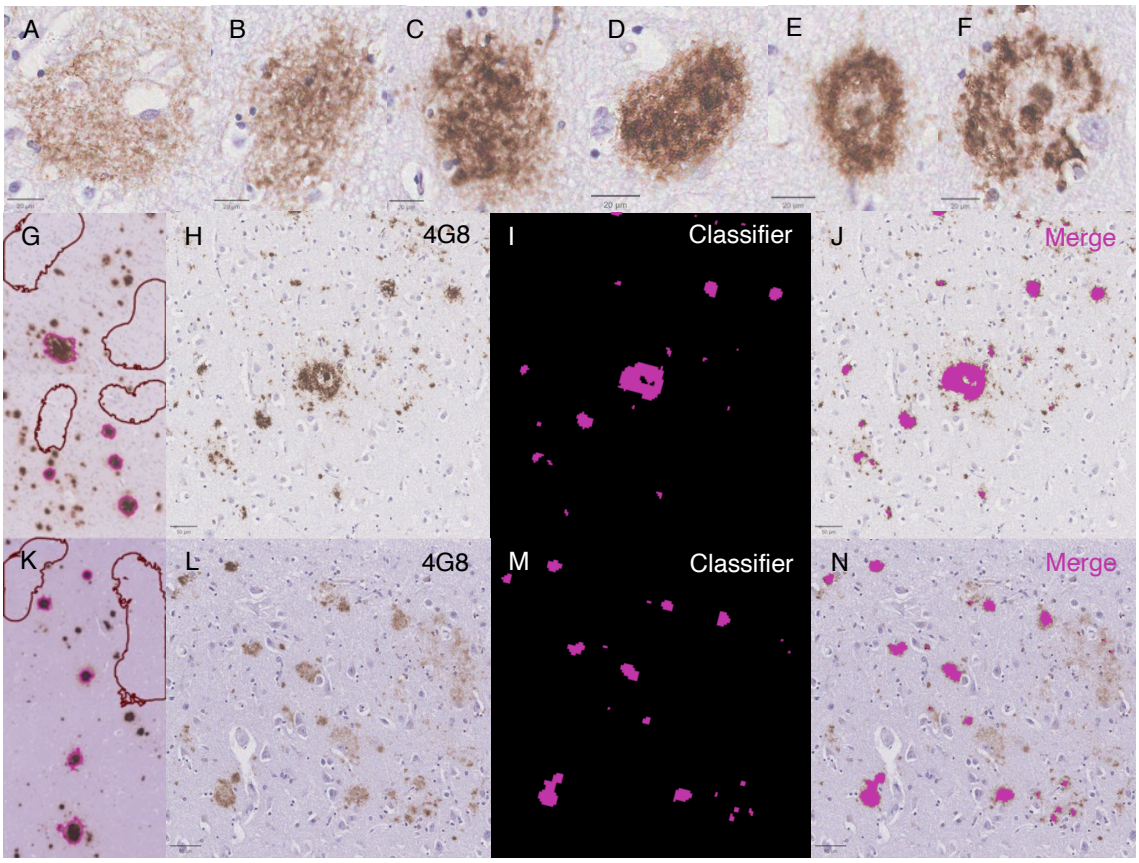


Figure 4.8 Features of Alzheimer's-related amyloid beta (aβ) pathology and pixel classification. A–N. 4G8 immunolabelling. **A–B.** Diffuse plaques. **C–D.** Compact plaques. **E–F.** Dense core plaques. **G,K.** Representative images from different cases used to train the pixel classifier in which compact and cored plaques are defined as positive (pink) and other areas are defined as negative (maroon). **H–J.** Application of the pixel classifier in one case showing detection of compact and dense core plaques. **L–N.** Application of the pixel classifier in another case showing detection of diffuse and compact plaques but not fleecy amyloid.

4.2.2.2 Anatomical methods

4.2.2.2.1 3D reconstruction from post-mortem photographs

3D reconstructions were generated from PM photographs and reports for each case by applying the method described in **Section 3.2.2.2.2** to all cases in the neuropathological study cohort. As described above, regions of brain damage were defined as tissue deficits, resulting either from trauma or surgical debridement, and regions of encephalomalacia. In each coronal section, the extent of the damaged region was identified independently by two neuropathologists and traced onto a labelled neuroanatomical template from 'Oppenheimer's Diagnostic Neuropathology'

[410]. In ITK-SNAP, a standard T1-weighted MRI brain volume (ch2better) was manually registered to the neuroanatomical template [407,408]. To do this, the brain volume was rotated (x = +5, y = 0, z = 0) to match major anatomical landmarks (e.g. optic chiasm, ventricles). The inferior limit of the brainstem was chosen as the centre of rotation (x = 151, y = 186, z = 1) on the basis that the underside of the brain would be placed on a flat surface to be cut into coronal sections at PM. The rotated brain volume was then re-sliced using linear interpolation at a standard thickness (one coronal section = 16 voxels).

Regions of brain damage were manually segmented in each coronal slice with reference to the annotated neuroanatomical templates, PM photographs, and PM reports. For each case, morphological interpolation using optimal slice alignment was applied to render a 3D volume from serial 2D coronal lesional segmentations. As above, anterior and posterior ends of the volume were rounded using a 3D paintbrush. To enable overlay onto brain atlases for anatomical analysis, the volume was registered to another standard T1-weighted MRI brain volume (mni152) by reversing the initial rotation (x = -5, y = 0, z = 0) and re-slicing using nearest neighbour interpolation. In MRIcroGL, any areas of the volume extending outside of the brain volume were removed using an intensity filter and the volume was smoothed using a smooth and refine tool [409]. This method was applied to generate a `.nii` volume for each case (**Figure 4.9**). These volumes were then overlaid onto brain atlases to quantify the extent of anatomical disconnection between regions of interest as outlined below.

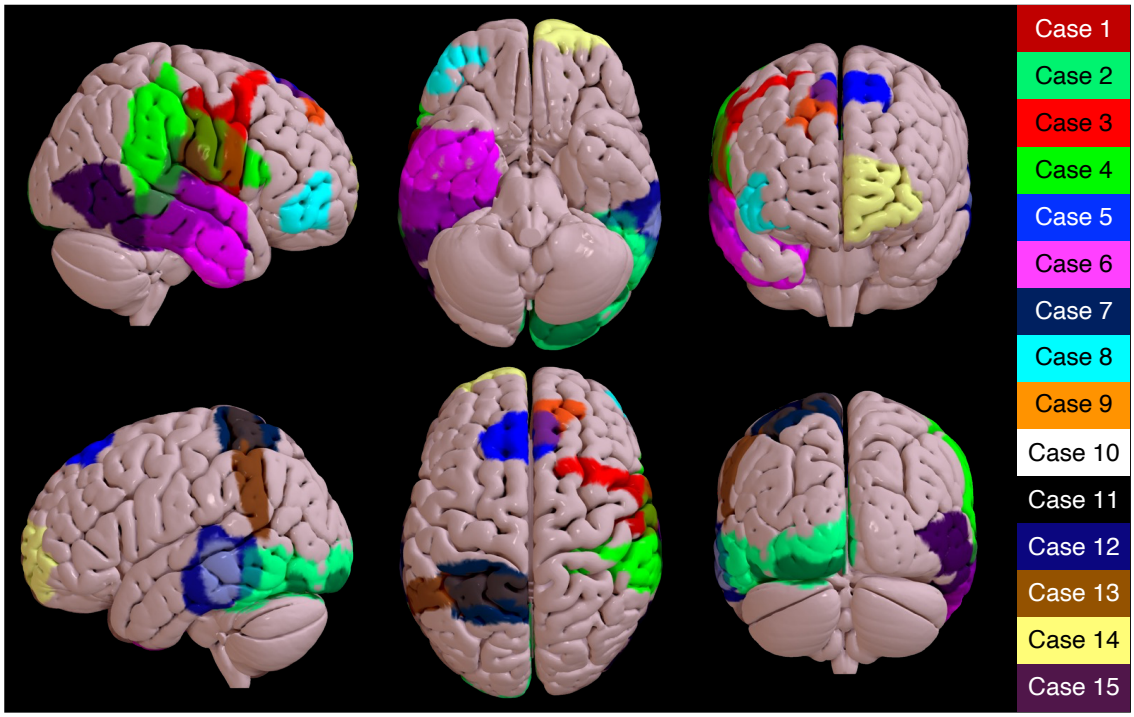


Figure 4.9 Lesional volumes reconstructed from post-mortem photographs (*n* = 15). Lesions varied in size and were distributed across all lobes in both cerebral hemispheres.

4.2.2.2.2 Analysis of anatomical disconnection

To determine the extent of disruption to cortico-cortical connections between the MTL and AC, lesional volumes were overlaid onto a white matter atlas derived from DTI tractography (natbrainlab) in MRIcroGL [411–413]. Descriptive volumetric data were extracted to quantify the proportion of voxels in an anatomical region included within the lesional volume (%damage).

With reference to a DTI-based hippocampal connectome, the connection between the MTL and the frontal lobe was defined as the cingulum and the connection between the MTL and the parietal lobe was defined as the cingulum and the inferior longitudinal fasciculus (ILF) [451]. Cases with less than 5% lesional damage to the ILF or cingulum were classified as not disconnecting the AC from the MTL. Cases with lesions involving the anterior pole of the brain were also classified as not disconnecting, given that the sampled region of AC could not be disconnected by the lesion. Remaining cases with

more than 5% damage to connecting structures between the AC and MTL were classified as having disconnecting lesions.

4.2.2.3 Statistical methods

Statistical analyses were performed using SPSS (v.29.0.2.0) and graphs were produced using GraphPad Prism (v.10.3.1).

4.2.2.3.1 Reliability analysis

Inter-rater reliability of %NFT was assessed using an intraclass correlation coefficient (ICC) from a two-way, mixed effects model using absolute agreement of single measures, which is appropriate for continuous data. Coefficients were interpreted as follows: <0.5: poor reliability, 0.5–0.75: acceptable reliability, 0.75–0.9: good reliability, >0.9: very good reliability [414].

4.2.2.3.2 Experimental analysis

Pathological data were considered to be clustered, with each case representing a cluster, to account for variation in the extent of AD pathology and FFT between cases [452]. Therefore, I performed a clustered form of linear regression using generalised estimating equation (GEE) models to evaluate the association and interaction between variables. Hemisphere (i.e. affected or unaffected), region (i.e. MTL or AC), and disconnection (i.e. anatomical disconnection of MTL and AC) were defined as independent variables and measures of AD pathology (%NFT, %Aβ) as dependent variables. Given the hypothesis that p-tau spreads from the MTL to the AC, %NFT in the AC was adjusted for %NFT in the ipsilateral MTL for each case.

4.2.3 Research ethics

Ethical approval for this study was provided by the National Health Service Health Research Authority (REC15/SC/0639, REC23/SC/0241).

4.3 Results

4.3.1 Baseline characteristics

After applying the inclusion and exclusion criteria, a study cohort was formed (*n* = 15) (**Figure 4.10**). Median age at injury was 26 years (IQR 22.0–29.5, range 20.0–36.0) and median age at death was 80 years (IQR 76.0–84.0, range 67.0–95.0) (**Table 4.1**, **Table A.4.2**). Average time from injury to death was more than 50 years (range 45.0–65.0). All injuries were caused by high velocity mechanisms and resulted in a significant burden of focal neurological impairments and post-traumatic seizures.

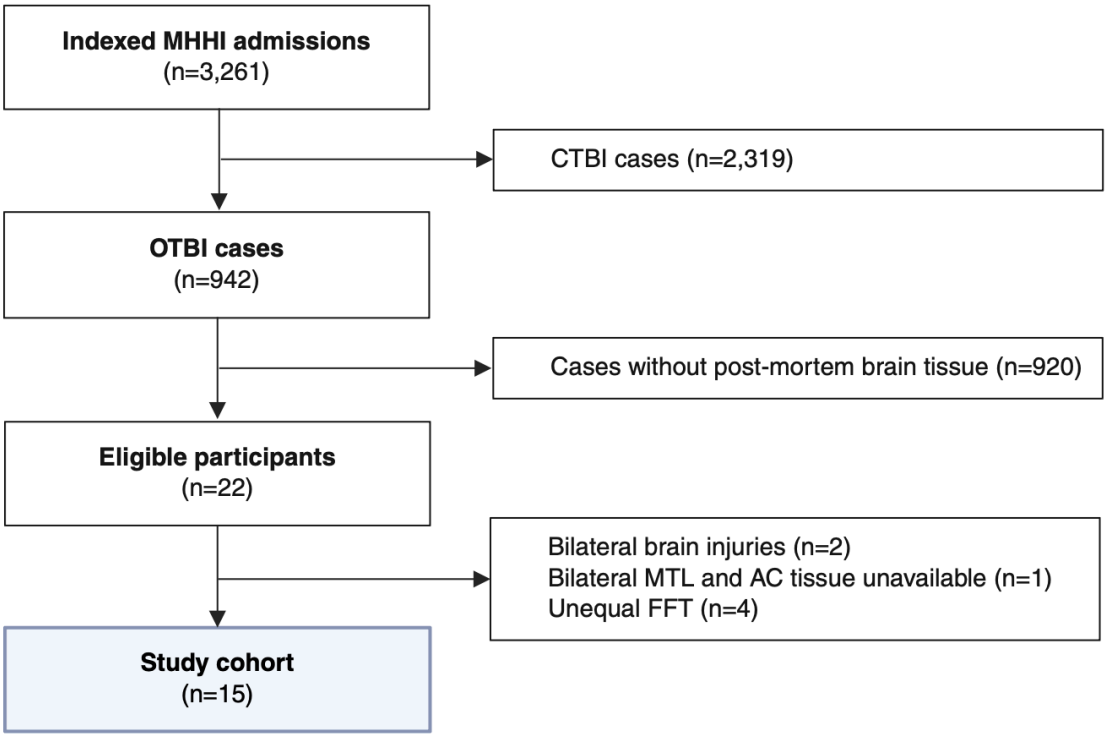


Figure 4.10 Neuropathology study cohort. The study cohort was formed by screening the group of veterans who donated post-mortem brain tissue between 1978–2012, who were a sub-group of the original Military Hospital for Head Injuries (MHHI) cohort. CTBI = closed traumatic brain injury. OTBI = open traumatic brain injury. MTL = mesial temporal lobe. AC = association cortex. FFT = formalin fixation time. Created with BioRender.com.

Table 4.1 Baseline characteristics and injury details for study cases (*n* = 15).

Baseline characteristics and injury details	
Age – median (IQR)	
Age at admission (years)	26.0 (22.0–29.5)
Age at death (years)	80.0 (76.0–84.0)
Sex – n (%)	
Male	15 (100.0)
Female	0 (0.0)
Hemisphere – n (%)	
Left	8 (53.3)
Right	7 (46.7)
Mechanism – n (%)	
Shell	10 (66.7)
Bullet	4 (26.7)
Mine	1 (6.7)
Post-traumatic amnesia – n (%)	
Nil	1 (6.7)
Less than 1 day	1 (6.7)
1–7 days	1 (6.7)
More than 7 days	1 (6.7)
Unknown	11 (73.3)
Retrograde amnesia – n (%)	
More than 30 mins	0 (0.0)
Up to 30 mins	4 (26.7)
None	9 (60.0)
Unknown	2 (13.3)
Early signs and symptoms – n (%)	
Dysphasia	6 (40.0)
Sensorimotor disturbance	7 (46.7)
Visual impairment	
Post-traumatic seizures – n (%)	9 (60.0)
Focal only	1 (6.7)
Generalised	8 (53.3)
None	6 (40.0)

According to diagnostic PM reports, the overall extent of AD pathology was relatively low, with two thirds of cases showing none or only mild NFT pathology (Braak 0–II) and a preponderance of diffuse plaques over NPs, commensurate with the advanced age of the cohort (**Table 4.2**, **Table A.4.3**). Two cases were affected by large vessel infarcts but were not excluded as the infarcts did not involve connections between the

MTL and AC (Case 7: left middle cerebral artery infarct affecting left middle and inferior frontal gyri, left parietal penetrating brain injury; Case 13: right posterior inferior cerebellar artery infarct affecting right medulla, left parieto-occipital penetrating brain injury). Cause of death was not available for a third of cases but where available no deaths were due to neurological causes. Post-mortem interval (PMI) was short but only available for a minority of cases (*n* = 6). FFT varied up to 22 years. The experimental control group was free from significant other neurodegenerative or cerebrovascular pathology (**Table 4.3**, **Table A.4.4**).

Table 4.2 Post-mortem findings for study cases (*n* = 15).

Post-mortem findings	
Braak NFT stage – n (%)	
0	3 (20.0)
I/II	7 (46.7)
III/IV	4 (26.7)
V/VI	1 (6.7)
Amyloid-β plaques – n (%)	
None	4 (26.7)
Diffuse plaques only	11 (73.3)
Diffuse and neuritic plaques	5 (33.3)
Cerebral amyloid angiopathy – n (%)	
Mild	1 (6.7)
Moderate	2 (13.3)
Severe	2 (13.3)
Other neurodegenerative pathology – n (%)	
Lewy bodies	2 (13.3)
Cerebrovascular disease – n (%)	
Lacunar infarct	4 (26.7)
Large vessel infarct	2 (13.3)
Haemorrhage	0 (0.0)
Underlying cause of death – n (%)	
Cancer	5 (33.3)
Ischaemic heart disease	3 (20.0)
Heart failure	2 (13.3)
Unknown	5 (33.3)
Post-mortem processing – median (IQR)	
Post-mortem interval (days)[a]	2.0 (2.0)
Formalin fixation time (years)	16.0 (3.0–18.0)

[a] Post-mortem interval only available for *n* = 6.

Table 4.3 Baseline characteristics and post-mortem findings for controls (*n* = 9).

Characteristic	
Age – median (IQR)	
Age at death (years)	82.0 (80.0–86.0)
Sex – n (%)	
Male	9 (100.0)
Female	0 (0.0)
Braak NFT staging – n (%)	
0	1 (11.1)
I/II	4 (44.4)
III/IV	1 (11.1)
V/VI	3 (33.3)
Amyloid-β plaques – n (%)	
None	0 (0.0)
None or diffuse plaques	3 (33.3)
Neuritic plaques	6 (66.7)
Cerebral amyloid angiopathy – n (%)	
Mild	0 (0.0)
Moderate	0 (0.0)
Severe	0 (0.0)
Other neurodegenerative pathology – n (%)	
Lewy bodies	0 (0.0)
Cerebrovascular disease – n (%)	
Lacunar infarct	0 (0.0)
Large vessel infarct	1 (11.1)
Haemorrhage	1 (11.1)

4.3.2 Anatomical disconnection

Seven cases were classified as having lesional deficits that disconnected the sampled regions of the AC and MTL in the affected hemisphere (Cases 2, 4, 6, 7, 11, 12, 15) and eight cases had lesions without disconnection of the areas of interest (Cases 1, 3, 5, 8, 9, 10, 13, 14, **Table 4.4**, **Figure 4.11**).

Table 4.4 Damage and anatomical disconnection of sampled mesial temporal lobe and association cortex regions.

Case	Hemisphere	AC lobe	ILF %damage	Cingulum %damage	AC lobe disconnected	Comments
1	Right	Frontal	0.0	0.0	No	
2	Left	Frontal	31.3	14.2	Yes	
3	Right	Frontal	0.0	0.7	No	<5% damage
4	Right	Parietal	16.6	8.7	Yes	
5	Left	Frontal	0.0	0.2	No	<5% damage
6	Right	Parietal	50.0	8.2	Yes	
7	Left	Parietal	0.0	7.3	Yes	
8	Right	Parietal	0.0	0.0	No	
9	Right	Frontal	0.0	0.0	No	
10	Left	Parietal	0.1	0.0	No	<5% damage
11	Left	Parietal	15.4	0.3	Yes	
12	Left	Parietal	25.1	0.0	Yes	
13	Left	Frontal	0.0	1.1	No	<5% damage
14	Left	Frontal	0.0	6.4	No	Anterior pole
15	Right	Parietal	69.3	2.3	Yes	

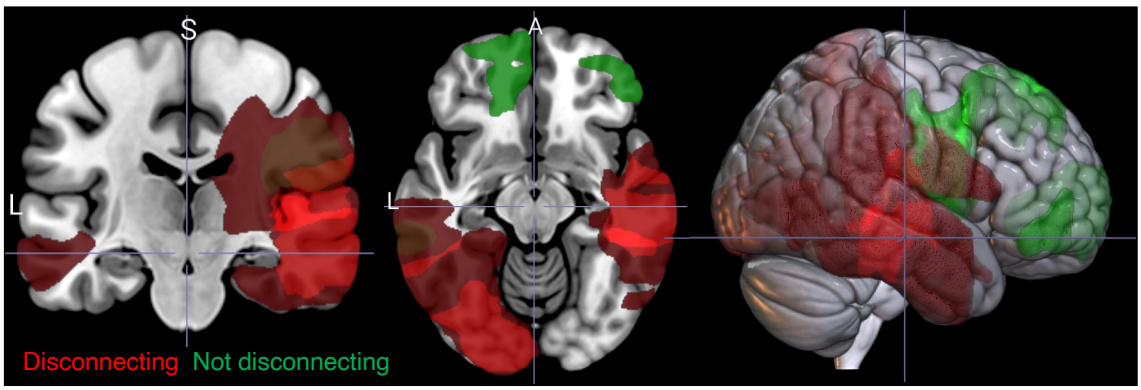


Figure 4.11 Anatomical classification of lesional disconnection. Lesional volumes classified as either disconnecting the mesial temporal lobe and association cortex in the affected hemisphere ($n = 7$, red) or not disconnecting these regions ($n = 8$, green). Cases with lesions involving the anterior pole of the brain or less than 5% lesional damage to the inferior longitudinal fasciculus or cingulum were classified as not disconnecting.

4.3.3 Qualitative description of p-tau and aβ pathology

Patterns of AT8-ir and 4G8-ir encompassed the morphology and distribution of pathology described in AD, CTE, and aging-related tau astrogliopathy (ARTAG).

4.3.3.1 Hyperphosphorylated tau

Three cases (Cases 7, 9, 12) showed minimal or no AT8-ir, consistent with diagnostic PM examination (**Table 4.2**, **Table A.4.3**). Exploratory analyses showed no difference in age at death (76.0 vs 81.0 years, $p = 0.114$, Mann-Whitney U) or FFT (19.0 vs 12.5 years, $p = 0.103$, Mann-Whitney U) compared to cases with AT8-ir.

