Traumatic Brain Injury in humans and animal models

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To Amir & Elmira

Human beings are members of a whole
In creation of one essence and soul
If one member is afflicted with pain
Other members uneasy will remain
If you've no sympathy for human pain
The name of human you cannot retain

Saadi Shirazi, Persian poet & scholar 1200CE,

The Persian calligraphy of Saadi's aphorism above is a generous contribution of the painter and calligrapher Mr. Kakayi. KakayiArt© Belgium

ABSTRACT

Traumatic brain injuries (TBI) are receiving increasing attention due to a combination of injuries related to war and sports, as well as to an increasing number of traffic accident survivors. Today the leading cause of death in young adults in industrialized nations is traumatic brain injury and in the population under 35 years, the death rate is 3.5 times that of cancer and heart disease combined. Despite a major improvement in the outcome of TBI in the acute setting, the assessment, therapeutic interventions and prevention of long-term complications remain a challenge. The challenges today are primarily related to a rapid diagnosis, identification of patient's pathophysiological heterogeneity and to limit the secondary injuries. TBI is a complex condition that can be caused by focal or diffuse primary impacts that may initiate complex secondary neurochemical processes that proceeds over hours and days. The major secondary events include neuronal death, ischemia, excitotoxicity, mitochondrial failure, oxidative stress, oedema and inflammation. In addition, the brain's restorative capacity involving neurotrophins, in particular brain derived neurotrophic factor (BDNF), is triggered. Animal models are necessary to gain a deeper insight into the events that follow a TBI, and to ultimately apply the findings to the clinical setting. The aim of this thesis was to identify distinct pathological processes in different types of TBI by using animal models that mimic distinct types of TBI found in patients. We investigated alterations in gene expression, serum biomarkers and secondary processes such as inflammatory response involving the complement cascade. In addition we aimed to assess the effects of heterogeneity of TBI patients, based on their genetic background, on the outcome of TBI, with specific focus on BDNF. We used animal models to mimic three major types of TBI; blast wave, penetrating and rotational acceleration TBI. We found distinct profiles of alteration in gene expression in these models. The histological findings in blast and rotational TBI indicated these injuries to be mild. The hallmark of the rotational TBI was axonal injuries found in anatomical locations comparable with clinical findings in diffuse axonal injuries (DAI) in humans. Despite the mild type of injury displayed in the histology and behavioural outcome, significant increases in the serum biomarkers Tau, S100B, NF-H and MBP were observed up to 2 weeks following the injury. The complement cascade was initiated in both penetrating and rotational TBI, detected by C1q and C3. However, the terminal pathway that generates cell death, detected by C5b9, was only activated in the penetrating TBI. This suggests that axonal injuries and secondary axotomy found in the rotational TBI are not complement mediated. In order to investigate whether genetic heterogeneity can be used to predict injury outcome and brain plasticity following TBI, we targeted the ApoE ε4 allele and the BDNF gene. We investigated whether there was an association between the presence of the ApoE ε4 allele and BDNF polymorphisms and cognitive outcome in veterans who had suffered penetrating head injury. We found that the genetic polymorphisms of BDNF predict general intelligence following penetrating TBI. Subsequently we investigated the expression of BDNF and its receptors TrkB-full length, TrkB-truncated and p75^{NTR}, in animals exposed to penetrating TBI. The expression of TrkB truncated and p75^{NTR} was altered in the chronic phase.

In summary, these results show the importance of categorizing the different types of TBI, not only through the use of animal models but also in the clinical setting. Each type of TBI shows distinct patterns of gene expression, behavioural outcome, and morphological changes that may be reflected in the release of serum biomarkers. In the clinical setting, the situation is further complicated by the coexistence of different types of injuries. In addition to this, the genetic background of each patient contributes to the heterogeneity of TBI pathology as well as their ability to recover. The use of distinct types of TBI models will provide essential information about the underlying pathology, which can then be applied to the clinical setting. This will contribute to the establishment of better diagnostic tools as well as more individualized treatment approaches.

SVENSK SAMMANFATTNING

Traumatiska hjärnskador (THS) är den ledande dödsorsaken bland unga vuxna i i-länder. Dödligheten är 3,5 gånger högre än för cancer och hjärtkärlsjukdomar tillsammans för de som är under 35 år. THS sker ofta i samband med olyckor, våldsbrott eller under sportutövande. Oavsett typ av primär skada så utlöser den olika neurokemiska reaktioner som kan leda till sekundära skador, såsom neuroinflammation vilket kan fortgå i dagar upp till månader och som kan leda till sekundära skador. Trots stora förbättringar i det akuta omhändertagandet finns det fortfarande stora brister i diagnostik, terapeutiska möjligheter och förebyggande av sekundära skador. THS är ett mångfacetterat och heterogent tillstånd som involverar flera olika patologiska processer. Lyckligtvis har hjärnan även en restaurerande förmåga där proteiner som neurotrofiner och Brain Derived Neurotrofic Factor (BDNF) spelar en avgörande roll.

Det övergripande målet med vårt arbete är att kunna förutsäga och förhindra sekundära skador samt att förstå vilken roll patienternas genetiska bakgrund spelar. Syftet med avhandlingen är att identifiera olika patologiska processer i olika typer av THS. Vi studerar förändringar i genuttryck, biomarkörer i blodet samt det inflammatoriska svaret vid TSH. Utöver detta undersöker vi vilken roll THS-patienters genetiska bakgrund spelar på den kognitiva förmågan efter en hjärnskada. Djurmodeller som efterliknar hjärnskador hos patienter är nödvändiga för att få kunskaper som kan föra oss närmare dessa mål. De använda djurmodellerna efterliknar tre huvudtyper av THS; tryckvågsorsakade TSH, penetrerande och rotations-accelerationskador. Vi fann tydliga förändringar i genuttryck som var specifika för de olika typerna av THS. De histologiska fynden i tryckvågs- och rotations-THS visade på en mild hjärnskada medan penetrationsskadan är en mer allvarlig form av THS. Kännetecknade för rotations-THS var axonala skador i hjärnbalken (corpus callosum), gränsen mellan vit och grå substans och i de centroaxiala strukturerna. Dessa är jämförbara med de kliniska fynden i diffusa axonala skador s.k. DAI hos människor. Trots att denna THS är en mild form av hjärnskada så kunde vi ändå detektera förhöjda nivåer av serumbiomarkörer som Tau, S100B, NF-H och MBP upp till två veckor efter skadan. Vår studie av det inflammatoriska svaret, med fokus på komplementkaskaden, visade att den celldödsmedierande delen av kaskaden, syntes av det terminala cytolytiska komplexet C5b9, initieras vid penetrerande skada, men inte vid rotationsskada. Detta tyder på att axonala skador och den sekundära axotomin som har en avgörande roll i patologin av TSH och främst rotationsskadan inte är komplementmedierade.

För att undersöka om den genetiska variationen kan förutspå resultatet av en hjärnskada gällande den kognitiva förmågan tittade vi på IQ och generna ApoE och BDNF. ApoE är den mest undersökta genen relaterad till hjärnskador och dess utfall. Detta studerades hos Vietnamveteraner med och utan penetrerande hjärnskador. Data för IQ både före kriget och vid ytterligare 2 tillfällen efter kriget och hjärnskadan erbjuder en lysande möjlighet att undersöka hjärnskadans effekt på kognitiv förmåga. Ingen effekt av allel ε4 av ApoE-genen kunde ses. Dock visar vår studie att polymorfism i den BDNF-producerande genen kan förutsäga IQ hos dessa patienter efter en penetrerande hjärnskada. Detta kan ha stor betydelse för potentiella behandlingar för patienter med hjärnskador. Detta har kunnat undersökas direkt i våra djurmodeller och är ett exempel på framgångsrik translatorisk forskning.

LIST OF PUBLICATIONS

This thesis is based on the following publications, which are referred to in the text by their roman numerals (Study I-V):

I. Risling M, Plantman S, Angeria M, **Rostami E**, Bellander BM, Kirkegaard M, Arborelius U, Davidsson J. Mechanisms of blast induced brain injuries, experimental studies in rats. *Neuroimage.* 2011 Jan; 54 Suppl 1:S89-97. Epub 2010 May 21.

II. **Rostami E**, Davidsson J, Ng KC, Lu J, Gyorgy A, Wingo D, Walker J, Plantman S, Bellander BM, Agoston D, Risling M. A model for mild traumatic brain injury that induces limited transient memory impairment and increased levels of axon related serum biomarkers.
Front Neurol. 2012; 3:115. Epub 2012 Jul 23

III. **Rostami E**, Davidsson J, Agoston DV, Gyorgy A, Risling M, Bellander BM
The complement terminal pathway is activated in focal penetrating but not in mild diffuse Traumatic Brain Injury. *Submitted*

IV. **Rostami E**, Krueger F, Zoubak S, Dal Monte O, Raymont V, Pardini M, Hodgkinson CA, Goldman D, Risling M, Grafman J. BDNF polymorphism predicts general intelligence after penetrating traumatic brain injury.
PLoS ONE. 2011; 6(11):e27389. Epub 2011 Nov 8.

V. **Rostami E**, Krueger F, Plantman S, Davidsson J, Agoston DV, Grafman J, Risling M. Alteration in BDNF and its receptors, full-length and truncated TrkB and p75^{NTR} following penetrating traumatic brain injury. *Submitted*

ADDITIONAL PUBLICATIONS NOT INCLUDED THE THESIS

Rostami E, Bondi M.
β-adrenoreceptor activation in brain, lung and adipose tissue, measured by microdialysis in pig.
Adv Med Sci. 2012 Jun 1;57(1):136-41.

Rostami E, Bellander BM.
Monitoring of glucose in brain, adipose tissue, and peripheral blood in patients with traumatic brain injury: a microdialysis study.
J Diabetes Sci Technol. 2011 May 1;5(3):596-604.

Risling M, Ochsman T, Carlstedt T, Lindå H, Plantman S, **Rostami E**, Angeria M, Sköld MK.
On acute gene expression changes after ventral root replantation.
Front Neurol. 2011 Jan 4;1:159.

Krueger F, **Rostami E**, Huey ED, Snyder A, Grafman J.
Evidence of an inferior total-order planning strategy in patients with frontotemporal dementia.
Neurocase. 2007 Oct;13(5):426-37.

Ungerstedt U, **Rostami E**.
Microdialysis in neurointensive care.
Curr Pharm Des. 2004; 10(18):2145-52. Review.

Rostami E, Rocksén D, Ekberg N, Goiny M, Ungerstedt U.
Hyperoxia in combination with hypoventilation decreases lactate and increases oxygenation in brain of non-injured pig.
In review

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LIST OF ABBREVIATIONS

ABLe	Analysis of Brain Lesions
AFQT	Army Force Qualification Test
AIMs	Ancestry information markers
ApoE	Apolipoprotein E
APP	Amyloid Precursor Protein
BBB	Blood brain barrier
BDNF	Brain Derived Neurotrophic Factor
CCI	Controlled cortical impact
CNS	Central Nervous System
COMT	Catechol-O-methyltransferase
cRNA	Complementary ribonucleic acid
CSF	Cerebrospinal fluid
CT	Computer tomography
DAI	Diffuse axonal injury
DAVID	Database for Annotation, Visualization and Integrated Discovery
DG	Dentate gyrus
DNA	Deoxyribonucleic acid
DTI	Diffusion tensor imaging
EPM	Elevated plus maze
FPI	Fluid percussion injury
GABA	Gamma-aminobutyric acid
GAPDH	Glyceraldehyde 3-phosphate dehydrogenase
GCS	Glasgow coma scale
ICP	Intracranial pressure
LD	Linkage disequilibrium
LFP	Lateral fluid percussion
LOC	Loss of Consciousness
MMSE	Mini-mental state examination test
MRI	Magnetic resonance imaging
mRNA	Messenger ribonucleic acid
mTBI	Mild traumatic brain injury
NF-H	Neurofilament heavy
NICU	NeuroIntensive Care Unit
Pen-TBI	Penetrating traumatic brain injury
PTA	Post-traumatic amnesia
Rot-TBI	Rotational traumatic brain injury
RPPM	Reverse Phase Protein Microarray
SNP	Single-nucleotide polymorphism
TAI	Traumatic axonal injury
TBI	Traumatic brain injury
Trk	Tropomyosin-receptor-kinase
VHIS	Vietnam Head Injury Study
WAIS	Wechsler Adult Intelligence Scale
WMS	Wechsler Memory Scale
WHO	World Health Organization

1 INTRODUCTION

The brain is the human body's most complex organ. This complexity, however, comes at the cost of a correspondingly high vulnerability for failure. Brain injuries have fascinated mankind since ancient times. Hippocrates (460BC-370BC) wrote extensively about head injuries and the clinical symptoms that are associated with it. Observations of the cognitive deficits following head injuries may underlie the brain hypothesis - the belief of ancient scientists, including Hippocrates, that all of our behaviour lies within the brain. However it was not until centuries later that the Persian physician Rhazes (Ibn Zakarya Razi, 865 – 925) became the first to describe brain injuries in more detail, in regards to both injury type and severity. In his observations he clearly distinguished concussion from severe brain injury, commencing the classification of traumatic brain injury (TBI). Many of the observations of heady injuries during this time were combat related. Findings in battlefield graves show crania with signs of trepanation, a method that may have been used to treat brain injuries in ancient times. The phenomenon of soldiers who have been "hit by the wave" and who come back from war without visible signs of injury but with devastating behavioural changes has been described and is well known among people who have experienced war. However, it is only recently that the victims of what is now called "blast wave injury" have come into focus (Moore and Jaffee, 2010; Risling and Davidsson, 2012). The combination of war injuries, an increasing number of traffic accident survivors and sports related injuries have contributed to bring the topic of brain injuries forward. We know that the leading cause of death in young adults in industrialized nations is TBI and in the population under 35 years, the death rate is 3.5 times that of cancer and heart disease combined (Ghajar, 2000). In the developing world, the WHO considers TBI a silent epidemic caused by an increasing number of traffic accidents. It is estimated that it will be the third greatest cause of the global burden of disease and injury by the year 2020 (Finfer and Cohen, 2001). The American Centre of Disease Control estimates that the incidence of TBI in the US is 2.1 million cases each year. In Sweden the corresponding figures are approximately 20.000 victims annually. Many years of productive life are lost, and countless people have to suffer years of disability after brain injury. In addition, it causes great economic costs for individuals, families and society. Despite the major improvement of TBI outcome in the acute setting in the past 20 years, the assessment, therapeutic interventions and prevention of long-term complications remain a challenge (Maas et al., 2008; Ling et al., 2010). The challenges lie mainly in the current concept of TBI classification, in identifying the interindividual pathophysiological heterogeneity and in limiting the processes involved in secondary damages. A way to approach these issues is to use better surrogate markers in an attempt to reach a better understanding of the pathology of the different types of TBI and the influence of the patient's genetic background. This would contribute to the establishment of more individualized treatment approaches. In order to make these critical progresses, bridges must be built over the barriers between laboratory experiments and patient care applications. The use of distinct types of TBI models will reveal information about the heterogeneous underlying pathology and possible distinct signature. This in combination with precise classification of clinical TBI will enable a more successful translational research in the field of TBI.

1.1   CLASSIFICATION OF TBI

Currently, in the clinical management of TBI the Glasgow Coma Scale (GCS), a clinical scale that assesses the level of consciousness after TBI, is used to divide the patients into the broad categories of mild, moderate and severe injury (Teasdale and Jennett, 1974) (Fig. 1). While GCS is the international standard for grading the severity of head injuries, the reaction level scale (RLS85) is a scale used in many clinics in Sweden. There is a good correlation between the GCS and RLS85 (Starmark et al., 1988)

Glasgow Coma Scale (GCS)

Eye Opening Response	Points
Spontaneous--open with blinking at baseline	4
To verbal stimuli, command, speech	3
To pain only (not applied to face)	2
No response	1
Verbal Response	
Oriented	5
Confused conversation, but able to answer questions	4
Inappropriate words	3
Incomprehensible speech	2
No response	1
Motor Response	
Obeys commands for movement	6
Purposeful movement to painful stimulus	5
Withdraws in response to pain	4
Flexion in response to pain (decorticate posturing)	3
Extension response in response to pain (decerebrate posturing)	2
No response	1

Reaction Level Scale (RLS85)

Clinical descriptor	Responsiveness Score	Points
Alert	No delay in response	1
Drowsy or confused	Responsive to light stimulation	2
Very drowsy or confused	Responsive to strong stimulation	3
Unconscious	Localizes but does not ward of pain	4
Unconscious	Withdrawing movements on pain stimulation	5
Unconscious	Stereotype flexion movements on pain stimulation	6
Unconscious	Stereotype extension movements on pain stimulation	7
Unconscious	No response on pain stimulation	8

Figure 1. Most common neurological scales for initial assessment of patients with TBI. The GCS comprises three tests: eye, verbal and motor responses. The three values separately as well as their sum are considered. The lowest possible GCS (the sum) is 3 (deep coma or death), while the highest is 15 (fully awake person). The RLS-85 coma scale is mainly used in Sweden. The main advantage of this over GCS is its reliable use in the management of patients who are difficult to assess, such as intubated patients and patients with swollen eyelids.

The 15-point GCS is classified as mild GCS 13-15, moderate GCS 9-13 and severe GCS 3-8. The GCS has proved to be extremely useful as a tool that assists neurosurgeons or trauma physicians in the early triaging of TBI patients and it also has a high inter-observer reliability (Narayan et al., 2002). It does however not provide any specific information about the pathophysiological mechanisms responsible for the neurological deficits (Saatman et al., 2008). A GCS $\leq$ 8 can cover the significant heterogeneity of pathological findings such as epidural hematoma, contusion, diffuse axonal injury or subarachnoid haemorrhage. It is given that a targeted therapy for severe TBI cannot effectively treat all of these different types of injury simultaneously. It has been shown that outcomes among patients with the same admission GCS were significantly different and were influenced by the mechanism of injury (Demetriades et al., 2004). Patients with penetrating TBI can present a deceptively high GCS, but still suffer a lethal TBI.   Furthermore, infants, young children and patients with pre-existing neurologic impairment are difficult to assess using the GCS. The GCS is also a poor discriminator for mild TBI, which account for 80–90% of all cases. It correlates poorly with the neuropsychiatric symptoms following a mTBI (McCullagh et al., 2001). Most importantly, the GCS must be applied as early as possible in particular in mTBI where most of the symptoms are present in the first few hours following TBI and can be transitory (Drake et al., 2006). In order to better assess mTBI the WHO Collaborating Centre for Neurotrauma Task Force on Mild Traumatic Brain Injury performed a comprehensive review on the epidemiology, diagnosis, prognosis and treatment of mild traumatic brain injury (Borg et al., 2004a; Borg et al., 2004b; Carroll et al., 2004a; Carroll et al., 2004b; Carroll et al., 2004c; Cassidy et al., 2004a; Cassidy et al., 2004b; Peloso et al., 2004). Their work resulted in many recommendations as well as the establishment of a set of diagnostic criteria. This diagnostic tool, which has become the most frequently used internationally, is recommended by the American Congress of Rehabilitation Medicine and the Centres for Disease Control and Prevention. The patient is considered to suffer a mTBI if displaying any of the following symptoms:

(1) Any period of loss of consciousness (LOC) of < 30 min and GCS of 13-15 after this period of LOC.

(2) Any loss of memory for events immediately before or after the accident, with posttraumatic amnesia.

(3) Any alteration in mental state at the time of the accident (e.g. feeling dazed)

(4) Focal neurological deficit(s) that may or may not be transient

1.2   IMAGING IN TBI

Following the first assessment of TBI patients with the GCS and the pupil reaction, the next step in the clinical setting is computed tomography (CT-scan) of the brain. Presently imaging is critical to both the diagnosis and management of TBI, as it identifies intracranial haemorrhage that warrants neurosurgical evacuation. CT identifies both extra-axial haemorrhage (epidural, subdural, and subarachnoid/intraventricular haemorrhage) and intra-axial haemorrhage (cortical contusion, intraparenchymal hematoma, and TAI or shear injury). The noncontrast CT can identify the progression of haemorrhaging as well as signs of secondary injuries such as cerebral swelling, herniation, and hydrocephalus.

However the non-haemorrhagic cortical contusions and traumatic axonal injuries are difficult to diagnose using CT. Patients with TAI can be comatose and suffer significant post-traumatic symptoms with cognitive impairment and poor functional outcome despite normal findings on a CT-scan. Although magnetic resonance imaging (MRI) has a better diagnostic sensitivity in these cases, the MRI images may also be normal in TAI patients. As such, there has been a need to develop more sensitive diagnostic tools for detecting TAI. One such imaging tool is diffusion tensor imaging (DTI). DTI maps out the microstructural characteristics of the brain based on the intrinsic diffusion properties of neurons by assessing diffusion in at least 6 but typically 25–30 directions. This yields a more complete set of diffusivity information that can be used to deduce axonal orientation and create maps of white matter tracts in the brain. DTI has shown promising results in blast-TBI and mTBI (Warden et al., 2009; Sharp and Ham, 2011) and have been shown to detect axonal injuries not seen with MRI (Mac Donald et al., 2007).

Although GCS and imaging are valuable assessment tools in the acute management of TBI patients, they do not reveal the heterogeneous underlying pathology, especially not for TAI and mild TBI. Furthermore, to obtain information about both insults at the time of impact and a deleterious secondary cascade of events, repetitive imaging combined with different types of imaging (CT, MRI or DTI) is required. This is a diagnostic and prognostic difficulty for TBI management and in many clinics and situations also a financial and practical challenge. Using biomarkers could be a way to fill this gap. Biomarkers could potentially be able to reveal the type of TBI, the severity of injury and even predict outcome.

1.3   SERUM BIOMARKERS

A biomarker is an indicator of a specific biological state that can be measured by samples taken from body fluids such as serum and CSF. The human serum proteome is comprised of approximately 100,000 proteins whose concentrations span over 12 orders of magnitude with 50% of the proteins being low in abundance, making the identification of biomarkers akin to identifying a precious diamond in a vast mine. There are two common approaches for biomarker discovery; a "top-down" and a "bottom-up" methodology. In the top-down approach, known biomarkers are used based on their involvement in the pathological state, mainly by protein profiling. In the bottom-up approach, changes in the examined tissue are linked to the pathology. This approach is usually used in biomarker discovery using high performance mass spectroscopy. In the present study we used a "top-down" approach by investigating known proteins involved in TBI pathology.

1.3.1   S100B

The most extensively studied biomarker in TBI is S100B (Rothermundt et al., 2003; Unden et al., 2007; Vos et al., 2010). It was identified in the mid-1960s as a protein fraction, which was then detectable only in the brain and not in non-neural extracts. It was named S100 because of its solubility in a 100% saturated solution of ammonium sulphate (Moore, 1965). S100B is a calcium-binding protein of 21kDA that glial-specific and primarily expressed by astrocytes.

S100B has been considered a promising biomarker of brain damage based on several studies showing a positive correlation between high S100B and severity and outcome of TBI patients (Raabe et al., 1999; Raabe and Seifert, 2000; Rothoerl et al., 2000; Pleines et al., 2001). Two studies have shown a correlation between high serum S100B levels and persistent neuropsychological deficits in patients following minor head injury (GCS >13) (Waterloo et al., 1997; Herrmann et al., 2001), however in the first study there are only 7 patients and in the second more than 50% of the patients had intracranial pathology shown in CT. Serum S100B has been reported to be variable, being high directly after TBI and normalized within 24h, even in patients with poor outcome (Jackson et al., 2000). A delayed increase on day 6 has been suggested to reflect secondary events (Raabe and Seifert, 2000). However, the interpretation of a high increase in S100B and its correlation to TBI severity or degree of tissue damage has been questioned in several ways; the initial increase may indicate increased passage through a disrupted BBB as opposed to being a reflection of brain damage due to trauma. Neurotrophic and neuroprotective properties of S100B might reflect a repair mechanism (Azmitia et al., 1990; Goncalves et al., 2000; Brewton et al., 2001). S100B is present in many different cell types (Zimmer et al., 1995) and high S100B has been shown in patients without TBI but with injuries such as bone fractures and thoracic contusions (Anderson et al., 2001a; Anderson et al., 2001b). However, an undetectable S100B serum level has been shown to have a negative predictive value of 0.99 predicting normal intracranial findings on a CT scan (Romner et al., 2000).

Despite many existing studies showing a good correlation between high serum levels of S100B, GCS and outcome, there is a lack of knowledge about the correlation between high serum levels of S100B and the underlying pathology of different types of TBI.

1.3.2   Tau

Proteins such as Tau and neurofilaments have been suggested appropriate to be used as axon specific biomarkers (Binder et al., 1985; Zemlan et al., 1999; Shaw et al., 2005). Tau is a microtubule-associated protein that is abundant in neurons in the CNS and it is primarily located in axons (Binder et al., 1985). One of Tau's main functions is to promote and modulate the stability of axonal microtubules, which are essential for the axonal transport in the neurons. Phosphorylation of Tau affects its function, it can disturb anterograde axonal transport (Mandelkow et al., 2003; Cuchillo-Ibanez et al., 2008) and reduce its ability to bind to tubuline (Schneider et al., 1999; Sun and Gamblin, 2009). Hyperphosphorylation of Tau can lead to self-assembly of tangles leading to tauopathies (Gendron and Petrucelli, 2009). In an experimental rat model using CCI TBI, C-Tau in cortex and hippocampus increased with increasing severity of TBI. The C-Tau in cortex increased as early as 6 h after TBI peaking at 168h post-injury. However, in the serum a significant increase was observed only 6h post-injury (Gabbita et al., 2005). Using microdialysis, high levels of interstitial total Tau were measured in the brain of TBI patients. Patients with focal lesion showed higher levels than patients with DAI (Marklund et al., 2009). In one study, the levels of C-Tau in the CSF of patients with TBI were compared to either neurologic or non-neurologic control patients. C-Tau increased 40000 fold in CSF of TBI patients. The initial increase was also correlated with elevated ICP and clinical outcome (Zemlan et al., 2002). The

correlation between increased serum Tau and outcome was demonstrated in patients with closed head injury where it also correlated with pathological findings on CT (Shaw et al., 2002).

1.3.3   Myelin basic protein

The myelin basic protein (MBP) is the most abundant protein in the white matter and it maintains compact assembly of the myelin. Following TBI, activation of calpains leads to degradation of myelin and further axonal vulnerability (Liu et al., 2006). In the mentioned study, MBP accumulated in the rat cortex at 2 hours after TBI, peaked at 1 day to 2 days, and returned to basal levels at 6 days to 7 days. In patients with TBI, a high serum level of MBP was correlated with high mortality rate (Yamazaki et al., 1995). In a study of 157 patients with head injuries, MBP was elevated significantly on admission and remained high for 2 weeks among patients with severe intracerebral damage (Thomas et al., 1978). An elevated serum level of MBP is believed to reflect the extent of myelin damage in the brain (Cohen et al., 1976).

1.3.4   Neurofilaments

Neurofilaments (NF) can be defined as the intermediate or 10nm filaments found specifically in neuronal cells (Julien and Mushynski, 1998). They are particularly abundant in axons and provide structural support for neurons and their synapses. Neurofilaments are composed of a mixture of subunits, usually including three neurofilament triplet proteins: neurofilament light, 68-70 kD (NF-L), neurofilament medium, 145-160 kD (NF-M) and neurofilament heavy, 200-220 kD (NF-H) (Ching and Liem, 1993). Disruption of NF after TBI is believed to play an important role in axonal injury. A biphasic increase of NF in serum has been shown in CCI in rats, the first phase occurring during the first hours and the second after 2 days (Anderson et al., 2008). It was suggested that these 2 peaks corresponds well with the biphasic opening of BBB following TBI.

Several additional biomarkers have been identified such as glial fibrillary acidic protein, neuron specific enolase, alpha-II-spectrin, ubiquitin C-terminal hydrolase, and many inflammatory markers such as interleukins and chemokines (Kovesdi et al., 2010; Sharma and Laskowitz, 2012). The use of microdialysis in the brain tissue and CSF sampling and its application in proteomics has opened a new exciting field in TBI research (Maurer et al., 2003). In particular, the 100-kDa molecular weight cut-off catheters provide the ability to investigate higher molecular weight biomarkers such as cytokines and chemokines (Hutchinson et al., 2005; Hutchinson et al., 2007; Helmy et al., 2009). However up until today, no single marker has been able to diagnose injury type or predict severity and outcome in TBI patients.

1.4 TBI MODELS

The classification of TBI based on GCS for trial inclusion and targeted therapies is important but mechanistic classification has great utility in modelling injuries and developing preventive measures. The seminal work of Holbourn made a first classification of a "localized injury due to scull distortion" and "injury due to rotation" (Holbourn, 1943). The physical mechanisms have been further developed and can be classified according to "impact loading" which usually results in focal injuries while "inertial loading" generally causes diffuse injuries. In an excellent review paper colleagues ask; *"do we really need to build a better mousetrap?"* (Morales et al., 2005). As a response to their question, I would answer *"maybe not better, but a bit different."*
The most commonly used TBI models can be classified as described below.