4.3.3.1.1 Neurofibrillary tangles

All 12 cases with significant AT8-ir showed features of AD pathology (i.e. Braak stage I or more), including NFTs, NPs, and NTs. In six cases, NFTs were limited to the MTL in both hemispheres (Cases 1, 6, 10, 13–15). In the remaining six cases, NFTs were present in both the MTL and AC (Cases 2–5, 8, 11). In several of these cases, the extent of NFTs and NTs appeared to be reduced in the AC of the affected hemisphere (**Figure 4.12.B,F**) compared to the AC in the unaffected hemisphere (**Figure 4.12.A,E**).

4.3.3.1.2 ARTAG

AT8-ir astrocytes were identifiable in several cases, especially in subpial, subependymal, and perivascular locations throughout the AC, consistent with ARTAG [453]. As this pattern is not thought to be associated with cognitive impairment, it was not evaluated further.

4.3.3.1.3 Chronic traumatic encephalopathy

CTE pathology was observed in one case, which is discussed further in **Appendix 4.5**.

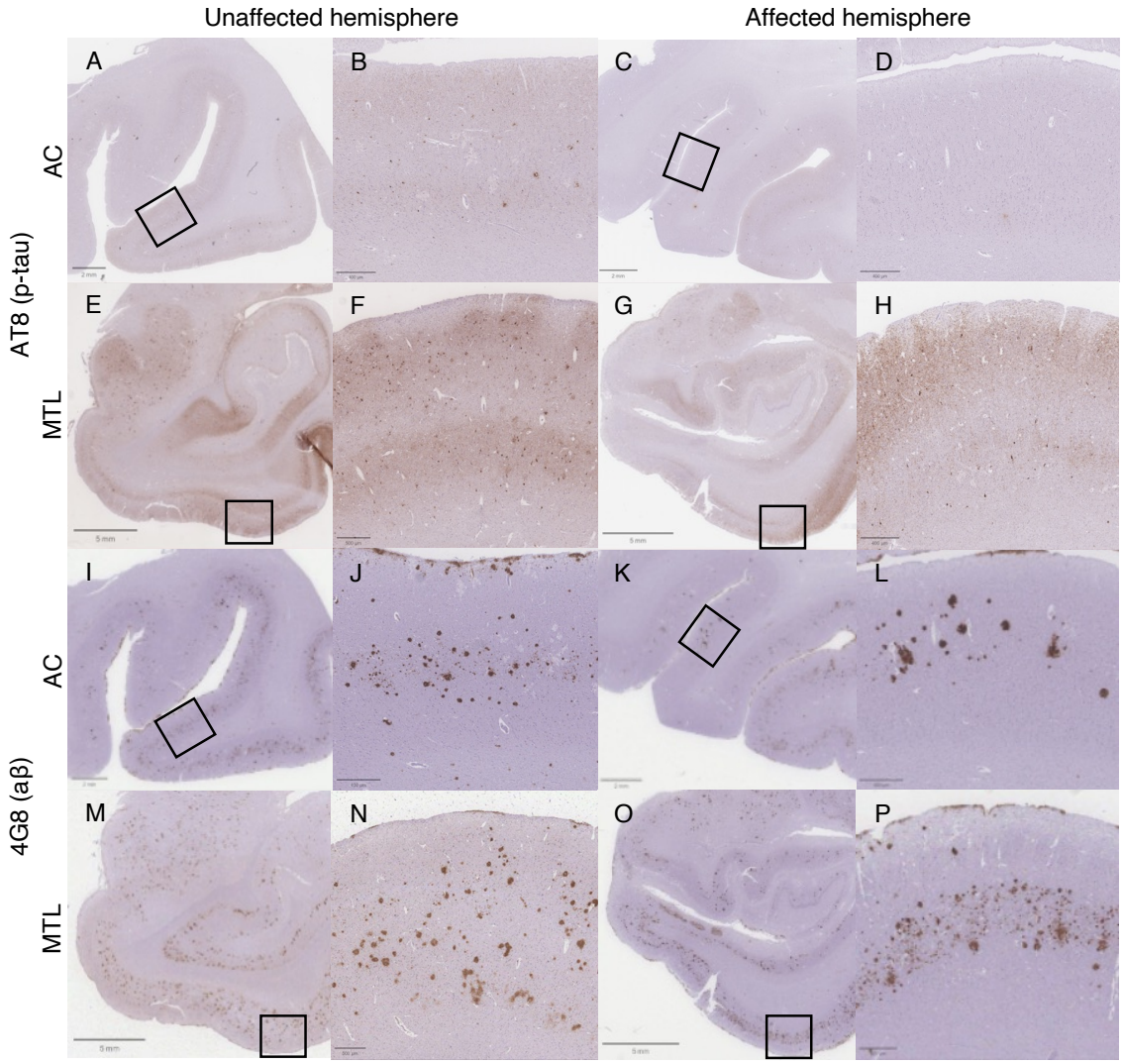


Figure 4.12 An illustrative case showing the extent of Alzheimer's-related pathology in the mesial temporal lobe (MTL) and association cortex (AC) of affected and unaffected hemispheres. A–H. AT8 immunolabelling. **I–P.** 4G8 immunolabelling. **A–H.** The extent of hyperphosphorylated tau (p-tau) pathology appeared reduced in the AC of the affected hemisphere (**C,D**) compared to the AC in the unaffected hemisphere (**A,B**). The level of p-tau pathology in the MTL of both hemispheres was similar (**E–H**). **I–P.** The extent of amyloid beta (aβ) pathology appeared symmetrical in the AC (**I–L**) and MTL (**M–P**) of affected and unaffected hemispheres.

4.3.3.2 Amyloid-β

Four cases (Cases 1–2, 7, 9) showed minimal or no 4G8-ir, consistent with diagnostic PM examination (**Table 4.2**, **Table A.4.3**). Exploratory analyses showed no difference in age at death (78.0 vs 81.0 years, $p = 0.115$, Mann-Whitney U) or FFT (16.0 vs 14.5 years, $p = 0.801$, Mann-Whitney U) compared to cases with 4G8-ir.

Collectively, therefore, there was no significant AT8- or 4G8-ir in two cases (Case 7, aged 67; Case 9, aged 76); two cases showed p-tau pathology without aβ (Case 1, aged 80; Case 2, aged 80) with NFTs limited to the MTL, consistent with PART; and one case (Case 12; aged 80) showed diffuse aβ plaques without p-tau pathology, typical of ageing. The remaining 10 cases showed AD-related patterns of AT8-ir and 4G8-ir.

4.3.3.2.1 Aβ plaques

Consistent with diagnostic PM reports, 11 cases displayed diffuse aβ plaques, and of these, five also showed NPs. In the AC, extracellular aβ plaques were seen in all cortical layers, especially layers III and V, and higher plaque density was associated with both sub-pial band-like aβ deposition and diffuse and globular plaques in the sub-cortical white matter. Endothelial aβ in the AC was in-keeping with the description of CAA in diagnostic reports. In the MTL, aβ plaques were seen in the EC (mostly in pre-β/-γ layers, fewest in pre-α), TEC, SUB, and CA1, with sub-pial fleecy amyloid in the EC and lake-like amyloid in the SUB. Overall, the extent and distribution of aβ pathology appeared symmetrical in the AC and MTL of affected and unaffected hemispheres (**Figure 4.12**), except for two cases with fewer diffuse plaques in the AC of the affected hemisphere (Cases 6 and 14).

4.3.4 Quantitative analysis

4.3.4.1 Reliability and validity of %NFT

Inter-rater reliability of %NFT was very good, when tested by two independent assessors counting NFTs and neurons in eight blocks (85 subregions, >3,500 neurons; ICC 0.907, 95% CI 0.674–0.960). Reliability of %NFT generated from AT8 immunostaining was also good when compared to %NFT from silver staining, after one

assessor counted NFTs in all cases stained using both methods (ICC 0.867, 95% CI 0.770–0.921). %NFT was also calculated in experimental controls to confirm its validity as a measure of the extent of AD-related p-tau pathology (**Figure 4.13**).

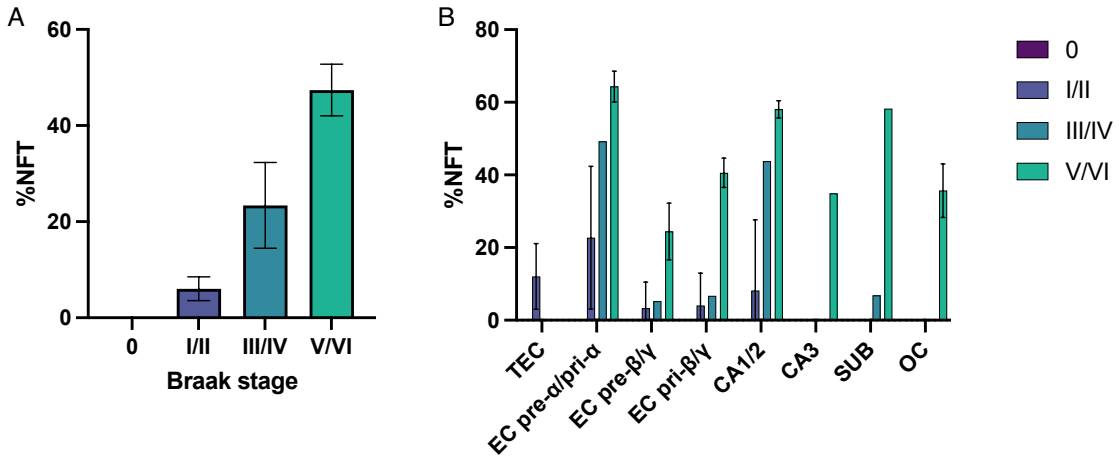


Figure 4.13 Validation of %NFT as a measure of Alzheimer's-related pathology. Average %NFT differentiated between Braak stages across controls (**A**) and within subregions (**B**), confirming validity of the score as a measure of the extent of AD-related p-tau pathology ($n = 9$). Graphs show mean ± standard error.

4.3.4.2 Hyperphosphorylated tau

In total, more than 1,300 NFTs and 15,000 neurons were counted (15 cases, 58 blocks, 128 subregions, 384 FOVs). Applying GEE models, %NFT in the MTL and AC combined was similar between the affected and unaffected hemispheres (affected: 7.89%, unaffected: 9.25%, $\chi^2 = 0.476$, $p = 0.490$, **Figure 4.14.A**). %NFT was significantly lower in the AC of both hemispheres combined compared to the MTL (AC: 3.46%, MTL: 10.5%, $\chi^2 = 11.360$, $p < 0.001$, **Figure 4.14.B**). The interaction between hemisphere and region was significant ($\chi^2 = 25.888$, $p < 0.001$), and the reduction in %NFT between MTL and AC was greater in the affected hemisphere (MTL: 10.19%, AC: 1.70%, $p < 0.001$, **Figure 4.14.C**) than in the unaffected hemisphere (MTL: 10.79%, AC: 5.09%, $p = 0.039$, **Figure 4.14.C**). Disconnection had a significant effect on %NFT in the AC, adjusting for %NFT in the ipsilateral MTL ($\chi^2 = 3.899$, $p = 0.048$),

and %NFT in AC disconnected from the MTL was significantly lower than in AC connected to the MTL (disconnected: 0.86%, connected: 3.16%, $p = 0.015$, **Figure 4.14.D**). Disconnection had no effect on %NFT in the MTL ($\chi^2 = 0.555$, $p = 0.456$, **Figure 4.14.E**).

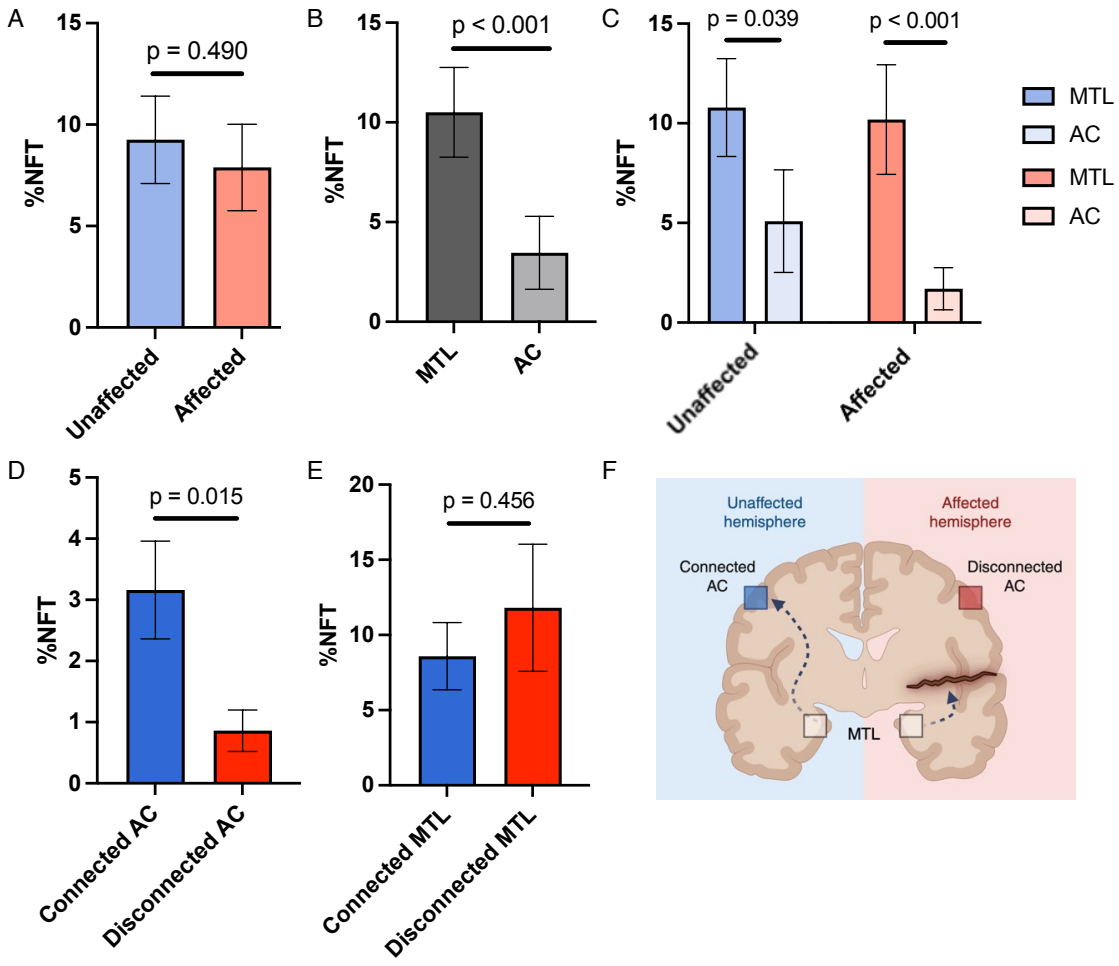


Figure 4.14 The effect of hemisphere, region, and disconnection on %NFT. A. %NFT in the mesial temporal lobe (MTL) and association cortex (AC) combined was similar between the affected and unaffected hemispheres. **B.** %NFT was significantly lower in the AC of both hemispheres combined compared to the MTL. **C.** The reduction in %NFT between MTL and AC was greater in the affected hemisphere than in the unaffected hemisphere. **D.** %NFT in AC disconnected from the MTL was significantly lower than in AC connected to the MTL. **E.** Disconnection had no effect on %NFT in the MTL. Graphs show mean ± standard error. Created with BioRender.com.

4.3.4.3 Amyloid-β

%Aβ in the MTL and AC combined was similar between the affected and unaffected hemispheres (affected: 1.23%, unaffected: 1.72%, $\chi^2 = 1.358$, $p = 0.244$, **Figure 4.15.A**).

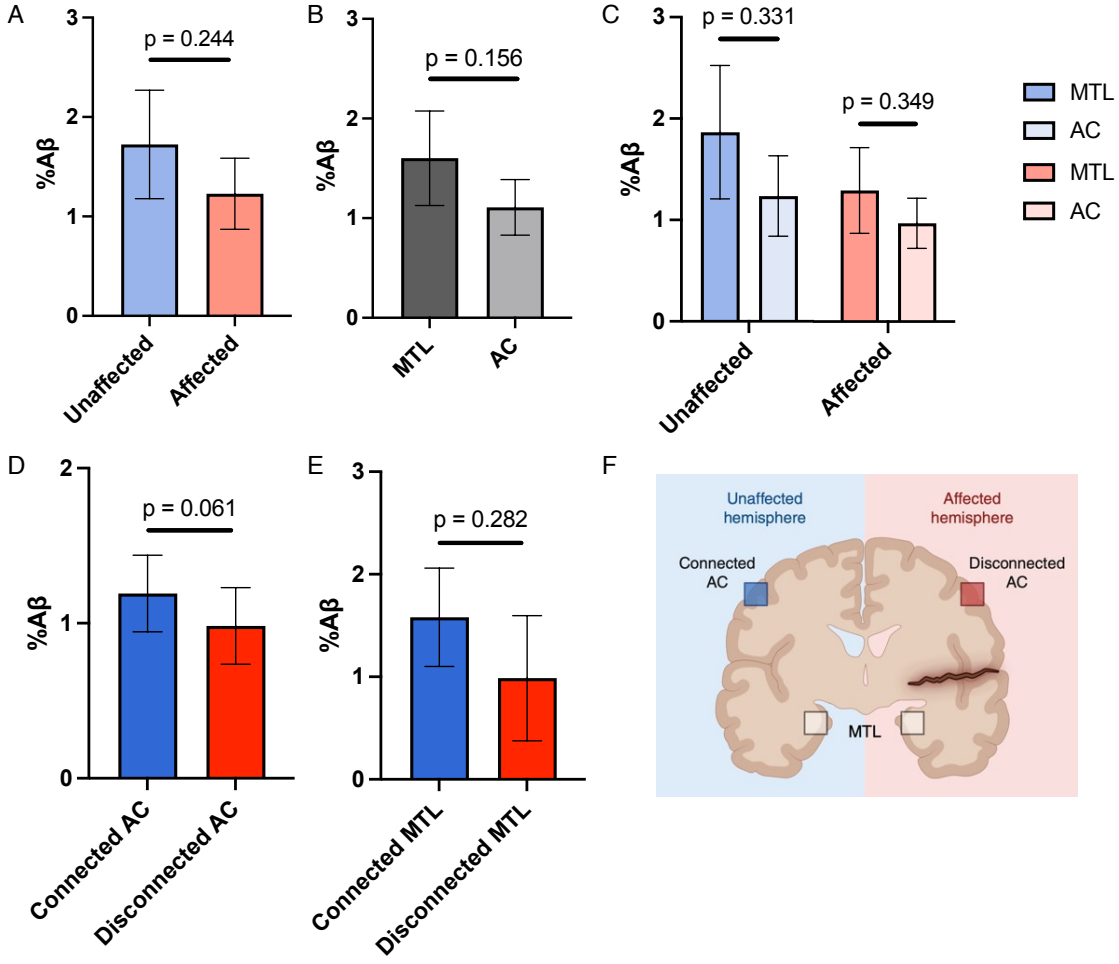


Figure 4.15 The effect of hemisphere, region, and disconnection on %Aβ. A. %Aβ in the mesial temporal lobe (MTL) and association cortex (AC) combined was similar between the affected and unaffected hemispheres. **B.** %Aβ in the AC of both hemispheres combined was similar to the MTL. **C.** The apparent reduction in %Aβ between MTL and AC was similar in the affected hemisphere and unaffected hemisphere. **D.** %Aβ in AC disconnected from the MTL and AC connected to the MTL trended to difference. **E.** Disconnection had no effect on %Aβ in the MTL. Graphs show mean ± standard error. Created with BioRender.com.

%Aβ in the AC of both hemispheres combined was similar to the MTL (AC: 1.11%, MTL: 1.60%, $\chi^2 = 2.009$, $p = 0.156$, **Figure 4.15.B**). The interaction between hemisphere and region was not significant ($\chi^2 = 4.496$, $p = 0.213$), and the apparent

reduction in %Aβ between MTL and AC was similar in the affected hemisphere (MTL: 1.31%, AC: 0.98%, $p = 0.349$, **Figure 4.15.C**) and unaffected hemisphere (MTL: 1.87%, AC: 1.24%, $p = 0.331$, **Figure 4.15.C**). Disconnection did not impact %Aβ in the AC, adjusting for %Aβ in the ipsilateral MTL ($\chi^2 = 0.462$, $p = 0.497$). %Aβ in AC disconnected from the MTL and AC connected to the MTL trended to difference (disconnected: 0.98%, connected: 1.19%, $p = 0.061$, **Figure 4.15.D**). Disconnection also had no effect on %Aβ in the MTL ($\chi^2 = 1.156$, $p = 0.282$, **Figure 4.15.E**).

4.4 Discussion

The objective of this study was to compare the extent and distribution of AD-related p-tau and aβ pathology between the cerebral hemispheres of individuals who sustained unilateral penetrating brain injuries in early adulthood. The main findings from this work are: i) reduced p-tau in AC disconnected from the MTL compared to AC that remained connected to the MTL, ii) no effect on aβ pathology in AC disconnected from the MTL, and iii) a relative lack of AD pathology decades after penetrating brain injury.

4.4.1 Reduced p-tau pathology in disconnected association cortex

The proportion of neurons affected by NFTs in AC disconnected from the MTL by a unilateral penetrating brain injury was lower than the proportion in AC connected to the MTL in the contralateral hemisphere (0.86% vs 3.16%, $p = 0.015$, **Figure 3.14.D**). This result supports the hypothesis that disrupting the connections between the MTL and AC is associated with reduced AD-related p-tau pathology in the disconnected AC, compared to the unaffected hemisphere. It can be inferred from this finding that the development of p-tau pathology in the AC is, to a significant extent, dependent on intact connections between the AC and MTL. The simplest explanation for this would be that p-tau is transported between the neurons that connect the MTL and AC, as has

been shown to occur in cell and animal models. This finding is consistent with previous descriptive human post-mortem studies, and its demonstration in this comparative post-mortem study represents a novel strand of support for the tau propagation hypothesis.

4.4.1.1 Future studies to confirm and extend this finding

The main strength of this study is the comparison of AD pathology between two hemispheres from the same individual, performed among a cohort who aged after surviving unilateral penetrating brain injuries. This was made possible by access to an exceptional cohort with mature multi-modal longitudinal data. The findings of this study could be extended by evaluating microglial expression in selected cases with evidence of reduced tau propagation to explore the relationship between lesional disruption, neuroinflammation, and tau transport. Individuals who have undergone neurosurgical procedures for treatment of brain tumours, epilepsy, or (rarely) severe psychiatric disorders could also be studied along these lines, although it may be difficult to fully discount the effects of the underlying conditions in these cases.