Focal *"impact loading"*:

- Weight drop model *(Fenney, Shohami)*
- Fluid Percussion Injury model *(Hayes, McIntosh)*
- Controlled Cortical Impact model *(Dixon, Hayes)*
- Missile and ballistic Injury models *(Carey, Williams. Tortella)*
- Penetrating TBI model *(Davidsson, Risling)*

Diffuse *"Inertial loading"*:
Impact

- Inertial acceleration model *(Ono)*
- Diffuse injury model *(Cernak, Vink)*
- Impact acceleration model *(Marmarou)*

Non-impact

- Inertial acceleration models *(Thibault, Genneralli, Meaney, Graham)*
- Rotational TBI model *(Davidsson, Risling)*
- Blast TBI models

1.4.1 Weight drop model

The weight drop model is considered the original TBI model (Dail et al., 1981; Feeney et al., 1981). The focal impact is produced by a free falling weight guided in a tube that is made to hit the exposed skull. The impact of the weight on the skull produces a contusion type injury. The severity of the injury can be adjusted by the height and the mass of the weight dropped and can be combined with or without craniotomy. The weight drop model is a fast and easy model, hence its popularity. However, there are limitations such as unintentional skull fractures, risk of a second rebound injury and inaccuracy with regards to the impact site.

1.4.2   Fluid percussion injury model

A model of closed head injury with fluid pressure was developed already in the 1960'ies by Lindgren et al in order to produce an "experimental brain concussion" (Lindgren and Rinder, 1969). A further development of the model, the fluid percussion injury (FPI) is one of the most frequently used focal injury models (Sullivan et al., 1976; Dixon et al., 1987; Hayes et al., 1987; McIntosh et al., 1987). In this model a craniotomy is made either centrally around the midline between the bregma and lambda or laterally. A cylindrical reservoir filled with saline is attached to a cap cemented on the place of craniotomy on the animal's skull. A strike of a pendulum at the other end of the cylindrical reservoir generates a pressure pulse that is delivered to the intact dura and causes deformation of the underlying brain. Different levels of injury severity can be produced by adjusting the height of the pendulum, which defines the force of the fluid pressure pulse transmitted through the saline reservoir. The injury caused by this model replicates clinical contusion without skull fracture mixed with diffuse injury characteristics (Thompson et al., 2005). The placement of the craniotomy has shown to be important in producing a localized ipsilateral injury or an additional contralateral injury and also affect the reproducibility and reliability of this model. The so-called lateral fluid percussion model is frequently used to generate both a focal and diffuse brain injury. A limitation of the fluid percussion model is to generate a reliable continuum of injury severity since it cannot reproduce prolonged unconsciousness. Furthermore there is a disproportional brainstem involvement and injury severity and neurogenic pulmonary oedema, adding to increased morbidity.

1.4.3   Controlled cortical impact model

The controlled cortical impact model (CCI) has been suggested to be superior to the FPI model due to a better control over mechanical factors such as time, velocity of impact and depth of resulting deformation of the brain. An additional strength of this model of TBI is the lack of risk of a rebound injury that can be seen in gravity-driven devices. A compressed air-driven metallic piston produces a controlled impact causing deformation of the brain parenchyma with an intact dura (Lighthall, 1988; Dixon et al., 1991). The model produces a focal injury similar to clinical contusions with the ability to control the severity of the injury. In addition, other features seen in clinical TBI such as subdural hematoma, increased ICP, axonal injury and coma have also been associated with CCI. However, there is a lack of brain stem deformation in this model and thus a low mortality rate.

1.4.4   Penetrating TBI models

Several models have been developed to mimic penetrating brain injuries that can be produced by missiles, gunshots or sharp objects in general (Crockard et al., 1977; Carey et al., 1989; Finnie, 1993; Tan et al., 1998). All of these models are in non-rodent animals and not in use today. With the exception of a ballistic brain injury model using a balloon inflation technique (Williams et al., 2005), no high-speed penetrating rodent

injury model has been available. The main obstacle has been the association of high mortality rate in high-speed penetrating TBI.

Recently we have reported on a newly developed rat model whereby a probe is driven into the brain parenchyma at approximately 90 m/s after being hit by a pellet accelerated from a specially designed air rifle. The speed and the depth of penetration is adjustable and highly reproducible (Plantman et al., 2012). The biomechanics of this TBI model enables survival of the animals following a high-speed penetration of brain tissue. Neurodegeneration was detected in the injured cortex 24h after injury and declined rapidly. The injured area showed a progressive expansion that had developed to a large cavity by day 14. The injured area showed also BBB defect and signs of extracellular perivascular oedema. The injured animals displayed sustained deficits in reference memory and transient attention and showed balance and motor disturbances.

1.4.5   Diffuse injury models

Diffuse brain injuries usually arise when the skull is accelerated and the brain mass, due to its inertia, lags behind or continues its motion relative to the skull (Holbourn, 1943).
Brain tissue is more likely to be injured due to rotational acceleration rather than linear because the brain is relatively incompressible while the shear modulus for the brain tissue is relatively low. The main pathological finding in rotational acceleration injury is diffuse axonal injuries (Gennarelli et al., 1982; Adams et al., 1989a; Adams et al., 1989b). Experiments on primates have demonstrated that the incidence and degree of diffuse axonal injury is strongly correlated with the direction of the head acceleration: coronal plane angular acceleration was the direction causing the longest lasting coma, while sagittal plane angular accelerations and oblique accelerations produced coma for a shorter period (Gennarelli et al., 1982).
The first models producing acceleration injury mass impacts were performed on the unconstrained head of primates (Gurdjian et al., 1954; Ommaya et al., 1971). The anesthetized animal is positioned prone on the injury device, the head is tightly fixed, and inertial loading is generated through a biphasic centroidal rotation for 110 degrees within 20 ms. These models reproduced the acceleration-deceleration force seen in human head injuries. Additional models generating acceleration brain injuries, in which different impactors stroke the head of primates, were developed by Ono (Ono et al., 1980). This model caused concussion by a frontal or occipital impact over a narrow contact area without the using of a head restraint. Haemorrhages were seen dependent on the severity of the concussion (Kanda et al., 1981). In contrast to the findings of Gennarelli et al. they did not find any correlation between concussion and angular acceleration.
Other models generated impact on the temporal region of the unrestrained skull of sheep (Lewis et al., 1996). Although the unrestrained head models may replicate some of the characteristics of human TBI, they lack injury reproducibility. There is no control over the biomechanical forces related to impact and head dynamic response. Additional acceleration models were developed to understand injuries following the movement of the head alone and these models expose the head to acceleration injuries

without any impact (Gennarelli et al., 1981; Ross et al., 1994; Smith et al., 1997; Xiao-Sheng et al., 2000; Gutierrez et al., 2001). The majority of these models were used on larger animals such as primates, pigs and rabbits. This nonhuman primate model uses a pneumatic shock tester to generate a nonimpact, controlled, single rotation, which displaces the head 60° within 10–20 ms (Gennarelli et al., 1981; Ross et al., 1994). In the model using swine, the head is secured to a pneumatic actuator through a snout clamp. The pneumatic actuator produces linear motion that is further converted to angular motion through a linkage assembly directly mounted to the device. Based on position of the head the motion can coronal or axial plane rotation (Smith et al., 1997). The cost and size of the animals in addition to limitations in behavioural outcome measures make their use difficult. Thus rodent models have been found more convenient.

In order to replicate human concussive and diffuse brain injury in rodents, without any focal damages, haemorrhages, skull fractures and bleeding, several animal models have been developed (Goldman et al., 1991; Marmarou et al., 1994; Cernak et al., 2004; Maruichi et al., 2009). These models all have in common that they are constrained impact acceleration models that can produce graded brain injury.

The one most frequently used is the Marmarou's weight drop model; it is inexpensive, easy to perform and can produce graded DAI. Despite these advantages there have been concerns regarding a second hit induced by the weight dropped on the skull. Also the movement of the weight during the fall in the Plexiglas can produce a lateralized impact with uneven distribution. Cernak et al developed a constrained impact acceleration model to improve the control and reproducibility of the impact. Although this model succeeded in this matter the impact cannot be graded (Cernak et al., 2004). Maruichi et al reported on a model based on the methodology used in the Cernak model mentioned above but made advancements to grade the impact. However, subarachnoid and intraventricular haemorrhages in addition to haemorrhages in corpus callosum were frequently observed (Maruichi et al., 2009).

1.4.6   Rotational TBI model

Despite the number of models mentioned above, none of them is able to produce a graded DAI without large quantities of contusion or haemorrhage injuries in rodents. Therefore a new model in which the heads of the rats are exposed to sagittal plane rotational accelerations resulting in graded levels of DAI have been developed (Davidsson and Risling, 2011). The range of rotational acceleration studied is 0.3 to 2.1 Mrad/s^2 and β-APP positive axons is seen in all animals exposed to head rotational trauma of 1.0 Mrad/s^2 or above. These β-APP positive axons were detected as early as 2h post-injury and were found in corpus callosum and the border of white and grey matter. In animals with high acceleration trauma β-APP positive axons were detected in the brain stem. There were also signs of axonal swelling and bulbs in the brain stem of these animals detected by FD silver staining With high acceleration trauma there was evidence of subdural and subarachnoid haemorrhages that could not be seen in low acceleration trauma. No signs of BBB changes could be detected. Serum S100B levels increased with head acceleration above 0.8 Mrad/s^2.

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Further studies on gene expression, serum biomarkers and behavioural analysis in this model will be presented in the current thesis.

1.4.7   Blast injury models

When an explosive is detonated it generates a high-pressure wave that travels outwards. This wave consists of a "shock wave" and a "blast wind". The "shock wave" that is a peak overpressure falls in a short time while the "blast wind" gives rise to a very large volume of gas, pushing air and debris outwards and acts over a longer time course. These are collectively called the "Blast wave". Recent studies have shown that despite the lack of a direct head injury, a blast trauma can cause significant brain damage (Cernak et al., 1996; Cernak et al., 2001; Kato et al., 2007; Saljo et al., 2008). In real life the blast injuries are classified according to the forces causing the injury. There are four main categories: primary, secondary and tertiary, with various additional injuries forming an additional (quaternary) group.

Primary blast injuries are caused by a shock wave hitting the body. Injuries are largely confined to the air-containing organs, such as the lungs, bowel and ears, often without external signs of injury. *Secondary blast injuries* result from the impact of fragments and larger missiles accelerated by the blast. Injuries caused by these fragments can further be categorized as penetrating or non-penetrating. *Tertiary blast injuries* result from the acceleration of the whole body or parts of the body by the blast wave causing translational impacts of the body with the ground or other fixed objects. *Quaternary blast injuries* represent a further group of various injuries including those not included in the first three groups such as: flash burns caused by the radiant and convective heat of the explosion, burns caused by the combustion of the environment, crush syndrome and/or the effects of noxious gaseous products, especially carbon monoxide, liberated in enclosed spaces.
Today there are both large-animal models of blast injury (Saljo et al., 2008; Bauman et al., 2009; Garner et al., 2009; Lu et al., 2012) as well as small-animal models (Cernak et al., 2001; Chavko et al., 2007; Elder and Cristian, 2009; Long et al., 2009) where chemical explosives are used as the source of the blast wave. Most of these models produce the primary blast injury type. The models can broadly be classified as open-field exposure, blast tube explosive and shock tube with compressed air or gas (Risling and Davidsson, 2012).

Open field exposure

The first studies of open field exposure to blast were carried out in the 1960'ies where both large and small animals were subjected to blasts with simple wave forms (White et al., 1965; Richmond et al., 1967). Although the open field experiments with large animals provide a more realistic and similar setting to that of the real scenario, they require large amounts of explosives. Furthermore, it is difficult to control the physiology of the experimental animals and reproduce the exact same experimental conditions. Today there are newly developed modified open- field models for primates with promising results (Lu et al., 2012).

Blast tubes

The blast tubes were developed by Clemendson at the Swedish FOA (Swedish Defense Research Establishment) in the 1950'ies (Clemedson and Criborn, 1955; Clemedson et al., 1957). Initially they used larger animals such as pigs. The tube was later developed for rodents. In the current rodent models of the blast tube, the anesthetized rats are fixed in special net holders to avoid movement of the body (Risling et al., 2011). The detonation charge is placed at the other end of the tube, 1m from the rat. The rats can also wear protection or be placed in a holder to avoid pulmonary injuries. The blast waves produced have short duration and a simple form. The blast tube generates mainly primary blast injuries, however the gas and smoke emission might generate a tertiary blast injury. A blast tube for larger animal such as swine is also currently in use (Bauman et al., 2009).

Shock tubes

The simplest form of shock tubes consists of two chambers, separated by a membrane called the diaphragm. One of the chambers is filled with compressed air or gas and the other chamber contains the animal. The diaphragm is ruptured and the compressed air or gas simulates a propagating blast wave. There are currently several research facilities using shock tubes (Saljo et al., 2000; Chavko et al., 2007; Long et al., 2009; Cernak et al., 2011). The one used by Cernak et al is complex, with a flexible, multi-chamber shock tube capable of reproducing complex shock waves.

1.5   PATHOLOGY OF TBI

Acute traumatic brain injury is characterized by a *primary* and a *secondary injury.* Primary brain injury is the direct injury to the brain parenchyma at the time of the initial impact with traumatic and diffuse axonal injury and neuronal disconnection. The secondary brain injury is caused by a combination of neuronal and vascular damage, proteolytic pathways, excitotoxicity, oxygen-free radicals, apoptosis, inflammatory processes and ischemia. The brain possesses several restorative properties such as neurogenesis, axonal remodelling and synaptogenesis that are induced after injury. Evidence suggests that neurotrophins and in particular BDNF play a prominent role in the cellular events that occur in these processes following TBI. BDNF may provide a neuroprotective and repair function and restore connectivity in disrupted areas by reconnection through fiber sprouting and synaptogenesis following TBI.

1.5.1   Primary injury

The primary traumatic brain injury is the result of mechanical forces that put a strain on the brain parenchyma at the time of impact. These forces may be of various forms e.g. penetrating injuries, rotational acceleration, compression and distension from acceleration or deceleration. This can lead to injuries to vessels, axons, neurons and glia in a focal or diffuse pattern. The vascular injuries may result in intracerebral, subdural,

extradural or subarachnoid haemorrhage. The damage to the parenchyma may lead to contusions or lacerations. Diffuse injuries may strike the vessels leading to multiple small haemorrhages throughout the brain or affect the white matter with no apparent focal injuries. The primary cause of diffuse injuries is head rotation and the most common microscopic pathology found is axonal injuries (Adams, 1992). One of the initial and acute changes induced by the primary injuries is alteration of gene expression. This triggers regulation of both harmful and beneficial factors that are part of the processes following the initial brain trauma (Fig.2).
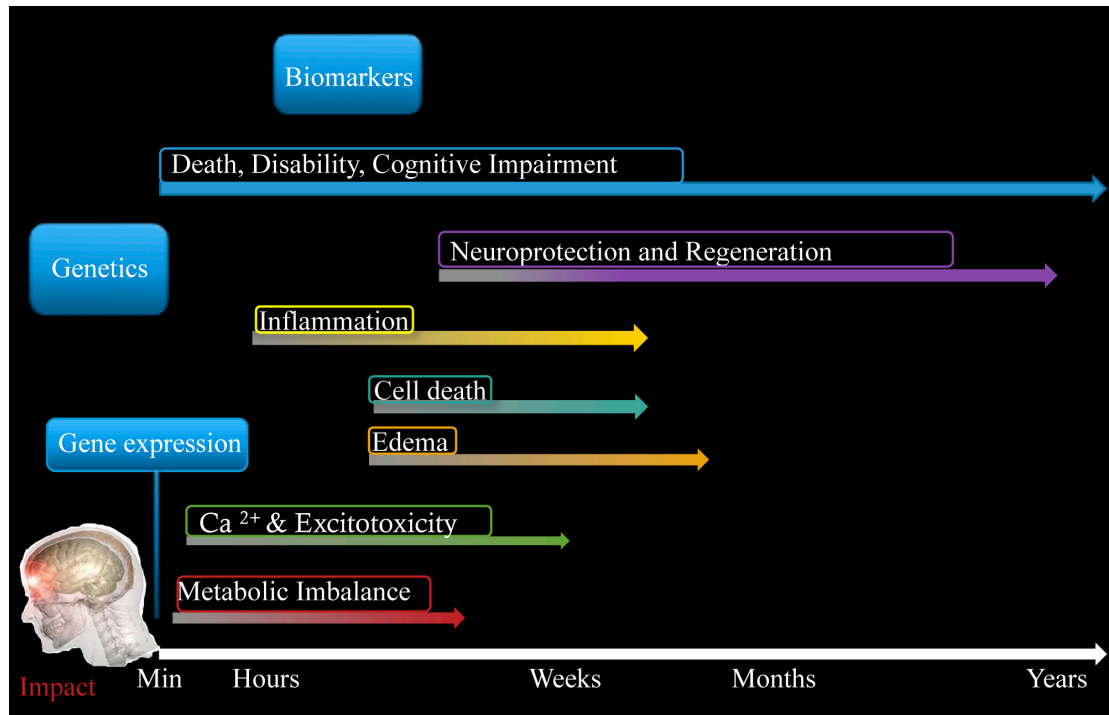

Figure 2. Basic concepts of the primary traumatic brain injury that initiates the secondary injuries. Minutes to hours following injury, ionic disturbances cause metabolic instability and mitochondrial dysfunction. Ca^{2+} plays a major role by affecting gene expression and excitotoxicity. Alteration in gene expression is one of the earliest events. Eventually these processes lead to cell swelling and cell death. Along with this, neuroinflammation and axonal injury occur. In parallel with these harmful events, a neuroprotective and regenerative process that involves neurotrophins develops. Biomarkers, in particular in the acute and subacute phase, indicating these pathological processes might help in diagnosing and even predicting the outcome of TBI patients. However, the effects of the TBI are also dependent on the genetic background of the patients.

1.5.2   Secondary injuries

An illustration of secondary injuries was made by Reilly where he described the "Walk and die" or "Talk and deteriorate" patients (Reilly et al., 1975). These patients with traumatic brain injuries showed no initial clinical signs but later developed serious intracranial complications. The secondary injuries are a result or complication that develops due to the different types of primary injuries. They can progress over hours, months and even years. Common pathways of neuronal death, posttraumatic ischemia, energy failure, excitotoxicity, mitochondrial failure, oxidative stress and release of free radicals, secondary cerebral swelling and inflammation, are all triggered by the primary

injury (Siesjo and Siesjo, 1996; Verweij et al., 2000; Morganti-Kossmann et al., 2001). In parallel with these harmful processes, an activation of neutrophins and growth factors that promote neuronal survival and plasticity occur.

The injured brain might also be subjected to *secondary insults* that are any event that may cause secondary injuries. These secondary insults are also referred to as *avoidable factors* in clinical setting e.g. hypoxia, hypercapnia, hypocapnia, arterial hypotension, hyperthermia, hyperglycaemia, hypoglycaemia and hyponatremia. A great challenge for the treatment of TBI patients in the NICU is to detect early signs of secondary injuries in order to prevent further advancement and deterioration of the brain tissue. Multimodal monitoring including ICP monitoring and microdialysis are widely used methods for detection of secondary events (Ungerstedt and Rostami, 2004). Microdialysis has been used in particular to detect ischemia and metabolic crises following TBI (Vespa et al., 2005; Nelson et al., 2011)

1.5.2.1   Ischemia

Ischemia plays a major role in the pathology of TBI; signs of ischemic brain damage are found on autopsy in more than 90% of TBI patients (Graham et al., 1978; Graham et al., 1989). Neurons are known to be very sensitive to periods of cerebral ischemia. Cerebral flow reduction of 25ml/100g/minute in rodents leads to cell death (Bolander et al., 1989). The brain has almost no capacity to survive without oxygen. Its reserved oxygen capacity will only last for a few seconds. Furthermore, the brain adenosine triphosphate (ATP), the fuel crucial for neuronal functioning, will only last for 40 seconds during ischemia (Siesjo, 1978). In addition to the severity of reduction in flow rate, the duration of ischemia is also determinant of the outcome (Heiss and Rosner, 1983). The degree of flow reduction following TBI correlates with injury severity (Graham and Adams, 1971; Dietrich et al., 1998). The level of ATP in brain tissue following TBI has also been shown to be related to the severity of the brain injury (Marklund et al., 2006). The areas most vulnerable to ischemia are the CA1 sector and dentate gyrus of the hippocampus, the dorsolateral striatum and the Purkinje neurons in the cerebellum (Kirino, 1982; Pulsinelli et al., 1982). In a severe TBI there may be both diffuse and focal injuries that can generate haemorrhagic contusions. This can directly damage blood vessels and lead to necrotic cellular elements in addition to damaging neuronal membranes in cell bodies and axonal processes. The primary injury can lead to damage of glial cells including astrocytes and oligodendrocytes. One of the earliest cellular changes observed after contusion injury is glial swelling. Regions exhibiting milder reductions in flow surround focal areas of reduced CBF following TBI (DeWitt et al., 1986; Dietrich et al., 1996). This border zone area contains scattered damaged neurons within an intact neuropil (Dietrich et al., 1994). This area is at risk for secondary insults but most importantly due to its viability it is sensitive to therapeutic interventions (Bramlett et al., 1999).

1.5.2.2 Ca^{2+} and Excitotoxicity

One of the consequential major factors that follow brain injury is a massive rise in intracellular Ca^{2+} due to failure of Ca^{2+} regulating mechanisms. This has a major effect on cell metabolism, gene expression and cell death. The fall in ATP levels impedes the ATP-utilizing Na^+-K^+ pumps, leading to a net outward leakage of K^+ that results in

progressive depolarization. Subsequently, a general depolarization occurs with a rapid outflow of K^+ and inflow of Ca^{2+} and Na^+. Na^+ is a co-transporter to remove glutamate from the extracellular space. A low extracellular Na^+ concentration in combination with increased intracellular Ca^{2+} leads to a massive release and an increase of extracellular glutamate. Increased levels of glutamate following TBI have been found both in rodents (Nilsson et al., 1990; Zweckberger et al., 2011) and humans and correlated with outcome (Hillered et al., 1992; Chamoun et al., 2010; Timofeev et al., 2011). Interestingly, areas in the brain with a high density of excitatory synapses such as the molecular layer of the hippocampus, CA1, are more sensitive to brain injury (Wang and Michaelis, 2010).

The intracellular concentration of glutamate is normally 1000 times higher than in the extracellular space. Disruption of this balance leads to additional inflow of Ca^{2+} and Na^+ with deleterious effects (Choi, 1987; Bonfoco et al., 1995). The final neuronal death is caused by:

- Activation of lytic processes (proteases, lipases and endonucleases) by Ca2+
- Cell swelling and lysis due to water accompanying the inflow of ions leading to increased intracranial pressure
- Elevated levels of free fatty acids and other lipids causing membrane damage
- Production of cytotoxic free radicals and aldehydes, especially upon reperfusion

Ca^{2+} can alter gene expression immediately after trauma. High levels of Ca^{2+} activate regulatory transcription factors such as cAMP response element binding (CREB) (Bito and Takemoto-Kimura, 2003). Furthermore, alterations in the levels of second messenger molecules lead to an immediate-early gene response. This in turn activates cellular immediate-early genes such as c-fos and c-jun which subsequently modifies transcription of target genes (Morgan and Curran, 1988). Identification of target genes involved in TBI pathophysiology will help in understanding the molecular mechanisms of neuronal damage after trauma and may lead to the development of new pharmacological and genetic therapies.

1.5.2.3 Inflammatory response

The extensive investigations on the underlying cause of TBI pathobiology during the past decades have shown neuroinflammation to be a hallmark of secondary processes in TBI (Morganti-Kossmann et al., 2001). It is characterized by glial activation, leukocyte recruitment, and upregulation and secretion of mediators such as cytokines, chemokines and complement proteins. Disruption of the BBB following TBI allows the entry of circulating neutrophils, lymphocytes and monocytes into the CNS, affecting neuronal survival and death (Clark et al., 1994; Kubes and Ward, 2000). However, the resident brain cells are capable of producing inflammatory proteins independent of peripheral immune cell activation and recruitment (Riva-Depaty et al., 1994). In the brain both glia and neurons can synthesize cytokines, chemokines and complement proteins and also express their receptors. Cytokines are intercellular signalling molecules synthesized by several immune system cells as well as by brain cells,

including microglia, astrocytes and neurons. Cytokines are key mediators in several CNS pathologies and can be broadly divided into pro- and anti-inflammatory. The pro-inflammatory cytokines interleukin-1 (IL-1), interleukin-6 (IL-6) and tumour necrosis factor a (TNF-a) increase within hours following TBI (Taupin et al., 1993; Holmin et al., 1995; Holmin and Hojeberg, 2004). IL-1 plays a major role in initiating the immune response and exacerbates neuronal injury, it also stimulates the release of chemokines (Rothwell, 1999). Inhibition of IL-1B following TBI has shown to improve histological and behavioural outcome in rats (Clausen et al., 2011). IL-6 however shows a beneficial effect following neurotrauma (Penkowa et al., 2003) and IL-6 deficiency decreases neuronal survival (Penkowa et al., 2000). TNF-a is mainly synthesized by activated microglia following injury. Studies show that the function of TNF-a may differ in the acute and the delayed phase after TBI. Inhibition of TNF-a in the acute phase following TBI in rats did not affect the behavioural outcome (Marklund et al., 2005). Initially, TNF-a seems to act as a potent immune mediator, but later as a protective neurotrophic factor that is required for repair (Ziebell and Morganti-Kossmann, 2010).

Chemokines are chemotactic cytokines, able to recruit and attract leukocytes. They are both homeostatic in normal cell processes and pro-inflammatory. IL-8 is a chemokine released by astrocytes and has been suggested to contribute to secondary injuries since high CSF levels were associated with increased mortality (Whalen et al., 2000) and severe BBB disruption (Morganti-Kossman et al., 1997).

1.5.2.3.1 Complement Cascade

The complement system, a powerful pillar of the innate immune system, has been shown to play a crucial role in many central nervous system pathologies such as Alzheimer's disease (Rogers et al., 1992), spinal cord injury (Anderson et al., 2004) and multiple sclerosis (Morgan et al., 1997). Furthermore, both experimental animal models and human studies provide convincing evidence that the complement system is activated following TBI (Bellander et al., 2001; van Beek et al., 2003; Schmidt et al., 2005).

The complement system can be activated through three different well-known pathways; the classical complement pathway, the alternative complement pathway, and the mannose-binding lectin pathway (Fig. 3). All three well known pathways converge at C3 which will generate C3b. Detection of C3b indicates the progression of the cascade towards the activation of C5 and further activation of complement factors down the cascade and formation of C5b9/MAC, finally leading to cell destruction.

The C1q complement that is a part of the classical pathway can be activated by antigen-antibody complexes or pentraxins that confirm the complement protein 1 (C1) leading to presentation of its subunit C1q that initiates the complement cascade. The next step in complement cascade that is crucial for synthesis of the end product membrane attack complex (MAC)/C5b9 is the cleavage of C component 3 to C3a and C3b. C3a is an anaphylatoxin that stimulate chemotaxis whereas C3b is a membrane-bound opsonin that enhances phagocytosis.
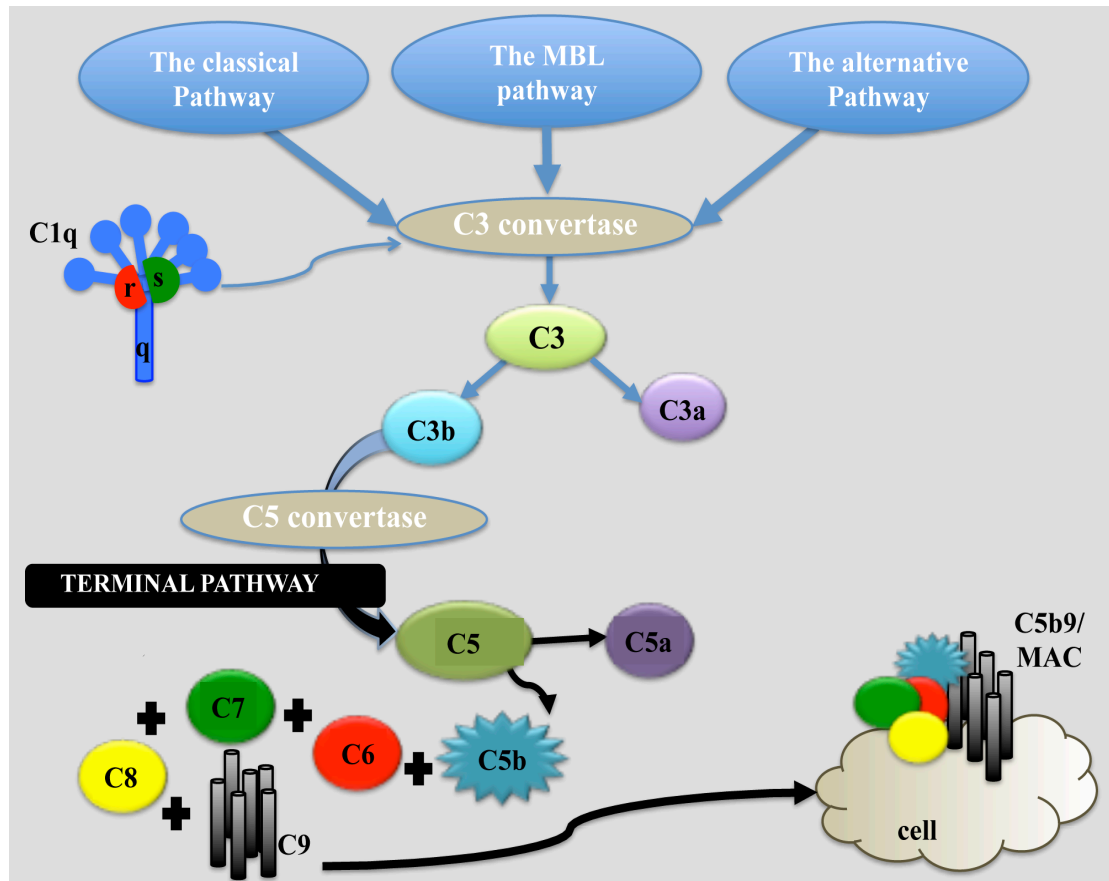
Figure 3. Three main pathways initiate the complement cascade, when upon activation all generate the protease C3-convertase. The classical pathway is activated through the C1-complex that consists of C1q, C1r and C1s. The lectin pathway is activated by mannose-binding lectin instead of C1q. The alternative pathway is continuously active at a low level but the C3b that is generated from C3 by the C3 convertase is rapidly inactivated. The generation of C3-convertase by all pathways leads to cleavage of C3 into C3a and C3b. All complement proteins with the initial (*a*) are anaphylotoxins while (*b*) stands for opsonins. Upon further activation of C5 convertase, it cleaves C5 into C5a and C5b and the terminal pathway is activated. The C5b assembles additional complement proteins and C9 and forms the membrane attack complex (MAC) or C5b9. This creates a hole in the cell membrane that eventually leads to cell death.