One of the limitations of this study is that it is cross-sectional and therefore cannot, in isolation, prove causal relationships or describe temporal processes. To support our conclusions, a longitudinal study using serial positron emission tomography (PET) imaging could be performed to reveal the progression of p-tau pathology over time among individuals with unilateral disruption to the connections between the MTL and AC, either from neurosurgical procedures or penetrating brain injuries. Such an approach could not only identify the distribution of NFTs, but could also investigate the effect of anatomical disconnection on p-tau oligomers using recently discovered radiotracers specific to these molecules [454]. Clinical assessments could also be

performed to investigate whether restricted p-tau was associated with the sparing of specific cognitive functions, which, if shown, would add strong support to the notion that the spread of p-tau drives cognitive impairment in AD. Unfortunately, while many cases in our study cohort underwent neuropsychological assessments between the 1960s–1980s, limited information about cognitive performance in the years before death was available.

4.4.1.2 A pathway towards clinical impact

In the search for disease-modifying AD treatments, attention is increasingly shifting away from aβ and towards p-tau [345,455]. Although drugs targeting p-tau have yielded disappointing results in early phase clinical trials, to date these candidates have largely targeted intracellular steps of p-tau aggregation. The tau propagation hypothesis proposes that the spread of oligomeric p-tau across the synaptic cleft is a critical step in AD pathogenesis [358]. Thus, the interception of extracellular p-tau oligomers, or interference with vesicular transport, may represent more promising targets for drug development. Several monoclonal antibodies (e.g. UCB0107, JNJ-63733657) and antisense oligonucleotides (e.g. ISIS814907/BIIB080) have now entered phase 2 trials with this aim [456]. Cell-to-cell transfer of pathogenic molecules is implicated in a range of neurodegenerative conditions, including others characterised by the aggregation of p-tau (e.g. frontotemporal dementia, corticobasal degeneration, progressive supranuclear palsy), α-synuclein (e.g. dementia with Lewy bodies, multiple system atrophy), and TDP-43 (e.g. frontotemporal lobar degeneration) [344,457]. Therefore, it is possible that treatments which prevent the transport of these proteins into the neocortex may be applicable across multiple disorders. The main finding of this study supports further research into the development of such treatments.

4.4.1.3 Challenges and limitations

4.4.1.3.1 What is the relevance of a reduced proportion of NFTs?

While the distribution of NFTs is associated with the severity of cognitive decline, and the formation of NFTs is associated with microtubule disassembly, impaired axonal transport, and synaptic dysfunction, NFTs themselves are not necessarily damaging. In fact, as discussed in **Section 1.4.5.1.2**, the formation of NFTs may be a protective response to the accumulation of neurotoxic p-tau oligomers [303]. The possibility that NFTs are a proxy marker of the concentration of p-tau oligomers warrants consideration. While an indirect role for NFTs does not change the interpretation of our results, it does highlight the need for more research into the roles of p-tau oligomers and NFTs in the pathogenesis of AD. Longitudinal studies combining advanced neuroimaging with clinical assessment, as described above, may serve to advance this frontier.

4.4.1.3.2 Why was p-tau pathology not completely absent in disconnected AC?

The fact that the proportion of neurons affected by NFTs was not reduced to zero in the disconnected AC does not violate our hypothesis. First, while the ILF and cingulate represent the most direct routes between the MTL and AC, multi-step connections between these regions exist and may also play a role in p-tau propagation. Second, while the route of tau propagation is proposed to pass from subcortical centres to the AC via subregions of the MTL (i.e. EC and pre-subiculum), the contribution of inter-connected regions (e.g. retrosplenial cortex) is not yet clear [363]. Finally, the lesion reconstruction method used in this study indicated that anatomical connections between the NFT and AC were not completely disrupted, meaning that some propagation along the proportion that remained intact may still be expected. However, given the limited accuracy of this method, the extent of disconnection should only be

considered to be approximate. As such, in this study, anatomical disconnection reflects partial disruption of the main connections between the MTL and AC, which is consistent with our results.

4.4.2 No effect on aβ pathology in disconnected association cortex

While this study showed a significant reduction in p-tau pathology in AC that was disconnected from the MTL, it did not show a significant effect on aβ pathology. This was expected, given that there is limited evidence that aβ proteins propagate along neurons in AD. However, it is worth noting that the results for %Aβ in this study followed the same trends as those of %NFT. The simplest interpretation of this finding is that aβ and p-tau are partially co-dependent. Consistent with reports that diffuse aβ plaques regress after CTBI, the total burden of plaques in the affected hemisphere was equivalent to the unaffected hemisphere. What governs the aggregation of aβ40 and aβ42 remains unclear, but catalysts of this process include a wide range of molecules, metal ions, and microbes. Effects of the penetrating lesion on chronic neuroinflammation and vascular disruption may therefore influence the distribution of aβ in these cases, along with many other individual factors. A limitation of this study was that the pixel classifier used to quantify %aβ was not trained to distinguish between diffuse and neuritic plaques, due to the limited functionality of the tools used. Refinement of this approach, or manually counting plaques with different morphologies, could be pursued to investigate the effect of anatomical disconnection on NPs, specifically.

In-keeping with the patterns of AD pathology described in the literature, NFTs were not observed in the AC without aβ plaques. This is consistent with aβ pathology being required for NFTs to form in the AC, and the recently reported finding that aβ can

trigger neuronal hyperactivity and promote hyperconnectivity between early and advanced regions of p-tau pathology [341–343]. Intriguingly, animal studies suggest that microglia may play a role in the spread of p-tau oligomers across synapses, and the phagocytosis of synaptic compartments affected by p-tau, which is associated with early cognitive impairment in AD [458–460]. One of the central mechanisms underlying AD pathogenesis, combining aβ and p-tau proteins, neuroinflammation, and neurodegeneration, may therefore be converging on the inflammatory response to the synaptic transport of p-tau.

4.4.3 Relative lack of Alzheimer's pathology decades after penetrating brain injury

The overall extent of AD pathology in the cohort was perhaps less than might be expected, especially given the advanced age of the individuals studied, and the epidemiological and pathological links between TBI and AD. However, as discussed in **Section 1.4.4**, while NFTs and aβ plaques are both chronic neuropathological features of TBI, they appear in the form of CTE and transient diffuse aβ plaques, not AD-related NFTs and NPs. Unfortunately, the pathophysiological links between TBI and AD remain unclear, and even less is known about the links between penetrating brain injury and AD.

Members of the cohort survived more than 50 years after penetrating brain injuries and, on average, lived until the age of 80, exceeding the life expectancy of an age- and sex-matched cohort according to life-table estimates, as discussed in Section **2.4.2.1**. Both of these characteristics imply strong resilience factors, which could mitigate the effects of ageing and neurodegeneration. Additionally, individuals in this cohort may have benefitted from more regular access to healthcare, which could have led to identification and treatment of co-morbid risk factors associated with AD and

neurodegeneration, including vascular risk factors. It is therefore difficult to comment on the prevalence of AD pathology after penetrating brain injury from the small and highly selected sample studied here, except perhaps to conclude that it is far from inevitable.

5 Synthesis

5.1 Thesis summary

Through this thesis, I aim to elucidate the long-term consequences of TBI by leveraging data from the MHHI cohort to not only advance the understanding of TBI but also to gain insights into structure-function relationships in the brain and the mechanisms driving AD. The research underpinning this thesis was structured around three core aims: to describe long-term outcomes of OTBI, to develop a method enabling digital lesion analysis from archival film-based CT scans and apply it to neuropsychological research, and to investigate the pathogenesis of AD through post-mortem analysis of individuals with unilateral penetrating brain injuries. These aims address key challenges in the fields of epidemiology, neuropsychology, and neuropathology, respectively, while also exploring how historical data can inform current scientific understanding.

In **Chapter 2**, I established that OTBI, while associated with significant early neurological morbidity, can be distinguished from CTBI by its specific pattern of long-term outcomes. I found that OTBI was associated with a higher incidence of seizures, visual impairment, and dysphasia, but with unexpectedly lower rates of psychiatric and diffuse cognitive symptoms compared to CTBI. These findings lend empirical support to the traditional clinical impression that CTBI is more likely to result in a diffuse cerebral syndrome, while OTBI typically leads to focal deficits. I also found that individuals with prolonged PTA were at increased risk of developing seizures after injury, reinforcing PTA as a clinically meaningful marker of injury severity in the context of OTBI. Sensorimotor and visual impairments were early predictors of long-term disability following OTBI, while seizures and dysphasia were associated with increased

disability in the long term. In contrast, headache was paradoxically linked to reduced disability levels, potentially reflecting reporting bias or differential engagement in employment. My analysis of mortality outcomes revealed that, while average life expectancy was reduced by approximately seven years compared to the general population, nearly a quarter of OTBI cases had neurological or psychiatric conditions listed as causes of death. However, full interpretation of these findings awaits the inclusion of updated mortality data and comparative cohorts.

In **Chapter 3**, I addressed the technical challenge of converting analogue CT radiographs into a format suitable for modern LSM by developing and validating a novel lesion reconstruction method. Using this approach, I then performed lesion studies using cases from the MHHI cohort. The first study replicated earlier findings linking damage to the left prefrontal cortex with insomnia and demonstrated that this association was not explained by co-occurring depression or anxiety symptoms, suggesting a direct role for this region in sleep regulation. The second study attempted to replicate findings regarding the clock-drawing test from a stroke lesion cohort but was limited by sample size. Nonetheless, the results were broadly consistent with established hemispheric specialisation, with time-setting errors more common after left hemisphere damage and visuospatial errors after right hemisphere lesions. This study also showed that impaired performance in the clock-drawing test can arise from a wide range of lesion locations, suggesting that a broad network may be involved in the accurate execution of this complex task. More generally, this work highlights the complementary strengths of archival CT and modern neuroimaging methods, as well as the distinct lesion profiles of penetrating versus ischaemic injury.

In **Chapter 4**, I examined the neuropathological consequences of penetrating brain injury with respect to AD pathogenesis. Using post-mortem samples from individuals with unilateral brain injuries, I observed a reduced burden of p-tau pathology in AC that was anatomically disconnected from the MTL. This strengthens the tau propagation hypothesis, adding a novel strand of neuropathological support to the growing body of evidence from cell and animal models. Notably, I did not observe this effect for aβ, raising the possibility that while aggregation aβ and p-tau may be co-dependent, their spatial patterns may be governed by distinct mechanisms, with p-tau showing greater dependence on network architecture. The overall burden of AD pathology in this cohort was unexpectedly low, suggesting potential resilience factors among long-term survivors of penetrating brain injury.

5.2 Limitations

The use of the MHHI cohort offered a rare opportunity to conduct high-resolution longitudinal research into the long-term effects of TBI, but reliance on historical and archival data introduced several challenges. Foremost among these was imprecision: medical practice in the 1940s, while impressively systematic at the MHHI, lacked the diagnostic granularity afforded by modern neuroimaging and clinical tools. In my epidemiological study, this limited the diagnostic accuracy of injury characteristics and post-injury complications, particularly for variables such as intracranial haematoma or retained foreign bodies. Furthermore, outcome data derived from postal questionnaires were susceptible to both recall and observer bias. These biases are compounded by potential underreporting due to stigma, especially for psychiatric outcomes, as well as variable involvement of third parties in completing questionnaires. Selection bias was another concern, potentially leading to overestimation of recovery and underestimation of morbidity. These limitations were partly mitigated through the use of pre-defined

inclusion criteria, comparison with a matched group, and multivariable regression models to control for confounding variables. In addition, analyses were restricted to incidence rates where appropriate to account for differential follow-up duration and reduce the impact of attrition bias.

The main limitation of my neuropsychological studies was the resolution of lesions reconstructed from archival CT films. Although validated structurally and functionally, the reconstructions had lower resolution than modern scans, limiting the sensitivity of voxel-wise LSM. Additionally, small lesion volumes and minimal overlap between cases reduced the statistical power of lesion studies, particularly when examining specific neuropsychological tasks such as the clock-drawing test. However, this limitation was offset by advantages of a relatively even distribution of lesions across cerebral hemispheres and lobes, which reduces the bias introduced by stereotyped lesion overlap and enables the study of brain-behaviour relationships in less frequently sampled regions.

For the post-mortem study, an effect was demonstrated despite a small sample size and lower than expected overall levels of AD pathology. The most notable limitation was the resolution of anatomical reconstructions used to define connectivity between the MTL and AC. The highly selected nature of participants in this study precludes inferences about the prevalence of AD after penetrating brain injury.

5.3 Future work

Building on this work, future directions span all three aims. First, I plan to extend the epidemiological study of morbidity outcomes up to 35 years after OTBI, and the study of mortality outcomes through linkage with NHS and ONS records up to 2024. The latter will allow for accurate estimation of average age at death, inclusion of CTBI and

peripheral nerve injury controls, and refined analysis of causes of death. In particular, this will facilitate an evaluation of whether OTBI is associated with increased long-term risk of cerebrovascular and neurodegenerative diseases. Given the recent literature linking OTBI with haemorrhagic stroke, it is hoped that this can help to inform future management guidelines for this condition.

Second, I intend to apply the validated lesion reconstruction method using cases from the MHHI cohort to test hypotheses regarding the neural correlates of anxiety, depression, and personality change, as originally proposed in the VHIS and anticipated by the original MHHI investigators. These analyses will benefit from the use of region-specific LSM approaches, which offer optimal specificity and sensitivity for this cohort. Lesion-network mapping techniques will also be explored, potentially revealing the functional connectivity disruptions underlying chronic neuropsychological impairments and psychiatric symptoms.

Third, my neuropathological work can be taken further through the application of advanced quantitative neuropathology tools to distinguish between diffuse and neuritic plaques and to evaluate the role of microglia in tau propagation. Further comparative studies among neurotrauma or neurosurgical patients with unilateral brain injuries could also confirm or challenge my findings related to p-tau propagation. Longitudinal PET imaging studies may help to characterise the temporal dynamics of oligomeric tau spread in relation to connectivity, while behavioural correlates could clarify whether disruption of p-tau propagation confers any protective cognitive effects.

The compiled and reconstructed MHHI dataset now offers exciting opportunities for data sharing and further research not yet imagined in this thesis. The unique characteristics of this cohort, including the controlled nature of the injuries, structured

follow-up, and eventual post-mortem examination, make it a valuable resource for contemporary neuroscientific research.

5.4 Concluding thoughts

This thesis demonstrates the continued scientific value of historical data when combined with modern analytical tools. The MHHI cohort, once a dormant collection of wartime medical records, has yielded novel insights into the long-term outcomes of TBI across a spectrum of neuroscientific research. From revealing the differential morbidity patterns of OTBI and CTBI, to mapping structure-function relationships in the brain using archival CT scans, and illuminating the mechanistic underpinnings of p-tau propagation in AD, each aim contributes uniquely to our understanding of brain injury and disease.

A key theme emerging from this work is that focal brain injuries occurring in individuals who are otherwise neurologically intact continue to offer powerful models for understanding the brain and mind. This potential can be realised across the spectrum of clinical neuroscience, from neuropathology to neuropsychology, to confirm, refine, or challenge hypotheses derived from other models.

Ultimately, the long-term consequences of TBI remain an area of urgent importance, with implications for patient care and public health. This thesis contributes to the foundational knowledge required to mitigate the effects of TBI, to understand the neural basis of cognition, and to advance the search for disease-modifying treatments in neurodegenerative disorders. In doing so, it aims to honour the sacrifices of those who served during WWII, and the foresight of those who preserved the records of their care for future discovery.

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Appendices

Appendix to Chapter 1: Introduction and Background

Appendix 1.1 Preparation of research materials

In this section, the steps taken to prepare and access the research materials used to meet these aims are briefly summarised.

Admission records, medical files, research records, and correspondence relating to all indexed cases at the MHHI were preserved in the Cairns Library at the Radcliffe Infirmary until 1995, when due to limited space, they were transferred to the Archive at St Hugh's College under the Public Records Act 1958 S.3(6) with the approval of the Public Record Office and the Principal of the College. In 2012, with the support of a Research Resources in Medical History Award from the Wellcome Trust, the complete records of the MHHI were catalogued, indexed, and re-packaged for their preservation and conservation as the HHA. Indexing linked the records of individual cases across nine collections spanning more than 70 years, enabling data collected longitudinally from each case to be identified. In 2021, the personal research records of Freda Newcombe were transferred to the Archive from her colleague, Professor Andy Young at the University of York. These included additional records relating to scientific publications involving the MHHI cohort and the original CT radiographs acquired at the Radcliffe Infirmary during the 1980s. To conduct the research reported in the following chapters, clinical and research records for all indexed cases at the MHHI were accessed with the support of the College Archivists and Librarians.[21]

[21] Records were accessed from the following collections: HHA/1 Case files for Patients and Research Subjects, 1939-2001; HHA/2 Research Datasets and Supplementary Materials, 1940-2003; HHA/4

Records relating to the establishment and operation of the MHHI were accessed from The National Archives (TNA) in Kew to provide valuable scientific and historical context. Specifically, the minutes of the Brain Injuries Committee (BIC) and reports from the MHHI were reviewed.[22]

Post-mortem brain tissue was donated to the Thomas Willis Oxford Brain Collection which subsequently became the Oxford Brain Bank (OBB). The OBB continues to preserve the tissue samples and diagnostic reports from the donations made by veterans treated at the MHHI. Anatomical photographs of post-mortem brain tissue from these cases are stored in more than 250 35mm projector slides and were made available by Professor Esiri. Slide scanning was performed by Oxford Medical Illustration to acquire digital images for the purpose of the neuropsychological and neuropathological studies. Formalin-fixed paraffin-embedded blocks of tissue were accessed with the approval of the OBB for the neuropathological study (OBB reference: TW170).

Correspondence, 1940-52, 1988-95; HHA/6 Photographs, c. 1940-52; HHA/9 Former Lists and Finding Aids, c. 1945-98; and the records donated by Professor Andy Young.

[22] Records were accessed from the following collections: FD 1/5269 Brain Injuries Committee; minutes of sixteen meetings (1940–42); FD 1/5342 Committee on Brain Injuries minutes of ten meetings (1940–42); FD 1/5343 Committee on Brain Injuries; minutes of five meetings (1941–42); FD 1/6137 Wounds and the nervous system: Head Injuries Centre, Oxford (1942–50); WO 222/845 Oxford Military Hospital (1940).

Appendix 1.2 Criteria for admission to the Military Hospital for Head Injuries

Individuals were admitted to the MHHI if they had one or more of the following indications after a head injury:

- Signs of neurological impairment (i.e. motor, sensory, language impairments)
- Signs of cognitive impairment (i.e. disorientation, memory loss)
- Symptoms of raised intracranial pressure
- Symptoms persisting beyond 6 weeks and producing functional impairment (e.g. headache, dizziness, nervousness)
- Frontal, maxillary, or mastoid sinus infection
- Skull osteomyelitis
- Depressed skull fractures
- Penetrating brain injuries

Individuals with injuries requiring urgent neurosurgical intervention (e.g. persistent low conscious level, epilepsy, intracranial haemorrhage, compound skull fracture) were transferred to the MHHI provided it could be reached within 2–3 hours and there were no contra-indications to transfer (e.g. cardiovascular shock, respiratory failure, uncontrolled bleeding).

Source: 'Indications for the Admission of Patients to the Oxford Military Hospital (Head Injuries)', TNA FD 1/5342.

Appendix 1.3 Assessment documents used at the Military Hospital for Head Injuries

Pre-hospital head injury assessment form

BIC.7

Form 2151

Cases of Head Injury

Date and time of injury .....

Date and time of examination .....

ˣNo external injury / ˣExternal injury

ˣNo fracture seen / ˣFracture seen (ˣFissured (ˣDepressed (ˣPenetrating

Mental state (a) (ˣAlert (ˣDrowsy (ˣComatose (b) (ˣLucid (ˣConfused (c) (ˣQuiet (ˣExcited (ˣIrritable

Pupils (ˣDilated (ˣPin point (ˣEqual (ˣRight larger (ˣLeft larger

Weakness or paralysis, right limbs, ˣAbsent / ˣPresent

Weakness or paralysis, left limbs, ˣAbsent / ˣPresent

Pulse rate .....

If unconscious, note (History of unconsciousness .....
(How long .....
(Duration of amnesia period .....
(Presence of headache .....

Treatment given.....
.....
.....
.....

Progress under observation (ˣImproved (ˣStationary (ˣWorse

Remarks (N. B. In cases of accident give details from witnesses and from patients if conscious)
.....
.....
.....

ˣ Delete where not applicable

Source: BIC 7, 'Case-taking scheme in use at Oxford Military Hospital (Head Injuries)', TNA FD 1/5342.

Case-taking scheme

CASE-TAKING SCHEME IN USE AT OXFORD MILITARY HOSPITAL (HEAD INJURIES)

Name Age Home address

Number Rank Unit

HISTORY.

A. 1. Place, date, time and nature of injury.
2. Last memory before injury.
3. First memory after injury.
4. Subsequent history to date. (a) From patient. (b) From notes.

B. Present complaints. 1. Spontaneous. 2. To direct questions.

C. Family History, to include
1. Mental or nervous instability in parents, uncles, aunts, sibs, and first cousins.
2. Epilepsy.
3. Migraine.

D. Personal History, to include
1. Synopsis of life story, i.e.
Standard of alcohol performance.
Affective type (cheerful, anxious, etc.)
Capacity for mixing.
Games.
Hobbies and intellectual interests.
Habits, alcohol, and tobacco.
Jobs held.
Marriage, children, domestic background.
Wage earned pre-war.
2. Brief record of past illness.
3. Detailed record of any previous
(a) History of head injury.
(b) Liability to fits, faints or dizziness.
(c) Liability to headache.
(d) Indication of neurotic tendency.
(e) Insomnia.

EXAMINATION.

A. Neurological routine.

B. General routine.

C. Mental state.
1. Behaviour
2. Spontaneous talk.- with sample, including any subjective amount of disturbance of thinking.
3. Mood (depressed, anxious, happy, irritable, suspicious, apathetic).
4. Pre-occupations, including obsessions, delusions and hallucinations.
5. Intellectual functions.
Orientation.
General Information.
Memory.
Name, address and flower test, 5 minutes (How long?)
Digit retention.
Calculation 100 – 7.
Comprehension.
Performance. (Letter)
Judgement.
6. Insight.

Source: 'Report from Oxford Military Hospital (Head Injuries), Appendix II, Routine scheme for examination and case records', TNA FD 1/5342.

Neurosurgical operation note

Field Card No.
B.I.C.6

Operation Card for Head Injuries.

(Field Card No. should be filled in before operation).

No. Name Rank

Unit

Date and time of operation

Situation of wounds (enter on diagrams on back of cards)

X-ray findings. Fracture ?_____ F.B. ?_____ Site of F.B. ?_____

(Mark X-ray findings on diagram overleaf).