Recent work has shown complement mediated synapse loss in the developing and adult brain (Stevens et al., 2007) which may play a crucial role in the early events of synapse loss in neurodegenerative diseases (Selkoe, 2002). C5a is a known anaphylatoxin and the most potent mediator of complement inflammatory functions. It is also a potent chemotactic factor in the CNS (Yao et al., 1990). It has been shown to modulate the activity of many cell types with a broad range of biological outcomes such as production of free oxygen radicals, production of chemokines and cytokines as well as delayed apoptosis and phagocytosis (Lee et al., 2008). C5a receptors (C5aR) are expressed in neurones (O'Barr et al., 2001), microglia (Lacy et al., 1995), astrocytes (Gasque et al., 1997), neuronal stem cells (Rahpeymai et al., 2006) and oligodendrocytes (Nataf et al., 2001). The C5aR has been shown to be upregulated in CNS pathology such as closed head injury (Stahel et al., 1997) and in the inflamed CNS (Gasque et al., 1997). A significant decrease of serum C5a and neuronal cell

death following TBI was observed in knockout mice lacking functional alternative complement pathway, suggesting the importance of C5a and the alternative pathway in secondary brain injuries (Leinhase et al., 2006). Knockout mice lacking CD59, a complement regulatory protein that prevents MAC assembly, showed a significant increase in neuronal cell death and impaired neurological outcome after TBI (Stahel et al., 2009). Following TBI, high levels of C5b9 have been detected in brain tissue (Bellander et al., 2001) and CSF (Stahel et al., 2001) and was correlated with BBB dysfunction and secondary insults (Bellander et al., 2011).

1.5.2.4   Axonal Injury

In a paper Strich demonstrated severe white matter injuries in patients with closed and uncomplicated head injuries (Strich, 1961). None of the patients showed laceration of the brain or signs of intracranial haemorrhages or increased intracranial pressure, but displayed severe neurological deficits. Strich's post-mortem study of the brain showed severe injuries in the corpus callosum and cerebral peduncles and most interestingly by using Marchi's method, she demonstrated a widespread diffuse degeneration of the white matter in the long-surviving cases. This suggested a widespread secondary or Wallerian degeneration in the absence of any focal lesions. Furthermore, Strich reported on widespread findings of retraction balls and clubs in patients with shorter survival time. It was put forward that the rotational acceleration of the head at the time of injury leads to shear stress that stretches or tears the axons, which subsequently undergo secondary degeneration. This has later been confirmed by others where axonal injuries have been shown to be a hallmark of closed head injuries and in particular rotational head injuries (Gennarelli et al., 1982; Adams et al., 1989a; Margulies et al., 1990; Margulies and Thibault, 1992). Extensive work has been performed on the pathology of axonal injuries which are characterized by perturbation to the axoplasmic transport along the length of the axons, which may lead to axonal swelling (Povlishock and Christman, 1995; Povlishock and Jenkins, 1995; Povlishock and Pettus, 1996).

Axonal *swelling* refers to focal increases in axonal diameter with remaining continuity of the axon at both ends. In contrast, the term axonal *bulb* refers to the terminal increases in axonal diameter found after axonal disconnection. Thus in damaged nerve fibers that will eventually undergo secondary axotomy, axonal swellings precede axonal bulbs.

Altered axolemmal permeability has long been thought to be a direct consequence of the shear and tensile forces evoked by mechanical injury. This altered axolemmal permeability will allow the intraaxonal influx of normally excluded ions such as Ca^{2+} (Siesjo et al., 1999). As previously mentioned, the Ca^{2+} induced proteolytic pathways are considered key players in ensuing axonal pathology. The local intraaxonal calcium dysregulation triggers activation of cysteine protease pathways that locally degrade the intraaxonal cytoskeletal network (Buki et al., 1999). Part of this cytoskeletal network, the microtubules and neurofilaments participate in local intraaxonal transport and the disruption of these results in the accumulation of organelles and vesicles. This leads to axonal lobulation and axonal swelling. Finally the axons detach and the proximal axonal segment continues to swell due to continued delivery of organelles via anterograde transport. This leads to the formation of retraction balls. However not all

injured or altered axons undergo axonal swelling and bulb formation (Stone et al., 2001; Suehiro et al., 2001). These studies demonstrated local cytoskeletal damage without coexisting impaired axonal transport. It has been shown that local axonal cytoskeleton morphological changes such as neurofilament compaction and loss of microtubules can occur, almost without altering the general structural integrity for several hours post injury (Povlishock and Pettus, 1996). This indicates that the Ca^{2+} induced proteolytic alterations triggers a more gradual rather than immediate damage to the axons.

Axonal swelling increase in size between 2 to 6 hours in animal models (Povlishock et al., 1983; Povlishock, 1992) and 3 and 12 hours in humans (Povlishock and Christman, 1995). β-APP labelled axonal swelling occurs between 1.75 (Blumbergs et al., 1995) and 2 h (McKenzie et al., 1996) after head injury and clear examples of axonal disconnection may be found at 12h (Christman et al., 1994) after human head injury. A delayed appearance of axonal swelling has been visualized up to 88h (Grady et al., 1993) and 99 days (Blumbergs et al., 1994; 1995) after the initial injury.

The morphologic sequence of TAI in experimental modes parallels that described in patients after blunt head injury. However, the rate of axonal change is greatest in small animals and occur most rapidly in the rodents with widespread axonal disconnection 2 to 4 h post-injury (Povlishock, 1992). In humans NF-L immunolabeling showed axonal disconnection first after 12h post-injury (Grady et al., 1993) however β-APP immunolabeling demonstrated axonal bulbs as early as 3h post-injury (McKenzie et al., 1996).

1.5.3   Restorative properties

Today there is clear evidence that the human brain is plastic and possess the capacity to repair and reorganize (Gross, 2000). The study of Eriksson et al. involving human cancer patients revealed human adult neurogenesis as a real phenomenon (Eriksson et al., 1998). Well-known imaging studies on the London taxi drivers and jugglers illustrated activity dependent response in brain structure volume and evidence of plasticity in the adult human brain (Maguire et al., 2000; Draganski et al., 2004). There is also accumulating evidence that neurogenesis, synaptogenesis and axonal sprouting take place following TBI (McKinney et al., 1997; Kernie et al., 2001; Richardson et al., 2007). In the mammalian subventricular zone (SVZ) and dentate gyrus (DG) of hippocampus new functional neurons are constantly generated from neural stem cells throughout life (Lie et al., 2004). There is also evidence that injury to the adult CNS induces neurogenesis, indicating the presence of an endogenous repair process which may potentially be used for therapeutic application (Parent, 2003). A key player in these restorative processes following TBI is neurotrophins and most importantly BDNF.

1.5.3.1   Neurotrophins
Neutrophins are a family of related proteins: Nerve Growth Factor (NGF), Brain-derived Neurotrophic Factor (BDNF), Neurotrophin-3 (NT-3) and Neurotrophin-4/5 (NT-4/5) (Levi-Montalcini and Hamburger, 1951; Barde et al., 1982; Phillips et al.,

1990; Ibanez et al., 1993). The neurotrophic properties of NGF were described by Nobel-laureate Rita-Levi Montalcini (Levi-Montalcini and Hamburger, 1951). It was shown that NGF inhibits nerve terminal death and that blocking axonal transport of NGF leads to sympathetic nerve cell death. Purified from pig brain, BDNF was the next neurotrophin to be discovered (Barde et al., 1982).

1.5.3.2 BDNF

BDNF is the most abundant neurotrophin in the brain (Leibrock et al., 1989; Lipsky and Marini, 2007), playing an important role in the survival, differentiation, synaptic plasticity and outgrowth of peripheral and central neurons throughout adulthood (Thoenen, 1995; Huang and Reichardt, 2001; Poo, 2001). The BDNF gene contains 11 exons and spans about 70 kb. Nine of these exons are transcription start points, each of which are associated with a functional promoter (Pruunsild et al., 2007). BDNF is synthesized as a precursor protein (35kDa) that is cleaved intracellulary to yield the mature, biologically active form of the protein (26kDa). The pro region of BDNF plays a significant biological role involving folding, secreting and p75^{NTR} receptor binding. BDNF knockout mice suffer severe neuronal deficits and an early postnatal death (Ernfors et al., 1994).

BDNF mediates its effect through high affinity tyrosine kinase receptor TrkB (Klein et al., 1991). There are several alternatively spliced isoforms of TrkB, including a full-length, catalytically active kinase receptor (TrkB-FL) and several truncated isoforms (TrkB-T), with TrkB-T1 being the most abundant. TrkB-T receptors bind neurotrophins with the same affinity as TrkB-FL receptors, but they lack the catalytic intracellular tyrosine kinase domain necessary to transduce signals via classical pathways (Middlemas et al., 1991). It has been shown that when the truncated form is co-expressed with the full-length it inhibits the BDNF signalling (Eide et al., 1996). Increased expression of TrkB mRNA for truncated receptors has been detected the site of injury following spinal cord injury (Frisen et al., 1993) and suggested that it might bind and present BDNF to axonal growth cones (Frisen et al., 1992). Additional function attributed to TrkB-T that these receptors isolate the available neurotrophins and prevent their interaction with trkB-FL receptors (Biffo et al., 1995). It was shown that truncated receptor on non-neuronal cells inhibit BDNF neurite outgrowth by internalizing BDNF (Biffo et al., 1995; Fryer et al., 1997). Making these receptors as a "molecular sponge" that soak and remove the BDNF necessary for axonal growth. However, recent evidence has shown that TrkB-T can activate intracellular signalling, regulate cytoskeletal changes in neurons and Ca^{2+} release in astrocytes, suggesting a more active and independent role of TrkB-T (Rose et al., 2003; Fenner, 2012).

BDNF also binds to a pan-neurotrophin receptor p75^{NTR}, that belongs to the tumour necrosis factor receptor (TNFR) family (Dechant and Barde, 2002). It was initially identified as a low-affinity receptor for nerve growth factor (LNGFR) but was later shown to bind all members of the neurotrophin family with similar affinity (Rodriguez-Tebar et al., 1990). The p75^{NTR} receptor has been suggested to have a dual role both as a facilitator of Trk-mediated neuronal survival and as a regulator of neuronal cell death. It facilitates the binding of neurotrophins to Trk receptors (Zaccaro et al., 2001) and

20

during development, p75^{NTR} is important for axon growth and innervation (Bentley and Lee, 2000). However, the binding of neurotrophins to p75^{NTR} receptor has been shown to cause cell death in the nervous system (Frade et al., 1996). In addition, it has also been shown to mediate axonal growth inhibition by myelin-derived neurite growth inhibitors (Wang et al., 2002).

Several hundred polymorphisms in the BDNF gene have been reported so far and two of them are known to impact the expression level of BDNF. The first one is BDNF-LCPR (BDNF-linked complex polymorphic region), a 5'-UTR located microsatellite polymorphism that is associated with an elevated risk of bipolar disorder and a lower transcription level of BDNF (Okada et al., 2006). The second BDNF polymorphism is the SNP rs6265, which produces a missense mutation Val66Met (196G/A) and affects the regulated secretion and neuroplastic effect of mature BDNF (Egan et al., 2003). Several studies have shown an association between the BDNF polymorphism and episodic memory (Egan et al., 2003) and hippocampal volumes (Hariri et al., 2003; Pezawas et al., 2004; Szeszko et al., 2005; Bueller et al., 2006). An association between a BDNF polymorphism and different neuropathological conditions such as Alzheimer's disease (Huang et al., 2007), obsessive-compulsive disorder (Hall et al., 2003), eating disorders (Ribases et al., 2003) and bipolar disorder (Neves-Pereira et al., 2002; Sklar et al., 2002) has also been documented, as well as an association with executive functions in TBI patients (Hicks et al., 1997).

There are a limited number of studies on the role of BDNF and its receptors following TBI (Yang et al., 1996; Hicks et al., 1997; Hicks et al., 1998a; Hicks et al., 1999a; Hicks et al., 1999b). Furthermore, these studies have been focused on the acute and subacute period following TBI and not the chronic state. In addition, there are no reports on the expression of TrkB-T1 or p75^{NTR} receptors following TBI.

1.5.3.3   Plasticity and TBI

A century ago, Cajal proposed that the brain possesses the ability to remodel and strengthen neuronal cortical connections following lesions, based on experience. This concept is now known as restorative plasticity (Stahnisch and Nitsch, 2002). The adult mammalian brain neurogenesis persists in distinct regions, the subgranular zone of the dentate gyrus (DG) in the hippocampus, in the forebrain subventricular zone (SVZ) and in the ependymal layer. Neurogenesis has been shown to occur in the adult human brain (Eriksson et al., 1998). Several in vivo studies have proposed the occurrence of lesion-induced cortical neurogenesis (Gu et al., 2000; Magavi et al., 2000; Jiang et al., 2001; Magavi and Macklis, 2001) and that functional improvement after permanent lesion is related to lesion-induced plasticity in the intact brain tissue (Jenkins and Merzenich, 1987; Johansson and Grabowski, 1994; Nudo and Milliken, 1996; Buonomano and Merzenich, 1998; Xerri et al., 1998; Hallett, 2001). Especially the ependymal cells have been shown to proliferate after injury and migrate to the site of injury where they differentiate to astrocytes (Johansson et al., 1999). The plasticity in the brain has received extensive interest during studies of the injured brain (Will et al., 2004; Pascual-Leone et al., 2005; Seitz et al., 2005; Raymont and Grafman, 2006) and there are several studies that demonstrate the central role of BDNF in brain plasticity. It regulates vesicle docking in on going synaptic functioning and enhances

synaptic transmission (Pozzo-Miller et al., 1999; Tyler and Pozzo-Miller, 2001). Furthermore, BDNF is essential for synaptic remodelling in the adult hippocampus (Heldt et al., 2007). At the synaptic level, plasticity can occur in relation to increased dendritic spine formation, pruning, and remodelling (Ethell and Pasquale, 2005); calcium channel regulation (Catterall et al., 2008); changes in N-methyl-d-aspartic acid (NMDA) receptors (Cull-Candy and Leszkiewicz, 2004); or changes in α-amino-3-hydroxyl-5-methyl-4-isoxazole-propionate (AMPA) receptor trafficking (Kessels and Malinow, 2009).

Over thirty-five years ago Blinzinger and Kreutzberg described the phenomena of "synaptic stripping" where transection of peripheral nerves lead to the pruning of synapses from the affected neuronal bodies (Blinzinger and Kreutzberg, 1968). It was suggested that microglia cells removed synapses from the injured neurons. In a more recent study, the role of complement proteins in synaptic removal have been suggested that synapses are tagged by C1q for phagocytosis (Stevens et al., 2007).

Many of the neuronal signals driving plasticity involve the activation of specific genes; hence the genetic variation in humans might influence plasticity-related events and subsequently also the outcome in TBI patients.

1.6   GENETICS AND TBI

In striving to explore the pathophysiological heterogeneity in TBI patients' genetic information becomes crucial. Recent studies on the genetic influence and outcome following TBI have identified several genes to be important.

The most extensively studied gene in TBI patients is the apolipoprotein E (ApoE), which encodes for a cholesterol carrier lipoprotein. ApoE is polymorphic and exists in three isoforms ε2, ε3, and ε4, where the ε4 allele has been identified as a susceptibility gene for late onset familial and sporadic Alzheimer's disease (Corder et al., 1993). Many neurobiological functions are associated with ApoE such as neurofibrillary tangle formation, antioxidant activity and mitochondrial damage, neuronal repair and neuroprotection, synaptic plasticity as well as memory function. TBI patients with the ApoE ε4 allele had longer hospital stays (Chiang et al., 2003), larger intracranial hematomas (Liaquat et al., 2002) and unfavourable outcome defined as death, vegetative state, or severe disability (Teasdale et al., 1997; Friedman et al., 1999). Several studies suggest that ApoE may influence cognitive and/or behavioural functioning following TBI (Crawford et al., 2002; Liberman et al., 2002; Sundstrom et al., 2004), however these results are mainly based on mild and non-penetrating TBI cases. Also, in sports related TBI with mild but repetitive impacts to the brain as in for example American football and boxing, a correlation between cognitive outcomes and the presence of the ApoE ε4 allele has been shown (Jordan et al., 1997; Kutner et al., 2000). ApoE and ApoE promoter genes may impact the outcome by influencing the brain's ability to repair and/or protect against injury and may involve mechanisms similar to those taking part in the pathophysiology of Alzheimer's disease.

The catechol-o-methyltransferase (COMT) gene encodes for the enzyme that inactivates dopamine (DA) and norepinephrine (NE) and exists in three isoforms: (COMT Val/Val, COMT Val/Met, and COMT Met/Met). These three functional polymorphisms differentially affect DA levels (Weinshilboum et al., 1999) and have

been shown to affect DA associated cognitive functions (Akil et al., 2003). Patients with TBI that present the high enzyme activity polymorphism (Val/Val), and presumably lower cortical DA levels, performed worse on cognitive tests compared to patients with the low activity polymorphism (Met/Met) and presumably higher cortical DA levels (Lipsky et al., 2005). However, conflicting results have been reported (Krueger et al., 2011). The COMT gene may modulate dopamine dependent cognitive processes such as executive/frontal lobe functions that are invariable affected by TBI.

Other candidate genes reported are the interleukin genes that mediate inflammation. They are prominent component in the pathophysiological cascade initiated by TBI. The polymorphisms of the p53 gene may modulate apoptosis that occurs as a consequence of TBI. The ACE gene may affect TBI outcome via mechanisms of cerebral blood flow and/or autoregulation and the CACNA1A gene may exert an influence via the calcium channel and its effect on delayed cerebral oedema.

1.7   CHALLENGES IN TBI RESEARCH

Traumatic brain injury is one of the most complex CNS disorder. Patients suffering from the consequences of TBI are heterogeneous in term of the type and severity of injury and their conditions are also dynamically changing. Currently however, there are only limited tools available identify the on going pathological processes, monitor progress and direct evidence based therapies. In the absence of such tools, clinicians need to treat a highly heterogeneous condition in a rather homogenous way.

Animal models that mimic a single type of primary impact resulting in TBI can help in identifying signatures that are characteristic or unique for the given type of TBI. Unfortunately, results from animal models have had limited diagnostic and therapeutic implications in the clinical setting.

The challenges for successfully translating data from bench to bedside include:

- The anatomical and physiological differences between the human and rodent body especially the heads and CNSs.
- The differences in our ability to compare neurobehavioral outcomes measured in humans vs. rodents especially higher cognitive functions.
- Connecting the pathological changes underlying the different types of TBI in animal models to human conditions and finding signature biomarkers for the different types of injuries.
- Comparing experimental data obtained in a genetically homogeneous animal population with clinical observations derived from patients with different genetic backgrounds in terms of response to injury and recovery.

2 AIMS

The overall aim of this thesis was to address some of the issues identified above by performing a translational study.

The **Specific Aims** were:

Aim 1: To identify the specific transcriptional responses and the histopathological changes to non-impact (blast), inertial loading (rotational) and impact loading (penetrating) types of traumatic brain injuries using rodent models *(Study I).*

Aim 2: To identify secondary injury mechanisms through analysing changes in serum protein biomarker levels and behavioural outcomes after inertial loading (rotational) and impact loading (penetrating) types of traumatic brain injuries using rodent models *(Study II)*.

Aim 3: To identify the inflammatory, especially the complement response after inertial loading (rotational) and impact loading (penetrating) traumatic brain injuries using rodent models *(Study III)*.

Aim 4: To identify the role of genetic background with a specific focus on BDNF polymorphisms on the long-term outcome following impact loading (penetrating) TBI in humans *(Study IV)*.

Aim 5: To investigate the effect of impact loading (penetrating) TBI on the temporal and spatial expression of BDNF and its receptors using a rodent model in order to better understand the molecular mechanisms of the clinical findings in study IV *(Study V)*.

3   MATERIALS AND METHODS

3.1   ANIMALS

In all the studies included in this thesis male Sprague-Dawley rats were used. The weight ranged between 250-500 g. All work was performed in accordance with the Swedish national guidelines for animal experiments, and was approved by the animal care and use ethics committee in Umeå and Stockholm.

The animals were deeply anaesthetized by using a 2.4 ml/kg intra-abdominal injection of a mixture of 1ml Dormicum® (5 mg/ml Midazolan, Roche), 1 ml Hypnorm® (Janssen) and 2 ml of distilled water. Thereafter the objects were given approximately 0.2 ml/kg intra-muscular injections every 0.5 hours until the trauma and following surgery had been performed. The animals were either exposed to the blast-injury, the penetration injury or the rotational injury model.

3.2   TBI MODELS

3.2.1   Blast TBI

A 1.5 m long metal blast tube for small animals was used for the primary blast experiments (Fig. 4). The tube was initially described by Clemedson (Clemedson and Criborn, 1955) but has since then been modified by Suneson for use with rats (Saljo et al., 2000). It was mounted with a holder that limited acceleration movements and employed a non-electrical ignition process for detonation. Briefly, the animal was placed in a metallic net holder at a distance of 1 m from the charge, which is usually 1–2 g of pentaerythritol tetranitrate (PETN) explosive, resulting in a shock wave at the surface of the animal with a duration of 1–2 ms and a peak pressure of 136–236 kPa. A pressure above 236 kPa results in lethal bleeding from the airways in more than 50% of the animals. The animal was mounted with the side facing the charge. A level of 196 kPa was used for the gene array experiments. This pressure level has been used in previous studies on cell death in the inner ear, as well as MRI and EEG analysis (Kirkegaard et al., 2006).
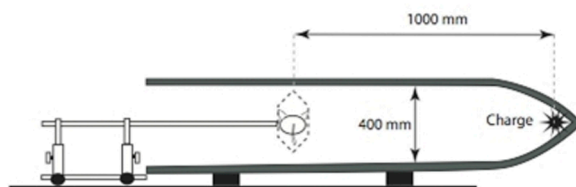

Figure 4. A schematic presentation of the blast tube.

3.2.2   Rotation TBI

For this injury a preparation of the skull of the animal is needed to attach a skullcap. A midline incision was made through the skin and periosteum on the skull vault, and parts of the frontal, nasal and parietal bones were freed from adherent tissue. The exposed

bone was treated with 15% phosphoric acid for 3 min, thoroughly rinsed with tempered distilled water, and dried for 3 min with an air drier providing air at 37 °C. The exposed bone was then gently sanded prior to gluing (Super-Bond C & B; Sun Medical Co., Shiga, Japan) of a curved aluminium plate, denominated the skullcap, shaped to match the contour of the exposed skull (35 mm long and 2–9 mm wide). The dental glue was allowed to cure for 15 min at 37 ° C. An attachment plate was fastened by means of two screws to the skullcap previously glued to the rat skull bone. The attachment plate was then inserted and secured to a revolving bar that can rotate freely around a horizontal axis. The resulting pre-trauma position of the head was slightly flexed and the brain centre of gravity was located about 6.5 mm above the centre of rotation (Fig. 5). This is equivalent to a centre of rotation located 1 mm below the head base and 5 mm forward of the front of the foramina magnum. A solid brass weight, denominated the striker (weight 10g), was accelerated by air pressure and hit the polyurethane bumper on the striker target (aluminium plate 6mm thick and 15 mm wide). The resulting impulse subjected the animal's heads to a short sagittal plane rearward rotational acceleration, which lasted about 0.4ms. This was followed by a rearward rotation at near constant velocity and, finally, the heads came to a stop by a deceleration of about 25% of the initial acceleration. The entire trauma caused the head and neck to extend 25°. The rotational acceleration magnitude was selected by modifying the striker speed that was varied by means of modifying the air pressure in a specially designed air driven accelerator. This method has previously been described in detail (Davidsson and Risling, 2011).
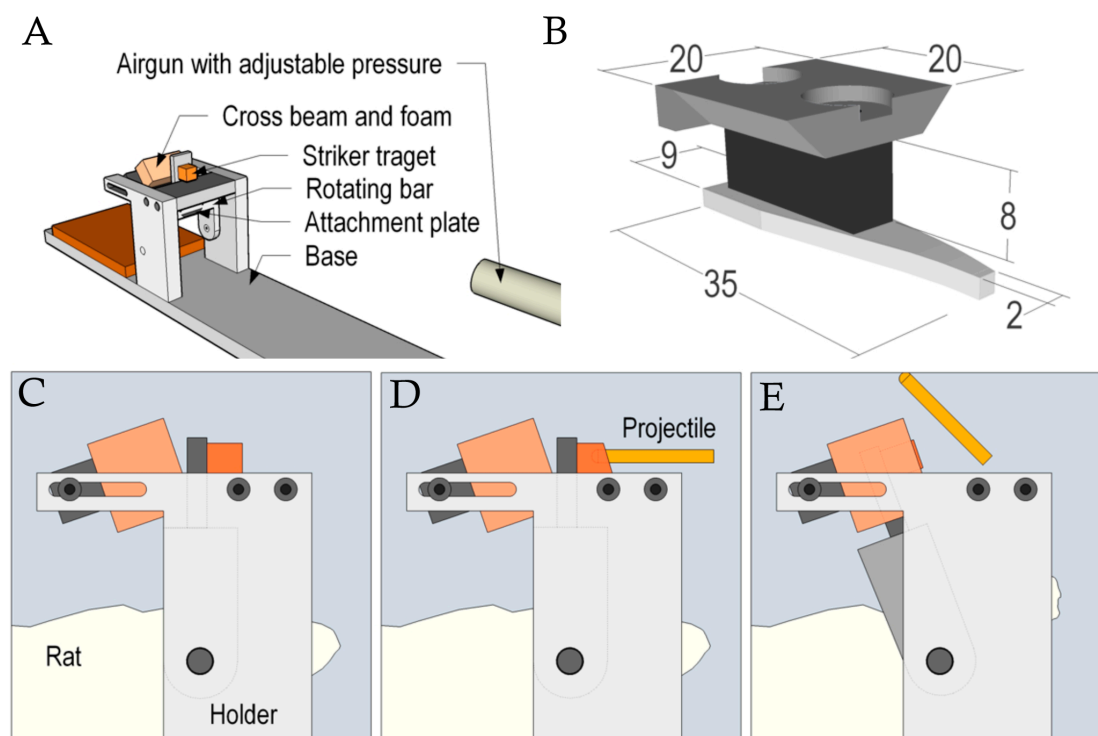

Figure 5. Schematic representation of the model for rotational TBI. In Figure (A), the relation between the accelerator (modified air gun) and the head holder is represented. The attachment plate for the rat head is shown in (B). A metallic level, which extends to the top of the test rig, is mounted on this attachment plate. (C–E) is a graphic representation of a schematic sequence which shows how the striker projectile hits the level in (D), which results in the backward rotation in (E).

3.2.3   Penetration TBI

A midline incision was made through the skin and periosteum, and a burr hole 2.7mm in diameter was drilled with its centre 3mm lateral and 3mm posterior to the bregma. Exposing the brain before the penetration prevents a secondary penetration from bone fragments. The head of the animal was fixed in a stereotactic frame in order to avoid acceleration injury. The impact probe was positioned directly above the dura exposed by the burr hole. The penetration speed could be adjusted between 1 and 100 m/s. A projectile consisting of a metal cylinder with an attached carbon fiber pin (length 24 mm, diameter 2 mm) was accelerated by the impact of a pendulum or an air pressure driven lead bullet (Fig. 6). A narrow tube guided the pin of the penetrating projectile and the penetration depth was limited to 5 mm by the use of a special cuff. This method has previously been described in detail (Plantman et al., 2012).
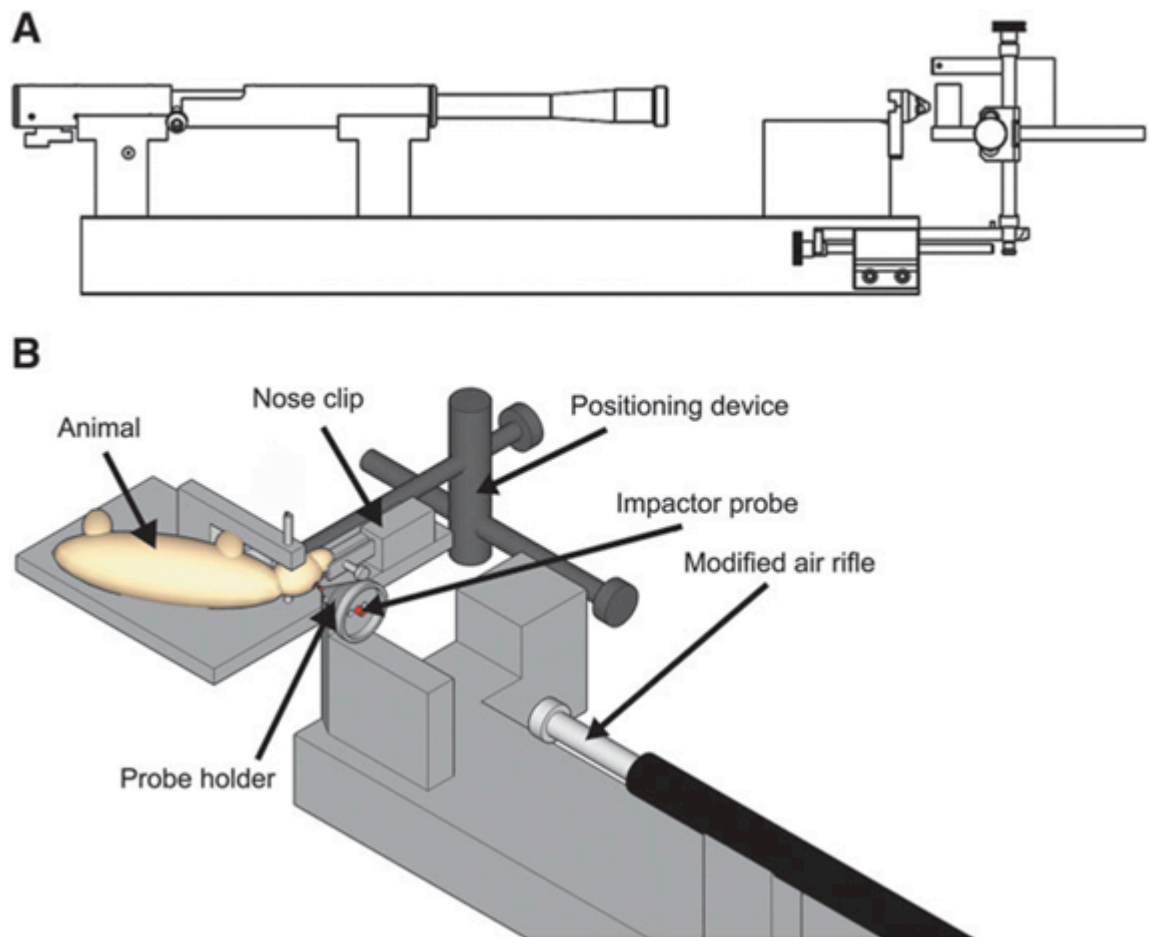

Figure 6. Schematic presentation of the penetration TBI. (A) Schematic side view of the rig showing the modified air rifle, probe holder, and stereotactic frame that holds the animal. (B) Oblique view of the rig showing the barrel, impact probe, probe holder, and the position of the animal.

3.3   AFFYMETRIX GENE MICROARRAY

Affymetrix microarrays are high throughput assays for measuring the expression levels of thousands of gene transcripts simultaneously. It uses the principle of hybridization of complementary nucleic acid sequences to specifically pair with each other by forming hydrogen bonds between complementary nucleotide base pairs. A microarray contains oligonucleotides or probes that can be a short section of a gene or other DNA element. These will hybridize with a cRNA/mRNA in the sample. The target mRNA is fluorescently labelled and the data is captured as an image. The image intensity is then correlated with the amount of mRNA in the sample. Microarrays use relative quantization in which the intensity of an e.g. control sample is compared to the intensity of a manipulated sample.

In Study I, twenty-four hours after the exposure the animals were anesthetized and sacrificed and the brains were removed. The hippocampus was dissected out and immersed in RNA later (Qiagen, Crawley, West Sussex, UK). For penetration TBI, the border zone around the cortical penetration was subjected to the same examination. RNA samples were brought to the Karolinska Institutet core facility for Bioinformatics and Expression Analysis (www.bea.ki.se), where target preparation and hybridization to the microarray was completed. RNA was labelled with biotin to produce the final target according to Affymetrix standard procedures (www.affymetrix.com). Labelled cRNA was then hybridized to each GeneChip® Rat Expression Array 230A (Affymetrix) (primary blast) or Affymetrix Rat Gene ST 1.0 array (rotation and penetration TBI). After probing and scanning, the quality of the images was checked. All arrays passed the Affymetrix quality control check. Following normalization, the change in gene expression between the three controls (anesthetized but not exposed), and the three exposed rats was compared using an unpaired t-test and fold change values. Lists of genes that passed the selected significance level were uploaded to the Database for Annotation, Visualization and Integrated Discovery (DAVID) (http://david.abcc.ncifcrf.gov) for functional annotation and detection of enriched functional-related gene groups and enriched biological themes, particularly GO terms (Dennis et al., 2003; Huang da et al., 2009). The 230A GeneChip were analysed using the Affymetrix® Microarray Suite (MAS) software. Following quality check and normalization, a 3 times 3 comparison was performed, comparing GeneChip from control animals (n = 3) to GeneChip from blast exposed animals (n = 3). The change p-value and an associated change (increased or decreased) as well as the signal log ratio were calculated using the algorithms of MAS. Probe sets that were either increased or decreased in at least 6 out of 9 comparisons were selected.

3.4   IMMUNOHISTOCHEMISTRY

The mid region of frozen brain tissue was cut by Cryo-Star HM 560 M (MICROM International GmbH) in coronal sections with a thickness of 14 μm and placed on Superfrost Plus slides. They were subsequently incubated in a humid chamber at 4° C for 24 h with primary antibodies that were diluted in a solution of 0.3% Triton, 5% bovine serum albumin and 0.1% sodium in 0.01 M PBS. Donkey serum (5%) was

added to minimize background staining. The list of all primary antibodies used is given in Table 1. The secondary antibodies were preabsorbed against the tissue as specified by the manufacturer. After the sections were rinsed in PBS, they were mounted in a mixture of glycerol and PBS (1:2) and cover slipped.

Sections were examined in a Nikon E600 microscope (Nikon, Shinjuku, Japan) using appropriate filter settings or a confocal C1 unit. Images were captured with a Nikon Digital Sight DS-U1 (5 megapixel) camera, controlled with Nikon NIS Elements software.

Table 1. List of all primary antibodies used in different Studies and methods.

Antibody	Tissue	Species	Manufacture	Dilution	Method	paper
Occludin	Brain	Goat polyclonal	Santa Cruz	1:100	Immuno	I
NF (PAN)	Brain	Mouse Monoclonal	Zymed	1:100	Immuno	I
GFAP	Brain	Rat Monoclonal	Invitrogen	1:100	Immuno	I
SMI71	Brain	Rat Monoclonal	SMI	1:100	Immuno	I
Glut-1	Brain	Goat polyclonal	Santa Cruz	1:100	Immuno	I
Nestin	Brain	Goat polyclonal	Santa Cruz	1:100	Immuno	I
APP	Brain	Rabbit polyclonal	Zymed	1:100	Immuno	I, II, III
S100B	Serum	Rabbit polyclonal	Abcam	1:20	RPPM	II
MBP	Serum	Rabbit monoclonal	Santa Cruz	1:20	RPPM	II
Tau	Serum	Goat polyclonal	Santa Cruz	1:20	RPPM	II
NF-H	Serum	Rabbit polyclonal	Sigma	1:20	RPPM	II
C3	Brain	Rat Monoclonal	Hycult BT	1:50	Immuno	II
C5b9	Brain	Mouse Monoclonal	Hycult BT	1:50	Immuno	II
ED1	Brain	Mouse Monoclonal	AbD Serotec	1:1000	Immuno	II
CD11b	Brain	Mouse Monoclonal	Merck Millipore	1:100	Immuno	II
NeuN	Brain	Mouse Monoclonal	Merck Millipore	1:100	Immuno	II
C3	Serum	Mouse Monoclonal	Santa Cruz	1:20	RPPM	III
C5b9	Serum	Mouse Monoclonal	Santa Cruz	1:20	RPPM	III
GADPH	Brain	Mouse Monoclonal	Novus Biologicals	1:1000	WB	III
C5b9	Brain	Mouse Monoclonal	Santa Cruz	1:500	WB	III
BDNF	Brain	Rabbit polyclonal	Santa Cruz	1:20	RPPM	V

3.5   IN SITU HYBRIDIZATION

The frozen brain tissue was cut by Cryo-Star HM 560 M (MICROM International GmbH) in coronal sections with a thickness of 14 microns (μm) and placed on Superfrost Plus slides. Synthetic oligonucleotides were synthesized (CyberGeneAB, Huddinge, Sweden). The sequence of the probes was checked in a GeneBank database search to exclude significant homology with unrelated genes. The sequences of all probes used are given in Table 2. The probes were labelled at the 3′-end with deoxyadenosine- alpha-(thio)triphosphate[33P] (NEN, Boston, MA, USA) using terminal deoxynucleotidyl transferase (Amersham-Pharmacia, Uppsala, Sweden) to a specific activity of $1.5–6 \cdot 10^5$ cpm ∕ lL and hybridized to the sections, without pre-

treatment, for 16–18 h at 42 C. The hybridization mixture contained: 50% formamide (Fluka ⁄ Sigma-Aldrich, Sweden), saline sodium citrate buffer (0.15 m NaCl and 0.015 m sodium citrate), Denhardt's solution (0.02% each of polyvinyl-pyrrolidone, bovine serum albumin and Ficoll), 1% sarcosyl (N-lauroylsarcosine; Sigma-Aldrich), 0.02 m phosphate buffer (pH 7.0), 10% dextran sulphate (Amersham-Pharmacia), 500 μg⁄mL sheared and heat-denatured salmon sperm DNA (Sigma-Aldrich) and 200 mM dithiothreitol (Sigma-Aldrich). Following hybridization, the sections were washed several times in saline sodium citrate buffer for 15 min at 60°C, rinsed in distilled water and dehydrated in ascending concentrations of ethanol. The sections were then coated with NTB2 nuclear track emulsion (Kodak, Rochester, NY, USA). After 2–4 weeks, the sections were developed in D-19 developer (Kodak) for 4 min at room temperature, fixed in AL-4 fixative (Kodak) for 5 min and cover slipped. Some of the slides were counterstained with cresyl violet (C5042, Sigma, USA), dehydrated in ascending concentrations of ethanol and mounted in Entellan (Histolab Products AB, Gothenburg, Sweden). Sense probes for the different probes were also used as negative controls. Photomicrographs were captured in a microscope in bright field or cold light dark field illumination (Nikon), digitized by using a digital camera (Digital Sight, U1, Nikon) and analysed U1 with Eclipsenet software.

Table 2. List of all probes and their sequences used in the different Studies.

Probe	Sequence	Paper
GFAP	TGA AGT GG AGG CAG TTG CGT TGA TGG TGG CTG TCT TGG TAG GCT GCG GG	I
VEGF	TCG ACG GTG ACG ATG GTG GTG TGG TGG TGA CAT GGT TAA TCG GCT TTT CC	I
C1q	CCA TCC CTG AGA GGT CTC CAT GAT GTT CCT GCC TCC GTG GTC CGC TCG	III
C3	CCT CCT TTC CAT CAA CTG CAC TGA GCG ACG GCG GCG GGC AGC TGG CTT	III
BDNF	AUTCCAGTGCCTFTTCTCTATGCCCCTGCAGCCTTCCTTCCTCTAACCC	V
TrkB tr	CCTTTCATGCCAAACTTGGAATGTCTCGCCAACTTGAGCAGAAGC	V
TrkB full	GAGAGGGCTGGCAGAGTCATCGTCGTTGCTGATGACGGAAGCTGGGC	V
p75NTR	ACAAGGCCCACGACCACAGCAGCCAAGATGGAGCAATAGACAAGGAATGAG	V

3.6   BEHAVIOURAL TESTS

The behavioural tests were used in study II, starting 3 days following injury. There might have been more behavioural deficits in the acute phase following TBI. However, we believe that using the subacute phase rather than the acute phase, excludes possible effects of anaesthesia and surgical intervention as contributing factors to behavioural impairment.

3.6.1   Beam walking test

Fine motor coordination was assessed by using a beam-walking test. This test essentially examines the ability of the animal to remain upright and to walk on an elevated and relatively narrow beam (Goldstein and Davis, 1990). The setup consisted of a rigid 2.5 cm wide flat beam of 1.5 m in length leading to a brightly decorated goal-box. The beam was placed 50 cm above the floor and foam cushions were placed underneath the beam. Three days before habituation, animals were deprived of food until their body weight was reduced to 85% of the initial free-feeding weight (approx. 3 days). In the habituation phase (2 days) the rat was first placed into the goal-box to feed on the reward pellets provided (45mg, Raspberry flavored, Testdiet, UK). Subsequently, progressive beam training was initiated, starting from the beam-end closest to the goal-box and progressing gradually to the opposite end. At testing, the time taken for the animals to cross the 1m length of the beam was recorded with a stopwatch. Animals were finally tested 1 day prior to surgery. Thereafter testing was performed at 3 through 7 days post surgery. Animals that were unable to perform the test were designated "immobile", and no time was entered. However, the animals were kept and re-assessed at each testing interval.

3.6.2   Elevated plus maze

Elevated Plus Maze (EPM) relies on the animal's preference for dark and enclosed spaces over bright, exposed areas. It involves a conflict between the desire to explore and the anxiety of exposure and height (Lau et al., 2008). The EPM has a centrally placed open platform (height above floor: 50 cm) from which four 30 cm long arms extend, two open (i.e. without walls) and two closed (i.e. with 30 cm high walls) (Walf and Frye, 2007). The experimental procedure was initiated by the placement of the rat on the central platform with its head facing a closed arm. The rat was then allowed to roam the maze without any visual or audio distractions for 5 minutes. The whole EPM exposure was video-recorded, and various behavioural patterns were subsequently counted and timed. This included the following, 1) time spent in open arms 2) time spent in closed arms 3) number of rearing and 4) number of central platform crossings. The rats were tested on the EPM at 3 days post-injury.

3.6.3   Radial arm maze

To measure working and reference memory, we used the radial arm maze (RAM). The maze (Panlab, Spain) consisted of an octagonal central platform with eight automated sliding guillotine doors giving access to eight radiating arms of equal lengths (measurements of the maze is as follows: width; 1690 mm, length; 1250 mm and height; 1450 mm). Each arm contains 2 pairs of photoelectrical cells mounted on the proximal and distal ends of the arm to differentiate between arm entries and visits. In addition, a food site is located at the end of each arm. The contents of the food sites are not visible from the central platform. Prominent extra-maze visual cues are present to allow spatial recognition of arm position. During habituation phase (2-3 days) the food-restricted rats were familiarized with the maze. All doors were open and food rewards

were scattered around the maze to entice the rats to explore. After the rats had been allowed to freely explore the maze and had consumed the food rewards at the food disc at the distal arm, the training phase started (span of 10 or more days). To measure reference memory, the rats were trained to retrieve 4 food pellets from each selected baited arm only once. To train the rats to do so, they were first placed in the centre arena with all doors closed. After 5 s, all eight doors to the radial arms were opened and the rats were allowed to explore the RAM until the entrance into one of the arms was detected. At that time, all doors closed, except to the arm being visited. After the animal returned to the central area, the open door was closed. This was followed by a confinement time (5 s) and then the doors reopened for a new round of choice. This cycle was repeated automatically until the rats had visited all four baited arms or after 10 min had elapsed. For each trial, the arm choice, latency to obtain all the pellets (i.e. response latency), and the number of visits to each arm was automatically recorded by the MazeSoft software (Panlab, Spain). After each run, the maze was cleaned with absorbing paper to prevent a bias due to olfactory cues. Over time, the rats would also learn that certain arms were not baited and avoid them accordingly. Animals were trained twice each day until a stable baseline performance (>75% accuracy) was reached. After surgery and exposure to trauma or sham exposure, rats were tested daily, starting at 72 hours post injury and ending at seven days.

3.7   REVERSE PHASE PROTEIN MICROARRAY (RPPM)

3.7.1   Preparation of samples

Sample preparation, printing, scanning, and data analysis was performed as described in detail (Gyorgy et al., 2010). Briefly, the blood was drained and centrifuged and serum was collected, aliquoted and frozen on dry ice for proteomic analysis. Fresh frozen brain samples were pulverized in liquid nitrogen; 200 mg of the powder was transferred into 1 ml of T-per lysis buffer (Thermo Fisher, Waltham, MA, USA) with protease and phosphatase inhibitors (Thermo Fisher) and then sonicated. Samples were centrifuged for 15 min at 4°C and the supernatants were aliquoted and stored at −80°C. Tissue samples were diluted in print buffer (10% glycerol, 0.05% SDS, 50 mM DTT in $1 \times$ TBS) to a final protein concentration of 1 mg/ml. Samples were then subjected to an 11-point serial 1:2 dilution and transferred into Genetix 384-well plates (X7022, Fisher Scientific, Pittsburg, PA, USA) using a JANUS Varispan Integrator and Expanded Platform Workstation (PerkinElmer, Waltham, MA, USA). Plates were transferred into an Aushon 2470 Arrayer (Aushon Biosytems, Billerica, MA, USA) to be printed on ONCYTE Avid (tissue samples) or ONCYTE Nova (serum samples) single-pad nitrocellulose coated glass slides (Grace Bio-Labs, Bend, OR, USA).

Printing parameters

The Aushon Arrayer was programmed to use 16 pins (4 × 4 pattern). Each sample was printed in 12 dilutions (12 rows) and in triplicate (3 columns), resulting in a block of 3

× 12 dots per sample. The Spot Diameter was set to 250 nm with a spacing of 500 nm between dots on the x-axis and 375 nm on the y-axis. Wash time was set at 2 s without delays. The printer was programmed for a single deposition per dot for printing serum and tissue extracts.

3.7.2   Immunochemical detection

Primary antibodies were diluted to 10× the optimal Western analysis concentration in antibody incubation buffer [0.1% bovine serum albumin (BSA), protease inhibitors (EDTA-free Halt protease and phosphatase inhibitor cocktail, Thermo Fisher, Waltham, MA, USA), 1 × TBS, 0.5%Tween 20]. The primary antibody solution was incubated overnight at 4°C with a cover slip (Nunc*mSeries LifterSlips, Fisher Scientific, Pittsburg, PA, USA). The following day slides were washed and then incubated with a secondary antibody for 1 h at room temperature (RT). After washing and drying, the fluorescent signals were measured by a Scan Array Express HT microarray scanner (Perkin Elmer, Waltham, MA, USA) using a 633-nm wavelength laser and a 647-nm filter; data were imported into a bioinformatics program.

3.7.3   Data analysis and bioinformatics

Data from the scanned images were imported into a Microsoft Excel-based bioinformatics program developed in house for analysis. The tool calculates total net intensity after local background subtraction for each spot. The intensity data from the dilution series of each sample was then plotted against dilution on a log–log graph. The linear regression of the log–log data was calculated after the removal of flagged data, which include signal to noise ratios of less than 2, spot intensities in the saturation range or noise range, or high variability between duplicate spots (>10−15%). The total amount of antigen is determined by the Y-axis intercept (Gyorgy et al., 2010).

3.8   HUMAN SUBJECTS

Veterans were drawn from Phase III of the W.F. Caveness Vietnam Head Injury Study (VHIS) registry. The VHIS is a prospective, long-term follow-up study of veterans with mostly focal penetrating TBIs, which has stretched over more than 3 decades (Raymont et al., 2008; Raymont et al., 2011). The VHIS registry was collected during the Vietnam conflict by William Caveness at the National Institutes of Health (NIH). Simple registry forms outlining demographic, injury and initial outcome data were completed by military physicians in Vietnam on head injured soldiers who had survived the first week following a severe head-injury including information about "type of penetrating head injury" and "classification of loss of consciousness". About 2,000 subjects were entered in the registry between 1967 and 1970. Phase I (PI) of the VHIS was a medical records review some 5 years post-injury using the military, VA medical and personnel records of 1221 of these men, for whom adequate field, hospital, rehabilitation and follow-up records were available.

Phase II (PII) was a collaborative project between the three Military Services; the Department of Veterans Affairs, the National Institutes of Health and the American Red Cross. It consisted of a comprehensive, multidisciplinary inpatient evaluation at Walter Reed Army Medical Centre. Approximately 520 head injured subjects from the original registry could be identified from VA records, thus these and 85 matched normal volunteers (recruited through veteran publications) were evaluated between 1981 and 1984, some 10–15 years post-injury.

At Phase III (PIII), of the 520 subjects who were assessed in PII, 484 were still alive and 182 attended PIII of the study (30–35 years post-injury). In addition, 17 subjects identified in PI who did not attend PII were assessed. The original 80 control subjects without head injuries recruited in PII, 32 attended PIII and a further 23 were recruited for PIII through advertisements in veteran publications. Therefore, a total of 199 subjects with head injuries attended PIII. No significant differences in age were observed between PIII attendees and non-attendees, in the head-injured or control groups. However, subjects (both head-injured and healthy controls) that attended PIII did have more years of education (t=3.06, P=<0.002), and higher AFQT scores (pre-injury: t=4.85, P<0.001, PII: t=6.15, P<0.001) than PIII non-attendees. Since those subjects attending PIII had a higher level of pre-injury intelligence than those attending PII, as well as more years of education, it is possible that those studied at PIII differed in other ways from PIII non-attendees, which may have affected the longitudinal results we report in this study.

A further reduction of sample size in this study is explained by several reasons: First, out of these 199 only 168 consented to genotyping. Second, from the remaining subjects those who did not complete all three phases of the study were excluded from the analyses (n=33). Third, as a majority of studied subjects were Caucasian in ethnicity, those subjects who had Caucasian ethnicity AIM scores <0.5 were also excluded (n=25). Finally, one subject had to be excluded as an outlier due to his massive brain volume loss. The final studied samples included male Caucasian combat veterans with focal penetrating TBIs (n=109) and non-head-injured normal control subjects who also served in Vietnam (n=38). Importantly, there were no significant differences in AFQT scores at PIII or educational level attained in the group of 109 we studied compared to the 90 excluded head-injured subjects from PIII (F(1,193)=0.10, P=0.919). The type of penetrating TBI injury was classified by neurosurgeons at the time of injury into the following categories: Fragment (69.1%), Gunshot (21.3%), Unclassified (1.5%) and Closed Head injury (8.1%). Further, loss of consciousness (LOC) was classified as following: No (42.6%) Yes, Momentary (17.6%), 1-15min (14%), 15min – 1 day (11.8%), > 1 day (11%), unknown (1.5%). The finals groups were matched with respect to age, level of education, and pre-injury intelligence. All participants gave their written informed consent, which was approved by the Institutional Review Board at the National Naval Medical Centre and the National Institute of Neurological Disorders and Stroke, Bethesda, MD, USA. Military personnel represent an ideal population when studying changes in cognitive functions after penetrating TBI, since pre- and post injury data are usually available in the form of the Army Force Qualification Test (AFQT) on which performance is associated highly with scores on the Wechsler Adult Intelligence Scale (WAIS) (Grafman et al., 1988).

3.8.1   Neuropsychological Testing in human subjects

Subjects were admitted at the National Naval Medical Centre in Bethesda, MD, over a 5-7 day period and underwent a wide variety of neuropsychological testing. The tests were designed to measure cognitive abilities such as memory, language, social cognition, and executive functioning. In this study, we focused on the AFQT (AFQT-7A, Department of Defence, 1960), which is a standardized multiple-choice test of cognitive aptitude measuring verbal ability, visual-spatial organization, arithmetic and functional associations via multiple choice questions. The total score range is 0 to 100 and the subtest scores range from 0 to 25. AFQT scores are reported as percentiles (1 to 99) and correlate highly with WAIS scores (Grafman et al., 1988). It was the only pre-injury cognitive assessment available in our sample and was also used in PII and PIII.
To determine the specific effect of BDNF genotype on the recovery of general cognitive intelligence, two additional cognitive control tasks were used in this study: First, the mini-mental state examination test (MMSE) from PIII was used, which is a well-validated standard test for cognitive impairment in adults, where scores <24 indicates cognitive impairment (Folstein et al., 1975). The purpose of its inclusion was to separate out issues of exacerbated cognitive decline from the onset of dementia (Raymont et al., 2008). Second, the delayed score of the logical memory subtest of the Wechsler Memory Scale, version III (WMS-III) was used to assess episodic memory, which reflects the amount of information from stories that a subject can recall after a 30 min delay (Wechsler, 1997).

3.8.2   Computed Tomography (CT) Acquisition and Analysis in human subjects

The axial CT scans were acquired without contrast in helical mode on a GE Electric Medical Systems Light Speed Plus CT scanner at the Bethesda Naval Hospital. Structural neuroimaging data was reconstructed with an in-plane voxel size of 0.4x0.4mm, an overlapping slice thickness of 2.5mm and a 1mm slice interval. The lesion location and volume were determined from CT images using the interactive Analysis of Brain Lesions (ABLe) software implemented in MEDx v3.44 (Medical Numerics) (Makale et al., 2002; Solomon et al., 2007). The analysis was performed on CT images from Phase III. Lesion volume was calculated by manually tracing the lesion in all relevant slices of the CT image in native space, and then summing the trace areas and multiplying by slice thickness. Manual tracing was performed by a trained psychiatrist (V.R.) with clinical experience of reading CT scans. The lesion tracing was then reviewed by an observer that was blind to the results of the clinical evaluation and neuropsychological testing (J.G.) enabling a consensus decision to be reached regarding the limits of each lesion. The CT image of each individual's brain was normalized to a CT template brain image in Montreal Neurological Institute (MNI) space. The spatial normalization was performed with the AIR algorithm (Woods et al., 1993), using a 12-parameter affine fit. Note that both the patient's brain and the MNI template's brain are first skull-stripped in order to maximize the efficacy of the AIR registration from native space to MNI space. In addition, voxels inside the traced lesion were not included in the spatial normalization procedure. Afterwards, the percentage of Automated anatomical labelling (AAL) structures that were intersected by the lesion was

determined by analysing the overlap of the spatially normalized lesion image with the AAL atlas (Tzourio-Mazoyer et al., 2002).

3.8.3   Genotyping and Haplotype Analysis

We used an addiction array designed by Hodgkinson et al (Hodgkinson et al., 2008). The array is built on the Illumina GoldenGate platform and allows for simultaneous genotyping of 1350 SNPs including 7 BDNF SNPs. The candidate genes for the array were selected on the basis of their roles in drug addictions and the related phenotypes of anxiety and depression. These are the genes important in signalling networks, stress/endocrine genes, and key neurotransmitter systems including dopamine, serotonin, glutamate, GABA and acetylcholine. As all these functional domains are involved in the majority of brain functions, the addiction array represents a very convenient tool for our study. For each gene (including BDNF), array contains SNPs that tag common haplotypes. In addition to 130 addiction-related genes, the array includes a panel of 186 ancestry information markers (AIMs) that allows for determining the subject's ethnicity background. Each marker represents a SNP with known frequencies of occurrence in different ethnical groups. The AIM panel covers seven major populations: African, European, Middle Eastern, Asian, Far East, Oceania and Native American. Following genotyping, the population assignment was performed for each individual according to obtained AIM scores. Only Caucasians with a "European" AIM score > 0.5 were included in this study. Genotyping was performed according to the Illumina protocol on 96 well-format Sentrix arrays. The completion rate of the array assay was > 99%. The error rate of the assay was determined by replicate genotyping, and was < 0.5%. Genotype frequencies were tested for the Hardy-Weinberg equilibrium (HWE) applying Fisher's exact tests. Haplotype analysis was performed using a Bayesian approach implemented with PHASE (Stephens et al., 2001). Haploview 4.2 (Broad Institute, USA,) was used to produce linkage disequilibrium (LD) matrices. Haplotype blocks were constructed by pairing the SNPs with the LD's greater than 0.85, as described by Gabriel et al (Gabriel et al., 2002). We also investigated the effect of the presence of the ApoE ε4 allele and COMT Val158Met (rs4680) on recovery of general intelligence to determine the relative specificity of any BDNF effect.