Name of operator

Anaesthetic employed

Dura Torn ?_____ Intact?_____

Brain Bruised ?_____ Lacerated?_____
Intact?_____
If infected ?_____ By what organism ?_____

Other findings _____

Operative Treatment _____ _____

What foreign bodies removed ? _____

Drainage ? _____

For distribution to Casualty Clearing Stations, Stationary and Base Hospitals.

0075 159/1/40.

Source: BIC 6, 'Case-taking scheme in use at Oxford Military Hospital (Head Injuries)', TNA FD 1/5342. F.B. = foreign body.

Fit assessment form

TO BE USED IN CASES OF FITS, FAINTING ATTACKS, etc. Observations on attack

As many as possible of these questions should be answered when actual observations are obtainable.

Name..... Date..... Time.....

1.	What was he doing just before the attack (i.e. job, any special emotional or physical stress)?	1.
2.	General condition before attack (asleep, awake, irritable, dull, etc.)	2.
3.	Onset – sudden or gradual> Falling? Injury?	3.
4.	Cry or noise?	4.
5.	Loss of consciousness? Its duration.	5.
6.	Colour of face (pale, flushed, blue, natural, etc.)	6.
7.	Movements, if any. (Rigidity, and if so, position of limbs. Jerking. Twitching. Purposive movements. Kicking. Fighting, etc.)	7.
8.	Turning of head, eyes or trunk? If so, to which side?	8.
9.	Course of attack. Which limbs involved? Generalised from onset? If not, which limb first affected? Order in which movements spread. Which side most affected?	9.
10.	Duration of movements?	10.
11.	Biting of tongue?	11.
12.	Incontinence or urine or faeces?	12.
13.	Symptoms afterwards? (Headache, sleep, vomiting, peculiar behaviour, crying or emotional disturbance.)	13.

Signature of Observer.....

Source: 'To be used in cases of fits, fainting attacks, etc.', TNA WO 222/845.

Form for medical discharge from military service

CONFIDENTIAL IV B.I.C.7

FORM FOR USE AT INVALIDING BOARDS.

Name Rank Number

Ship or Establishment, Regiment, or Unit.

Home address

Approved Society Membership Number

Age

History.

1. Date of injury.

2. Nature of injury. (Please tick against appropriate category or categories)

1. Head injury without lesion of scalp or of cranium.
2. Had injury without lesion of scalp, but with fracture of skull
   (a) vault (b) base
3. Scalp wound with intact cranium and dura.
4. Wounds with local cranial fracture, dura intact. (a) with or
   (b) without depression of external table.
5. Local depressed fracture with puncture of the dura.
6. Wounds with detached bone fragments driven into the brain.
7. Penetrating wounds with lodgment of projectiles.
8. Cranio-cerebral wounds, involving (a) orbito-nasal
   (b) auro-petrosal region.
9. Through and through cranio-cerebral wounds.
10. Cranio-cerebral injuries with massive fracture of the skull.

3. Complications

Meningitis		
Abscess		site
Brain fungus		site
Retained missile		site
Haemorrhage		extradural
		subdural
		subarachnoid
		intracerebral
C.S.F. leak.	nose	ear
Wounds or injuries elsewhere		site

P.T.O.

-2-

4. Clinical Story. (Loss of memory, i.e., from last memory before injury to first memory after injury: duration)

Duration of absolute unconsciousness

Fits focal generalised major minor

Evidence of focal injury (e.g. aphasia; hemianopia; motor, sensory or reflex abnormality; cranial nerve palsies; diabetes insipidus)

Unusual features

5. Treatment

1. Operative
2. Other: Dehydration Regine Yes/No
   Regular phenobarbitone Yes/No
   Sulphonamides Yes/No

6. Present State

1. Complaints
2. Mental state (e.g. intelligence, behaviour, orientation, memory, emotional state)
3. Speech
4. Special senses
5. Cranial nerve
6. Motor
7. Sensory
8. Reflex

Name of hospital…… Signature of Medical Officer in charge of case

Date…… ……

Source: BIC 7, 'IV: Form for use at invaliding boards', TNA FD 1/5269.

Appendix 1.4 Glossary of terms used at the Military Hospital for Head Injuries

Term	Definition
Coma	A state of absolute unconsciousness as judged by the absence of any psychologically understandable response (including, for example, change of expression) to external stimuli or inner need.
Semicoma	A state in which psychological understandable responses are elicited only by painful or other disagreeable stimuli, e.g. pinching the skin, shaking the patient violently.
Confusion	Disturbance of consciousness, characterised by impaired capacity to think clearly and with customary rapidity and to perceive, respond to and remember current stimuli; there is also disorientation.
Delirium	A state of much disturbed consciousness (confusion) with motor restlessness, transient hallucinations, disorientation and perhaps delusions.
Psychoneurosis	It is inevitable that in a certain number of patients a psychoneurosis should masquerade in the guise of the effect of injury to the head ('shell shock', etc.) For this reason a number of cases of stupor, amnesia, depression, and neurotic headache or fits have gained admission.
Traumatic intellectual impairment	Impairment of the intellectual functions which may be persistent or recoverable, but is not progressive, resulting from structural damage to the brain.
Traumatic personality disorder	Alteration of temperament and character, which may be persistent or recoverable, but is not progressive, resulting from structural damage to the brain.
Mood	The patient's appearance may be described, so far as it is indicative of his mood. His answers to 'How do you feel in yourself?', 'What is your mood?', 'How about your spirits?', or some similar enquiry should be recorded. Many variations of mood may be present, not merely happiness or sadness, but such states as irritability, suspicion, fear, unreality, worry, restlessness, bewilderment, and many more which it is convenient to include under this heading. Observe the constancy of the mood, the influences which change it; the appropriateness of the patient's apparent emotional state to what he says.
Orientation	Record the patient's answers to questions about his own name, and identity, the place where he is, the time of day and the date.
Memory	This may be tested by comparing the patient's account of his life with that given by others, or examining his account for intrinsic evidence of gaps or inconsistencies…There should be special inquiry for recent events, such as those of his admission to hospital and happenings in the ward since…Give the patient the 'Cowboy' or 'Gilded Boy' stories and ask him to repeat it in his own words…Give him digits to repeat.
Grasp of general information and calculation	Ask the following questions related to general information: i) The name of the King, and his immediate predecessors ii) The name of the prime minister iii) The capitals of France, Germany, Italy, and the US iv) The date of Armistice Day v) What happened at Dunkirk? vi) The names of three large seaports vii) What are the colours of traffic lights? What are they for? viii) The 100-7 test ix) Write a letter describing a day in their life.

Source: BIC 20a, 'Glossary of psychological terms commonly used in cases of head injury', TNA, FD 1/5342 and 'Comments on treatment and disposal' and 'Mental state', TNA, WO 222/845. US = United States.

Appendix 1.5 Approach to head injury management at the Military Hospital for Head Injuries

Pre-hospital management

Patients with reduced conscious level were laid on their side to avoid aspiration and were kept nil by mouth unless able to swallow water safely. Agitation was managed with sedation (chloral hydrate 20g PO or 40g PR, paraldehyde 2 drams PO or 4 drams PR) or morphine (167mg IM). Scalp wounds were cauterised using artery forceps and packed with antiseptic-soaked gauze (euflavine 1:2,000, azochloramide 1:3,000) before hair around the wound was clipped or shaved. After removing the gauze, the wound was examined and bone fragments or foreign bodies under the scalp were removed if this could be done without touching the brain. For scalp wounds, healthy wound edges were closed with interrupted silk stitches and unhealthy edges were left unsutured, awaiting urgent excision. Skull fractures were packed with antiseptic gauze. If there was no bleeding after forceps were removed, the wound was left unsutured. If bleeding continued, the scalp was sutured loosely over the gauze. An external dressing and a firm bandage were applied. An antibacterial (sulfapyridine 4g PO or 1g IM, codename 'M&B 693') was given as prophylaxis against meningitis, when available.

Closed head injuries

Medical management of closed head injuries

Reduced conscious level after closed head injury was attributed to cerebral contusions. Patients with reduced conscious level were positioned on their side, secretions were suctioned, and nasogastric feeding was used to avoid aspiration. They were either laid flat or with the head lowered if suction was not available. Agitation was managed using the same medications as described above. If the conscious level remained low after 36 hours, lumbar puncture (LP) was performed. If the pressure was above 300mmH_2O, fluid was removed until the pressure was reduced below 150mmH_2O. If the pressure was above 200mmH_2O,

the head was elevated (Fowler position). Those who survived the first 48 hours usually regained consciousness.

Temporary loss of consciousness, headache, dizziness, concentration difficulty, irritability, insomnia, and 'nervous instability' after head injury were attributed to concussion. It was understood that 'the brain may be severely damaged in the absence of fracture and sometimes without any external injury of importance'.

For those with contusions or concussion, the aims of treatment were 'to provide adequate rest of body and mind for the relief of symptoms as long as this is necessary' and 'to encourage the patient, as he improves, to discover how much he can do without aggravation'. A period of physical and cognitive rest was intended to allow the most severe symptoms to abate and was followed by graduated convalescence and psychological support with the aim of returning to military service. Management of psychoneurosis was similar, involving 'explanation, firm encouragement, and occupation therapy, with more rapid grading to full activity than in the purely organic cases'. The following guidance was issued to the management of these cases:

> 'For young persons, recovery with return to duty is the rule, even if the injury has been a severe one (traumatic amnesia of several days). Many patients after simple concussion (unconsciousness only for a few moments) will be fit to return to duty after 48 hours complete rest and 48 hours convalescence. In such a case, the patient should <u>not</u> be warned to look out for further symptoms (headache, etc.).…One of the comment causes of persistent disability following head injuries under Service conditions is neurosis. It is therefore important that the patient, whether he has been unconscious or not, should be reassured and encouraged from the first.…For patients with injuries of moderate or severe degree whose symptoms have improved

rapidly under hospital treatment, a period of graduated convalescence is an essential prelude to return to duty. A patient who is symptomless on leaving hospital will often relapse if the transition to duty is too abrupt.'

Surgical management of closed head injuries

Closed head injuries were not operated on, except when a progressive deterioration in conscious level, loss of pupil reactivity, and hemiparesis signalled the possibility of extradural, subdural, or intracerebral haemorrhage. In these cases, exploration and clot evacuation via a burr hole in the temporal crest were performed. Linear or depressed skull fractures without damage to the overlying scalp were not operated on, unless the x-ray showed an in-driven fragment that was likely to have penetrated the dura.

Open head injuries

Medical management of open head injuries

Topical application of antibiotics was explored, especially for cases where surgery was going to be delayed for 12 hours or more. Antibacterial prophylaxis (sulfapyridine 1g every four hours PO or IM) was given for 48 hours to all patients at risk of intracranial infection (including those with compound fracture and base of skull fracture) and continued if there was evidence of infection. All patients with penetrating brain injuries or prolonged low conscious level (>1 hour) were prescribed preventative anti-seizure medication (phenobarbital 'luminal' 1 grain twice daily).

Surgical management of open head injuries

Excision and primary suture of the wound was performed as early as possible, preferably within 10 hours of injury, to prevent infection. The approach to anaesthesia was adapted to the conscious level and degree of agitation in each case. Morphine was used for analgesia, novocaine (1% with adrenaline) for local and regional anaesthesia, and pentothal (0.5–1.0g

intravenous) for general anaesthesia. Paraldehyde (0.3g intravenous) and inhaled nitrous oxide with ether were used for severe agitation. Skin was sterilised with antiseptic (euflavine or azochloramide) and shaved

Scalp wounds and linear skull fractures

It was recognised that scalp wounds caused by projectiles during wartime were associated with greater surrounding tissue damage and higher risk of infection than typical scalp wounds. As such, scalp shaving and exploration under general anaesthesia were encouraged. Scalp wounds were irrigated with antiseptic (euflavine or azochloramide). Dirty wound edges were excised sparingly, unless infection was already established, or the wound was completely clean. The surface of the skull was carefully examined, and linear fractures were not disturbed. Clean scalp wounds were closed with two layers of interrupted silk stitches in the aponeurosis and skin. Infected scalp wounds were loosely closed with a single layer of interrupted silk stitches and drainage via a thin strip of rubber or gutta-percha.

Compound skull fractures from blunt injury

Linear fractures with overlapping edges or compound fractures were understood to be a common source of intracranial infection. In these cases, the skin wound was enlarged, and skull fragments were elevated by means of one or more nearby burr holes made using a Hudson perforator. The extradural space was then cleared of clot and foreign bodies and the dura were examined. If the dura was intact, it was not opened except to relieve massive underlying subdural haemorrhage, before being sutured closed. If the dura was torn, foreign bodies, bone, and clot were gently removed from the surface of the brain. Dural adhesions to the brain were disturbed as little as possible. The torn dural edges were often too retracted to be closed, but, if possible, they were closed with interrupted silk stitches. Wound drains were inserted down to, but not through, the dura.

Penetrating brain injuries

Before operating, skull x-rays were used to identify the number, position, and size of metallic foreign bodies and skull fragments in the brain parenchyma. The scalp and skull were explored, cleaned, and excised as above. Scalp and skull wounds were extended to expose the full extent of the dural tear with a 1cm margin. The dural tear was not enlarged and dural adhesions were not disturbed. The missile track was gently explored using a rubber catheter. Diffluent brain tissue was removed through the catheter by suction. If foreign bodies could be reached without inflicting further brain damage, they were removed by forceps under direct vision. Careful haemostasis was performed along the missile track using wool pledglets soaked in Ringer's solution, diathermy, or Cushing's silver clips, followed by antiseptic irrigation (euflavine or azochloramide). The scalp was closed and a drain was placed, as described above. Wounds were re-dressed daily. The brain often bulged through the skull defect, in which case, LP was used to relieve the tension. CSF culture, cell counts, and protein were used to monitor the course of intracranial infection. Raised polymorphonuclear leukocytes was associated with meningitis and increased protein with cerebral abscess. Antibacterial dosage was doubled to treat infection.

Source: BIC 3, 'Memorandum on the treatment of head injuries in war', TNA FD 1/5342.

Appendix 1.6 Characteristics of the Military Hospital for Head Injuries cohort

The average age at admission was 26 years (range: 14–60 years) and more than 99% of those admitted were male (**Table A.1.1**). Most of the women admitted were members of the Women's Auxiliary Air Force. The majority were serving in junior ranks within the British or Irish armed forces (**Figure A.1.1**, **Table A.1.2**), although many nations across Europe and the Commonwealth were represented (**Figure A.1.2**, **Table A.1.3**). Compared to the constitution of the British armed forces during WWII, those serving in the army were over-represented (78% vs ~70%) and those in the navy were under-represented (2% vs ~12%) [461,462]. The reported prevalence of pre-existing headache or migraine was less common than might be expected based on current population estimates [463]. Given that the prevalence of epilepsy appears to be representative, under-representation of headache may be because diagnostic criteria for headache disorders were not standardised before 1988, rather than pre-enlistment screening or under-reporting [464,465]. One in ten cases were said to have a prior 'predisposition to mental disorder' which, although consistent with the estimated prevalence of mental health conditions today, is perhaps surprising given the evolution of public awareness and diagnosis of mental illness since 1940. Indeed, the selection criteria used in relation to this characteristic are revealing, as they included 'backwardness', 'any functional nervous illness', and a 'poor work record' [369]. This statistic is therefore a poor indicator of the extent of pre-existing mental illness in the cohort. After excluding extreme outliers (i.e. more than 3 times the IQR above the upper quartile, $n = 237$, 7.3%), median time from injury to admission was 19 days (IQR: 3–86 days, range: 0–454 days).

Table A.1.1. Baseline characteristics of indexed admissions to the MHHI.

Characteristic	
Median age at admission (IQR), years	26.0 (22.0–30.0)
Sex – n (%)	
Male	3,238 (99.3)
Female	23 (0.7)
Military force – n (%)	
Army	2,545 (78.0)
Air force	581 (17.8)
Navy	57 (1.7)
Unknown	78 (2.4)
Military rank – n (%)	
Enlisted	1,893 (58.0)
Non-commissioned officer	928 (28.3)
Commissioned officer	324 (9.9)
Unknown	116 (3.6)
Personal history – n (%)	
Epilepsy	60 (1.8)
Headache/migraine	50 (1.5)
Predisposition to mental disorder	317 (9.7)
Family history – n (%)	
Epilepsy	122 (3.7)
Headache/migraine	73 (2.2)
Predisposition to mental disorder	299 (9.2)

IQR = interquartile range.

Table A.1.2. Military ranks of indexed cases admitted to the MHHI.

Military rank (army equivalent) – n (%)	
Private	1,886 (57.8)
L/Corporal	328 (10.1)
Corporal	266 (8.2)
Sergeant	257 (7.9)
S/Sergeant	42 (1.3)
Warrant Officer	35 (1.1)
2/Lt	85 (2.6)
Lieutenant	124 (3.8)
Captain	19 (0.6)
Major	42 (1.3)
Lt/Col	4 (0.1)
Colonel	47 (1.4)
Brigadier	0 (0.0)
Maj/General	3 (0.1)
Lt/General	0 (0.0)
General	0 (0.0)
Marshal	0 (0.0)
Unknown[a]	123 (3.8)

Table A.1.3. Nationalities of the armed forces represented by indexed admissions to the MHHI.

Nationality – n (%)	
Britain and Ireland	3,129 (96.0)
Poland	17 (0.5)
Canada	16 (0.5)
Australia	11 (0.3)
New Zealand	6 (0.2)
Czech Republic	4 (0.1)
Belgium	3 (0.1)
Germany	2 (0.1)
India	2 (0.1)

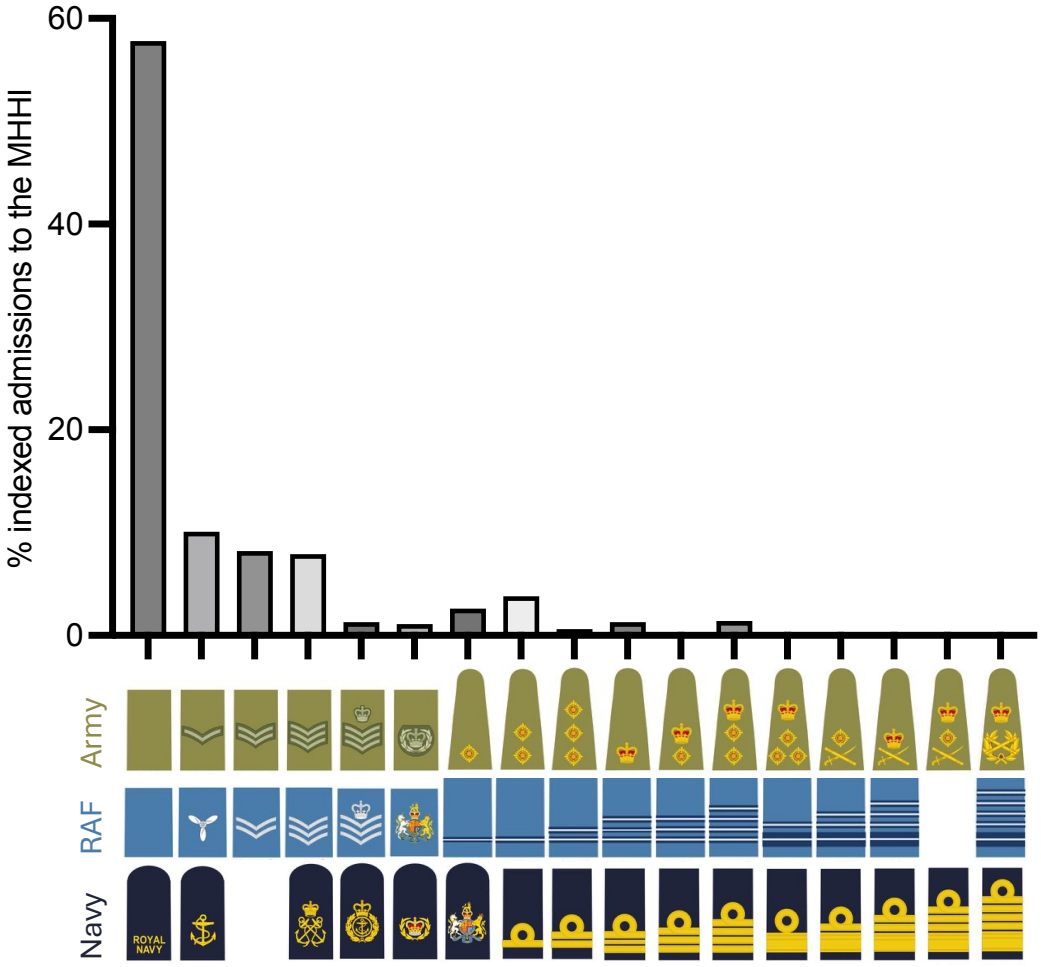


Figure A.1.1. Military ranks of indexed cases admitted to the Military Hospital for Head Injuries (MHHI). Insignia from the British Army, Royal Air Force (RAF), and Royal Navy show the following rank equivalents (left to right): Private, Lance Corporal, Corporal, Sergeant, Staff sergeant, Warrant Officer, Second Lieutenant, Lieutenant, Captain, Major, Lieutenant Colonel, Colonel, Brigadier, Major General, Lieutenant General, General, Marshall.