3.9   STATISTICAL ANALYSIS

3.9.1   Study II

The percent increase of each biomarker in sham and injured animals compared to normal controls was analysed. These values were used in a two-way analysis of variances (ANOVA) with Time (1-, 3- and 14 days) as a within-subject factor and Group (sham, injured) as a between-subjects factor. For behavioural analysis a one-way analysis of variances (ANOVA) was performed with Group (sham, injured) as a between-subjects factor. All the ANOVA analyses were followed up by pairwise

comparison based on estimated marginal means and Bonferroni correction was included in all analyses. All statistical analyses were carried out using SPSS 20.0 with an alpha level set to p<0.05 (two-tailed).

3.9.2   Study III

Statistical analysis was carried out using SPSS 19.0 and alpha was set to p<0.05 for all analyses. For each of the biomarkers, a one-way analysis of variance (ANOVA) was performed to compare values of sham and normal controls. After that no significant differences were obtained between sham and controls the log10 values obtained for each biomarker in injured animals were normalized (z-transformation) in comparison to values obtained from normal controls. For each type of TBI and the biomarkers, a two-way ANOVA with time and group was performed. The ANOVA analysis was followed up by pairwise comparison based on estimated marginal means and Bonferroni correction was included in all analyses.

3.9.3   Study IV

Behavioural data analysis was carried out using SPSS 15.0 with an alpha level set to p<0.05 (two-tailed). Multiple comparisons with Bonferroni correction were included in all analyses. The relationship between variations in the BDNF genotype and the recovery of general cognitive intelligence was analysed in several ways:
First, the demographic variables between the injured and controls groups were compared to ensure that the groups were matched with respect to age, education, and pre-injury AFQT using one-way analysis of variances (ANOVAs) with Group (injured, control) as a between-subjects factor.
Second, the AQFT percentile score of the injured group was normalized (z-transformation) in comparison to the performances of the control group. For each of the 7 SNPs of the BDNF gene, a mixed 3 x 3 analysis of variance (ANOVA) on AQFT z-scores was performed with Time (pre-injury, PII, PIII) as a within-subjects factor and Genotype (TT, CT, CC) as a between-subjects factor. In planned follow-up analyses, the AFQT z- scores among the different allele carriers in each SNP were compared using between-subjects t-tests. In addition, effect sizes (Cohen's d) that represent the observed difference in the AFQT performance between genotype groups were calculated (d=0.2 indicates a small effect size, d=0.5 a medium effect size and d=0.8 a large effect size) (Cohen, 1988).
Third, the specificity of the BDNF genotype effect on the recovery of general cognitive intelligence was determined. Since the BDNF polymorphism has been shown to modulate episodic memory and hippocampal function (Egan et al., 2003; Dempster et al., 2005), episodic memory scores were compared among the different allele carriers in the injured and normal control groups applying a 2 x 3 ANOVA with Group (injured, control) and Genotype (TT, CT, CC) as between-subjects factors. In planned follow-up analyses, the episodic memory scores among the different allele carriers in each group were compared using between-subjects t tests. Subjects within the genotype groups did not differ in age, education, lesion size or pre-injury AFQT.

Fourth, the relative contribution of the BDNF genotype on the recovery of general intelligence was estimated for PII and PIII. A stepwise multiple linear regression analysis was applied including the AFQT z-score as the dependent variable and BDNF genotype, pre-injury intelligence, age, education, degree of atrophy, percentage of total brain volume loss and brain volume loss within each hemisphere as independent variables. This analysis allowed for an estimation of the relative contribution of each predictor to general intelligence. At the same time, it controls for potential confounding factors that may influence general intelligence.

Fifth, the influence of the ApoE ε4 allele or COMT Val158Met genotype on the recovery of general intelligence was determined applying a mixed 3 x 3 analysis of variance (ANOVA) on AQFT z-scores with Time (pre-injury, PII, PIII) as a within-subjects factor and Genotype as a between-subjects factor.

Finally, we performed a haplotype analysis to increase the chances of capturing gene-disease association by applying an ANOVA on AQFT z-scores with Time (pre-injury, PII, PIII) as a within-subjects factor and haplotypes (111222, 112122, 222211, 222212) as a between-subjects factor. The ANOVA was done for 2 haplotype blocks: block 1 included rs1519480, rs7124442, and rs6265, whereas block 2 included rs7934165, rs11030121, and rs908867.

3.9.4   Study V

Statistical analysis was performed in SPSS version 20.0 (IBM). First, a two-way analysis of variance (ANOVA) was applied on BDNF protein levels analysing the main effects of treatment (injury versus sham) and survival time as well as its interaction. Second, Bonferroni post hoc analyses were performed for pairwise comparisons with a significant threshold of $p < 0.05$ (two-tailed).

4   RESULTS AND DISCUSSION

4.1   STUDY I

Aim: "To identify gene expression differences and similarities following blast-TBI, rotational-TBI and penetrating-TBI. Furthermore, to investigate the histopathological changes following each different type of injury."

Identification of target genes involved in TBI pathophysiology will help in understanding the molecular mechanisms of neuronal damage after trauma. In this study we used gene microarray to identify similarities and discrepancies in altered gene expression following three different TBI models. Although there is a combination of types of injuries in the clinical situations, decomposition of the injury is necessary to understand the distinct pathological processes.

The gene array analysis at 24 h after TBI revealed dissimilarity in the 3 different types of TBI. In the hippocampus of animals subjected to penetrating TBI, the majority of the responding genes were found to be downregulated. A similar response was found in the hippocampus after the primary blast. However, in the rotational TBI the majority of the gene expression changes were upregulated in the hippocampus. A similar trend was observed in the border zone of the cortex in the penetrating TBI.

Using the Database for Annotation, Visualization and Integrated Discovery (DAVID) it was possible to group responding genes into functional-related gene groups (GO—Gene Ontology search terms). This analysis generated a distinctive profile for each of the experimental models with families of genes related to development, differentiation, the inflammatory response, apoptosis, neurogenesis and synaptic transmission (Fig. 7). A number of genes related to neurogenesis were found to be downregulated in the hippocampus after primary blast but not after rotation or penetration TBI. A response linked to wounding and inflammation was more obvious after rotation TBI than after primary blast. Conversely, low activity of genes associated with apoptosis and cell death. The most evident response in genes related to apoptosis and response to stress were seen in the border zone of the cortex in the penetrating TBI.
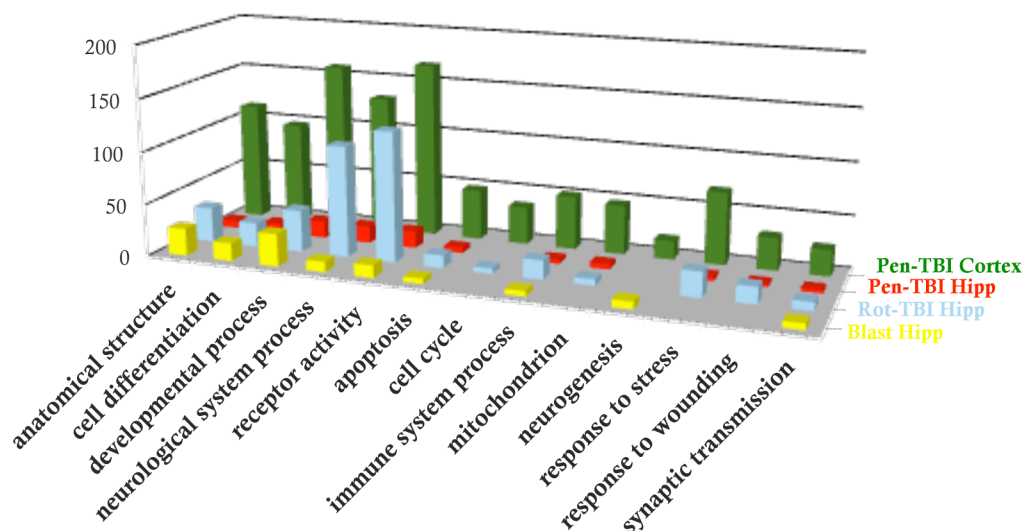
Figure 7. A diagram illustrating the number of significant genes in selected categories after primary blast, in the hippocampus after rotational TBI, in the hippocampus after penetrating TBI and in the border zone after penetrating TBI. The reader should concentrate on the difference in the profiles between the different materials and not on absolute numbers.

Our histological investigation showed no evidence of apoptosis or bleeding in the rotational TBI. There were no signs of BBB disruption. An increased number of β-APP-positive axons were detected at the border between the corpus callosum and the subcortical white matter 2h after high acceleration trauma, indicating axonal injury. Intracellular oedema was also detected in this type of injury. This is in contrast to the penetrating TBI, where the observed oedema was mainly perivascular. Fluoro-Jade and TUNEL labelling revealed numerous dying cells in the penumbra with a maximum occurring between day 1 and day 3.

In the blast TBI no signs of cell death or bleeding were detected. Nor were there any signs of inflammation or oedema observed in the primary blast injury model.

The results of this study indicate that the blast injury model produces a mild injury with no evident structural changes. The limited morphological changes observed could partially depend on the low peak level blast pressure that was limited to below 236 kPa. The main reason for this was to limit animal mortality caused by haemorrhaging in the lungs. Furthermore, the pressure wave in a blast tube like the one used in the present study results in a primary positive peak with comparatively short duration (less than 1 ms), a simple biphasic configuration with only a small underpressure component. In real events related to detonation of improvised explosive devices much more complex waveforms can be expected (Risling and Davidsson, 2012). Furthermore, this model does not expose the head to rotation, which is a part of blast wave TBI and may further aggravate the injury (Clayton et al., 2012).

The morphological changes found in the rotational TBI were mainly axonal injuries with no findings of apoptosis or BBB disruption. However, there was an evident increase in the number of responding genes with the majority being upregulated.

By using gene microarray we found distinct gene expression profile in the three different TBI models. This provides information about genes and biological processes

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involved in the pathophysiology of different types of TBI. However, it should be noted that although gene microarrays are powerful tools they are limited by their insensitivity to identify transcripts of low abundance, i.e. genes expressed at low levels or in a small fraction of the cells studied. Thus, for detailed studies on single genes aiming to create timetables other methods such as PCR or in situ hybridization are preferred over this type of analysis. Moreover, the increased expressions of genes do not necessarily induce direct functional change in affected cell populations.

4.2   STUDY II

Aim: "To identify release of serum biomarkers and behavioural outcome in a rotational TBI model that produces axonal injuries and whether these finding are distinct from the penetrating TBI."

In this following study we investigated the behavioural outcome following the rotational TBI in addition to serum analysis in order to identify possible biomarkers of axonal injuries. The axonal injuries were the main histopathological findings demonstrated in Study I and additional studies on this model (Davidsson and Risling, 2011). Animals were exposed to the rotational TBI with a severity above the injury level where gene expression alterations were found. No macroscopic hematomas or contusions were observed. In order to verify our previous finding of axonal injury in brain tissue in this TBI model we used β-APP. Positive β-APP immunoreactivity (IR) was found in the corpus callosum and at the border zone of the grey-white matter of the corpus callosum and the hippocampus (Fig. 8). No β-APP-IR was found in the sham-exposed animals. These findings were in accordance with our previous reports on β-APP-IR in this TBI model.
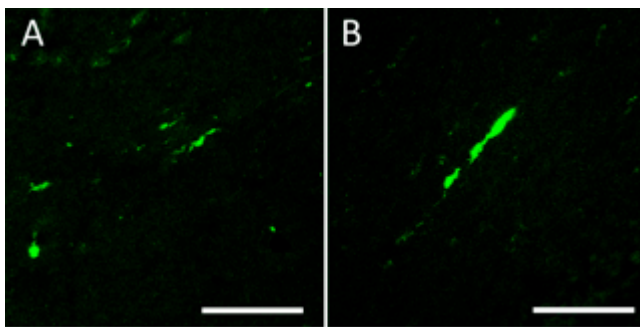

Figure 8. Confocal images of β-APP staining. β-APP positive axons can be detected at the border between the corpus callosum and subcortical white matter (A). In addition swollen axons could be found in the corpus callosum as represented in the image (B). Both pictures present 24h following injury. Scale bar = 50 μm.

4.2.1   Behavioural tests

All animals survived the trauma and were able to remain upright and to walk on the beam at the initial trial following injury. All the animals continued to complete the test at all the following time points up to 7 days and no significant differences could be detected between the injured and the sham animals.

The results from the Elevated Plus Maze (EPM) revealed that the animals did not experience any increase in anxiety. There were no differences between the shams and the injured group in time spent in closed or open arms or at the centre platform. No significant changes in the number of total arm entries, rearing activity or the number of crossings were observed. However, the radial arm maze revealed a limited but statistically significant increase in working memory errors at day 3 ($F1,126 = 5.9$, $P= 0,016$) and reference memory errors at day 5 ($F1,126 = 5.5$, $P= 0,021$) in injured animals compared to sham. No changes could be detected in visit response latency (time taken to correctly find all four baited arms) or the number of visits in the baited arms or all arms, at any time point following injury.

Memory deficits are the most common cognitive measure displayed in patients with minor or mild TBI (Bazarian et al., 1999; King et al., 1999; Bazarian and Atabaki, 2001; Bazarian et al., 2006). In the current study, we found transient deficits in working and reference memory but no alterations in other behavioural parameters measured. Using the same set of behavioural testing used in the current study in a penetrating TBI model, no impairment of working memory could be found (Plantman et al., 2011). However, a persistent increase of errors in reference memory was found in the injured animals. Furthermore in the penetrating TBI, we found that none of the animals could balance or move on the beam walk 3 days following injury. The penetrating TBI is classified as a severe type of TBI using same behavioural test setting as in the current study, thus the results obtained in the present study show that our model produces a mild type of TBI. A correlation between the TBI severity and behavioural deficit has been reported in other animal TBI models (Yu et al., 2009). Furthermore, the persistence of this deficit in severely injured animals has been documented (Pierce et al., 1998).

4.2.2   Serum biomarkers

The result of RPPM values obtained in log10. The percentage increase of each biomarker in both sham and injured animals compared to normal controls are illustrated in Figure 9. There was a significant difference between the sham and injured animals in all the biomarkers; S100B ($F1,16 = 24.3$, $P=<0.001$. MBP ($F1,16 = 57.7$, $P=<0.001$). NF-H ($F1,16 = 19.3$, $P=<0.001$). Tau ($F1,16 = 27.5$, $P=<0.001$).
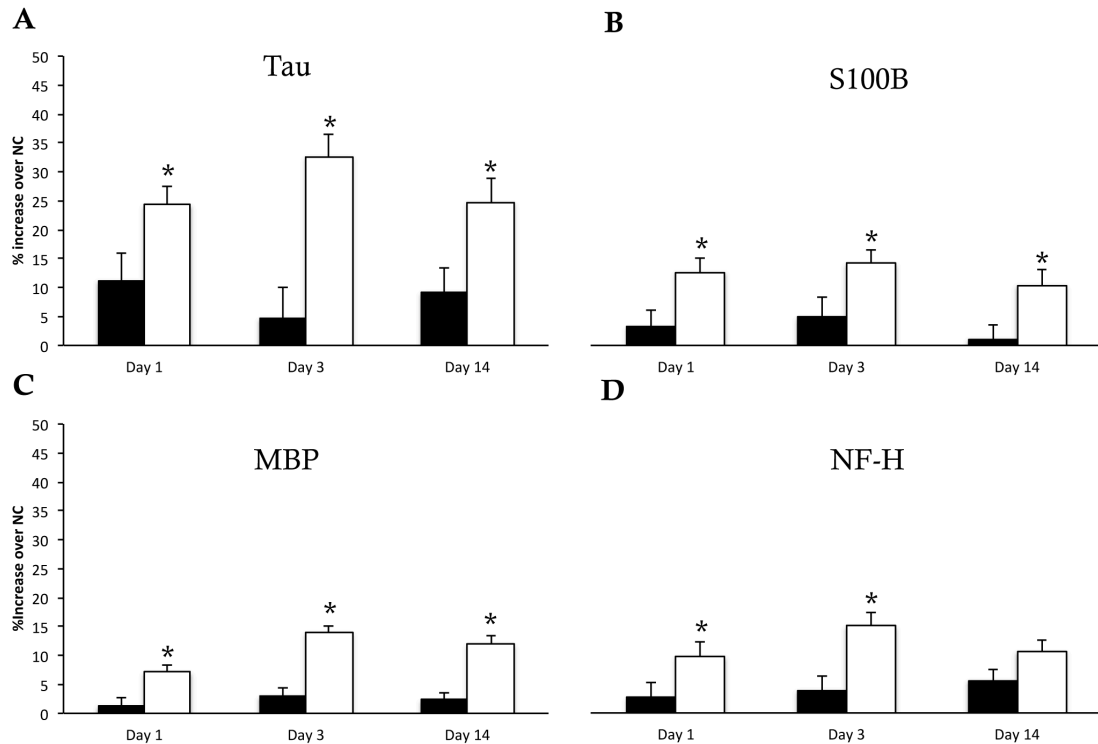
Figure 9. Serum biomarkers. Y-intercept values percentage the increase of each biomarker in sham animals (black bars) and injured animals (white bars) compared to normal controls (NC), given as mean ± SD. The biomarker Tau (A) showed the most pronounced increase following injury. The multiple comparison showed a significant increase in Tau, S100B (B), MBP (C) at all time points compared to sham animals indicated by (*). The NF-H (D) showed significant increase in injured animals compared to sham at day 1 and 3 but not at 14 days following injury.

The most pronounced increase was found in Tau at all time points. Tau has been suggested as a marker of axonal injury. High serum levels of Tau following mTBI have been reported both in patients (Bulut et al., 2006; Kavalci et al., 2007) and in rats (Liliang et al., 2010) with conflicting results. The temporal changes were significant in MBP (P=0.022) with the highest values recorded at day 3. The values remained elevated at day 15. Myelin degradation can be expected to occur secondary to axonal injury and has been shown in brain tissue of patients with DAI (Ng et al., 1994). In addition, the same trend of serum MBP, analysed by RPPM, was shown in pigs exposed to blast TBI (Gyorgy et al., 2011). High levels of MBP were present 2 weeks following injury. Also NF-H, S100B showed the trend of a peak at day 3. Release of NF-H in serum has been previously studied in TBI (Anderson et al., 2008), the phosphorylated form of NF-H was analysed and increased significantly in moderate and severe TBI but not in mTBI. Using RPPM for NF-H analysis in a blast TBI model, showed that NF-H is correlated with injury severity and that in the mild injury group it peaked 3 days after injury (Gyorgy et al., 2011). The findings in the latter study are in line with the results described in this thesis, indicating that axonal injury can be detected by NF-H up until day 3. No significant differences were found between sham and injured animals at day 14.

In a previous study using the present TBI model, we have shown an increase of serum S100B as early as 2-3h following trauma, measured by ELISA (Davidsson and Risling, 2011). In this study using RPPM, we could confirm the release of S100B in serum for

up to 14 days following injury. The current communication and several previous studies indicate the usefulness of RPPM as a screening tool for known biomarkers (Spurrier et al., 2008).

In summary, the transient memory impairment in combination with the absence of other behavioural deficits in this model indicates that the TBI produced corresponds to a mild type of TBI. Furthermore, the main histological findings using this model were axonal injuries. Interestingly, this could also be detected by biomarkers in serum in the absence of evident BBB defects.

4.3   STUDY III

Aim: "To identify the inflammatory response in the rotational and penetrating TBI models in serum and brain tissue. Specifically, the aim is to investigate whether the secondary axotomy is complement mediated."

One of the major mechanisms contributing to secondary injuries following TBI is the inflammatory response. Here we examined this process focusing on the complement cascade in the penetrating and rotational TBI. We investigated the inflammatory response both in serum and in the brain tissue and studied whether secondary axotomy is complement mediated in the rotational TBI.

4.3.1   Serum analysis

The serum CRP measurements for all rodents were within the normal range, indicating no systemic inflammatory response following the isolated TBIs.

There was no significant increase in serum C3 in animals exposed to trauma compared to sham operated animals in any of the TBI models (rot-TBI p=0.051, pen-TBI p=0.913), although the p-value in rot-TBI was close to significant. Serum levels of C5b9 on the other hand were significantly increased in rot-TBI compared to sham operated animals at all time points (p=<0.001). No significant differences could be detected in pen-TBI (p=0.359). The peripheral increase in C5b9 in rot-TBI might be due the mechanical injuries of the neck muscles at the time of impact. Another possible source could be injury to the cardiac muscle at the time of serum sampling (Yasuda et al., 1990). It should be noted that in the clinical setting, many TBI patients suffer from multitrauma. These other injuries may trigger other systemic inflammatory responses, which can aggravate the TBI.

4.3.2   Histology

The activation of microglial and/or invasion of macrophages were investigated using CD11b and ED1. A few CD11b positive cells were found at day 3 post injury in the rot-TBI model. However, in the pen-TBI many positive cells were detected in the area surrounding the cavity already on day 1, and by day 3 there was massive CD11b immunoreactivity (IR). In the rot-TBI only few cells with changed morphology and

positive ED1-IR were found in the cortex. This was detected on day 1 and did not increase by day 5. The ED1-IR in the pen-TBI showed many positive cells on day 1 and a prominent labelling in the area surrounding the cavity by day 3. The penetrating TBI is a focal injury with a vascular lesion while the rotational TBI is characterized by diffuse axonal injury (DAI) without apparent vascular lesions. Our previous studies show a transient disturbed integrity of the BBB in the pen-TBI model with a maximum at 1 to 3 days following injury, while no disturbed integrity of the BBB was found in the rot-TBI model (Davidsson and Risling, 2011; Plantman et al., 2011; Risling et al., 2011). This distinction between the models explains the recruitment of macrophages from the circulation into the injured area found in the pen-TBI but not in the rot-TBI.

4.3.3   Complement proteins

Activation of the classical complement pathway was shown by in situ hybridization for C1q mRNA and the progression of the complement cascade by C3 mRNA. In the rot-TBI model, an increased expression of C1q was found in the cingulate cortex, the hippocampus, the corpus callosum, the thalamus and the amygdala. This activation decreased over time, and by day 5 the C1q activation could mainly be seen in the centroaxial line structures and the cingulate cortex. In the hippocampus positive cells were mainly found in the neurons of DG.

The time course for C3 expression in the different areas varied. The hippocampus showed a clear expression of C3 in the polymorph and granular layer of the DG on day 1 that could not be observed on day 3 or 5. There was also a clear expression of C3 in the centroaxial line structures including the different thalamic nuclei and the amygdala. Contrary to what occurred in the hippocampus, this expression increased overtime. The anatomical distribution of increased complement expression corresponded well with the findings in patients suffering DAI (Adams, 1982; Adams et al., 1989a). These areas are also known to be involved in memory disturbance, Post Traumatic Stress Disorder and cognitive dysfunctions seen in patients with DAI (Fork et al., 2005; Koenigs et al., 2008). Recent work has shown complement mediated synapse loss in both the developing and adult brain (Stevens et al., 2007). This generates a tantalizing postulation that complement mediated synapse loss in the affected area caused by rotational injury could play a role in the manifested clinical symptoms in patients.

In the pen-TBI model an increased expression of C1q was detected in the corpus callosum and hippocampus ipsilateral and, to a lesser extent, contralateral to the injury. On day 1 and day 3 the C1q increased, peaking on day 5 in the area surrounding the cavity. The expression of C3 mRNA increased in the area surrounding the cavity. There was a distinct increase in expression of C3 mRNA on day 3, reaching a maximum by day 5. C3 was found in the corpus callosum and the hippocampus contralateral to the injury site by day 1. A summary of the anatomical localisation of APP and complement proteins is given in Figure 10.
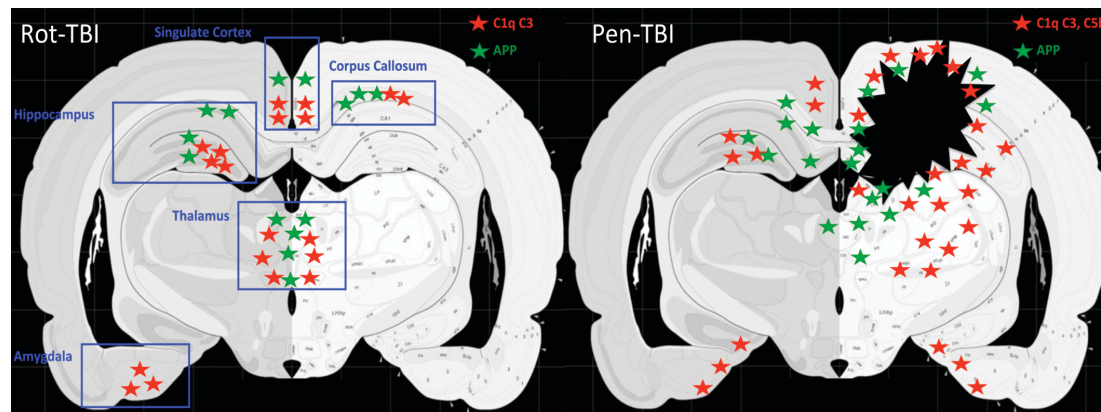
Figure 10. Localization of APP and complement proteins: A summary of localization of APP and complement proteins in the rot-TBI model (left) and the pen-TBI model (right). Green stars represent APP while red stars represent complement proteins. In the rot-TBI model APP was found in the corpus callosum and the hippocampus. The complement proteins C1q and C3 were expressed in the centroaxial lines, in addition to the hippocampus, amygdala, thalamus and cingulated cortex. In the pen-TBI model there was a local expression of APP and the complement proteins C1q, C3 and C5b9 in the border zone of the injury as well as in the thalamus and hippocampus ipsilateral to the injury.

Activation of the terminal complement pathway was assessed using an antibody targeting C5b9. There was a positive C5b9-IR in the area surrounding the cavity and in the remote area of the cavity on day 3 and 5. However no C5b9-IR was observed in the rot-TBI model at any time point. We investigated the expression of C5b9 by Western blot and could see positive bands in the pen-TBI model on day 1 and 3 but not in the rot-TBI model (Fig 11).

A penetrating focal injury caused a massive induction of inflammatory response detected by microglia activation, macrophage and neutrophil infiltration and complement protein expression. MAC/C5b9 positive cells were detected in the injured area and also in the border zone of the injury. This suggests that the terminal pathway of the complement cascade is initiated and that complement mediated cell death occurs. However, this could not be detected in the rot-TBI. The key complement proteins C1q and C3 were detected but not C5b9, indicating that although the rot-TBI triggers the complement cascade it does not fully progress to the terminal pathway and formation of C5b9.
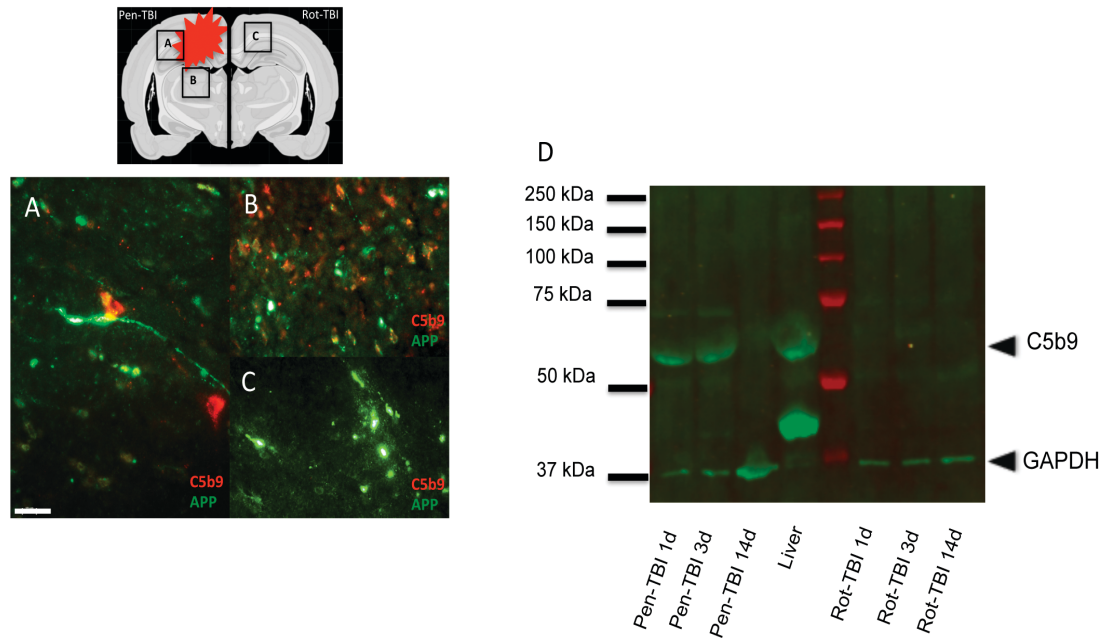
Figure 11. A schematic picture of the brain is illustrated in the upper left corner to provide an overview of where the images are taken. (A) Illustrates the area surrounding the cavity in Pen-TBI showing APP-IR axons and neurons with double labelling of C5b9. Also image (B) illustrates APP-IR and C5b9-IR in Pen-TBI in the penumbra zone 3 days following injury. Image (C) is APP-IR and negative C5b9 in corpus callosum of Rot-TBI 3 days following injury. Scale bar = 10 μm.

The findings were confirmed by Western blot (D) that showed positive bands (~65kDa) in the pen-TBI model at 1 and 3 days post injury, while in the rot-TBI model no bands was revealed at all around ~65kDa. In order to check that the lanes in the gel have been evenly loaded with samples, control loading was performed using GAPDH (~37kDa).

Here we demonstrate that in the pen-TBI model, the initiation of the complement cascade leads to the activation of the terminal pathway, detected by C5b9 using immunohistochemistry and Western blot. This is in line with previous findings of C5b9 in TBI with contusions (Bellander et al., 2001; Bellander et al., 2004). However, C5b9 could not be detected in the rot-TBI. In this model, we induce a sagittal acceleration-deceleration force that generates TAI. The severe level of the impact generates a distribution comparable to its clinical manifestation, DAI, in the corpus callosum, subcortical white matter and the brain stem (Davidsson and Risling, 2011). In Study II we demonstrated a serum peak of Tau and neurofilament by day 3 in the rot-TBI model, indicating occurrence of a secondary axotomy. These findings are also confirmed by silver staining showing disconnected axons following rot-TBI (Davidsson and Risling, 2011). Despite the findings of axonal injury, both with APP staining, silver staining and serum Tau and neurofilament, there was no activation of the complement terminal pathway in the present rot-TBI model. This indicates that the axonal injuries per se do not activate the complement terminal pathway and furthermore, an attack of C5b9 on the axon leading to a secondary axotomy seems unlikely.

The findings of this study suggest and support the previous findings that complement proteins may be a possible target in treatment and aid of recovery after TBI, but indicates that the secondary axotomy following DAI is not complement mediated.

4.4   STUDY IV

Aim: "To identify the impact of genetic background on the outcome of penetrating TBI in humans. Specifically investigate whether BDNF polymorphisms have an impact on general intelligence, and if so, how that impact differs during different post-injury stages. "

In parallel with the harmful processes following TBI there is also neuroregenerative and restorative processes that affect the recovery and plasticity of the brain. Our experimental results from study I showed involvement and upregulation of many genes involved in neurogenesis following penetrating TBI. One of the key players in these processes is BDNF. Furthermore, the genetic polymorphism of BDNF has shown to affect the function and cognitive ability in humans. We investigated the importance of genes previously found to be significant for the outcome following TBI. The outcome measure was general intelligence measured at 3 time points: pre-injury, 10-15 years post-injury (PII) and 30-35 years post-injury (PIII). We specifically studied ApoE, COMT and the BDNF polymorphisms in patients with penetrating TBI. Seven BDNF SNPs were analysed.
Our ANOVA analysis showed no significant differences between the injured and control groups regarding age, education, and preinjury AFQT.

4.4.1   Association of BDNF and general intelligence

The association between BDNF genotype and general cognitive intelligence at each evaluation Phase and for each of the 7 BDNF SNPs was evaluated. The mixed 3×3 ANOVAs on AQFT z-scores with Time (pre-injury, PII, PIII) and Genotype (TT, CT, CC) revealed a significant main effect of Time ($P<0.001$) but no significant main effect of Genotype for all SNPs. The interaction effect for Time × Genotype was only significant for SNPs rs7124442 and rs1519480. For both SNPs, no differences were found regarding type of injury (rs1519480: $F(2,108) = 0.105$, $P = 0.900$; rs7124442: $F(2,108) = 0.228$, $P = 0.797$) or loss of consciousness (rs1519480: $F(2,106) = 1,101$, $P = 0.336$; rs7124442: $F(2,106) = 1,043$, $P = 0.356$). Figure 12 illustrates the changes in AFQT z-scores across time (pre-injury, PII, PIII) among carriers of different genotypes of those SNPs rs7124442 and rs1519480. The genotype groups that performed best were CT (n = 51), in both SNP rs7124442 and rs1519480, and the groups performing worst were genotype CC (n = 8). For SNPs rs7124442 and rs1519480, the ANOVAs analysis showed a significant decline of general cognitive intelligence from pre-injury to PII but not from PII to PIII, indicating that those SNPs are more prominent during the early post-injury period. For both SNPs at PII, follow up t-tests revealed only significant differences between the CC and CT genotypes.
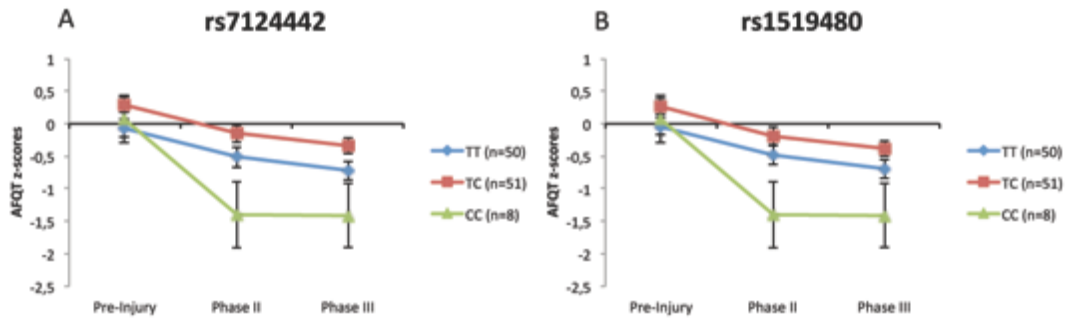
Figure 12. Association analysis of significant SNPs. The z-transformed (normalized) AFQT-scores of pre-injury, phase II and III for SNP are illustrated for the significant SNPs, rs7124442 (A) and SNP rs1519480 (B). Note that zero-line represents our normal control.

Furthermore, the specificity of the BDNF genotype effect on the recovery of general cognitive intelligence was determined and no significant differences were observed for any of the SNPs (rs7124442, rs1519480) regarding demographic variables, volume loss, degree of atrophy, episodic memory and MMSE. None of the subjects with penetrating head injury had MMSE scores lower than 25. Scores below 25 are associated with mild dementia and age-related cognitive impairment (Folstein et al., 1975).

The relative contribution of the significant SNPs to the recovery of general cognitive intelligence at PII and PIII was investigated using stepwise multiple linear regression analyses. For phase II, variance in cognitive intelligence was explained by pre-injury intelligence (47.0%) to SNP rs7124442 and genotype CC (4.9%), percentage of total brain volume loss (2.1%) and education (1.5%). For phase III, variance in cognitive intelligence was explained by pre-injury intelligence (41.8%), percentage of total brain volume loss (6.4%) and SNP rs7124442 and genotype CC (2.4%). No significant associations were found for age, education, atrophy ratings or right or left hemisphere brain volume loss for either time-point. As found in prior studies, we confirmed that pre-injury intelligence is the most consistent and vital predictor of cognitive outcome after TBI (Grafman et al., 1986; Gao et al., 2000; Kesler et al., 2003; Raymont et al., 2008). The next best predictor at the Phase II evaluation was the BDNF genotype.

4.4.2   Effect of ApoE and COMT

We determined whether the presence of the ApoE ε4 allele or COMT Val158Met genotype has an effect on the recovery of general cognitive intelligence. This genetic analysis for ApoE and COMT was available for 94 of the subjects. In the injured group, 22 subjects showed presence of the ApoE ε4 allele. The ANOVA revealed that there was a main effect on Time ($F_{(2,182)} = 25.2$ $P<0.001$) but no main effect for Genotype ($F_{(1,91)} = 0.95$, $P<0.332$) and no interaction effect for Time × Genotype ($F_{(2,182)} = 1.11$, $P<0.328$). The influence of COMT polymorphism (Val/Val = 23, Val/Met = 38, Met/Met = 33) was similar: significant main effect for Time ($F_{(2,182)} = 34.4$, $P<0.001$)

but no significant main effect for Genotype $(F(4,182) = 2.5, P<0.086)$ and no significant interaction effect for Time $\times$ Genotype $(F(2,91) = 1.4, P<0.247)$. Presence of the ApoE ε4 allele has been shown to be one of the strongest genetic predictors of poor outcome following TBI (Teasdale et al., 1997; Lichtman et al., 2000; Nathoo et al., 2003). Several association studies have investigated the role of the ApoE gene polymorphism in subjects with TBI, but overall the findings are inconclusive (Jordan, 2007; Dardiotis et al., 2010). One explanation could be that ApoE plays a role in the initial survival of TBI patients while BDNF plays a greater role in the cognitive outcome in those who did survive.

4.4.3   Lesion location

We performed a VSLM analysis to investigate the effect of lesion location on AFQT performance for either PII or PIII. We repeated this analysis with subgroups based on the different genotypes for SNP rs7124442 and SNP rs1519480 and did not find an association with lesion location. Previous studies have shown lesion location to be the least predictive of performance outcome of overall post injury intelligence (Grafman et al., 1986; Grafman et al., 1988). Correlations have mainly been found between subtest outcomes and specific injured lobe or hemisphere and not full-scale IQ or general intelligence (Warrington et al., 1986; Grafman et al., 1988; Glascher et al., 2010). An overlay lesion map of all the 109 subjects are presented in Figure 13.
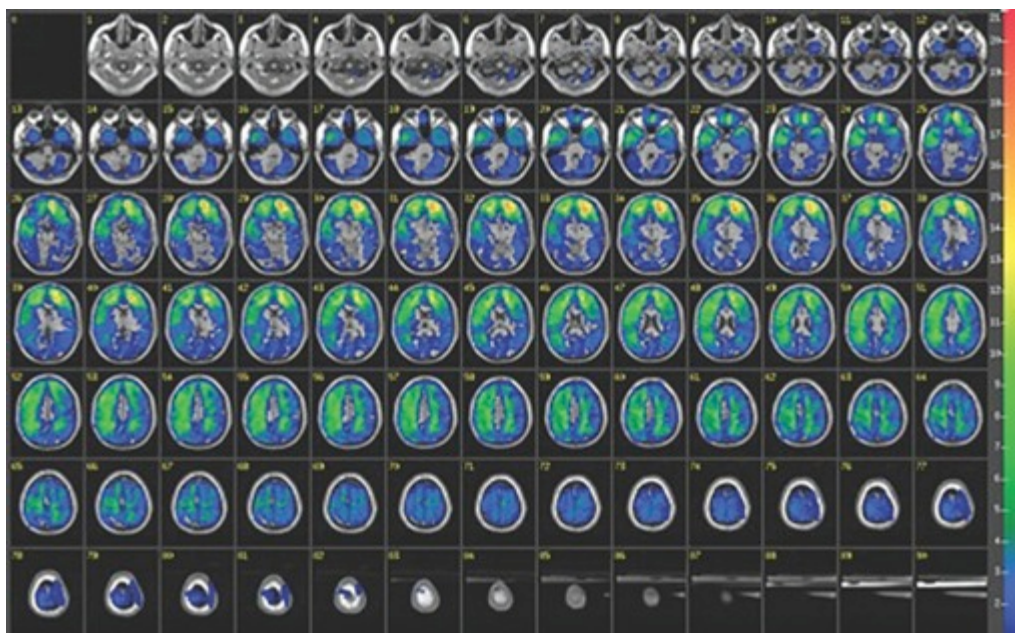

Figure 13. Lesion density map of all 109 subjects, lesions are overlaid on a standard brain template analysed in ABLe. Colour indicates the number of overlapping lesions at each voxel with red indicating more subjects and blue fewer.

4.4.4   Haplotype analysis

A haplotype analysis was carried out since the haplotype association analysis of polymorphisms in strong LD can potentially be more suitable for identifying gene–

disease associations than single locus tests. Four haplotypes with frequencies >0.09 were used for the analyses and no significant associations were found for these haplotypes. We further divided the main haplotype block into two short sub-blocks of 4 kb and 13 kb long. By Haploview software the blocks were defined using the pairs of SNPs with an LD >0.85. The presence of a recombination region between SNPs 3 and 4 suggested 2 blocks. Block 1 included rs1519480, rs7124442 and rs6265 and Block 2 consisted of rs7934165, rs11030121 and rs908867. Our ANOVA analysis showed a significant association for haplotype block 1 (combined) (P<0.017).

Here we provide evidence that the BDNF SNPs rs7124442 and rs1519480 have an effect on recovery of general cognitive intelligence. This influence is probably a lesion-induced plasticity that decreases over time. Our haplotype analysis confirmed this significant association. The rs7124442 SNP sequence is located in the 3′-UTR region. These sequences are a section of mRNA that follows a coding region and contains several regulatory sequences. 3′-UTR sequences might affect the activity-dependent BDNF mRNA targeting, translation and/or degradation at active synaptic sites and may also influence the proBDNF transcript. The SNP rs1519480 is located in the 3′-flanking region located in highly conserved regions, suggesting that this SNP plays an important functional role. Given that BDNF and its high affinity receptor tropomycin-related kinase B (TrkB) play a crucial role in axon guidance and growth as well as synapse formation and plasticity (Frisen et al., 1992; Huang and Reichardt, 2003; Lu, 2003; Luikart and Parada, 2006), it is straightforward to think that polymorphisms at this gene would influence recovery of function after a brain injury, particularly in the early phase when extensive synaptic remodelling might occur. Using the lateral fluid percussion model in rats, a decrease of BDNF and its receptor TrkB could be detected in the injured area while there was an increase in remote areas hours post-injury (Hicks et al., 1999a). Increased expression of BDNF and TrkB has also been observed in the hippocampus hours after injury (Hicks et al., 1999b) and interestingly, physical exercise following TBI has been shown to lead to increase in BDNF in CA1 of the hippocampus (Hicks et al., 1998a). However, we know little about the consequences of these alterations following TBI: are they compensatory responses, what role do they play in cell death and synaptic plasticity and in particular what are the long-term effects?

In conclusion, the results described in this Study indicate that BDNF polymorphism plays a modest but significant role in lesion-induced plasticity, which declines over time. Furthermore, the findings show the impact of two specific SNPs on the recovery of general intelligence. It is possible that polymorphisms in the BDNF gene contribute to functional enhancement or reconstruction of impaired neuronal circuitry or that endogenous neurogenesis is enhanced. Identifying the underlying mechanisms of this BDNF effect should provide insight into an important aspect of cognitive recovery after TBI and the value of assessing genotype risk in TBI subjects.

4.5   STUDY V

Aim: "To investigate the temporal and spatial expression of BDNF and its receptors in the penetrating TBI model in order to attempt to understand the molecular mechanisms in the clinical findings in study IV."

To gain a deeper insight into the underlying mechanism of the influence of BDNF in penetrating TBI, we investigated its expression and also its receptors expression in the brain, using the penetrating TBI model. We focused the analysis mainly on the hippocampus for several reasons. The BDNF mRNA is widely distributed in the brain but with the highest levels in the hippocampus. Our results from Study I showed significant alteration of genes related to neurogenesis in the hippocampus (Risling et al., 2011). Furthermore, the polymorphism of the BDNF has shown to affect cognitive functions such as general intelligence following TBI (Rostami et al., 2011) and executive function (Krueger et al., 2011). Hippocampus is a region with high potential of plasticity and important in cognitive outcome and recovery. We used in situ hybridization and investigated the mRNA expression of BDNF and its receptors, the full-length and the truncated TrkB and p75^{NTR}, from 1 day to 8 weeks following penetrating TBI. In addition, the protein level of BDNF in the cortex and hippocampus was measured by reverse phase protein microarray (RPPM).

Hybridization with the BNDF probe demonstrated an increase in the labelling intensity in the cortex and thalamus. The labelling was predominantly found in neuronal cells. The mRNA expression of BDNF in the hippocampus contralateral (CL) to the injury showed a significant increase up to 2 weeks compared to sham operated animals. This increase was found in all 3 areas examined; DG, CA3 and CA1. The values returned to the same level as sham at 8 weeks following injury (Fig 14).

The mRNA expression of TrkB full-length receptor was significantly increased at day 3 following injury and was not significantly altered at later time points. This receptor has the catalytically active kinase domain and the increased expression could be a response to increased BDNF in the contralateral side of the injury, mediating BDNFs full effect. The consistent low level of Trk-FL in the penumbra zone corresponds to the low levels of BDNF, indicating low activity of BDNF promoting recovery.

We found a significant increase in expression of mRNA TrkB truncated receptor, but not until after 2 weeks following injury. The expression decreased to the level of sham operated animals by week 8. Interestingly, looking at the injury cavity compared to the penumbra zone, the expression of TrkB truncated receptor was increased up to 8 weeks. At this time point, the expression of BDNF, TrkB-FL and p75^{NTR} in the injury cavity was low.
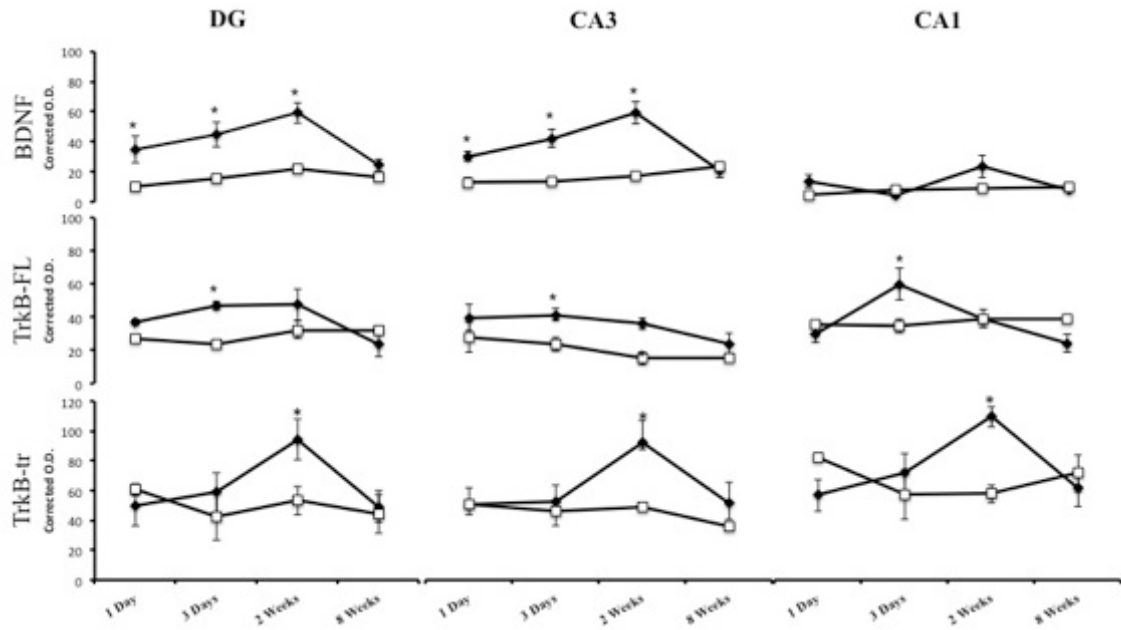
Figure 14. Graphs showing optical density (OD) measurements of hybridization for BDNF, TrkB full-length and TrkB truncated mRNA in the contralateral side of injury in the penumbra zone. The comparison was made between the injured animals (filled squares) and sham (empty squares) with equivalent survival time (injured n=5, sham n=3). The analysis was performed in the DG, CA3 and CA1. The BDNF mRNA showed a significant increase in DG and CA3 up to 2 weeks. The TrkB-FL showed a significant increase in all three areas measured at day 3 while this increase appeared at later time point, 3 weeks, in the TrkB truncated. The values are presented as mean ± SD and a significant level of $p < 0.05$, indicated by (*).

The expression of p75[NTR] in normal and sham operated animals was very low. In the injured animals the mRNA expression of p75[NTR] increased overtime in the area surrounding the cavity and this could be seen up to 8 weeks following injury. In the hippocampus of the penumbra zone only few p75[NTR] probes could be seen at 2 and 8 weeks following injury (Fig 15).
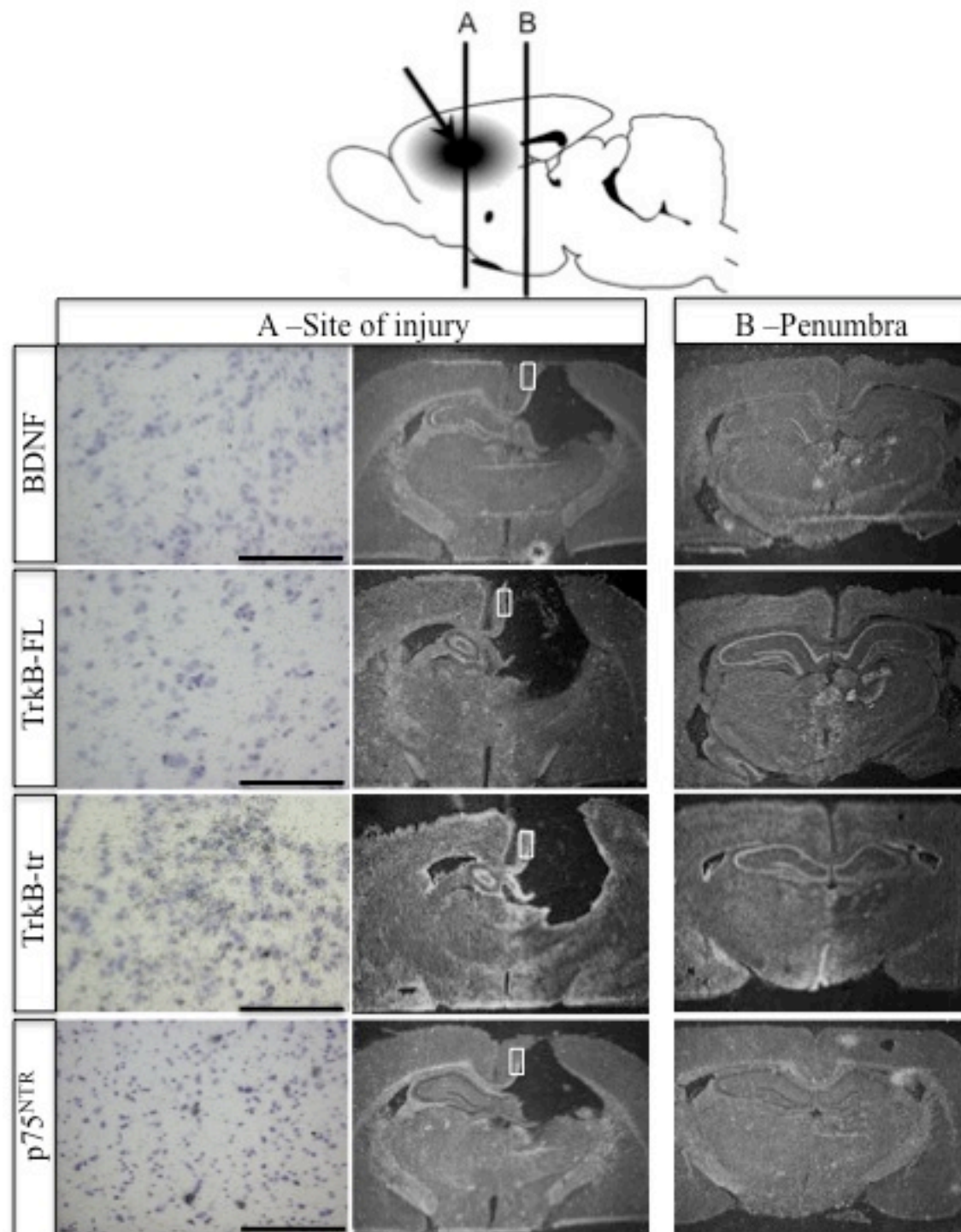
Figure 15. The schematic image presents the site of penetration and where the sections were taken. The section (B) represents the penumbra zone. All the pictures are taken from animals with survival time 8 weeks following injury. The TrkB truncated receptor showed an intense labelling in the area surrounding the cavity (encircled) 8 weeks following injury. This was not seen for BDNF mRNA or TrkB-FL.

For the frontal cortex, the ANOVA analysis of BDNF protein levels revealed a significant main effect of injury, indicating an increase in the injured animals compared to sham condition (p<0.001). The main effect of time and the interaction of injury and time were not significant. For the hippocampus, a significant main effect of injury was observed, indicating an increase in injured animals compared to the sham condition

(p<0.001). However, follow-up post-hoc analyses showed only significant increases for day 1 (p=0.009) and day 14 (p<0.001) but not for day 3 (p=0.058). No significant main effect for group and no significant interaction of injury and group were found. No significant difference between the levels of BDNF protein in hippocampus compared to frontal cortex was found (p=0.249).

Our study is the first to investigate the expression of these receptors following TBI during the chronic state. Our results demonstrate that the alteration in expression of BDNF mRNA is dependent on the distance to the cavity, with a decrease in the penumbra zone and an increase in the area contralateral to the injury. In addition, the TrkB-Truncated and p75^{NTR} mRNA expression was increased at the site of injury 8 weeks following TBI.

Previous experimental TBI studies investigating the expression of BDNF (Yang et al., 1996; Hicks et al., 1997; Hicks et al., 1999a; Hicks et al., 1999b) and TrkB-FL (Mudo et al., 1993; Hicks et al., 1998b; Hicks et al., 1999a; Hallam et al., 2004) have used models arguably less relevant to penetrating TBI such as lateral fluid percussion (LFP) and controlled cortical impact. Furthermore, all these studies focused on a time window of 1 hour up to 72 hours. Here we tried to mimic the penetrating TBI found in the Vietnam combat veterans and study this injury during a chronic state.

We found an increased BDNF expression in the contralateral side of the injury lasting up to 2 weeks. The mRNA expression of BDNF in the CA1 was not affected and the BDNF expression in the penumbra zone of the injury was decreased. This might be a compensatory increase of BDNF expression in the non-injured side to promote recovery or plasticity following injury and must likely be causing the increase of BDNF protein levels detected in the hippocampus (Schallert et al., 2000; Johansson, 2004; Keyvani et al., 2004). In patients with different BDNF polymorphisms there might be altered BDNF production or secretion, possibly affecting the protein levels or receptor bindings (Egan et al., 2003). This variation could have a greater impact in the injured brain.

Looking at the BDNF receptors, TrkB-FL showed a significant and consistent decrease in the penumbra zone up to 8 weeks following injury, while in the contralateral side there was a significant increase in the hippocampus 3 days following injury. This receptor has the catalytically active kinase domain and could be a response to increased BDNF in the contralateral side of injury, mediating BDNFs full effect. The consistent low level of Trk-FL in the penumbra zone corresponds to the low levels of BDNF, indicating low activity of BDNF promoting recovery.

The present study is the first to investigate the truncated forms of TrkB and p75^{NTR} following TBI. Importantly, these receptors were altered at a later time point than BDNF and TrkB-FL. The TrkB-truncated mRNA expression was significantly decreased in CA3 and CA1 8 weeks following injury while a significant increase was seen in the contralateral hippocampus 2 weeks after injury. In addition, this receptor showed a strong labelling in the area surrounding the cavity 8 weeks after injury. Similar expression was detected for p75^{NTR}, where it increased in the area surrounding the cavity up to 8 weeks after injury with no major effect in the hippocampus.

The neuronal and glial expression of p75^{NTR} has been shown to increase after injury (Taniuchi et al., 1988; Ernfors et al., 1989; Hayes et al., 1992; Chao, 2003; Oderfeld-

Nowak et al., 2003; Underwood and Coulson, 2008) and in spinal cord injury this increase lasted up to 8 weeks (Risling et al., 1992). The binding of neurotrophins to $p75^{NTR}$ receptor has been shown to cause cell death in the nervous system (Frade et al., 1996; Huang and Reichardt, 2001; Shulga et al., 2012) and deletion of this receptor prevents apoptosis (Naumann et al., 2002). Moreover, it has been shown that BDNF prevents axotomy-induced neuronal loss and atrophy (Giehl et al., 2001) and that endogenous BDNF is needed to overwhelm the death signalling from $p75^{NTR}$ receptor (Shulga et al., 2008). This may indicate that the low levels of BDNF expression and increased expression of $p75^{NTR}$ receptor observed in the cavity of the penetrating TBI 8 weeks following injury mediate cell death. However, the role of the $p75^{NTR}$ receptor following injury is complex and not fully elucidated (Chen et al., 2009). It is also possible that other neurotrophic factors than BDNF, such as NT-3 and NT-4, who also act on both TrkB- truncated and $p75^{NTR}$ receptors play a role in this chronic phase following TBI. It may be that the alterations at this late stage following TBI postulate a possibility to influence the plasticity and regenerative recovery of the injured brain tissue at a later stage than in the acute and subacute phase.

Studies have shown that while the TrkB-FL is essential for activating the BDNF signalling cascade, the TrkB-truncated inhibits this (Eide, F.F., et al. 1996). It acts as a BDNF-scavenging receptor (Biffo et al., 1995). Interestingly, the increased expression of TrkB truncated and $p75^{NTR}$ in our study was not accompanied by BNDF or TrkB-FL at 8 weeks after injury. This indicates that these receptors may play a role in the chronic phase following TBI, without the involvement of BDNF. Recent evidence suggests an independent role of TrkB-truncated in several functions, such as activation of intracellular signalling pathways, regulation of cytoskeletal changes in neurons and Ca^{2+} release in astrocytes (Rose et al., 2003; Fenner, 2012). Altogether, these findings may suggest an active role of TrkB-truncated following injury.