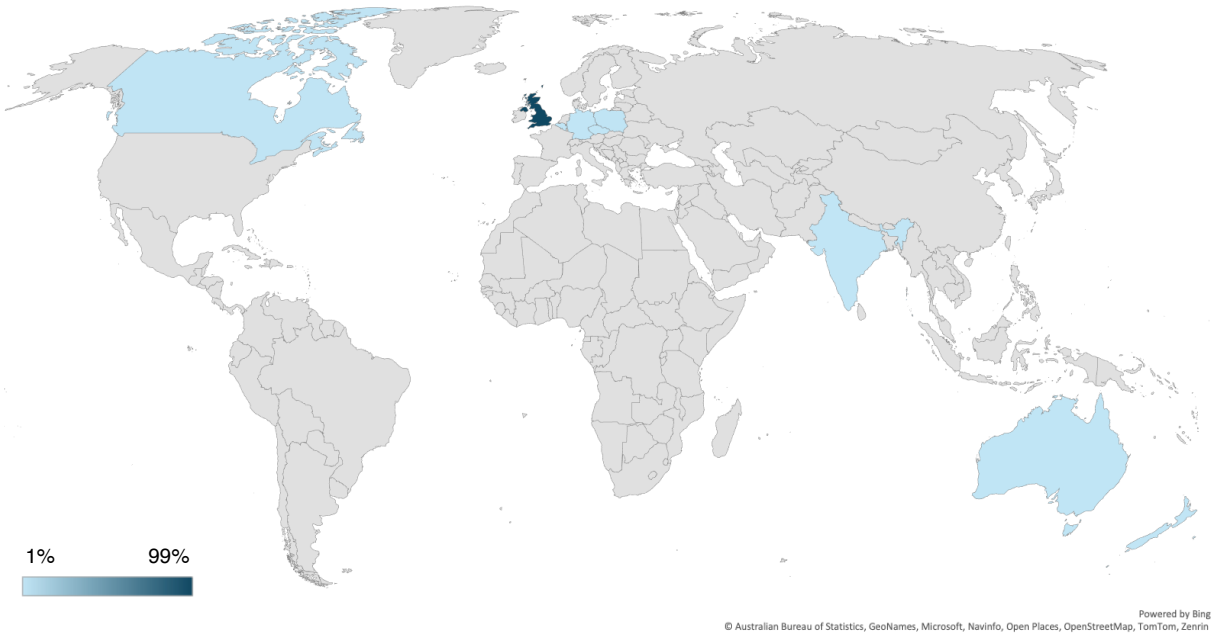


Figure A.1.2 Nationalities of the armed forces represented by indexed admissions to the Military Hospital for Head Injuries. Colours represent the proportion of admissions from the armed forces of each nation.

Appendix 1.7 Aetiology of traumatic brain injuries during WWII

The most common cause of CTBIs among both civilian and military groups during WWII was road traffic collisions, followed by falls (**Table A.1.4**) [466]. Among military personnel, there was a significant increase in the frequency of injuries caused by collisions involving motorcycles compared to the period before the war [467]. The majority of OTBI cases at the MHHI were caused by indirect fire (e.g. shell explosions), with a smaller number surviving injuries from direct fire (e.g. gunshot wounds), and much fewer sustaining low-velocity injuries (e.g. knife wounds) (**Table A.1.4**) [75].

OTBIs from indirect fire were caused by fragments of steel shrapnel weighing up to 20g or more that were propelled by the explosion of a mortar, shell, grenade, landmine, or aerial bomb (**Figure A.1.3.A–B**, **Table A.1.5**) [468,469]. While projectiles from these explosive weapons travelled at a higher initial velocity (>1,000 m/s), their irregular shape and aerodynamic inefficiency would result in a lower impact velocity than injuries from direct fire, although this would also have varied with distance from the explosion [43,34]. Injuries from indirect fire would also have involved overpressure caused by the blast wave [469]. A swine model has shown that blast injury causes prominent axonal injury in periventricular regions, in a pattern that appears to be distinct to other forms of brain injury [470].

OTBIs from direct fire were caused by bullets with a steel or lead core covered by a full metal jacket composed of copper, nickel, or zinc alloys (**Figure A.1.3.C**, **Table A.1.5**) [469]. This contrasts with bullets used by civilians, which are not required to have a full metal jacket and are therefore more likely to fragment in tissue and produce larger ‘mushroom-shaped’ wound cavities [37]. Bullets used in standard service weapons during WWII weighed between 7–12g and were either round-nose or boat-tail shaped for aerodynamic efficiency [468]. The initial projectile velocity was lower from pistols and submachine guns (300–400 m/s) than from rifles and machine guns (600–900 m/s), although impact velocity would have

varied with distance over which the weapon was used [469]. Higher impact velocities are associated with larger permanent and temporary cavities [43,34]. The extent of remote axonal injury in gunshot wounds caused by high velocity firearms used at intermediate and distant ranges in a military context is unclear but may be lower due the negligible effects of a blast wave at longer range and the differences between military and civilian ordnance.

Table A.1.4. Mechanisms of injury for admissions recorded using the early punch cards.

Mechanism of injury – n (%)	
	n=2,173
Motorcycle collision	417 (19.2)
Fall	224 (10.3)
Pedestrian collision	192 (8.8)
Car collision	151 (6.9)
Aircraft crash	150 (6.9)
Blunt impact	147 (6.8)
Shell explosion	136 (6.3)
Bullet	124 (5.7)
Bomb explosion	104 (4.8)
Bicycle collision	89 (4.1)
Lorry collision	78 (3.6)
Mortar explosion	39 (1.8)
Boxing	18 (0.8)
Football	17 (0.8)
Mine explosion	15 (0.7)
Grenade explosion	15 (0.7)
Truck collision	11 (0.5)
Horse	9 (0.4)
Other vehicle collision	6 (0.3)
Cricket	6 (0.3)
Other blast	5 (0.2)
Other sport	4 (0.2)
Rugby	4 (0.2)
Bus collision	4 (0.2)
Tank crash	4 (0.2)
Parachute injury	4 (0.2)
Stabbing	3 (0.1)
Train crash	2 (0.1)
Hockey	2 (0.1)
Van collision	2 (0.1)
Cow	1 (0.1)
Torpedo	1 (0.1)
Unspecified	189 (8.7)

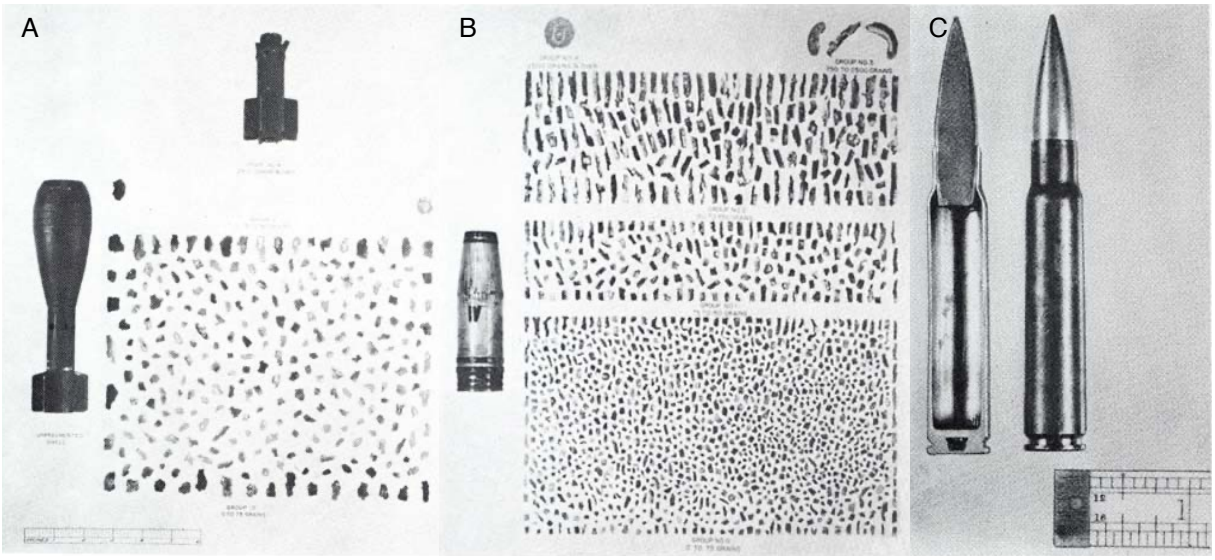


Figure A.1.3. The range of ordnance responsible for OTBIs during WWII. A. A mortar and the fragments of shrapnel that would form projectiles on its explosion. **B.** A high-explosive shell and the collection of shrapnel formed on its explosion. **C.** A rifle cartridge that would contain a bullet. Adapted from Beyer et al. (1962) [468].

Table A.1.5. Ordnance used by the German military during WWII.

Weapon	Cartridge	Projectile	Material	Shape	Weight[a] (g)	Velocity[b] (m/s)
Pistol e.g. Luger	9mm 'Parabellum'	Standard ball rounds	Lead/steel core, copper /nickel/zinc alloy jacket	Round-nose	7–8	300–400
Submachine gun e.g. MP40	9mm 'Parabellum'	Standard ball rounds	Lead/steel core, copper /nickel/zinc alloy jacket	Round-nose	7–8	350–400
Rifle e.g. K98k	8mm 'Mauser'	Spitzer with iron core	Steel core, lead/copper-zinc alloy jacket	Boat-tail	12	640–880
Machine gun e.g. MG34	8mm 'Mauser'	Spitzer with iron core	Steel core, lead/copper-zinc alloy jacket	Boat-tail	12	740–760
Anti-tank gun e.g. PaK 40	88mm explosive shell	Shrapnel	Steel casing, copper-zinc alloy band	Irregular	5	1,000–1,400
Mortar or artillery e.g. GrW 34	51–120mm explosive shell	Shrapnel	Steel casing, steel/aluminium fins	Irregular	2–3	1,200–1,800
Grenade e.g. stick grenade	n/a	Shrapnel	Steel	Irregular	2–3	900–1,000
Landmine e.g. S-mine	n/a	Shrapnel	Steel	Irregular	1–3	1,000–1,200
Explosive aerial bomb e.g. SC 250	n/a	Shrapnel	Steel	Irregular	>20	850–2,250
Fragment aerial bomb e.g. SD 2	n/a	Shrapnel	Steel	Irregular	>20	850–2,250

Source: French & Callender (1962) and Beyer et al. (1962) [468,469]. [a] Projectile weight. [b] Initial projectile velocity.

Appendix 1.8 Follow-up questionnaires

Questionnaire sent to Unit Medical Officers

HCO/A/

CONFIDENTIAL

To
Medical Officer i/c Unit

In all communications please quote serial no.

Name…… Rank……Number……Unit……

Was treated at the Military Hospital (Head Injuries) Oxford from …… to……Diagnosis:
Special features of the case:

In order that an accurate follow-up may be obtained it is requested that you will report on the patient's present condition and answer the questions below on……194.

Date of examination……

Q. Has he reported sick? If so, how often and with what symptoms? A.

Q. Has he been absent from duty on account of sickness? If so, how long? A.

Q. Has he complained of headaches? A.

Q. Has he suffered from giddiness, faints or fits? A.

Flying personnel
He has been performing flying/ground* duty efficiently/inefficiently

for……months

His present category is A* B*

Flying personnel
He has been performing light/full* duty efficiently/inefficiently

for……months

* strike out words not applicable

Remarks:

'M.O.'s name in BLOCK CAPITALS……
Station……

To be returned to: Officer Commanding,
Military Hospital (Head Injuries)
OXFORD

Source: 'HCO/A/', TNA, WO 222/845.

Questionnaire sent to individuals discharged from military service

If you are still in the Army, please state your medical category and the duties you perform. If discharged, please give reason and date of discharge.

Hospital No. H.N.
Military Hospital for Head Injuries,
Wheatley,
Nr. Oxford.

Date Sent: .....

Date Returned: .....

Dear Sir,

I am anxious to know how you are getting on Since you were discharged from this hospital on . Would you be kind enough to answer the following questions:

Do you suffer from:

(a) Headache? .....

How often? .....

+(b) Giddiness? .....

How often? .....

+(c) Faints? .....

How often? .....

+(d) Fits? .....

How often? .....

(e) Difficulty in concentration? .....

(f) Nervousness? .....

(g) Any other complaints? .....

(h) In receipt of a Pension, give amount: .....

(i) Are you at work? .....

(j) If so, since when? .....

(k) What kind of work? .....

(l) Give particulars of other work since discharge: .....

(m) Are you under the care of a doctor? .....

(n) If so, give his name and address: .....

(o) Have you attended any hospitals? .....

Which ones? .....

Give a general report on your condition and the effect, if any, which the injury has had on your health. A report from a relative, friend, or doctor would be helpful. Kindly write your report on the back of this sheet.

Date.....

Signed. W. RITCHIE RUSSELL, F.D. F.R.C.P.,
Consulting Neurologist.

Source: HHA/1/4.

Appendix to Chapter 2: Epidemiology

Appendix 2.1 Outcome variables

Table A.2.1. Outcome variable definitions.

Variable	Standard terms recorded on punch cards
Seizures	Seizures, fits, epilepsy
Headache	Headache, migraine
Giddiness or faints	Giddiness, faints
Motor weakness	Arm/leg weakness
Somatosensory disturbance	Arm/leg numbness, paraesthesia, tingling
Visual impairment	Blindness, visual field, diplopia
Auditory impairment	Deafness, tinnitus
Sleep disturbance	Insomnia
Dysphasia	Dysphasia
Memory impairment	Memory
Intellectual impairment	Intelligence
Concentration impairment	Concentration
Anxiety	Anxiety, nervousness
Depression	Depression, low mood
Personality change	Irritable, impulsive, aggressive, disinhibited, temper
Disability	Disablement (%)

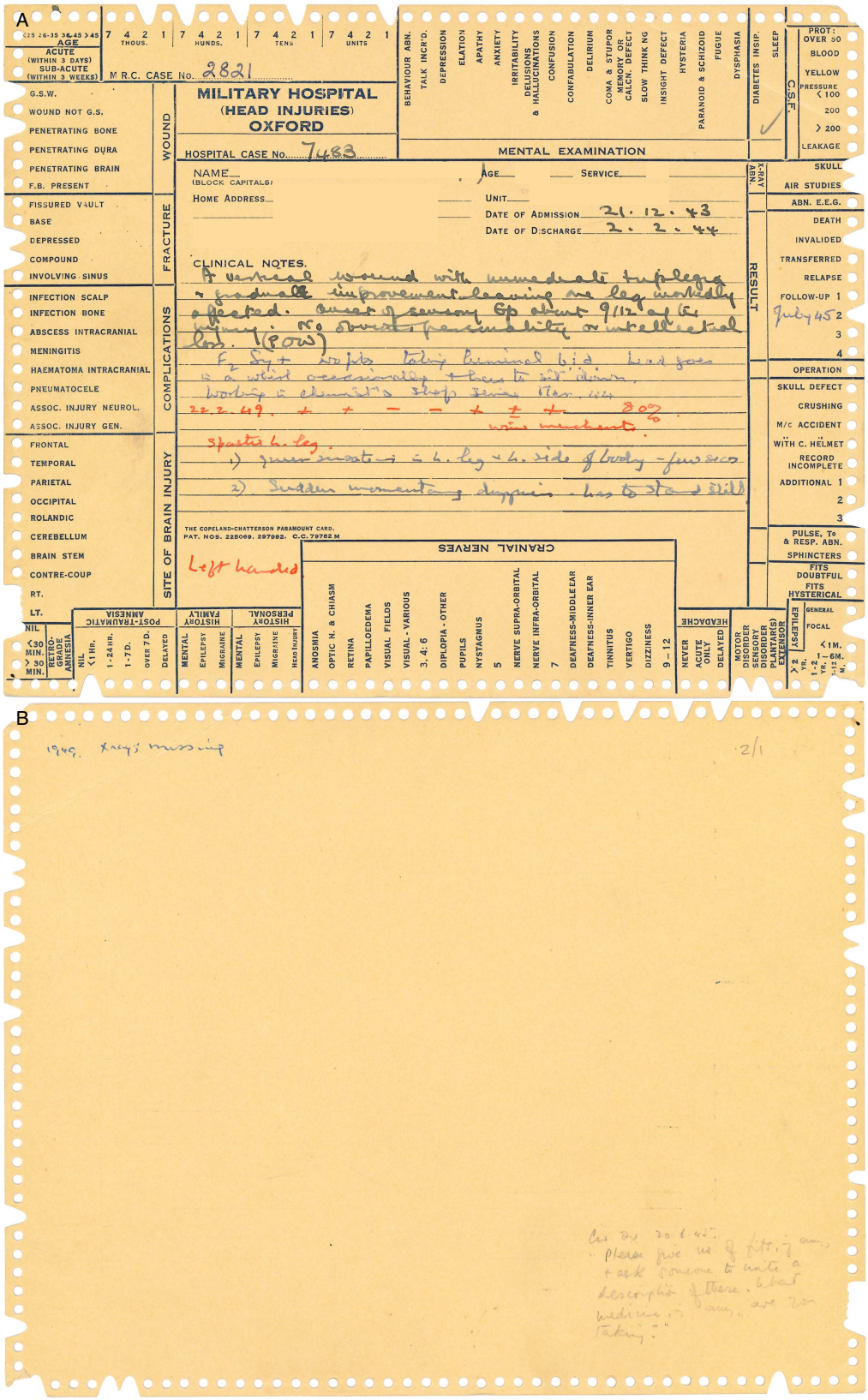


Figure A.2.1. Early version of the punch cards.

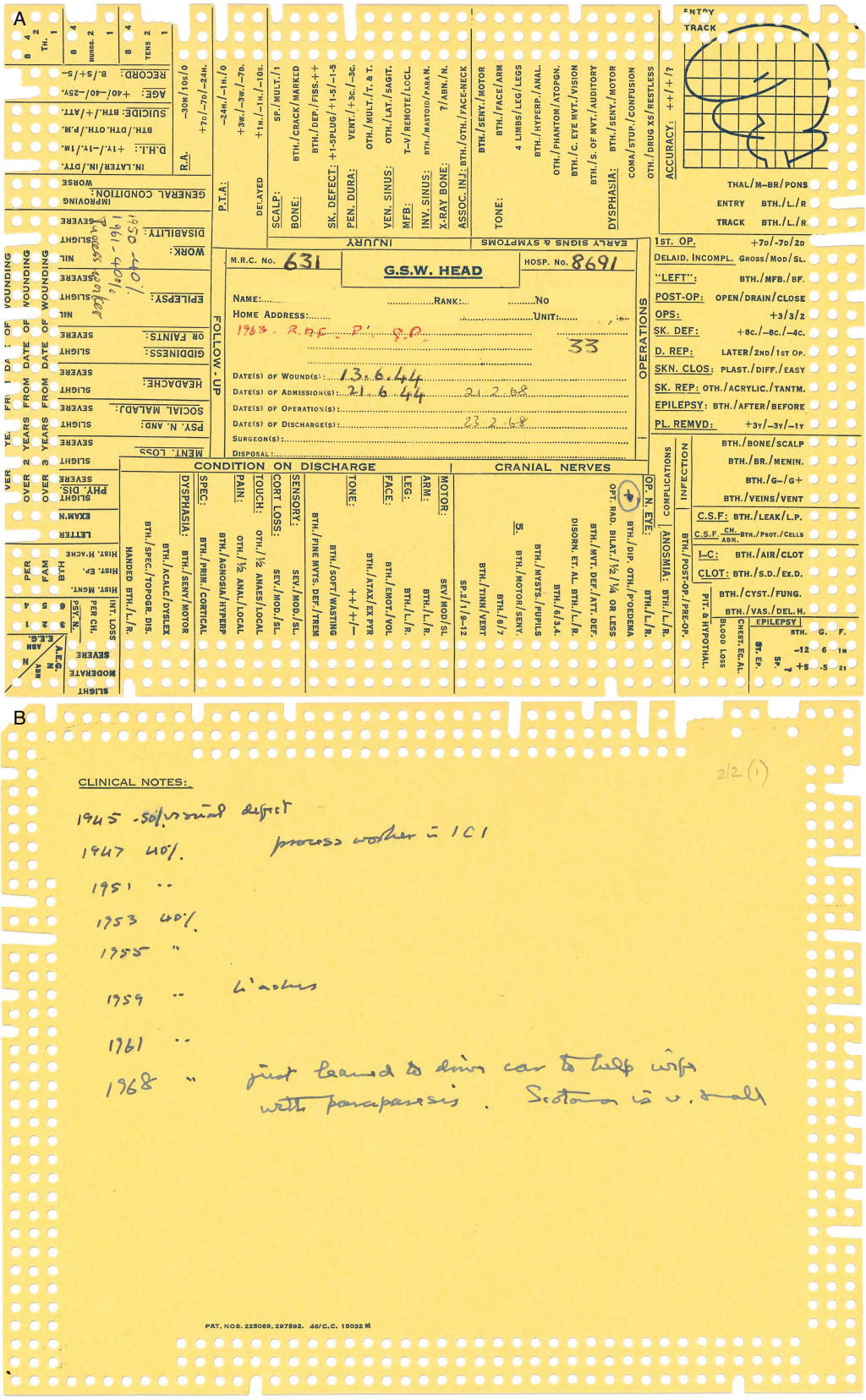


Figure A.2.2. Later version of the punch cards.

Appendix 2.3 Data merging

Table A.2.2. Data merging from labels used in two versions of the punch cards.

Category	Variable	Merge?	'MILITARY HOSPITAL' term	'G.S.W. HEAD' term
IDs	MRC number	Yes	M.R.C CASE NO.	M.R.C. NO.
	Hospital number	Yes	HOSPITAL CASE NO.	HOSP. NO.
Demographics	Age at admission (years)	Yes	CLINICAL NOTES	AGE
	Sex	Yes	CLINICAL NOTES	UNIT
History	Personal history of epilepsy	Yes	HISTORY PERSONAL	HIST. EP.
	Family history of epilepsy	Yes	HISTORY FAMILY	HIST. EP.
	Personal history of headache	Yes	HISTORY PERSONAL	HIST. H'ACHE.
	Family history of headache	Yes	HISTORY FAMILY	HIST. H'ACHE.
Military details	Military rank	Yes	CLINICAL NOTES	RANK
	Military division	Yes	CLINICAL NOTES	UNIT
Lesion	Lesion depth	No		PEN. DURA
Injury severity	Associated injuries	Yes	ASSOC. INJURY. NEUROL. ASSOC. INJURY. GEN.	ASSOC. INJ.
	Conscious level	Yes	COMA & STUPOR	COMA / STUP. / CONFUSION
	PTA duration	Yes	POST-TRAUMATIC AMNESIA	P.T.A.
	RA duration	Yes	RETROGRADE AMNESIA	R.A.
Early signs	Dysphasia	Yes	DYSPHASIA	DYSPHASIA
	Sensorimotor impairment	Yes	MOTOR DISORDER SENSORY DISORDER	BTH. / SENY. / MOTOR
	Visual impairment	Yes	CRANIAL NERVES: OPTIC N. & CHIASM RETINA PAPILLOEDEMA VISUAL FIELDS VISUAL – VARIOUS 3, 4, 6 DIPLOPIA – OTHER PUPILS	CRANIAL NERVES: OP. N. EYE: BTH . / L. / R. BTH. / DIP. OTH. / P'OEDEMA OPT. RAD. BILAT. / ½ / ¼ LESS BTH. / 6 / 3,4
	Auditory impairment	Yes	CRANIAL NERVES: DEAFNESS – MIDDLE EAR DEAFNESS – INNER EAR TINNITUS	CRANIAL NERVES: BTH. / 8 BTH. / TINN
	Vestibular impairment	Yes	CRANIAL NERVES: VERTIGO	CRANIAL NERVES: BTH. / VERT
Investigations	EEG abnormal	Yes	ABN. E.E.G.	E.E.G.: ABN.
	CSF protein >50g/dL	Yes	CSF PROT. >50	C.S.F CH. / ABN. PROT.
	CSF cells >2cells/µL	No		C.S.F CH. / ABN. CELLS
Complications	Infection	Yes	ABSCESS INTRACRANIAL MENINGITIS	INFECTION BTH. / BR. / MENIN.
	Retained MFB	No		"LEFT"
	Retained BF	No		"LEFT"
	ICH	Yes	HAEMATOMA INTRACRANIAL	CLOT
	Neuroendocrine dysfunction	Yes	DIABETES INSIP.	PIT. & HYPOTHAL.
Discharge	Invalided	Yes	INVALIDED	IN. LATER / IN. / DTY.
Time intervals	Injury to admission	Yes	CLINICAL NOTES DATE OF ADMISSION	DATE(S) OF WOUND(S) DATE(S) OF ADMISSION(S)
	Length of stay	Yes	DATE OF ADMISSION DATE OF DISCHARGE	DATE(S) OF ADMISSION(S) DATE(S) OF DISCHARGE(S)
	Follow-up duration	Yes	CLINICAL NOTES RESULT	DATE(S) OF WOUND(S) FOLLOW-UP

Appendix 2.4 Data categorisation

Table A.2.3. Data categorisation from ICD-9 codes.

ICD-9 term	ICD-9 code	COD grouping
Chapter II: neoplasms	140–239	
Malignant neoplasm of other and unspecific sites	190–199	
Malignant neoplasms of the brain	191	CNS neoplasia
Malignant neoplasm of other and unspecified parts of nervous system	192	CNS neoplasia
Benign neoplasms	210–229	
Benign neoplasm of brain and other parts of nervous system	225	CNS neoplasia
Benign neoplasm of pituitary	227.3	CNS neoplasia
Benign neoplasm of pineal gland	227.4	CNS neoplasia
Chapter V: mental disorders	290–319	
Psychosis	290–299	
Senile and presenile organic psychotic conditions	290	Neurodegenerative
Other organic psychotic conditions	294	Psychiatric
Schizophrenic psychoses	295	Psychiatric
Affective psychoses	296	Psychiatric
Neurotic disorders, personality disorders, and other mental disorders	300–316	
Neurotic disorders	300	Psychiatric
Specific nonpsychotic mental disorders following organic brain damage	310	Psychiatric
Depressive disorder, not elsewhere classified	311	Psychiatric
Chapter VI: diseases of the nervous system and sense organs	320–389	
Inflammatory diseases of the central nervous system	320–326	
Bacterial meningitis	320	CNS infection
Meningitis due to other organisms	321	CNS infection
Meningitis, NOS	322	CNS infection
Encephalitis, myelitis, and encephalomyelitis	323	CNS infection
Intracranial and intraspinal abscess	324	CNS infection
Phlebitis and thrombophlebitis of intracranial venous sinuses	325	CNS infection
Late effects of intracranial abscess or pyogenic infection	326	CNS infection
Hereditary and Degenerative diseases of the CNS	330–337	
Other cerebral degenerations (including Alzheimer’s disease)	331	Neurodegenerative
Parkinson's disease	332	Neurodegenerative
Other extrapyramidal disease and abnormal movement disorders	333	Neurodegenerative
Spinocerebellar disease	334	Other
Anterior horn cell disease (including motor neuron disease)	335	Neurodegenerative
Disorders of the autonomic nervous system	337	Autonomic
Other disorders of the central nervous system	340–349	
Multiple sclerosis	340	Demyelinating
Other demyelinating diseases of central nervous system	341	Demyelinating
Hemiplegia	342	Other
Other paralytic syndromes	344	Other
Epilepsy	345	Epilepsy
Migraine	346	Other
Cataplexy and narcolepsy	347	Other
Other conditions of brain	348	Other
Other and unspecified disorders of the nervous system	349	Other
Chapter VII: diseases of the circulatory system	390–459	
Cerebrovascular disease	430–438	
Subarachnoid haemorrhage	430	Cerebrovascular
Intracerebral haemorrhage	431	Cerebrovascular
Other and unspecified intracranial haemorrhage	432	Cerebrovascular
Occlusion and stenosis of precerebral arteries	433	Cerebrovascular
Occlusion of cerebral arteries	434	Cerebrovascular
Transient cerebral ischemia	435	Cerebrovascular

ICD-9 term	ICD-9 code	COD grouping
Acute but ill-defined cerebrovascular disease	436	Cerebrovascular
Other and ill-defined cerebrovascular disease	437	Cerebrovascular
Late effects of cerebrovascular disease	438	Cerebrovascular

Appendix 2.5 Baseline characteristic comparisons for morbidity outcomes

Table A.2.4. Baseline characteristic comparisons according to injury-admission interval.

Characteristic	Injury–admission <3 months	Injury–admission ≥3 months/unknown	χ^2	Mann-Whitney U	p-value
Age – median (IQR)	n=1,957	n=1,056			
Age at admission	25.0 (21.0–30.0)	26.0 (23.0–31.0)	n/a	9.328×10^5	<0.001
Personal history – n (%)	n=1,957	n=1,056			
Epilepsy	24 (1.2)	35 (3.3)	15.576	n/a	<0.001
Headache/migraine	29 (1.5)	20 (1.9)	0.728	n/a	0.394
Family history – n (%)	n=1,957	n=1,056			
Epilepsy	42 (2.1)	76 (7.2)	46.500	n/a	<0.001
Headache/migraine	29 (1.5)	43 (4.1)	19.727	n/a	<0.001
Military rank – n (%)	n=1,944	n=1,043	2.168	n/a	0.338
Enlisted	1,151 (59.2)	646 (61.9)			
Non-commissioned officer	584 (30.0)	295 (28.3)			
Commissioned officer	209 (10.8)	102 (9.8)			
Military division – n (%)	n=1,935	n=1,051	4.593	n/a	0.101
Army	1,523 (78.7)	849 (80.8)			
Air force	382 (19.7)	179 (17.0)			
Navy	30 (1.6)	23 (2.2)			

Table A.2.5. Baseline characteristic comparisons according to follow-up.

Characteristic	Follow-up	No follow-up	χ^2	Mann-Whitney U	p-value
Age – median (IQR)	n=1,568	n=389			
Age at admission	25.0 (21.0–30.0)	26.0 (22.0–31.0)	n/a	2.947×10^5	0.301
Personal history – n (%)	n=1,568	n=389			
Epilepsy	18 (1.1)	6 (1.5)	0.400	n/a	0.527
Headache/migraine	23 (1.5)	6 (1.5)	0.012	n/a	0.912
Family history – n (%)	n=1,568	n=389			
Epilepsy	38 (2.4)	4 (1.0)	2.889	n/a	0.089
Headache/migraine	23 (1.5)	6 (1.5)	0.012	n/a	0.912
Military rank – n (%)	n=1,537	n=379	0.889	n/a	0.641
Enlisted	229 (60.4)	903 (58.8)			
Non-commissioned officer	114 (30.1)	463 (30.1)			
Commissioned officer	36 (9.5)	171 (11.1)			
Military division – n (%)	n=1,554	n=381	4.434	n/a	0.109
Army	1,220 (78.5)	303 (79.5)			
Air force	314 (20.2)	68 (17.8)			
Navy	20 (1.3)	10 (2.6)			

Appendix 2.6 Supplementary analyses for predictors of seizures

Table A.2.6. Univariable logistic regression analyses to identify predictors of seizures.

Covariate	x/n	β	SE	Wald χ^2	p-value	OR	95% CI
Age							
Age at admission	n=552	0.017	0.017	1.104	0.298	1.018	0.985–1.051
History							
Personal history of epilepsy	1/558	21.934	4.019×10^4	0.000	1.000	3.356×10^9	n/a
Family history of epilepsy	3/558	0.033	1.228	0.001	0.979	1.033	0.093–11.469
Military rank							
Enlisted	347/552	ref	ref	1.156	0.561	ref	ref
Non-commissioned officer	152/552	0.140	0.207	0.462	0.497	1.151	0.768–1.725
Commissioned officer	53/552	0.294	0.306	0.919	0.338	1.341	0.736–2.444
Military division							
Army	518/546	ref	ref	3.028	0.220	ref	ref
Air force	20/546	0.769	0.457	2.834	0.092	2.159	0.881–5.287
Navy	8/546	-0.329	0.822	0.160	0.689	0.720	0.144–3.603
Lesion							
Depth >3cm	471/540	-0.370	0.264	1.956	0.162	0.691	0.412–1.160
Post-traumatic amnesia							
Nil	88/276	ref	ref	11.413	0.010	ref	ref
Less than 1 day	104/276	0.433	0.336	1.660	0.198	1.542	0.798–2.981
1–7 days	46/276	0.463	0.412	1.262	0.261	1.589	0.708–3.563
More than 7 days	38/276	1.395	0.416	11.270	<0.001	4.035	1.787–9.111
Early signs and symptoms							
Dysphasia	181/558	0.615	0.189	10.579	0.001	1.850	1.277–2.681
Sensorimotor impairment	308/558	0.660	0.187	12.423	<0.001	1.935	1.341–2.794
Visual impairment	255/558	0.060	0.181	0.110	0.740	1.062	0.745–1.514
Auditory impairment	36/558	-0.245	0.384	0.408	0.523	0.783	0.369–1.660
Vestibular impairment	4/558	-20.488	2.010×10^4	0.000	0.999	0.000	n/a
Investigations							
EEG abnormal	208/558	0.350	0.185	3.585	0.058	1.419	0.988–2.039
CSF protein >50g/dL	24/558	0.034	0.443	0.006	0.939	1.034	0.434–2.464
CSF >2 WBCs/µL	14/546	0.125	0.565	0.049	0.825	1.133	0.374–3.432
Complications							
Infection	518/558	0.115	0.345	0.111	0.739	1.112	0.571–2.204
Retained MFB	142/546	0.303	0.204	2.217	0.137	1.354	0.909–2.017
Retained BF	69/546	0.093	0.271	0.118	0.731	1.097	0.645–1.866
Intracranial haematoma	16/546	-0.081	0.547	0.022	0.822	0.922	0.315–2.695
Neuroendocrine dysfunction	3/558	1.427	1.228	1.350	0.245	4.167	0.375–46.254
Discharge status							
Invalided	435/558	1.049	0.259	16.346	<0.001	2.854	1.717–4.745
Time intervals							
Injury to admission	n=558	0.011	0.006	3.459	0.063	1.011	0.999–1.022
Length of stay	n=466	0.001	0.001	0.784	0.376	1.001	0.999–1.003
Follow-up duration	n=558	0.000	0.000	0.789	0.374	1.000	1.000–1.000

WBCs = white blood cells.

Table A.2.7. Multivariable logistic regression analyses to identify predictors of seizures.

Covariate	x/n	β	SE	Wald χ^2	p-value	Adjusted OR	95% CI
Age							
Age at admission	n=258	0.031	0.027	1.353	0.245	1.032	0.979–1.088
Lesion							
Depth >3cm	230/258	-0.529	0.461	1.316	0.251	0.589	0.238–1.455
Post-traumatic amnesia							
Nil	87/258	ref	ref	ref	ref	1.000	ref
<1 day	95/258	0.521	0.356	2.140	0.143	1.683	0.838–3.383
1–7 days	40/258	0.514	0.450	1.301	0.254	1.671	0.691–4.040
>7 days	36/258	1.012	0.464	4.764	0.029	2.751	1.109–6.824
Early signs and symptoms							
Dysphasia	77/258	0.029	0.324	0.008	0.928	1.030	0.546–1.941
Sensorimotor impairment	145/228	0.306	0.295	1.075	0.300	1.358	0.762–2.421
Visual impairment	109/258	0.054	0.299	0.033	0.856	1.056	0.588–1.895
Complications							
Retained MFB	67/258	0.184	0.319	0.333	0.564	1.202	0.644–2.244
Retained BF	35/258	0.225	0.405	0.309	0.578	1.253	0.566–2.722
Intracranial haematoma	8/258	0.708	0.793	0.798	0.372	2.031	0.429–9.611
Discharge status							
Invalided	197/258	0.669	0.386	3.011	0.083	1.953	0.917–4.158

Adjusted for age at admission and discharge status.

Table A.2.8. Sensitivity multivariable logistic regression analyses to identify predictors of seizures.

Covariate	x/n	β	SE	Wald χ^2	p-value	Adjusted OR	95% CI
Age							
Age at admission	n=258	0.031	0.027	1.286	0.257	1.031	0.978–1.088
Lesion							
Depth >3cm	230/258	-0.503	0.446	1.270	0.260	0.605	0.252–1.450
Post-traumatic amnesia							
Nil	87/258	ref	ref	ref	ref	1.000	ref
<1 day	95/258	0.515	0.358	2.073	0.150	1.674	0.830–3.375
1–7 days	40/258	0.474	0.450	1.108	0.293	1.606	0.665–3.881
>7 days	36/258	0.951	0.451	4.454	0.035	2.588	1.070–6.259
Early signs and symptoms							
Dysphasia, sensorimotor, or visual impairment	208/258	0.925	0.447	4.277	0.039	2.521	1.050–6.056
Complications							
Retained MFB	67/258	0.144	0.321	0.202	0.653	1.155	0.616–2.166
Retained BF	35/258	0.219	0.405	0.291	0.589	1.244	0.562–2.753
Intracranial haematoma	8/258	0.724	0.800	0.818	0.366	2.062	0.430–9.890
Discharge status							
Invalided	197/258	0.651	0.386	2.846	0.092	1.917	0.900–4.082

Adjusted for age at admission and discharge status.

Appendix 2.7 Supplementary analyses for predictors of headache

Table A.2.9. Univariable logistic regression analyses to identify predictors of headache.

Covariate	x/n	β	SE	Wald χ^2	p-value	OR	95% CI
Age							
Age at admission	n=552	-0.003	0.023	0.019	0.891	0.997	0.953–1.043
History							
Personal history of headache	2/558	-19.360	28420.722	0.000	0.999	0.000	0.000–0.000
Family history of headache	2/558	-19.360	28420.722	0.000	0.999	0.000	0.000–0.000
Military division							
Army	518/546	ref	ref	5.367	0.068	ref	ref
Air force	20/546	1.231	0.481	6.540	0.011	3.424	1.333–8.795
Navy	8/546	0.825	0.827	0.996	0.318	2.283	0.451–11.547
Military rank							
Enlisted	347/552	ref	ref	0.817	0.665	ref	ref
Non-commissioned officer	152/552	-0.226	0.294	0.590	0.442	0.798	0.449–1.419
Commissioned officer	53/552	-0.277	0.460	0.362	0.547	0.758	0.308–1.867
Lesion							
Depth >3cm	471/540	0.126	0.400	0.099	0.753	1.134	0.518–2.486
Post-traumatic amnesia							
Nil	88/276	ref	ref	1.607	0.658	ref	ref
Less than 1 day	104/276	-0.364	0.410	0.785	0.376	0.695	0.311–1.553
1–7 days	46/276	0.169	0.467	0.130	0.718	1.184	0.474–2.959
More than 7 days	38/276	0.094	0.505	0.035	0.852	1.099	0.408–2.959
Early signs and symptoms							
Dysphasia	181/558	-0.582	0.292	3.986	0.046	0.559	0.315–0.989
Sensorimotor impairment	308/558	-0.549	0.249	4.855	0.028	0.578	0.355–0.941
Visual impairment	255/558	-0.760	0.266	8.176	0.004	0.468	0.278–0.787
Auditory impairment	36/558	0.460	0.440	1.092	0.296	1.585	0.668–3.758
Vestibular impairment	4/558	0.756	1.161	0.423	0.515	2.129	0.219–20.735
Investigations							
EEG abnormal	208/558	0.171	0.252	0.464	0.496	1.187	0.725–1.945
CSF protein >50g/dL	24/558	-0.103	0.630	0.027	0.870	0.902	0.262–3.101
CSF >2 WBCs/µL	14/546	0.146	0.775	0.035	0.851	1.157	0.253–5.282
Complications							
Infection	518/558	-0.705	0.613	1.320	0.251	0.494	0.149–1.645
Retained metallic foreign body	142/546	-0.267	0.310	0.744	0.388	0.766	0.417–1.405
Retained bone fragment	69/546	-0.469	0.448	1.094	0.296	0.626	0.260–1.506
Intracranial haemorrhage	16/546	-0.081	0.547	0.022	0.882	0.922	0.315–2.695
Neuroendocrine dysfunction	3/558	-19.362	23205.422	0.000	0.999	0.000	n/a
Discharge status							
Invalided	435/558	-0.580	0.271	4.566	0.033	0.560	0.329–0.953
Time intervals							
Injury to admission	n=558	0.006	0.008	0.645	0.422	1.006	0.991–1.021
Length of stay	n=466	-0.003	0.002	2.589	0.108	0.997	0.993–1.001
Follow-up duration	n=558	0.000	0.000	1.618	0.203	1.000	1.000–1.000

Table A.2.10. Exploration of potential predictors, modifiers, and confounders of headache.

Covariate	Pre-specified predictor	χ^2	Mann-Whitney U	Kruskal-Wallis H	p-value
Military division	PTA	15.362	n/a	n/a	0.081
Dysphasia	PTA	22.139	n/a	n/a	<0.001
Sensorimotor impairment	PTA	5.470	n/a	n/a	0.140
Visual impairment	PTA	4.291	n/a	n/a	0.232

Table A.2.11. Multivariable logistic regression analysis to identify predictors of headache.

Covariate	x/n	β	SE	Wald χ^2	p-value	Adjusted OR	95% CI
Age							
Age at admission	n=273	-0.008	0.034	0.050	0.823	0.992	0.929–1.060
Military division							
Army	259/273	ref	ref	ref	ref	1.000	ref
Air force	11/273	1.549	0.693	4.996	0.025	4.709	1.121–18.324
Navy	3/273	0.552	1.288	0.184	0.668	1.737	0.139–21.687
Post-traumatic amnesia							
Nil	88/273	ref	ref	ref	ref	1.000	ref
<24 hours	102/273	-0.234	0.427	0.300	0.584	0.791	0.342–1.829
>24 hours	45/273	0.277	0.505	0.300	0.584	1.319	0.490–3.549
>7 days	38/273	0.445	0.595	0.560	0.454	1.561	0.486–5.011
Early signs and symptoms							
Dysphasia	79/273	-0.959	0.482	3.957	0.047	0.383	0.149–0.986
Sensorimotor impairment	152/273	-0.390	0.355	1.208	0.272	0.677	0.338–1.357
Visual impairment	111/273	-0.482	0.379	1.616	0.204	0.617	0.294–1.299
Discharge status							
Invalided	203/273	-0.273	0.392	0.485	0.486	0.761	0.353–1.640

Adjusted for age and discharge status.

Appendix 2.8 Supplementary analyses for predictors of disability

Table A.2.12. Univariable linear regression analyses to identify predictors of disability.

Covariate	x/n	B	SE	95% CI	Wald χ^2	p-value
Age						
Age at admission	n=531	0.007	0.002	0.003 to 0.012	9.857	0.002
History						
Personal history of headache	2/531	0.083	0.202	-0.314 to 0.479	0.166	0.684
Family history of headache	0/531	n/a	n/a	n/a	n/a	n/a
Personal history of epilepsy	1/531	0.032	0.286	-0.529 to 0.593	0.013	0.910
Family history of epilepsy	2/531	0.116	0.203	-0.281 to 0.513	0.328	0.567
Military division						
Army	499/520	ref	ref	ref	ref	ref
Air force	13/520	-0.037	0.080	-0.194 to 0.121	0.210	0.647
Navy	8/520	-0.185	0.129	-0.437 to 0.067	2.071	0.150
Military rank						
Enlisted	334/525	ref	ref	ref	ref	ref
Non-commissioned officer	143/525	0.005	0.029	-0.051 to 0.061	0.031	0.861
Commissioned officer	48/525	-0.091	0.016	-0.177 to -0.005	1862.609	<0.001
Lesion						
Depth >3cm	457/526	-0.139	0.034	-0.210 to -0.068	542.939	<0.001
Post-traumatic amnesia						
Nil	82/254	ref	ref	ref	ref	ref
Less than 1 day	97/254	0.044	0.042	-0.037 to 0.126	1.127	0.288
1–7 days	41/254	0.077	0.053	-0.027 to 0.182	2.121	0.145
More than 7 days	34/254	0.261	0.057	0.150 to 0.372	21.197	<0.001
Early signs and symptoms						
Dysphasia	173/531	0.121	0.026	0.070 to 0.172	21.766	<0.001
Sensorimotor impairment	300/531	0.116	0.025	0.068 to 0.164	22.349	<0.001
Visual impairment	250/531	0.151	0.024	0.104 to 0.198	39.928	<0.001
Auditory impairment	34/531	-0.049	0.051	-0.148 to 0.050	0.934	0.334
Vestibular impairment	3/531	-0.035	0.166	-0.359 to 0.290	0.044	0.834
Investigations						
EEG abnormal	208/558	0.038	0.026	-0.013 to 0.088	2.129	0.145
CSF protein >50g/dL	24/558	0.094	0.061	-0.025 to 0.213	2.404	0.121
CSF >2 WBCs/µL	14/546	0.133	0.077	-0.018 to 0.285	2.983	0.084
Complications						
Infection	40/531	0.112	0.047	0.020 to 0.204	5.729	0.017
Retained MFB	141/531	0.122	0.028	0.068 to 0.176	19.497	<0.001
Retained BF	68/531	0.076	0.037	0.003 to 0.148	4.197	0.041
Intracranial haematoma	16/531	-0.083	0.073	-0.225 to 0.059	1.304	0.254
Neuroendocrine dysfunction	2/531	0.333	0.202	-0.062 to 0.729	2.725	0.099
Discharge status						
Invalided	422/531	0.331	0.027	0.278 to 0.384	148.861	<0.001
Time intervals						
Injury to admission	n=531	0.001	0.001	-0.001 to 0.003	1.041	0.308
Length of stay	n=440	0.001	<0.001	0.001 to 0.001	38.967	<0.001
Follow-up duration	n=531	-1.524×10^{-5}	7.984×10^{-6}	-3.089×10^{-5} to 4.087×10^{-7}	3.643	0.056

Table A.2.13. Exploration of potential predictors, modifiers, and confounders of disability.