Further studies are needed to investigate whether these late expressions of these receptors are beneficial or deleterious. In either case, it raises the possibility to influence the recovery of brain injury during the chronic phase and the development of treatments that may improve the outcome in TBI patients.

5 GENERAL DISCUSSION

The clinicians and the neuroscientists face a great challenge when they attempt to understand how the most complicated organ in the human body responds to a complex disease. In order to comprehend the pathology of TBI, animal models are necessary. However, no single TBI model can mimic all aspects of the clinical TBI. In the present thesis we have used animal models to investigate three major components of TBI: blast wave-, penetrating- and rotational-acceleration TBI. We have shown that different types of TBI generate distinct patterns of alterations in gene expression in the brain. This highlights that although there are common molecular and biological cascades triggered by different types of primary TBI, there are also TBI type specific pathological processes induced that contribute to the secondary injuries.

The blast injury displayed lower number of responding genes compared to rot-TBI as well as pen-TBI, and these genes were mainly downregulated. No signs of cell death, inflammation, bleeding or oedema were observed in the primary blast injury model. This type of blast TBI model produces the primary blast wave injury and the histological findings indicate it to be a mild type of TBI (Risling and Davidsson, 2012). The blast waves produced have short duration and a simple form. It produces mainly primary blast injuries, however the gas and smoke emission might generate a tertiary blast injury. It is similar to an explosive loading on a close distance such as the blast wave of a landmine. The majority of the sufferers of blast TBI studied, mainly war veterans, display mild TBI which has been referred to as the "signature injury" of the recent wars (Okie, 2006). This model would be suitable for performing further studies to mimic the mild form of blast TBI. However, the behavioural outcome of this model must be elucidated in order to truly assess the severity of the injury. It would also be interesting to investigate the release of serum biomarkers following this injury. Serum biomarkers are feasible compared to imaging and neuropsychiatric tests. Furthermore, they have the possibility to predict a delayed onset of functional changes and long-term disabilities that may occur (Agoston and Elsayed, 2012).

A penetrating traumatic brain injury can occur as part of a blast wave injury, which occurs frequently in war zones. In addition, penetrating traumatic brain injuries can be the result of high-speed traffic accidents and they are also prevalent in areas with a high incidence of gun-related violence (Meyer et al., 2008; Coronado et al., 2011). The current penetrating TBI model provides the opportunity to investigate penetration with different velocity, depth and projectile shapes with high reproducibility. Current studies on the penetrating TBI showed a significant change in gene expression in the border zone of the injured cortex with mainly an upregulation of the responding genes and included genes involved in response to stress, inflammation and apoptosis. Neurodegeneration, macrophage invasion, haemorrhage and BBB disintegration during the first three days was demonstrated in the current and a previous study (Plantman et al., 2012). Signs of gliosis were evident at 3 days following injury up to 14 days, associated with a large cavity. The animals exposed to pen-TBI suffered balance disturbances and persistent reference memory deficits, reflecting the severity of the injury. Furthermore, our serum biomarker analysis in this injury has shown a peak of

S100B, Tau and NF-H on day 1 and 14 following injury. This second peak was not observed in the rot-TBI and might indicate secondary injuries in the pen-TBI (Rostami, 2011). The second peak can be the result of an aggravation of the injury caused by the haemorrhage or BBB breakdown that was not seen in rot-TBI. The haemorrhage and BBB disintegration allow for recruitment of macrophages from the circulation into the injured area in the pen-TBI but not in the rot-TBI. As was demonstrated in Study III, there was a massive labelling of macrophages and/or microglia in the injured area in the pen-TBI. In addition, complement proteins from the circulation can enter the site of injury, initiating/aggravating the phagocytosis of injured neurons, myelin debris (Koski et al., 1996) and injured axons (Bruck, 1997). We did indeed detect the protein C5b9 of complement terminal pathway in the pen-TBI and not in the rot-TBI, indicating that complement mediated cell death is present and may aggravate the injury. This is in line with previous findings in animal and human TBI (Bellander et al., 2001; Bellander et al., 2004). Interestingly, the BDNF receptors TrkB truncated and p75^{NTR} was increased in the area surrounding the cavity up to 8 weeks after injury. This was not followed by an increase in BDNF. Increased expression of TrkB mRNA for truncated receptors has been detected at the site of injury weeks after spinal cord lesion (Frisen et al., 1993). It was suggested that the truncated TrkB might bind and present BDNF to axonal growth cones (Frisen et al., 1992). It has also been shown that truncated receptor on non-neuronal cells inhibit BDNF neurite outgrowth by internalizing BDNF (Biffo et al., 1995; Fryer et al., 1997). Making this receptor as a "molecular sponge" that soak and remove the BDNF necessary for axonal growth. However, recent evidence has shown that TrkB truncated can activate intracellular signalling, regulate cytoskeletal changes in neurons and Ca^{2+} release in astrocytes, suggesting a more active and independent role of TrkB truncated (Rose et al., 2003; Fenner, 2012). It is possible that these receptors play a BDNF independent role at chronic phase of injury but if this effect is deleterious or beneficial needs to be elucidated.

The neuronal and glial expression of p75^{NTR} has previously been shown to increase after injury (Taniuchi et al., 1988; Ernfors et al., 1989; Hayes et al., 1992) and in spinal cord injuries this increase lasted up to 8 weeks (Risling et al., 1992). The binding of neurotrophins to the p75^{NTR} receptor has shown to cause cell death in the nervous system (Frade et al., 1996; Shulga et al., 2012) and deletion of this receptor prevents apoptosis (Naumann et al., 2002). Moreover, it has been shown that BDNF prevents axotomy-induced neuronal loss and atrophy (Giehl et al., 2001) and that endogenous BDNF is needed to overwhelm the death signalling from p75^{NTR} receptor (Shulga et al., 2012). This may indicate that the low levels of BDNF expression and the increased expression of p75^{NTR} receptor observed in the cavity of the penetrating TBI 8 weeks following injury mediate cell death and is harmful. However, the role of the p75^{NTR} receptor following injury is complex and not fully understood (Chen et al., 2009). It is also possible that other neurotrophic factors other than BDNF, such as NT-3 and NT-4, who act on both the TrkB-truncated and the p75^{NTR} receptor, play a role in this chronic phase following TBI.

We found an increased BDNF expression in the contralateral side of the injury lasting up to 2 weeks. The mRNA expression of BDNF in the CA1 was not affected and the BDNF expression in the penumbra zone of the injury was decreased. This finding is in line with previous studies using LFP, where mRNA expression of BDNF increased

bilaterally in the dentate gyrus and CA3 but not in CA1 (Hicks et al., 1997). The different expression of BDNF in relation to the distance to the injured area has been shown previously where the BDNF mRNA expression was significantly decreased in the injured area of the cortex in contrast to an increase in the adjacent cortex (Hicks et al., 1999a). This might be a compensatory increase of BDNF expression in the non-injured side to promote recovery or plasticity following injury, most likely causing the increase of BDNF protein levels detected in the hippocampus (Schallert et al., 2000; Johansson, 2004; Keyvani et al., 2004). The lack of BDNF increase in CA1 might be due to its vulnerability to injury that prohibits an increase in BDNF production.

BDNF is essential for synaptic remodelling in the adult hippocampus, which is crucial for plasticity (Heldt et al., 2007). In patients with different BDNF polymorphisms there might be altered BDNF production or secretion that could affect the protein levels or receptor bindings (Egan et al., 2003). This variation could have a greater impact in the injured brain. We did indeed show that in humans suffering from penetrating TBI the BNDF polymorphisms plays a significant role in predicting the cognitive outcome measured by general intelligence. The significant decline in the inferior group was seen in the first period following injury (10-15 years), and was not altered in the second period (30-35 years). There were no differences in the normal controls indicating that the influence of BDNF polymorphism is injury induced and plays a major role in the plasticity and recovery of the injured brain. The spontaneous recovery following TBI is most prominent during the first 30 days, but continues for at least 6 months in patients suffering TBI (Ruttan et al., 2008). Several in vivo studies have proposed the occurrence of lesion-induced cortical neurogenesis (Gu et al., 2000; Magavi et al., 2000; Jiang et al., 2001; Magavi and Macklis, 2001) and that functional improvement after permanent lesion is related to lesion-induced plasticity in the intact brain tissue (Jenkins and Merzenich, 1987; Johansson and Grabowski, 1994; Nudo and Milliken, 1996; Buonomano and Merzenich, 1998; Xerri et al., 1998; Hallett, 2001). Interestingly, the result of the human study showed that the lesion volume had a lower contributing factor than the genetic polymorphism. There was no difference between the groups regarding lesion volume. This indicates that the BDNF polymorphism plays a crucial role in the lesion-induced plasticity in the remaining intact brain tissue.

The alterations BDNF and its receptor demonstrated in the penetrating TBI in animals 8 weeks post-injury and the lesion-induced changes in human subjects, support the suggestion that it is possible to influence the plasticity and regenerative recovery of the injured brain tissue at a later stage than the acute and subacute phase, the so called third time window (Witte, 1998).

Inflammation is a hallmark of TBI and has been shown to have both a harmful and neuroprotective effect (Alexander et al., 2008). It has been suggested that the secretion of BDNF from immune cells generates the neuroprotective effect of CNS inflammation following injury (Hohlfeld et al., 2007). In Study III, we detected a massive inflammatory response in the penetrating TBI as early as day 1 post-injury. Correspondingly, the BDNF mRNA and protein levels were also increased, suggesting the immune cells as a possible source for BDNF secretion. The penetrating TBI displayed also increased expression of complement proteins including C5b9. There was strong APP labelling in adjacent to C5b9, both in the injured area and the border zone.

Indicating that the axonal injury could be complement mediated or aggravated by complement terminal pathway. However, no C5b9 could be detected in rotational TBI.

The signature of the rotational TBI was axonal injuries indicated by APP labelling. Positive APP immunoreactivity was found in the corpus callosum, the borderline of grey and white matter and centroaxial structures. The distribution of axonal injury corresponded well with the clinical findings in DAI. The axonal injury could also be detected in serum by increase levels of Tau and NF-H. There was also an increase in S100B indicating glial injury and a sustained high level of MBP suggesting slow myelin breakdown. Despite the axonal injuries and significant increase of biomarkers in serum, we did not see signs of apoptosis, BBB disturbances or C5b9. There were no signs of hematomas or contusions. Furthermore, the behavioural test in the rotational TBI showed only transient memory deficits. These results suggest that this model can produce a mTBI with axonal injuries as the underlying pathology. Moreover, it suggests that the axonal injuries per se do not activate the complement terminal pathway and furthermore, an attack of C5b9 on the axon leading to a secondary axotomy seems unlikely. It has been previously reported that not all traumatized axons undergo secondary axotomy or cell death (Singleton et al., 2002; Buki and Povlishock, 2006) and it has been suggested that some injured axons may recover. In addition the Wallerian degeneration seen following axonal injury in CNS is very slow and may take months to years. One of the main reasons is the lack of extensive opening of the BBB in the CNS that inhibits entry of large numbers of peripheral opsonins and macrophages (Vargas and Barres, 2007). A distorted BBB as seen in pen-TBI may allow entry and involvement of macrophages and complement terminal pathway proteins that accelerate Wallerian degeneration and cell death. The lack of BBB disturbances in the rot-TBI may prohibit this. It would be highly relevant to study longer survival times in addition to repetitive injuries, as seen in sport related TBI, using this model to investigate the long term effects of these initial axonal injuries. In addition, further investigation regarding the synaptology following rot-TBI, both in the acute and chronic state, would be highly interesting in order to elucidate the role of the C1q and C3 in TBI.

One of the two anatomical areas in the adult mammalian brain showing neurogenesis is the subgranular zone of the dentate gyrus in the hippocampus, which plays a crucial role in plasticity. The hippocampus of animals exposed to rotational TBI showed an upregulation of genes involved in neurogenesis and synaptic transmission. Interestingly, C1q and C3 mRNA was increased in the hippocampus of the injured animals. C1q and C3b are known as opsonins that tag targeted cells to enhance recognition, recruitment and phagocytosis by cells such as macrophages and microglia (Trouw et al., 2008). There is convincing evidence that complement activation plays an important role in the pathogenesis of brain injury and neurodegenerative diseases (van Beek et al., 2003; Bonifati and Kishore, 2007). There are strong indications that synapse loss is an early event in neurodegenerative disease (Selkoe, 2002). Recent work has shown complement mediated synapse loss in the developing and adult brain (Stevens et al., 2007). Our findings of increased expression of C1q and C3 in the corpus callosum, the cingulate cortex, the hippocampus, the thalamus and the amygdala,

generate a tantalizing postulation that rotational injury may cause complement mediated synapse loss. Since this process might be very slow due to the lack of entry of peripheral macrophages and modest phagocytic activity of microglia in CNS, the outcome of this synaptic loss may be detected a significant time following injury. This synaptic loss could play a role in the manifested clinical symptoms in patients with mTBI. Furthermore, it has been shown that C1q knockout mice had more epileptiform activity than the wild type. It was suggested that the C1q knockout mice fail to prune excessive excitatory synaptic connections during development (Chu et al., 2010). It is highly possible that this function becomes important following TBI and affects the presence of posttraumatic epilepsy. It has indeed previously been shown that more than 50% of the Vietnam veterans studied in this thesis exhibit post-traumatic epilepsy (Raymont et al., 2010). It is possible that the polymorphism of C1q plays a role in determining the patients predisposed to posttraumatic epilepsy.

Although TBI has been described since time of Hippocrates and Rhazes we still have a long way ahead of us to understand and treat this widespread disease. But with more successful translation from animal models to humans and understanding the heterogenetic background of the patients and its application, we may pave the path for effective diagnostic and therapeutic tools.

6   MAIN CONCLUSIONS

We found distinct gene expression profiles in the three different TBI models. This provides information about genes and biological processes involved in the pathophysiology of different types of TBI *(Study I)*.

The blast injury displayed lower numbers of responding genes compared to rot-TBI as well as pen-TBI, and these genes were mainly downregulated. No signs of cell death, inflammation, bleeding or oedema were observed in the primary blast injury model. This type of blast TBI model produces the primary blast wave injury and the histological findings indicate it to be a mild type of TBI *(Study I)*.

The hallmark of the rotational TBI was axonal injuries found in anatomical locations comparable with clinical findings in DAI in humans. This was detected both by APP immunoreactivity and serum biomarkers. Serum biomarkers Tau, NF-H, S100B and MBP were increased despite the fact that no BBB disturbances were detected. There were no signs of apoptosis or any hematomas or contusions. The behavioural analysis showed only transient memory deficits. Indicating this rotational TBI to be mild *(Study II)*.

The penetrating TBI model showed a massive inflammatory response in combination with axonal injury at the injury site. The complement proteins including the terminal pathway were activated following TBI. In addition BDNF was also increased at the site of injury. Neurodegeneration and apoptosis were found in the border zone of injury *(Study III & V)*.

Serum biomarkers Tau, NF-H, S100B and MBP were increased in the rotational TBI from day 1 until 2 weeks following injury with a peak on day 3. Serum biomarkers were detected despite the lack of BBB disturbances. Increased serum levels of Tau, NF-H, S100B and MBP were also noted in the penetrating TBI, however the peak was found at day 1 and 14. This indicates signs of secondary injuries and generates distinct temporal patterns of these biomarkers for each type of TBI *(Study II)*.

The mRNA expression of C1q and C3 is increased in the rotational TBI and the anatomical localization corresponds well with areas affected in patients suffering DAI. However, activation of the terminal pathway could not be detected, targeting C5b9. Despite the axonal injury being the hallmark of rotational TBI the lack of C5b9 indicates that secondary axotomy is not complement mediated *(Study III)*.

The presence of the ApoE ε4 allele or COMT polymorphism did not affect the cognitive outcome following penetrating TBI in humans *(Study IV)*.

BDNF polymorphisms were significantly associated with post-injury recovery of general intelligence with the most pronounced effect at the Phase II time point, indicating lesion-induced plasticity. The genotypes accounted for 5% of the variance of the AFQT scores, independent of other significant predictors such as pre-injury

intelligence and percentage of brain volume loss. These data indicate that genetic variations in BDNF play a significant role in lesion-induced recovery following penetrating TBI *(Study IV).*

The BDNF mRNA and protein increased in the hippocampus up to 2 weeks following penetrating injury. The increase of mRNA TrkB full length and truncated receptor showed different temporal expression in the hippocampus, indicating their distinct role following injury *(Study V).*

Following penetrating TBI the expression of TrkB truncated and p75^{NTR} in the injured area was increased 8 weeks following injury without an increase in BDNF. Suggesting that these receptors play a BDNF independent role at the chronic phase of injury that could have therapeutic implications *(Study V)*.

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<div align="right">Isaac Newton.</div>

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8   REFERENCES

Adams, J.H. (1982). Diffuse axonal injury in non-missile head injury. *Injury* 13**,** 444-445.

Adams, J.H. (1992). "Head injury," in *Greenfield's Neuropathology,* ed. D.L. Adams Jh. 5th ed (London: Edward Arnold), 106-152.

Adams, J.H., Doyle, D., Ford, I., Gennarelli, T.A., Graham, D.I., and Mclellan, D.R. (1989a). Diffuse axonal injury in head injury: definition, diagnosis and grading. *Histopathology* 15**,** 49-59.

Adams, J.H., Doyle, D., Ford, I., Graham, D.I., Mcgee, M., and Mclellan, D.R. (1989b). Brain damage in fatal non-missile head injury in relation to age and type of injury. *Scott Med J* 34**,** 399-401.

Agoston, D.V., and Elsayed, M. (2012). Serum-Based Protein Biomarkers in Blast-Induced Traumatic Brain Injury Spectrum Disorder. *Front Neurol* 3**,** 107.

Akil, M., Kolachana, B.S., Rothmond, D.A., Hyde, T.M., Weinberger, D.R., and Kleinman, J.E. (2003). Catechol-O-methyltransferase genotype and dopamine regulation in the human brain. *J Neurosci* 23**,** 2008-2013.

Alexander, J.J., Anderson, A.J., Barnum, S.R., Stevens, B., and Tenner, A.J. (2008). The complement cascade: Yin-Yang in neuroinflammation--neuro-protection and -degeneration. *J Neurochem* 107**,** 1169-1187.

Anderson, A.J., Robert, S., Huang, W., Young, W., and Cotman, C.W. (2004). Activation of complement pathways after contusion-induced spinal cord injury. *J Neurotrauma* 21**,** 1831-1846.

Anderson, K.J., Scheff, S.W., Miller, K.M., Roberts, K.N., Gilmer, L.K., Yang, C., and Shaw, G. (2008). The phosphorylated axonal form of the neurofilament subunit NF-H (pNF-H) as a blood biomarker of traumatic brain injury. *J Neurotrauma* 25**,** 1079-1085.

Anderson, R.E., Hansson, L.O., Nilsson, O., Dijlai-Merzoug, R., and Settergren, G. (2001a). High serum S100B levels for trauma patients without head injuries. *Neurosurgery* 48**,** 1255-1258; discussion 1258-1260.

Anderson, R.E., Hansson, L.O., Nilsson, O., Liska, J., Settergren, G., and Vaage, J. (2001b). Increase in serum S100A1-B and S100BB during cardiac surgery arises from extracerebral sources. *Ann Thorac Surg* 71**,** 1512-1517.

Azmitia, E.C., Dolan, K., and Whitaker-Azmitia, P.M. (1990). S-100B but not NGF, EGF, insulin or calmodulin is a CNS serotonergic growth factor. *Brain Res* 516**,** 354-356.

Barde, Y.A., Edgar, D., and Thoenen, H. (1982). Purification of a new neurotrophic factor from mammalian brain. *EMBO J* 1**,** 549-553.

Bauman, R.A., Ling, G., Tong, L., Januszkiewicz, A., Agoston, D., Delanerolle, N., Kim, Y., Ritzel, D., Bell, R., Ecklund, J., Armonda, R., Bandak, F., and Parks, S. (2009). An introductory characterization of a combat-casualty-care relevant swine model of closed head injury resulting from exposure to explosive blast. *J Neurotrauma* 26**,** 841-860.

Bazarian, J.J., and Atabaki, S. (2001). Predicting postconcussion syndrome after minor traumatic brain injury. *Acad Emerg Med* 8**,** 788-795.

Bazarian, J.J., Blyth, B., and Cimpello, L. (2006). Bench to bedside: evidence for brain injury after concussion--looking beyond the computed tomography scan. *Acad Emerg Med* 13**,** 199-214.

Bazarian, J.J., Wong, T., Harris, M., Leahey, N., Mookerjee, S., and Dombovy, M. (1999). Epidemiology and predictors of post-concussive syndrome after minor head injury in an emergency population. *Brain Inj* 13**,** 173-189.

Bellander, B.M., Bendel, O., Von Euler, G., Ohlsson, M., and Svensson, M. (2004). Activation of microglial cells and complement following traumatic injury in rat entorhinal-hippocampal slice cultures. *J Neurotrauma* 21**,** 605-615.

Bellander, B.M., Olafsson, I.H., Ghatan, P.H., Bro Skejo, H.P., Hansson, L.O., Wanecek, M., and Svensson, M.A. (2011). Secondary insults following traumatic brain injury enhance complement activation in the human brain and release of the tissue damage marker S100B. *Acta Neurochir (Wien)* 153**,** 90-100.

Bellander, B.M., Singhrao, S.K., Ohlsson, M., Mattsson, P., and Svensson, M. (2001). Complement activation in the human brain after traumatic head injury. *J Neurotrauma* 18**,** 1295-1311.

Bentley, C.A., and Lee, K.F. (2000). p75 is important for axon growth and schwann cell migration during development. *J Neurosci* 20**,** 7706-7715.

Biffo, S., Offenhauser, N., Carter, B.D., and Barde, Y.A. (1995). Selective binding and internalisation by truncated receptors restrict the availability of BDNF during development. *Development* 121**,** 2461-2470.

Binder, L.I., Frankfurter, A., and Rebhun, L.I. (1985). The distribution of tau in the mammalian central nervous system. *J Cell Biol* 101**,** 1371-1378.

Bito, H., and Takemoto-Kimura, S. (2003). Ca(2+)/CREB/CBP-dependent gene regulation: a shared mechanism critical in long-term synaptic plasticity and neuronal survival. *Cell Calcium* 34**,** 425-430.

Blinzinger, K., and Kreutzberg, G. (1968). Displacement of synaptic terminals from regenerating motoneurons by microglial cells. *Z Zellforsch Mikrosk Anat* 85**,** 145-157.

Blumbergs, P.C., Scott, G., Manavis, J., Wainwright, H., Simpson, D.A., and Mclean, A.J. (1994). Staining of amyloid precursor protein to study axonal damage in mild head injury. *Lancet* 344**,** 1055-1056.

Blumbergs, P.C., Scott, G., Manavis, J., Wainwright, H., Simpson, D.A., and Mclean, A.J. (1995). Topography of axonal injury as defined by amyloid precursor protein and the sector scoring method in mild and severe closed head injury. *J Neurotrauma* 12**,** 565-572.

Bolander, H.G., Persson, L., Hillered, L., D'argy, R., Ponten, U., and Olsson, Y. (1989). Regional cerebral blood flow and histopathologic changes after middle cerebral artery occlusion in rats. *Stroke* 20**,** 930-937.

Bonfoco, E., Krainc, D., Ankarcrona, M., Nicotera, P., and Lipton, S.A. (1995). Apoptosis and necrosis: two distinct events induced, respectively, by mild and intense insults with N-methyl-D-aspartate or nitric oxide/superoxide in cortical cell cultures. *Proc Natl Acad Sci U S A* 92**,** 7162-7166.

Bonifati, D.M., and Kishore, U. (2007). Role of complement in neurodegeneration and neuroinflammation. *Mol Immunol* 44**,** 999-1010.

Borg, J., Holm, L., Cassidy, J.D., Peloso, P.M., Carroll, L.J., Von Holst, H., and Ericson, K. (2004a). Diagnostic procedures in mild traumatic brain injury: results of the WHO Collaborating Centre Task Force on Mild Traumatic Brain Injury. *J Rehabil Med***,** 61-75.

Borg, J., Holm, L., Peloso, P.M., Cassidy, J.D., Carroll, L.J., Von Holst, H., Paniak, C., and Yates, D. (2004b). Non-surgical intervention and cost for mild traumatic brain injury: results of the WHO Collaborating Centre Task Force on Mild Traumatic Brain Injury. *J Rehabil Med***,** 76-83.

Bramlett, H.M., Green, E.J., and Dietrich, W.D. (1999). Exacerbation of cortical and hippocampal CA1 damage due to posttraumatic hypoxia following moderate fluid-percussion brain injury in rats. *J Neurosurg* 91**,** 653-659.

Brewton, L.S., Haddad, L., and Azmitia, E.C. (2001). Colchicine-induced cytoskeletal collapse and apoptosis in N-18 neuroblastoma cultures is rapidly reversed by applied S-100beta. *Brain Res* 912**,** 9-16.

Bruck, W. (1997). The role of macrophages in Wallerian degeneration. *Brain Pathol* 7**,** 741-752.

Bueller, J.A., Aftab, M., Sen, S., Gomez-Hassan, D., Burmeister, M., and Zubieta, J.K. (2006). BDNF Val66Met allele is associated with reduced hippocampal volume in healthy subjects. *Biol Psychiatry* 59**,** 812-815.

Buki, A., and Povlishock, J.T. (2006). All roads lead to disconnection?--Traumatic axonal injury revisited. *Acta Neurochir (Wien)* 148**,** 181-193; discussion 193-184.

Buki, A., Siman, R., Trojanowski, J.Q., and Povlishock, J.T. (1999). The role of calpain-mediated spectrin proteolysis in traumatically induced axonal injury. *J Neuropathol Exp Neurol* 58**,** 365-375.

Bulut, M., Koksal, O., Dogan, S., Bolca, N., Ozguc, H., Korfali, E., Ilcol, Y.O., and Parklak, M. (2006). Tau protein as a serum marker of brain damage in mild traumatic brain injury: preliminary results. *Adv Ther* 23**,** 12-22.

Buonomano, D.V., and Merzenich, M.M. (1998). Cortical plasticity: from synapses to maps. *Annu Rev Neurosci* 21**,** 149-186.

Carey, M.E., Sarna, G.S., Farrell, J.B., and Happel, L.T. (1989). Experimental missile wound to the brain. *J Neurosurg* 71**,** 754-764.

Carroll, L.J., Cassidy, J.D., Holm, L., Kraus, J., and Coronado, V.G. (2004a). Methodological issues and research recommendations for mild traumatic brain injury: the WHO Collaborating Centre Task Force on Mild Traumatic Brain Injury. *J Rehabil Med***,** 113-125.

Carroll, L.J., Cassidy, J.D., Peloso, P.M., Borg, J., Von Holst, H., Holm, L., Paniak, C., and Pepin, M. (2004b). Prognosis for mild traumatic brain injury: results of the WHO Collaborating Centre Task Force on Mild Traumatic Brain Injury. *J Rehabil Med***,** 84-105.

Carroll, L.J., Cassidy, J.D., Peloso, P.M., Garritty, C., and Giles-Smith, L. (2004c). Systematic search and review procedures: results of the WHO Collaborating Centre Task Force on Mild Traumatic Brain Injury. *J Rehabil Med***,** 11-14.

Cassidy, J.D., Carroll, L., Cote, P., Holm, L., and Nygren, A. (2004a). Mild traumatic brain injury after traffic collisions: a population-based inception cohort study. *J Rehabil Med*, 15-21.

Cassidy, J.D., Carroll, L.J., Peloso, P.M., Borg, J., Von Holst, H., Holm, L., Kraus, J., and Coronado, V.G. (2004b). Incidence, risk factors and prevention of mild traumatic brain injury: results of the WHO Collaborating Centre Task Force on Mild Traumatic Brain Injury. *J Rehabil Med*, 28-60.

Catterall, W.A., Dib-Hajj, S., Meisler, M.H., and Pietrobon, D. (2008). Inherited neuronal ion channelopathies: new windows on complex neurological diseases. *J Neurosci* 28, 11768-11777.

Cernak, I., Merkle, A.C., Koliatsos, V.E., Bilik, J.M., Luong, Q.T., Mahota, T.M., Xu, L., Slack, N., Windle, D., and Ahmed, F.A. (2011). The pathobiology of blast injuries and blast-induced neurotrauma as identified using a new experimental model of injury in mice. *Neurobiol Dis* 41, 538-551.

Cernak, I., Savic, J., Malicevic, Z., Zunic, G., Radosevic, P., Ivanovic, I., and Davidovic, L. (1996). Involvement of the central nervous system in the general response to pulmonary blast injury. *J Trauma* 40, S100-104.

Cernak, I., Vink, R., Zapple, D.N., Cruz, M.I., Ahmed, F., Chang, T., Fricke, S.T., and Faden, A.I. (2004). The pathobiology of moderate diffuse traumatic brain injury as identified using a new experimental model of injury in rats. *Neurobiol Dis* 17, 29-43.