Covariate	Pre-specified predictor	χ^2	Mann-Whitney U	Kruskal-Wallis H	p-value
Military rank	Depth	0.441	n/a	n/a	0.802
	PTA	4.696	n/a	n/a	0.583
	Dysphasia	1.252	n/a	n/a	0.535
	Sensorimotor impairment	3.031	n/a	n/a	0.220
	Visual impairment	1.228	n/a	n/a	0.541
Infection	Depth	2.022	n/a	n/a	0.155
	PTA	5.967	n/a	n/a	0.113
	Dysphasia	0.129	n/a	n/a	0.719
	Sensorimotor impairment	0.197	n/a	n/a	0.657
	Visual impairment	1.502	n/a	n/a	0.220
Retained MFB	Depth	17.228	n/a	n/a	<0.001
	PTA	7.057	n/a	n/a	0.133
	Dysphasia	1.159	n/a	n/a	0.282
	Sensorimotor impairment	3.615	n/a	n/a	0.057
	Visual impairment	3.781	n/a	n/a	0.052
Retained BF	Depth	9.984	n/a	n/a	0.002
	PTA	3.837	n/a	n/a	0.280
	Dysphasia	0.042	n/a	n/a	0.838
	Sensorimotor impairment	0.014	n/a	n/a	0.906
	Visual impairment	0.640	n/a	n/a	0.424
Invalided	Depth	7.322	n/a	n/a	0.007
	PTA	11.015	n/a	n/a	0.012
	Dysphasia	12.027	n/a	n/a	<0.001
	Sensorimotor impairment	9.353	n/a	n/a	0.002
	Visual impairment	11.043	n/a	n/a	<0.001
Length of stay	Depth	n/a	8684.000	n/a	0.007
	PTA	n/a	n/a	17.716	<0.001
	Dysphasia	n/a	21282.500	n/a	0.198
	Sensorimotor impairment	n/a	22746.500	n/a	0.004
	Visual impairment	n/a	22479.000	n/a	0.002

Table A.2.14. Multivariable linear regression analysis to identify predictors of disability.

Covariate	x/n	B	SE	95% CI	Wald χ^2	p-value
Age						
Age at admission	n=213	0.013	0.003	0.007–0.019	18.805	0.000
Military rank						
Enlisted	137/213	0.000	ref	ref	ref	ref
Non-commissioned officer	58/213	-0.013	0.036	-0.084–0.058	0.127	0.721
Commissioned officer	18/213	-0.109	0.061	-0.229–0.011	3.158	0.076
Lesion depth						
>3cm	190/213	-0.019	0.054	-0.125–0.086	0.126	0.723
Post-traumatic amnesia						
Nil	75/213	0.000	ref	ref	ref	ref
<1 day	82/213	0.050	0.036	-0.02–0.121	1.962	0.161
1–7 days	34/213	0.009	0.048	-0.084–0.103	0.036	0.850
>7 days	22/213	0.059	0.060	-0.059–0.177	0.953	0.329
Early signs and symptoms						
Dysphasia	56/213	0.009	0.037	-0.063–0.082	0.064	0.801
Sensorimotor impairment	120/213	0.073	0.032	0.01–0.136	5.206	0.023
Visual impairment	89/213	0.096	0.033	0.032–0.161	8.552	0.003
Complications						
Infection	12/213	0.124	0.069	-0.011–0.259	3.241	0.072
Retained MFB	56/213	0.022	0.036	-0.049–0.094	0.375	0.540
Retained BF	25/213	0.093	0.050	-0.005–0.19	3.450	0.063
Discharge status						
Discharged from military	164/213	0.263	0.040	0.184–0.342	42.393	<0.001
Time						
Length of stay	n=213	0.000	0.000	0–0.001	7.615	0.006

Adjusted for age at admission.

Appendix 2.9 Supplementary analyses for associations with disability

Table A.2.15. Univariable linear regression analyses to explore associations with disability.

Covariate	x/n	B	SE	95% CI	Wald χ^2	p-value
Neurological						
Seizures	175/531	0.190	0.025	0.141 to 0.239	57.517	<0.001
Headache	65/531	-0.161	0.037	-0.233 to -0.088	18.622	<0.001
Giddiness/faints	50/531	-0.065	0.042	-0.148 to 0.018	2.343	0.126
Sensorimotor impairment	79/531	0.091	0.035	0.023 to 0.159	6.926	0.008
Visual impairment	66/531	0.124	0.037	0.052 to 0.197	11.187	<0.001
Auditory impairment	10/531	-0.022	0.091	-0.200 to 0.056	0.056	0.814
Sleep disturbance	16/531	-0.038	0.073	-0.180 to 0.105	0.269	0.604
Cognitive						
Dysphasia	70/531	0.143	0.036	0.072 to 0.214	15.620	<0.001
Memory impairment	59/531	0.016	0.040	-0.062 to 0.093	0.155	0.694
Intellectual impairment	12/531	0.134	0.083	-0.029 to 0.297	2.584	0.108
Concentration impairment	60/531	-0.065	0.039	-0.141 to 0.012	2.755	0.097
Psychiatric						
Anxiety	21/531	-0.030	0.064	-0.155 to 0.095	0.226	0.635
Depression	30/531	-0.023	0.054	-0.128 to 0.082	0.183	0.669
Personality change	59/531	0.012	0.040	-0.066 to 0.089	0.089	0.765

Table A.2.16. Multivariable linear regression analyses to explore associations with disability.

Covariate	x/n	B	SE	95% CI	Wald χ^2	p-value
Neurological						
Seizures	175/531	0.163	0.025	0.115 to 0.212	43.616	<0.001
Headache	65/531	-0.104	0.036	-0.175 to -0.033	8.326	0.004
Giddiness/faints	50/531	-0.021	0.040	-0.099 to 0.057	0.280	0.597
Sensorimotor impairment	79/531	0.054	0.032	-0.009 to 0.117	2.804	0.094
Visual impairment	66/531	0.112	0.035	0.044 to 0.179	10.471	0.001
Auditory impairment	10/531	0.000	0.084	-0.164 to 0.163	0.000	0.997
Sleep disturbance	16/531	0.022	0.068	-0.112 to 0.155	0.102	0.749
Cognitive						
Dysphasia	70/531	0.109	0.034	0.041 to 0.176	10.005	0.002
Memory impairment	59/531	-0.008	0.039	-0.084 to 0.068	0.046	0.830
Intellectual impairment	12/531	-0.049	0.038	-0.122 to 0.025	1.668	0.196
Concentration impairment	60/531	0.151	0.078	-0.001 to 0.304	3.775	0.052
Psychiatric						
Anxiety	21/531	-0.032	0.052	-0.133 to 0.069	0.375	0.540
Depression	30/531	0.027	0.059	-0.088 to 0.143	0.214	0.644
Personality change	59/531	0.040	0.039	-0.037 to 0.117	1.022	0.312

Adjusted for age at admission.

Appendix 2.10 Baseline characteristic comparisons for mortality outcomes

Table A.2.17. Baseline characteristic comparisons according to death certification.

Characteristic	Death certificate	No death certificate	χ^2	Mann-Whitney U	p-value
Age – median (IQR)	n=400	n=503			
Age at admission	26.0 (22.0–30.0)	25.0 (21.0–29.0)	n/a	9.112×10^4	0.015
Personal history – n (%)	n=400	n=503			
Epilepsy	0 (0.0)	2 (0.4)	1.594	n/a	0.207
Headache/migraine	0 (0.0)	4 (0.8)	3.195	n/a	0.074
Family history – n (%)	n=400	n=503			
Epilepsy	1 (0.3)	3 (0.6)	0.606	n/a	0.436
Headache/migraine	0 (0.0)	3 (0.6)	2.394	n/a	0.122
Military rank – n (%)	n=386	n=489	0.096	n/a	0.953
Enlisted	237 (61.4)	296 (60.5)			
Non-commissioned officer	107 (27.7)	137 (28.0)			
Commissioned officer	42 (10.9)	56 (11.5)			
Military division – n (%)	n=387	n=483	5.520	n/a	0.063
Army	367 (94.8)	452 (93.6)			
Air force	11 (2.8)	26 (5.4)			
Navy	9 (2.3)	5 (1.0)			

Appendix 2.11 Supplementary analyses for mortality outcomes

Table A.2.18. Exploratory comparison of causes of death.

Underlying cause of death – n (%)	MHHI OTBI cohort	Population cohort[a]
	n=400	n=540,529
Cerebrovascular disease	43 (10.8)	52,888 (9.8)
Unspecified	33 (8.3)	43,253 (8.0)
Haemorrhagic	8 (2.0)	4,121 (0.8)
Ischaemic	2 (0.5)	5,514 (1.0)
Neurodegenerative disease	1 (0.3)	10,271 (1.9)
Dementia, unspecified	0 (0.0)	3,275 (0.6)
Alzheimer's disease	0 (0.0)	1,608 (0.3)
Neurodegeneration, unspecified	0 (0.0)	170 (0.0)
Presenile dementia	0 (0.0)	430 (0.1)
Parkinson's disease	0 (0.0)	3,973 (0.7)
Motor neuron disease	1 (0.3)	815 (0.2)
Epilepsy	1 (0.3)	190 (0.0)
Generalised convulsive	0 (0.0)	9 (0.0)
Grand mal status	0 (0.0)	29 (0.0)
Petit mal status	0 (0.0)	0 (0.0)
Epilepsy, unspecified	1 (0.3)	152 (0.0)
CNS infection	3 (0.8)	131 (0.0)
CNS neoplasia	2 (0.5)	1,245 (0.2)
Malignant neoplasm of brain	2 (0.5)	1,245 (0.2)
Psychiatric	0 (0.0)	130 (0.0)
Demyelinating disease	1 (0.3)	168 (0.0)
Autonomic disease	1 (0.3)	7 (0.0)
Neurological or psychiatric disease, all	52 (13.0)	65,030 (12.0)

[a] Causes of death registered in England and Wales between 1994–2000 for men aged 70 years or older. Source: The 20th Century Mortality Files - 1994-2000 ICD9c, Office for National Statistics [388].

Appendix to Chapter 3: Neuropsychology

Appendix 3.1 Structural validation

Table A.3.1. Structural validation anatomical data.

Case	Region name	Region volume – voxels	PM lesion – voxels (%)	CT lesion – voxels (%)
1	Frontal_Inf_Oper_R	11174	0 (0.0)	67 (0.6)
1	Frontal_Inf_Orb_R	13747	24 (0.2)	28 (0.2)
1	Rolandic_Oper_R	10733	0 (0.0)	141 (1.3)
1	Insula_R	14128	233 (1.6)	393 (2.8)
1	Hippocampus_R	7606	5141 (67.6)	2966 (39.0)
1	ParaHippocampal_R	9028	5507 (61.0)	1539 (17.0)
1	Amygdala_R	1965	1517 (77.2)	1047 (53.3)
1	Fusiform_R	20227	6609 (32.7)	2019 (10.0)
1	Caudate_L	7682	10 (0.1)	0 (0.0)
1	Putamen_L	7942	1070 (13.5)	0 (0.0)
1	Putamen_R	8510	207 (2.4)	75 (0.9)
1	Pallidum_L	2285	132 (5.8)	0 (0.0)
1	Pallidum_R	2188	11 (0.5)	0 (0.0)
1	Temporal_Sup_R	25258	4904 (19.4)	7198 (28.5)
1	Temporal_Pole_Sup_R	10654	5534 (51.9)	6032 (56.6)
1	Temporal_Mid_R	35484	10922 (30.8)	10125 (28.5)
1	Temporal_Pole_Mid_R	9470	7002 (73.9)	3765 (39.8)
1	Temporal_Inf_R	28468	13492 (47.4)	5568 (19.6)
1	Cerebelum_4_5_R	6763	56 (0.8)	0 (0.0)
2	Frontal_Sup_L	28915	930 (3.2)	147 (0.5)
2	Frontal_Sup_Orb_L	7654	2216 (29.0)	2428 (31.7)
2	Frontal_Mid_L	38722	2247 (5.8)	557 (1.4)
2	Frontal_Mid_Orb_L	7112	6119 (86.0)	5663 (79.6)
2	Frontal_Inf_Tri_L	20104	1310 (6.5)	2368 (11.8)
2	Frontal_Inf_Orb_L	13590	10985 (80.8)	12756 (93.9)
2	Olfactory_L	2262	271 (12.0)	381 (16.8)
2	Frontal_Med_Orb_L	5792	0 (0.0)	3 (0.1)
2	Rectus_L	6864	437 (6.4)	746 (10.9)
2	Insula_L	15025	1706 (11.4)	2005 (13.3)
2	Hippocampus_L	7469	203 (2.7)	203 (2.7)
2	ParaHippocampal_L	7891	500 (6.3)	898 (11.4)
2	Amygdala_L	1733	665 (38.4)	315 (18.2)
2	Fusiform_L	18333	0 (0.0)	593 (3.2)
2	Caudate_L	7682	0 (0.0)	352 (4.6)
2	Putamen_L	7942	19 (0.2)	311 (3.9)
2	Temporal_Pole_Sup_L	10228	344 (3.4)	2794 (27.3)
2	Temporal_Mid_L	39353	0 (0.0)	60 (0.2)
2	Temporal_Pole_Mid_L	5984	0 (0.0)	615 (10.3)
2	Temporal_Inf_L	25647	0 (0.0)	66 (0.3)
3	Calcarine_L	18157	2784 (15.3)	2244 (12.4)
3	Cuneus_L	12133	0 (0.0)	6 (0.0)
3	Lingual_L	16932	7423 (43.8)	5278 (31.2)
3	Occipital_Sup_L	10791	0 (0.0)	239 (2.2)
3	Occipital_Mid_L	25989	137 (0.5)	2087 (8.0)
3	Occipital_Inf_L	7536	2490 (33.0)	2725 (36.2)
3	Fusiform_L	18333	4011 (21.9)	2170 (11.8)
3	Precuneus_L	28358	0 (0.0)	6 (0.0)
3	Cerebelum_Crus1_L	20667	843 (4.1)	1440 (7.0)
3	Cerebelum_Crus2_L	15216	0 (0.0)	1 (0.0)
3	Cerebelum_6_L	13672	640 (4.7)	694 (5.1)
4	Rolandic_Oper_R	10733	1739 (16.2)	2526 (23.5)
4	Insula_R	14128	270 (1.9)	406 (2.9)
4	Hippocampus_R	7606	2685 (35.3)	2818 (37.0)
4	ParaHippocampal_R	9028	1380 (15.3)	3117 (34.5)
4	Amygdala_R	1965	0 (0.0)	463 (23.6)

Case	Region name	Region volume – voxels	PM lesion – voxels (%)	CT lesion – voxels (%)
4	Calcarine_R	14885	355 (2.4)	10 (0.1)
4	Cuneus_R	11323	210 (1.9)	2046 (18.1)
4	Lingual_R	18450	393 (2.1)	71 (0.4)
4	Occipital_Sup_R	11149	22 (0.2)	94 (0.8)
4	Occipital_Mid_R	16512	3512 (21.3)	1742 (10.5)
4	Occipital_Inf_R	7929	2012 (25.4)	50 (0.6)
4	Fusiform_R	20227	5259 (26.0)	6388 (31.6)
4	Postcentral_R	30652	435 (1.4)	1558 (5.1)
4	SupraMarginal_R	15770	1128 (7.2)	870 (5.5)
4	Angular_R	14009	0 (0.0)	232 (1.7)
4	Precuneus_R	26083	2 (0.0)	820 (3.1)
4	Thalamus_R	8399	403 (4.8)	0 (0.0)
4	Heschl_R	1936	1024 (52.9)	27 (1.4)
4	Temporal_Sup_R	25258	14434 (57.1)	11553 (45.7)
4	Temporal_Pole_Sup_R	10654	494 (4.6)	274 (2.6)
4	Temporal_Mid_R	35484	20614 (58.1)	24161 (68.1)
4	Temporal_Pole_Mid_R	9470	337 (3.6)	74 (0.8)
4	Temporal_Inf_R	28468	17044 (59.9)	15007 (52.7)
4	Cerebelum_Crus1_R	21017	229 (1.1)	4 (0.0)
4	Cerebelum_4_5_R	6763	137 (2.0)	5 (0.1)
4	Cerebelum_6_R	14362	256 (1.8)	102 (0.7)

Table A.3.2. Structural validation summary statistics.

Case	PM lesion – cm^3	CT lesion – cm^3	CT & PM lesion overlap – cm^3 (%)
1	78.98	49.00	43.37 (88.51)
2	33.35	41.05	25.32 (61.68)
3	21.84	20.66	12.28 (59.44)
4	95.89	94.55	63.00 (66.63)
Average	57.52	51.32	35.99 (69.07)

Appendix 3.2 Region-specific lesion-symptom mapping for insomnia

Table A.3.3. Region-specific lesion-symptom mapping restricted to the left prefrontal region.

Region ID[a]	Region name[a]	z-score	z-threshold	$p < 0.05$
1	L G frontal superior-1	-0.712	< -0.741	No
2	L G frontal superior-2	-0.965	< -0.931	Yes
3	L G frontal superior-3	-0.864	< -0.868	No
4	L S sup frontal-1	0.355	< -0.802	No
5	L S sup frontal-2	0.496	< -1.179	No
6	L S sup frontal-3	-0.846	< -1.152	No
7	L S sup frontal-4	-1.067	< -1.010	Yes
8	L S sup frontal-5	-0.942	< -0.934	Yes
9	L S sup frontal-6	-0.936	< -0.929	Yes
104	L G frontal superior medial-1	-1.025	< -1.036	No
105	L G frontal superior medial-2	-0.875	< -0.874	Yes
106	L G frontal superior medial-3	-0.713	< -0.893	No

[a] Region ID and region name from the AICHA atlas.

Appendix to Chapter 4: Neuropathology

Appendix 4.1 Supplementary immunohistochemistry methods

Table A.4.1. Immunohistochemistry methods.

Antigen	Primary antibody	Supplier (cat no.)	Classification	Dilution	Incubation	Antigen retrieval	Blocker	Secondary	Chromogen
PHF-tau	AT8	Thermo Fisher (MN1020)	Monoclonal mouse	1:300 TBST	1 hour at RT	None	None	Envision 45 min at RT	1:50 DAB
Amyloid-β	4G8	Biolegend (SIG-39220)	Monoclonal mouse	1:24,000 5% FCS in TBST	20 hours at RT	80% formic acid	10% FCS in TBST for 30 mins at RT	Envision 45 min at RT	1:50 DAB
α-synuclein	Anti-α-synuclein	BD Biosciences (610787)	Monoclonal mouse	1:1,000 5% FCS in TBST	1 hour at RT then 20 hours at 4°C	80% formic acid	10% FCS in TBST for 20 mins at RT	Envision 45 min at RT	1:50 DAB
pTDP-43	Phospho-TDP43 (Ser409/410)	Proteintech (66318-1-IG)	Monoclonal mouse	1:250 TBST	20 hours at 4°C	AC citrate	DAKO serum-free protein block for 20 mins at RT	Envision 45 min at RT	1:50 DAB

RT = room temperature. DAB = 3,3'-diaminobenzidine. TBST = Tris-buffered Saline with Tween. FCS = foetal calf serum. AC = autoclave.

Appendix 4.2 Baseline characteristics

Table A.4.2. Study cohort demographics and injury details.

Case	Age at injury	Age at death	Hemisphere	Mechanism	Division	PTA	PTE	Motor/sensory
1	21	80	Right	Shell	Army	None	None	Motor
2	20	80	Left	Bullet	Unknown	Unknown	None	None
3	27	87	Right	Shell	Army	None	Generalised	None
4	24	83	Right	Bullet	Army	Unknown	Generalised	Sensorimotor
5	30	95	Left	Shell	Army	None	Generalised	Motor
6	33	81	Right	Shell	Army	None	Generalised	Motor
7	21	67	Left	Bullet	Army	<30 mins	Focal only	Sensorimotor
8	29	76	Right	Shell	Army	None	Generalised	None
9	26	76	Right	Shell	Unknown	None	None	None
10	36	88	Left	Shell	Army	<30 mins	None	Motor
11	33	85	Left	Shell	Army	<30 mins	None	None
12	26	80	Left	Shell	Army	None	None	None
13	28	81	Left	Shell	Army	None	Generalised	None
14	20	73	Left	Bullet	Army	None	Generalised	None
15	23	68	Right	Mine	Army	<30 mins	Generalised	Motor

PTA = post-traumatic amnesia. PTE = post-traumatic epilepsy.

Appendix 4.3 Post-mortem details

Table A.4.3. Study cohort post-mortem details.

Case	Hemisphere affected	PMI (days)	FFT (yrs)	Braak NFT stage	Diffuse plaques	Neuritic plaques	CAA	LBs	CVD	Comments	COD
1	Right	2	0	I/II	No	No	No	No	No		Unknown
2	Left	1	10	I/II	No	No	No	No	No	L hippocampal sclerosis and mamillary body atrophy	Unknown
3	Right	2	0	III/IV	Yes	Yes	Moderate	Yes	No	R amygdala atrophy and LC pallor	Unknown
4	Right	Unknown	0	III/IV	Yes	Yes	Severe	Yes	No	Posterior CC and R thalamus atrophy	Unknown
5	Left	Unknown	6	V/VI	Yes	Yes	Moderate	No	No	L amygdala atrophy and LC pallor	Unknown
6	Right	Unknown	22	I/II	Yes	No	No	No	L caudate (lacune)	R mamillary body and thalamic atrophy	Lung cancer
7	Left	Unknown	22	0	No	No	No	No	L frontal (MCA) and R putamen (lacune)		Bladder cancer
8	Right	2	22	III/IV	Yes	Yes	No	No	No		Heart failure
9	Right	2	19	0	No	No	No	No	No		Ischaemic heart disease
10	Left	Unknown	17	I/II	Yes	No	Mild	No	L putamen (lacune)	L hippocampal sclerosis	Heart failure
11	Left	Unknown	17	I/II	Yes	No	No	No	No	Carcinomatosis of leptomeninges	Lung cancer
12	Left	Unknown	17	0	Yes	No	No	No	L putamen (lacune)		Cancer NOS
13	Left	Unknown	16	III/IV	Yes	Yes	Severe	No	R medulla (PICA)		Ischaemic heart disease
14	Left	Unknown	15	I/II	Yes	No	No	No	No		Bladder cancer
15	Right	3	0	I/II	Yes	No	No	No	No	R hippocampus and mamillary body sclerosis	Ischaemic heart disease

PMI = post-mortem interval. FFT = formalin fixation time. CAA = cerebral amyloid angiopathy. LBs = Lewy bodies. CVD = cerebrovascular disease. COD = cause of death. L = left. R = right. LC = locus coeruleus. CC = corpus callosum. MCA = middle cerebral artery. PICA = posterior inferior cerebellar artery. NOS = not otherwise specified.

Table A.4.4. Experimental control post-mortem details.

Control	Group	Sex	Age at death	PMI (hrs)	Braak NFT stage	Diffuse plaques	Neuritic plaques	CAA	CERAD rating	NIA-Reagan	LBs	CVD	Comments
1	Negative	M	85	16	0	None	None	No	Normal brain	N/A	No	None	
2	Negative	M	80	30	I/II	None	None	No	Normal brain	N/A	No	None	
3	Negative	M	88	38	I/II								
4	Mild AD	M	80	60	III/IV	Yes	Yes	No	Probable AD	Intermediate	No	None	Hippocampal neuronal loss, gliosis
5	Mild AD	M	82	40	I/II	Yes	Yes	No	Possible AD	Low	No	Single Infarct	
6	Mild AD	M	86	90	I/II	Yes	Yes	No	Possible AD	Low	No	L midbrain microhaemorrhage	
7	Severe AD	M	73	29	V/VI	Yes	Yes	No	Definite AD	High	No	None	
8	Severe AD	M	75	28	V/VI	Yes	Yes	No	Definite AD	High	No	None	
9	Severe AD	M	92	112	V/VI	Yes	Yes	No	Definite AD	High	No	None	

PMI = post-mortem interval. NFT = neurofibrillary tangle. CAA = cerebral amyloid angiopathy. CERAD = Consortium to Establish a Registry for Alzheimer's Disease. NIA = National Institute on Aging. LBs = Lewy bodies. CVD = cerebrovascular disease. N/A = not applicable. AD = Alzheimer’s disease. L = left.

Appendix 4.4 Further discussion of chronic traumatic encephalopathy

Background

CTE is a neuropathological diagnosis defined by the presence of p-tau aggregates within neurons and astrocytes surrounding small blood vessels in the deep layers of cortical sulci, and in superficial layers elsewhere in the cortex [26]. CTE was first described in boxers and later characterised through postmortem examination of former American football players [261–264]. CTE has subsequently been identified in former players of other contact and collision sports, including ice hockey [471], association football (soccer) [472], rugby [473], and Australian rules football [474], as well as military personnel [264], individuals exposed to intimate partner violence [475], and people with epilepsy [476]. In these groups, the extent of CTE pathology is closely associated with the duration of exposure to RHIs [26]. However, the strength of this association is difficult to interpret given the risk of selection bias in post-mortem studies of highly selected cohorts, and the prevalence of individuals without CTE pathology after significant RHI exposure remains unclear [477,478]. Nevertheless, it is thought that RHI exposure can cause CTE through incremental neuronal damage sustained during each impact, without any one impact producing a clinically detectable injury [26,479,480]. Animal models show that axonal injury can precipitate p-tau pathology [481], and the fact that computational models of biomechanical forces during head impact predict maximal strain in sulcal regions also supports this theory [482]. Moreover, strain is greatest in proximity to blood vessels, and can itself cause BBB disruption, which may contribute to the predominant perivascular distribution of CTE pathology [26].

NFTs in a pattern resembling CTE have been described in one study of long-term survivors of single closed TBIs [270]. However, this study predated the formulation of formal CTE diagnostic criteria, which were therefore not applied, and co-incidental exposure to RHI was not assessed, meaning that the effect of a single closed TBI on CTE remains unclear. CTE has also been reported in a handful of cases with complete axonal disruption caused by

penetrating brain injuries, including surgical leucotomy for refractory schizophrenia (n = 5) and a shotgun wound (n = 1) [483,484]. However, there is ongoing debate about whether these cases meet CTE diagnostic criteria [26]. Specifically, authors of the criteria have suggested that the pathology described is more in-keeping with the non-specific changes of ARTAG and lacks the cardinal features of CTE [26,453]. Additionally, it has been argued that the assessment of co-incidental RHI exposure was insufficient [26]. For example, in the group with schizophrenia, their history of falls, fits, and other high-risk behaviours, such as head-banging were described in limited detail [26]. As such, it remains uncertain whether axonal disruption caused by a single penetrating brain injury can cause CTE.

While conducting this study, we identified features of CTE in the AC of the affected hemisphere from one case (Case 4), who died at the age of 83 with severe AD and CAA. Exploratory assessments were undertaken to assess the extent and distribution of CTE pathology in this case.

Methods

IHC was performed to label alpha-synuclein (α-syn, BD Biosciences 610787, 1:1,000) and phosphorylated TAR DNA-binding protein 43 (pTDP-43, Proteintech 66318-1, 1:250). Immunofluorescent (IF) triple-labelling was also performed to confirm the identity of cells containing p-tau (AT8, ThermoFisher MN1020, 1:200; NeuN, Merck ABN90P, 1:500; GFAP, Abcam ab4674, 1:1,000).

Results

CTE pathology was evident in the AC of the affected hemisphere, adjacent to the lesional deficit in the right inferior parietal lobe (**Figure A.4.1**). In this section, subcortical white matter showed severe pallor and rarefaction secondary to the nearby wound (**Figure A.4.1.A–D**). Two sulci can be seen superficial to the damaged white matter (**Figure**

A.4.1.E). One of these sulci contained a CTE lesion, with NFTs and dot-like neurites surrounding small blood vessels in the deep cortical layers of the sulcus (**Figure A.4.1.E–G**) [26,127]. Thorn-shaped p-tau immunoreactive astrocytes can also be seen, as have been described in older CTE cases and in ARTAG [453]. Immunofluorescent staining for AT8, NeuN, and GFAP confirmed co-localisation of p-tau within neurons as well as astrocytes in this region (**Figure A.4.1.I–L**).

To assess this case in more detail, FFPE blocks from the following regions were immunostained for p-tau, aβ, α-synuclein, and pTDP-43: brainstem (medulla, pons, midbrain), mesial and lateral temporal lobe, inferior parietal lobule, and superior frontal gyrus in both hemispheres. In the left superior frontal section, distant from the wound, a second CTE lesion was seen (**Figure A.4.2.E–L**). The underlying subcortical white matter in this section showed significantly less pallor (**Figure A.4.2.A–D**). No CTE lesions were seen in the other sections containing cerebral cortex (left inferior parietal lobule, right superior frontal gyrus, bilateral lateral temporal lobes).

In addition to CTE, this case showed neuropathological features of severe AD (Braak stage V/VI) and widespread CAA. NFTs were abundant in the hippocampus and temporal neocortex of both hemispheres, and more sparsely distributed in frontal and parietal cortex (layers II–V). Diffuse and neuritic aβ plaques were found throughout all layers of the cerebral cortex and in the endothelium of leptomeningeal and cortical blood vessels. Supportive features of CTE could be identified in these regions, although these are difficult to distinguish from the features of AD (**Figure A.4.3**). These included NFTs in the temporal neocortex (layer II) (**Figure A.4.3.A**), CA2 and CA4 hippocampal subregions (**Figure A.4.3.B**), amygdala (**Figure A.4.3.C**), and substantia nigra (**Figure A.4.3.D**). pTDP-43 immunostaining revealed sparse neuronal inclusions and dystrophic neurites throughout the

cerebral cortex and subcortical white matter. Immunostaining for α-synuclein showed occasional neuronal inclusions in the substantia nigra but not the locus coeruleus or cerebral cortex.

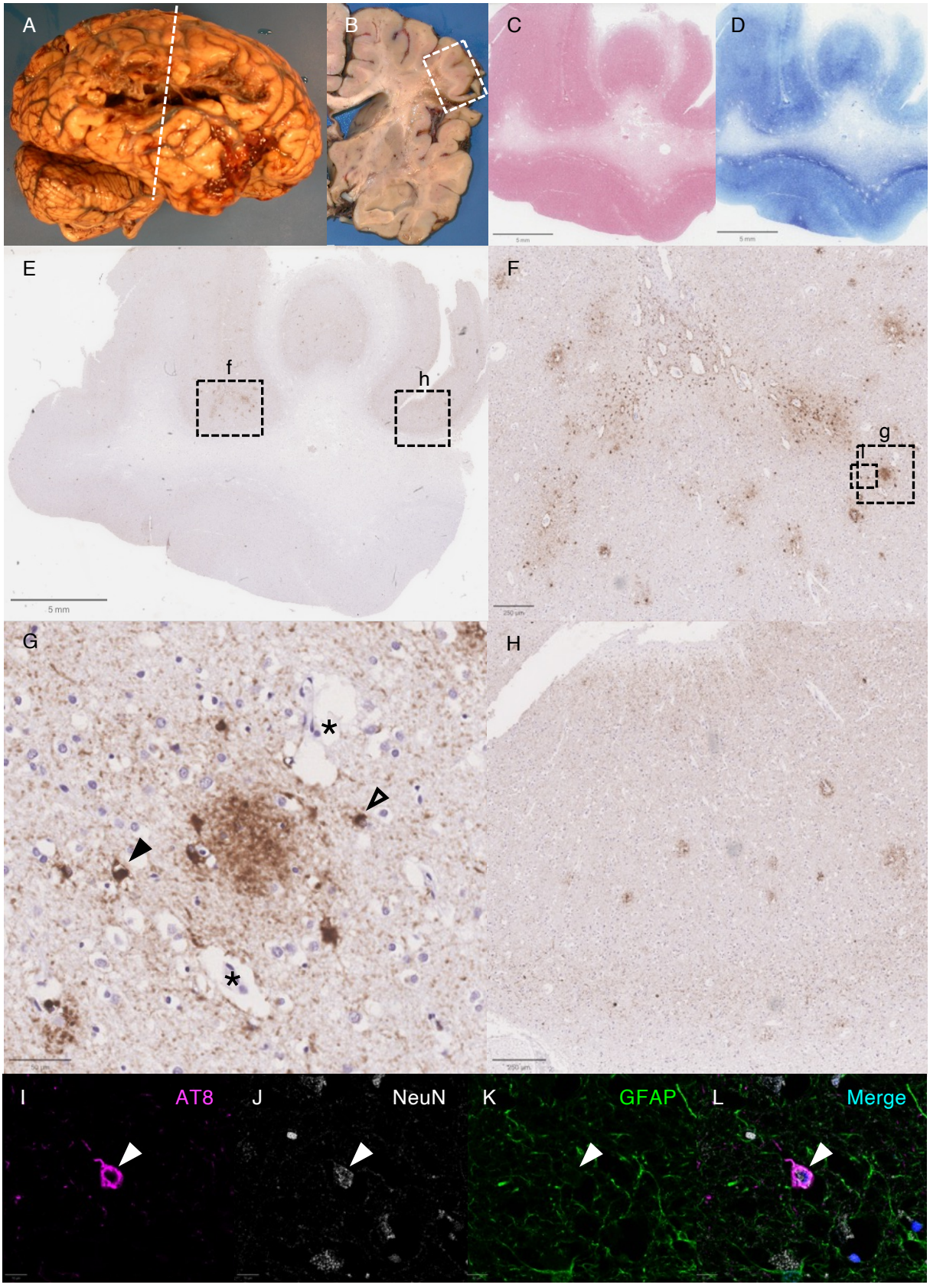
Discussion

CTE pathology was not identified in any of the blocks from other cases, but the sampling strategy used in this study did not follow the systematic approach recommended in current CTE diagnostic criteria, so we cannot draw conclusions about the prevalence of CTE after penetrating brain injury [127,26]. To explore this case in more detail, we studied additional FFPE blocks sampled from brainstem (medulla, pons, midbrain), mesial and lateral temporal lobe, inferior parietal lobule, and superior frontal gyrus in both hemispheres at post-mortem. Interestingly, we also identified CTE in the superior frontal gyrus of the unaffected hemisphere. CTE was therefore found both adjacent to and distant from a penetrating brain injury caused by a gunshot wound.

The presence of CTE adjacent to a gunshot wound could be interpreted as evidence that CTE can be caused by complete axonal disruption. However, according to MHHI records, this individual was also exposed to RHIs through participation in collision sport as a schoolboy. It is therefore also possible that the lesion developed in this area through incremental axonal damage prior to the injury. The fact that CTE developed in only one of the two sulci in this region of AC supports the interpretation that CTE was not caused by the gunshot wound. The CTE lesion in the unaffected hemisphere, distant from the wound and not associated with subcortical white matter destruction, seems more likely to have been caused by RHI exposure than complete axonal disruption.

This case illustrates how multiple forms of brain injury may coincide in the same individual, and the complexity of untangling downstream effects when this occurs. To overcome this

complexity, it is vital that post-mortem studies seeking to investigate the exposures associated with CTE are informed by detailed characterisation of relevant exposures and risks throughout life.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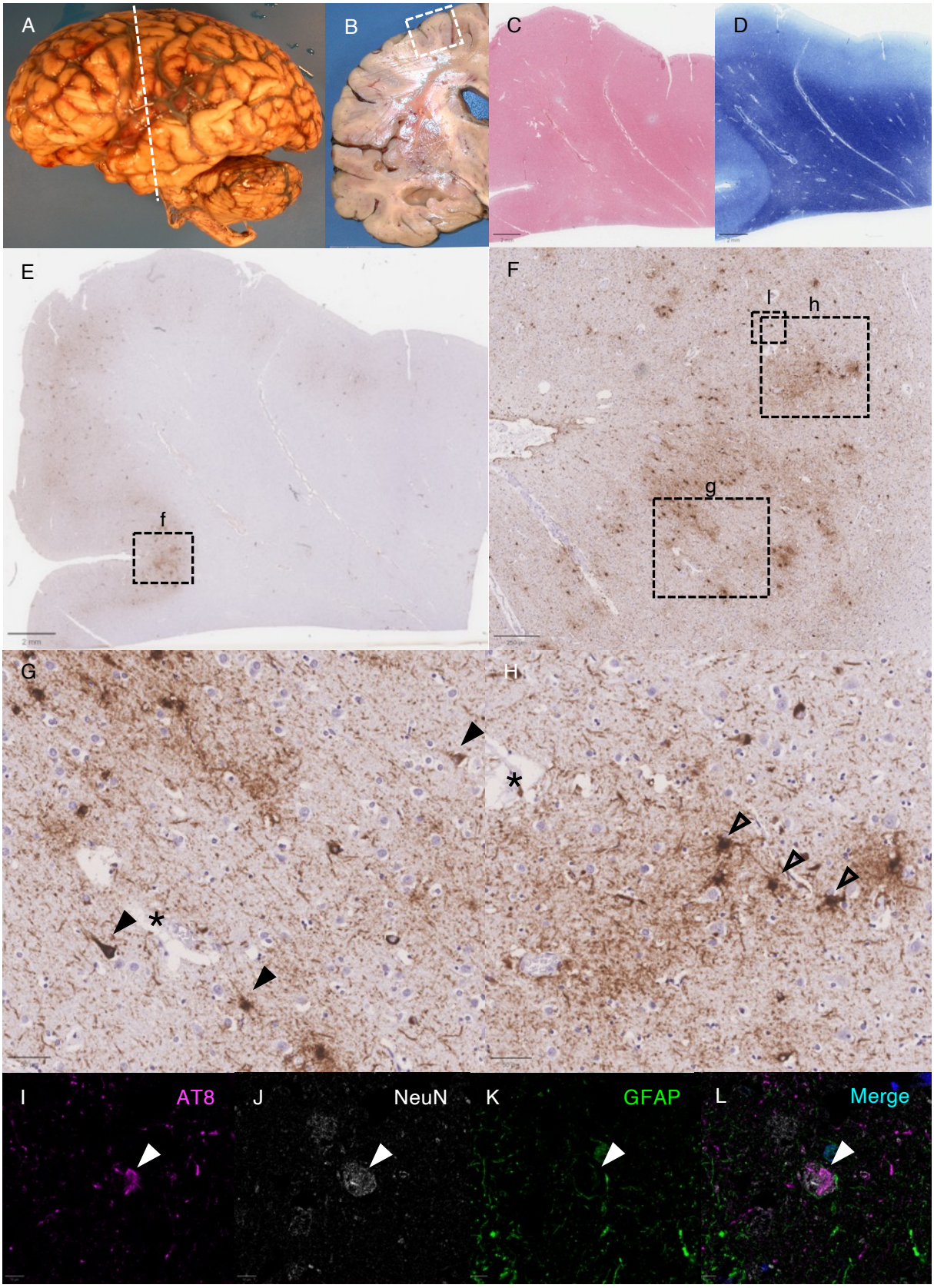
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Figure A.4.1. Chronic traumatic encephalopathy (CTE) in the right parietal lobe of Case 4, adjacent to the gunshot wound. A–B. Post-mortem photographs. **C.** Haematoxylin and eosin (H&E) staining. **D.** Luxol fast blue and cresyl violet (LBCV) staining. **E–H.** AT8 immunostaining. **I–L.** AT8-NeuN-GFAP immunofluorescence. **A.** The gunshot wound resulted in a large deficit involving the right inferior frontal gyrus, basal ganglia, insular cortex, superior temporal gyrus, superior and inferior parietal lobules, and inferolateral occipital gyrus (line represents the coronal slice shown in **B**). **B.** Tissue was sampled from the right inferior parietal lobule, adjacent to the wound (box represents the right inferior parietal section shown in **C–L**). **C–D.** Sub-cortical white matter shows severe pallor and rarefaction secondary to the nearby wound. **E–F.** AT8 immunoreactivity is clustered around small blood vessels in the deep cortical layers of one sulcus. **G.** NFTs (black triangle), dot-like neurites, and thorn-shaped astrocytes (white triangle) can be identified around blood vessels (✶). **H.** No features of CTE are present in the other sulcus adjacent to the wound. **I–L.** Co-localisation of AT8 and NeuN confirms the presence of neurofibrillary tangles**.**

Figure A.4.2. Chronic traumatic encephalopathy (CTE) in the left frontal lobe of Case 4, distant from the gunshot wound. A–B. Post-mortem photographs. **C.** H&E staining. **D.** LBCV staining. **E–H.** AT8 immunostaining. **J–L.** AT8-NeuN-GFAP immunofluorescence. **A.** The left hemisphere shows no external signs of damage (line represents the coronal slice shown in **B**). **B.** Tissue was sampled from the left superior frontal gyrus, distant from the wound (box represents the left superior frontal section shown in **C–L**). **C–D.** Sub-cortical white matter shows significantly less pallor compared to the right parietal section. **E–F.** AT8 immunoreactivity is clustered around small blood vessels in the deep cortical layers of a sulcus. **G–H.** NFTs (black triangle), dot-like neurites, and thorn-shaped astrocytes (white triangle) can be identified around blood vessels (✶). **I–L.** Co-localisation of AT8 and NeuN confirms the presence of neurofibrillary tangles.

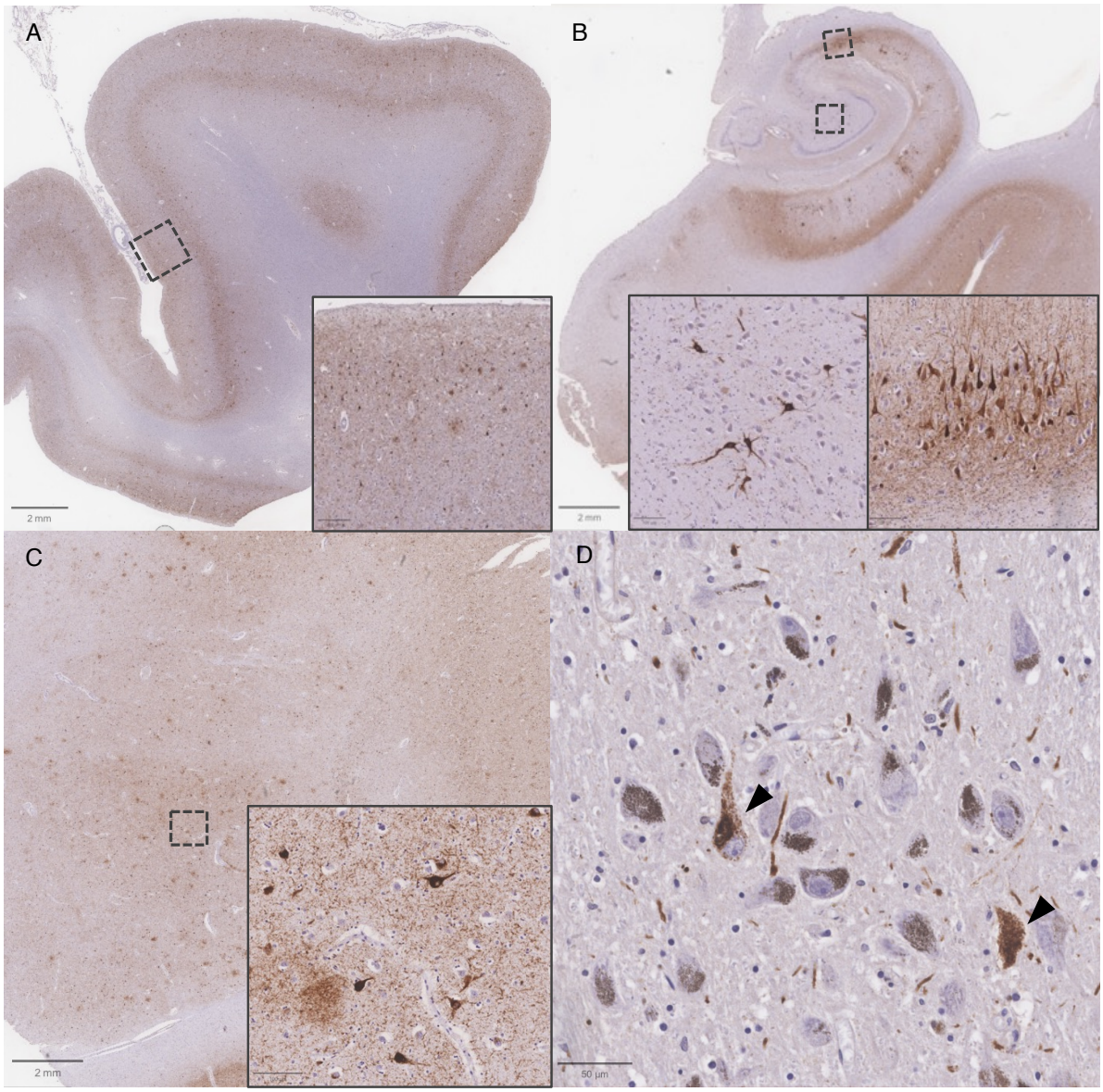

Figure A.4.3. Features which may be supportive of chronic traumatic encephalopathy (CTE) but also Alzheimer's disease in Case 4. A–D. AT8 immunostaining. **A.** Prominent AT8 immunoreactivity and NFTs in layer II of right temporal neocortex. **B.** In the right hippocampus, NFTs and pre-tangles are widespread in CA4 (left inset) and CA2 (right inset), as well as CA1, subiculum, entorhinal, and transentorhinal cortex. **C.** NFTs and neurites are also abundant in the left amygdala. **D.** NFTs (black triangle) are seen alongside pigmented neurons in the substantia nigra.