Cernak, I., Wang, Z., Jiang, J., Bian, X., and Savic, J. (2001). Ultrastructural and functional characteristics of blast injury-induced neurotrauma. *J Trauma* 50, 695-706.

Chamoun, R., Suki, D., Gopinath, S.P., Goodman, J.C., and Robertson, C. (2010). Role of extracellular glutamate measured by cerebral microdialysis in severe traumatic brain injury. *J Neurosurg* 113, 564-570.

Chao, M.V. (2003). Neurotrophins and their receptors: a convergence point for many signalling pathways. *Nat Rev Neurosci* 4, 299-309.

Chavko, M., Koller, W.A., Prusaczyk, W.K., and Mccarron, R.M. (2007). Measurement of blast wave by a miniature fiber optic pressure transducer in the rat brain. *J Neurosci Methods* 159, 277-281.

Chen, Y., Zeng, J., Cen, L., Chen, Y., Wang, X., Yao, G., Wang, W., Qi, W., and Kong, K. (2009). Multiple roles of the p75 neurotrophin receptor in the nervous system. *J Int Med Res* 37, 281-288.

Chiang, M.F., Chang, J.G., and Hu, C.J. (2003). Association between apolipoprotein E genotype and outcome of traumatic brain injury. *Acta Neurochir (Wien)* 145, 649-653; discussion 653-644.

Ching, G.Y., and Liem, R.K. (1993). Assembly of type IV neuronal intermediate filaments in nonneuronal cells in the absence of preexisting cytoplasmic intermediate filaments. *J Cell Biol* 122, 1323-1335.

Choi, D.W. (1987). Ionic dependence of glutamate neurotoxicity. *J Neurosci* 7, 369-379.

Christman, C.W., Grady, M.S., Walker, S.A., Holloway, K.L., and Povlishock, J.T. (1994). Ultrastructural studies of diffuse axonal injury in humans. *J Neurotrauma* 11, 173-186.

Chu, Y., Jin, X., Parada, I., Pesic, A., Stevens, B., Barres, B., and Prince, D.A. (2010). Enhanced synaptic connectivity and epilepsy in C1q knockout mice. *Proc Natl Acad Sci U S A* 107, 7975-7980.

Clark, R.S., Schiding, J.K., Kaczorowski, S.L., Marion, D.W., and Kochanek, P.M. (1994). Neutrophil accumulation after traumatic brain injury in rats: comparison of weight drop and controlled cortical impact models. *J Neurotrauma* 11, 499-506.

Clausen, F., Hanell, A., Israelsson, C., Hedin, J., Ebendal, T., Mir, A.K., Gram, H., and Marklund, N. (2011). Neutralization of interleukin-1beta reduces cerebral edema and tissue loss and improves late cognitive outcome following traumatic brain injury in mice. *Eur J Neurosci* 34, 110-123.

Clayton, E.H., Genin, G.M., and Bayly, P.V. (2012). Transmission, attenuation and reflection of shear waves in the human brain. *J R Soc Interface*.

Clemedson, C.J., and Criborn, C.O. (1955). A detonation chamber for physiological blast research. *J Aviat Med* 26, 373-381.

Clemedson, C.J., Hartelius, H., and Holmberg, G. (1957). The effect of high explosive blast on the cerebral vascular permeability. *Acta Pathol Microbiol Scand* 40, 89-95.

Cohen, J. (1988). Statistical power analysis for behavioral sciences. *NJ:* Lawrence Earlbaum Associates.

Cohen, S.R., Herndon, R.M., and Mckhann, G.M. (1976). Myelin basic protein in cerebrospinal fluid as an indicator of active demyelination. *Trans Am Neurol Assoc* 101, 45-47.

Corder, E.H., Saunders, A.M., Strittmatter, W.J., Schmechel, D.E., Gaskell, P.C., Small, G.W., Roses, A.D., Haines, J.L., and Pericak-Vance, M.A. (1993). Gene dose of apolipoprotein E type 4 allele and the risk of Alzheimer's disease in late onset families. *Science* 261, 921-923.

Coronado, V.G., Xu, L., Basavaraju, S.V., Mcguire, L.C., Wald, M.M., Faul, M.D., Guzman, B.R., and Hemphill, J.D. (2011). Surveillance for traumatic brain injury-related deaths--United States, 1997-2007. *MMWR Surveill Summ* 60, 1-32.

Crawford, F.C., Vanderploeg, R.D., Freeman, M.J., Singh, S., Waisman, M., Michaels, L., Abdullah, L., Warden, D., Lipsky, R., Salazar, A., and Mullan, M.J. (2002). APOE genotype influences acquisition and recall following traumatic brain injury. *Neurology* 58, 1115-1118.

Crockard, H.A., Brown, F.D., Johns, L.M., and Mullan, S. (1977). An experimental cerebral missile injury model in primates. *J Neurosurg* 46, 776-783.

Cuchillo-Ibanez, I., Seereeram, A., Byers, H.L., Leung, K.Y., Ward, M.A., Anderton, B.H., and Hanger, D.P. (2008). Phosphorylation of tau regulates its axonal transport by controlling its binding to kinesin. *FASEB J* 22, 3186-3195.

Cull-Candy, S.G., and Leszkiewicz, D.N. (2004). Role of distinct NMDA receptor subtypes at central synapses. *Sci STKE* 2004, re16.

Dail, W.G., Feeney, D.M., Murray, H.M., Linn, R.T., and Boyeson, M.G. (1981). Responses to cortical injury: II. Widespread depression of the activity of an enzyme in cortex remote from a focal injury. *Brain Res* 211, 79-89.

Dardiotis, E., Fountas, K.N., Dardioti, M., Xiromerisiou, G., Kapsalaki, E., Tasiou, A., and Hadjigeorgiou, G.M. (2010). Genetic association studies in patients with traumatic brain injury. *Neurosurg Focus* 28, E9.

Davidsson, J., and Risling, M. (2011). A new model to produce sagittal plane rotational induced diffuse axonal injuries. *Front Neurol* 2, 41.

Dechant, G., and Barde, Y.A. (2002). The neurotrophin receptor p75(NTR): novel functions and implications for diseases of the nervous system. *Nat Neurosci* 5, 1131-1136.

Demetriades, D., Kuncir, E., Murray, J., Velmahos, G.C., Rhee, P., and Chan, L. (2004). Mortality prediction of head Abbreviated Injury Score and Glasgow Coma Scale: analysis of 7,764 head injuries. *J Am Coll Surg* 199, 216-222.

Dempster, E., Toulopoulou, T., Mcdonald, C., Bramon, E., Walshe, M., Filbey, F., Wickham, H., Sham, P.C., Murray, R.M., and Collier, D.A. (2005). Association between BDNF val66 met genotype and episodic memory. *Am J Med Genet B Neuropsychiatr Genet* 134B, 73-75.

Dennis, G., Jr., Sherman, B.T., Hosack, D.A., Yang, J., Gao, W., Lane, H.C., and Lempicki, R.A. (2003). DAVID: Database for Annotation, Visualization, and Integrated Discovery. *Genome Biol* 4, P3.

Dewitt, D.S., Jenkins, L.W., Wei, E.P., Lutz, H., Becker, D.P., and Kontos, H.A. (1986). Effects of fluid-percussion brain injury on regional cerebral blood flow and pial arteriolar diameter. *J Neurosurg* 64, 787-794.

Dietrich, W.D., Alonso, O., Busto, R., Prado, R., Dewanjee, S., Dewanjee, M.K., and Ginsberg, M.D. (1996). Widespread hemodynamic depression and focal platelet accumulation after fluid percussion brain injury: a double-label autoradiographic study in rats. *J Cereb Blood Flow Metab* 16, 481-489.

Dietrich, W.D., Alonso, O., Busto, R., Prado, R., Zhao, W., Dewanjee, M.K., and Ginsberg, M.D. (1998). Posttraumatic cerebral ischemia after fluid percussion brain injury: an autoradiographic and histopathological study in rats. *Neurosurgery* 43, 585-593; discussion 593-584.

Dietrich, W.D., Alonso, O., and Halley, M. (1994). Early microvascular and neuronal consequences of traumatic brain injury: a light and electron microscopic study in rats. *J Neurotrauma* 11, 289-301.

Dixon, C.E., Clifton, G.L., Lighthall, J.W., Yaghmai, A.A., and Hayes, R.L. (1991). A controlled cortical impact model of traumatic brain injury in the rat. *J Neurosci Methods* 39, 253-262.

Dixon, C.E., Lyeth, B.G., Povlishock, J.T., Findling, R.L., Hamm, R.J., Marmarou, A., Young, H.F., and Hayes, R.L. (1987). A fluid percussion model of experimental brain injury in the rat. *J Neurosurg* 67, 110-119.

Draganski, B., Gaser, C., Busch, V., Schuierer, G., Bogdahn, U., and May, A. (2004). Neuroplasticity: changes in grey matter induced by training. *Nature* 427, 311-312.

Drake, A.I., Mcdonald, E.C., Magnus, N.E., Gray, N., and Gottshall, K. (2006). Utility of Glasgow Coma Scale-Extended in symptom prediction following mild traumatic brain injury. *Brain Inj* 20, 469-475.

Egan, M.F., Kojima, M., Callicott, J.H., Goldberg, T.E., Kolachana, B.S., Bertolino, A., Zaitsev, E., Gold, B., Goldman, D., Dean, M., Lu, B., and Weinberger, D.R. (2003). The BDNF val66met polymorphism affects activity-dependent secretion of BDNF and human memory and hippocampal function. *Cell* 112, 257-269.

Eide, F.F., Vining, E.R., Eide, B.L., Zang, K., Wang, X.Y., and Reichardt, L.F. (1996). Naturally occurring truncated trkB receptors have dominant inhibitory effects on brain-derived neurotrophic factor signaling. *J Neurosci* 16, 3123-3129.

Elder, G.A., and Cristian, A. (2009). Blast-related mild traumatic brain injury: mechanisms of injury and impact on clinical care. *Mt Sinai J Med* 76, 111-118.

Eriksson, P.S., Perfilieva, E., Bjork-Eriksson, T., Alborn, A.M., Nordborg, C., Peterson, D.A., and Gage, F.H. (1998). Neurogenesis in the adult human hippocampus. *Nat Med* 4, 1313-1317.

Ernfors, P., Henschen, A., Olson, L., and Persson, H. (1989). Expression of nerve growth factor receptor mRNA is developmentally regulated and increased after axotomy in rat spinal cord motoneurons. *Neuron* 2, 1605-1613.

Ernfors, P., Lee, K.F., and Jaenisch, R. (1994). Mice lacking brain-derived neurotrophic factor develop with sensory deficits. *Nature* 368, 147-150.

Ethell, I.M., and Pasquale, E.B. (2005). Molecular mechanisms of dendritic spine development and remodeling. *Prog Neurobiol* 75, 161-205.

Feeney, D.M., Boyeson, M.G., Linn, R.T., Murray, H.M., and Dail, W.G. (1981). Responses to cortical injury: I. Methodology and local effects of contusions in the rat. *Brain Res* 211, 67-77.

Fenner, B.M. (2012). Truncated TrkB: beyond a dominant negative receptor. *Cytokine Growth Factor Rev* 23, 15-24.

Finfer, S.R., and Cohen, J. (2001). Severe traumatic brain injury. *Resuscitation* 48, 77-90.

Finnie, J.W. (1993). Pathology of experimental traumatic craniocerebral missile injury. *J Comp Pathol* 108, 93-101.

Folstein, M.F., Folstein, S.E., and Mchugh, P.R. (1975). "Mini-mental state". A practical method for grading the cognitive state of patients for the clinician. *J Psychiatr Res* 12, 189-198.

Fork, M., Bartels, C., Ebert, A.D., Grubich, C., Synowitz, H., and Wallesch, C.W. (2005). Neuropsychological sequelae of diffuse traumatic brain injury. *Brain Inj* 19, 101-108.

Frade, J.M., Rodriguez-Tebar, A., and Barde, Y.A. (1996). Induction of cell death by endogenous nerve growth factor through its p75 receptor. *Nature* 383, 166-168.

Friedman, G., Froom, P., Sazbon, L., Grinblatt, I., Shochina, M., Tsenter, J., Babaey, S., Yehuda, B., and Groswasser, Z. (1999). Apolipoprotein E-epsilon4 genotype predicts a poor outcome in survivors of traumatic brain injury. *Neurology* 52, 244-248.

Frisen, J., Verge, V.M., Cullheim, S., Persson, H., Fried, K., Middlemas, D.S., Hunter, T., Hokfelt, T., and Risling, M. (1992). Increased levels of trkB mRNA and trkB protein-like immunoreactivity in the injured rat and cat spinal cord. *Proc Natl Acad Sci U S A* 89, 11282-11286.

Frisen, J., Verge, V.M., Fried, K., Risling, M., Persson, H., Trotter, J., Hokfelt, T., and Lindholm, D. (1993). Characterization of glial trkB receptors: differential response to injury in the central and peripheral nervous systems. *Proc Natl Acad Sci U S A* 90, 4971-4975.

Fryer, R.H., Kaplan, D.R., and Kromer, L.F. (1997). Truncated trkB receptors on nonneuronal cells inhibit BDNF-induced neurite outgrowth in vitro. *Exp Neurol* 148, 616-627.

Gabbita, S.P., Scheff, S.W., Menard, R.M., Roberts, K., Fugaccia, I., and Zemlan, F.P. (2005). Cleaved-tau: a biomarker of neuronal damage after traumatic brain injury. *J Neurotrauma* 22, 83-94.

Gabriel, S.B., Schaffner, S.F., Nguyen, H., Moore, J.M., Roy, J., Blumenstiel, B., Higgins, J., Defelice, M., Lochner, A., Faggart, M., Liu-Cordero, S.N., Rotimi, C., Adeyemo, A., Cooper, R., Ward, R., Lander, E.S., Daly, M.J., and Altshuler, D. (2002). The structure of haplotype blocks in the human genome. *Science* 296, 2225-2229.

Gao, B., Jiang, S., Wang, X., and Chen, J. (2000). The role of pre-injury IQ in the determination of intellectual impairment from traumatic head injury. *J Neuropsychiatry Clin Neurosci* 12, 385-388.

Garner, J.P., Watts, S., Parry, C., Bird, J., and Kirkman, E. (2009). Development of a large animal model for investigating resuscitation after blast and hemorrhage. *World J Surg* 33, 2194-2202.

Gasque, P., Singhrao, S.K., Neal, J.W., Gotze, O., and Morgan, B.P. (1997). Expression of the receptor for complement C5a (CD88) is up-regulated on reactive astrocytes, microglia, and endothelial cells in the inflamed human central nervous system. *Am J Pathol* 150, 31-41.

Gendron, T.F., and Petrucelli, L. (2009). The role of tau in neurodegeneration. *Mol Neurodegener* 4, 13.

Gennarelli, T.A., Adams, J.H., and Graham, D.I. (1981). Acceleration induced head injury in the monkey.I. The model, its mechanical and physiological correlates. *Acta Neuropathol Suppl* 7, 23-25.

Gennarelli, T.A., Thibault, L.E., Adams, J.H., Graham, D.I., Thompson, C.J., and Marcincin, R.P. (1982). Diffuse axonal injury and traumatic coma in the primate. *Ann Neurol* 12, 564-574.

Ghajar, J. (2000). Traumatic brain injury. *Lancet* 356**,** 923-929.

Giehl, K.M., Rohrig, S., Bonatz, H., Gutjahr, M., Leiner, B., Bartke, I., Yan, Q., Reichardt, L.F., Backus, C., Welcher, A.A., Dethleffsen, K., Mestres, P., and Meyer, M. (2001). Endogenous brain-derived neurotrophic factor and neurotrophin-3 antagonistically regulate survival of axotomized corticospinal neurons in vivo. *J Neurosci* 21**,** 3492-3502.

Glascher, J., Rudrauf, D., Colom, R., Paul, L.K., Tranel, D., Damasio, H., and Adolphs, R. (2010). Distributed neural system for general intelligence revealed by lesion mapping. *Proc Natl Acad Sci U S A* 107**,** 4705-4709.

Goldman, H., Hodgson, V., Morehead, M., Hazlett, J., and Murphy, S. (1991). Cerebrovascular changes in a rat model of moderate closed-head injury. *J Neurotrauma* 8**,** 129-144.

Goldstein, L.B., and Davis, J.N. (1990). Beam-walking in rats: studies towards developing an animal model of functional recovery after brain injury. *J Neurosci Methods* 31**,** 101-107.

Goncalves, D.S., Lenz, G., Karl, J., Goncalves, C.A., and Rodnight, R. (2000). Extracellular S100B protein modulates ERK in astrocyte cultures. *Neuroreport* 11**,** 807-809.

Grady, M.S., Mclaughlin, M.R., Christman, C.W., Valadka, A.B., Fligner, C.L., and Povlishock, J.T. (1993). The use of antibodies targeted against the neurofilament subunits for the detection of diffuse axonal injury in humans. *J Neuropathol Exp Neurol* 52**,** 143-152.

Grafman, J., Jonas, B.S., Martin, A., Salazar, A.M., Weingartner, H., Ludlow, C., Smutok, M.A., and Vance, S.C. (1988). Intellectual function following penetrating head injury in Vietnam veterans. *Brain* 111 (Pt 1)**,** 169-184.

Grafman, J., Salazar, A., Weingartner, H., Vance, S., and Amin, D. (1986). The relationship of brain-tissue loss volume and lesion location to cognitive deficit. *J Neurosci* 6**,** 301-307.

Graham, D.I., and Adams, J.H. (1971). Ischaemic brain damage in fatal head injuries. *Lancet* 1**,** 265-266.

Graham, D.I., Adams, J.H., and Doyle, D. (1978). Ischaemic brain damage in fatal non-missile head injuries. *J Neurol Sci* 39**,** 213-234.

Graham, D.I., Ford, I., Adams, J.H., Doyle, D., Teasdale, G.M., Lawrence, A.E., and Mclellan, D.R. (1989). Ischaemic brain damage is still common in fatal non-missile head injury. *J Neurol Neurosurg Psychiatry* 52**,** 346-350.

Gross, C.G. (2000). Neurogenesis in the adult brain: death of a dogma. *Nat Rev Neurosci* 1**,** 67-73.

Gu, W., Brannstrom, T., and Wester, P. (2000). Cortical neurogenesis in adult rats after reversible photothrombotic stroke. *J Cereb Blood Flow Metab* 20**,** 1166-1173.

Gurdjian, E.S., Lissner, H.R., Webster, J.E., Latimer, F.R., and Haddad, B.F. (1954). Studies on experimental concussion: relation of physiologic effect to time duration of intracranial pressure increase at impact. *Neurology* 4**,** 674-681.

Gutierrez, E., Huang, Y., Haglid, K., Bao, F., Hansson, H.A., Hamberger, A., and Viano, D. (2001). A new model for diffuse brain injury by rotational acceleration: I model, gross appearance, and astrocytosis. *J Neurotrauma* 18**,** 247-257.

Gyorgy, A., Ling, G., Wingo, D., Walker, J., Tong, L., Parks, S., Januszkiewicz, A., Baumann, R., and Agoston, D.V. (2011). Time-dependent changes in serum biomarker levels after blast traumatic brain injury. *J Neurotrauma* 28**,** 1121-1126.

Gyorgy, A.B., Walker, J., Wingo, D., Eidelman, O., Pollard, H.B., Molnar, A., and Agoston, D.V. (2010). Reverse phase protein microarray technology in traumatic brain injury. *J Neurosci Methods* 192**,** 96-101.

Hall, D., Dhilla, A., Charalambous, A., Gogos, J.A., and Karayiorgou, M. (2003). Sequence variants of the brain-derived neurotrophic factor (BDNF) gene are strongly associated with obsessive-compulsive disorder. *Am J Hum Genet* 73**,** 370-376.

Hallam, T.M., Floyd, C.L., Folkerts, M.M., Lee, L.L., Gong, Q.Z., Lyeth, B.G., Muizelaar, J.P., and Berman, R.F. (2004). Comparison of behavioral deficits and acute neuronal degeneration in rat lateral fluid percussion and weight-drop brain injury models. *J Neurotrauma* 21**,** 521-539.

Hallett, M. (2001). Plasticity of the human motor cortex and recovery from stroke. *Brain Res Brain Res Rev* 36**,** 169-174.

Hariri, A.R., Goldberg, T.E., Mattay, V.S., Kolachana, B.S., Callicott, J.H., Egan, M.F., and Weinberger, D.R. (2003). Brain-derived neurotrophic factor val66met polymorphism affects human memory-related hippocampal activity and predicts memory performance. *J Neurosci* 23**,** 6690-6694.

Hayes, R.C., Wiley, R.G., and Armstrong, D.M. (1992). Induction of nerve growth factor receptor (p75NGFr) mRNA within hypoglossal motoneurons following axonal injury. *Brain Res Mol Brain Res* 15**,** 291-297.

Hayes, R.L., Stalhammar, D., Povlishock, J.T., Allen, A.M., Galinat, B.J., Becker, D.P., and Stonnington, H.H. (1987). A new model of concussive brain injury in the cat produced by extradural fluid volume loading: II. Physiological and neuropathological observations. *Brain Inj* 1**,** 93-112.

Heiss, W.D., and Rosner, G. (1983). Functional recovery of cortical neurons as related to degree and duration of ischemia. *Ann Neurol* 14**,** 294-301.

Heldt, S.A., Stanek, L., Chhatwal, J.P., and Ressler, K.J. (2007). Hippocampus-specific deletion of BDNF in adult mice impairs spatial memory and extinction of aversive memories. *Mol Psychiatry* 12**,** 656-670.

Helmy, A., Carpenter, K.L., Skepper, J.N., Kirkpatrick, P.J., Pickard, J.D., and Hutchinson, P.J. (2009). Microdialysis of cytokines: methodological considerations, scanning electron microscopy, and determination of relative recovery. *J Neurotrauma* 26**,** 549-561.

Herrmann, M., Curio, N., Jost, S., Grubich, C., Ebert, A.D., Fork, M.L., and Synowitz, H. (2001). Release of biochemical markers of damage to neuronal and glial brain tissue is associated with short and long term neuropsychological outcome after traumatic brain injury. *J Neurol Neurosurg Psychiatry* 70**,** 95-100.

Hicks, R.R., Boggs, A., Leider, D., Kraemer, P., Brown, R., Scheff, S.W., and Seroogy, K.B. (1998a). Effects of Exercise Following Lateral Fluid Percussion Brain Injury in Rats. *Restor Neurol Neurosci* 12**,** 41-47.

Hicks, R.R., Li, C., Zhang, L., Dhillon, H.S., Prasad, M.R., and Seroogy, K.B. (1999a). Alterations in BDNF and trkB mRNA levels in the cerebral cortex following experimental brain trauma in rats. *J Neurotrauma* 16**,** 501-510.

Hicks, R.R., Martin, V.B., Zhang, L., and Seroogy, K.B. (1999b). Mild experimental brain injury differentially alters the expression of neurotrophin and neurotrophin receptor mRNAs in the hippocampus. *Exp Neurol* 160**,** 469-478.

Hicks, R.R., Numan, S., Dhillon, H.S., Prasad, M.R., and Seroogy, K.B. (1997). Alterations in BDNF and NT-3 mRNAs in rat hippocampus after experimental brain trauma. *Brain Res Mol Brain Res* 48**,** 401-406.

Hicks, R.R., Zhang, L., Dhillon, H.S., Prasad, M.R., and Seroogy, K.B. (1998b). Expression of trkB mRNA is altered in rat hippocampus after experimental brain trauma. *Brain Res Mol Brain Res* 59**,** 264-268.

Hillered, L., Persson, L., Carlson, H., Ungerstedt, U., Ronne-Engstrom, E., and Nilsson, P. (1992). Studies on excitatory amino acid receptor-linked brain disorders in rat and man using in vivo microdialysis. *Clin Neuropharmacol* 15 Suppl 1 Pt A**,** 695A-696A.

Hodgkinson, C.A., Yuan, Q., Xu, K., Shen, P.H., Heinz, E., Lobos, E.A., Binder, E.B., Cubells, J., Ehlers, C.L., Gelernter, J., Mann, J., Riley, B., Roy, A., Tabakoff, B., Todd, R.D., Zhou, Z., and Goldman, D. (2008). Addictions biology: haplotype-based analysis for 130 candidate genes on a single array. *Alcohol Alcohol* 43**,** 505-515.

Hohlfeld, R., Kerschensteiner, M., and Meinl, E. (2007). Dual role of inflammation in CNS disease. *Neurology* 68**,** S58-63; discussion S91-56.

Holbourn, H. (1943). Mechanics of head injuries. *The Lancet* 2**,** 438-441.

Holmin, S., and Hojeberg, B. (2004). In situ detection of intracerebral cytokine expression after human brain contusion. *Neurosci Lett* 369**,** 108-114.

Holmin, S., Mathiesen, T., Shetye, J., and Biberfeld, P. (1995). Intracerebral inflammatory response to experimental brain contusion. *Acta Neurochir (Wien)* 132**,** 110-119.

Huang Da, W., Sherman, B.T., and Lempicki, R.A. (2009). Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. *Nat Protoc* 4**,** 44-57.

Huang, E.J., and Reichardt, L.F. (2001). Neurotrophins: roles in neuronal development and function. *Annu Rev Neurosci* 24**,** 677-736.

Huang, E.J., and Reichardt, L.F. (2003). Trk receptors: roles in neuronal signal transduction. *Annu Rev Biochem* 72**,** 609-642.

Huang, R., Huang, J., Cathcart, H., Smith, S., and Poduslo, S.E. (2007). Genetic variants in brain-derived neurotrophic factor associated with Alzheimer's disease. *J Med Genet* 44**,** e66.

Hutchinson, P.J., O'connell, M.T., Nortje, J., Smith, P., Al-Rawi, P.G., Gupta, A.K., Menon, D.K., and Pickard, J.D. (2005). Cerebral microdialysis methodology--evaluation of 20 kDa and 100 kDa catheters. *Physiol Meas* 26**,** 423-428.

Hutchinson, P.J., O'connell, M.T., Rothwell, N.J., Hopkins, S.J., Nortje, J., Carpenter, K.L., Timofeev, I., Al-Rawi, P.G., Menon, D.K., and Pickard, J.D. (2007). Inflammation in human brain injury:

intracerebral concentrations of IL-1alpha, IL-1beta, and their endogenous inhibitor IL-1ra. *J Neurotrauma* 24**,** 1545-1557.

Ibanez, C.F., Ernfors, P., Timmusk, T., Ip, N.Y., Arenas, E., Yancopoulos, G.D., and Persson, H. (1993). Neurotrophin-4 is a target-derived neurotrophic factor for neurons of the trigeminal ganglion. *Development* 117**,** 1345-1353.

Jackson, R.G., Samra, G.S., Radcliffe, J., Clark, G.H., and Price, C.P. (2000). The early fall in levels of S-100 beta in traumatic brain injury. *Clin Chem Lab Med* 38**,** 1165-1167.

Jenkins, W.M., and Merzenich, M.M. (1987). Reorganization of neocortical representations after brain injury: a neurophysiological model of the bases of recovery from stroke. *Prog Brain Res* 71**,** 249-266.

Jiang, W., Gu, W., Brannstrom, T., Rosqvist, R., and Wester, P. (2001). Cortical neurogenesis in adult rats after transient middle cerebral artery occlusion. *Stroke* 32**,** 1201-1207.

Johansson, B.B. (2004). Functional and cellular effects of environmental enrichment after experimental brain infarcts. *Restor Neurol Neurosci* 22**,** 163-174.

Johansson, B.B., and Grabowski, M. (1994). Functional recovery after brain infarction: plasticity and neural transplantation. *Brain Pathol* 4**,** 85-95.

Johansson, C.B., Momma, S., Clarke, D.L., Risling, M., Lendahl, U., and Frisen, J. (1999). Identification of a neural stem cell in the adult mammalian central nervous system. *Cell* 96**,** 25-34.

Jordan, B.D. (2007). Genetic influences on outcome following traumatic brain injury. *Neurochem Res* 32**,** 905-915.

Jordan, B.D., Relkin, N.R., Ravdin, L.D., Jacobs, A.R., Bennett, A., and Gandy, S. (1997). Apolipoprotein E epsilon4 associated with chronic traumatic brain injury in boxing. *JAMA* 278**,** 136-140.

Julien, J.P., and Mushynski, W.E. (1998). Neurofilaments in health and disease. *Prog Nucleic Acid Res Mol Biol* 61**,** 1-23.

Kanda, R., Nakamura, N., Sekino, H., Sakai, H., Masuzawa, H., Mii, K., Aoyagi, N., Aruga, T., Kono, H., Sugimori, T., Sugiura, M., Mori, N., Kikuchi, A., Ono, K., and Kobayashi, H. (1981). Experimental head injury in monkeys --concussion and its tolerance level. *Neurol Med Chir (Tokyo)* 21**,** 645-656.

Kato, K., Fujimura, M., Nakagawa, A., Saito, A., Ohki, T., Takayama, K., and Tominaga, T. (2007). Pressure-dependent effect of shock waves on rat brain: induction of neuronal apoptosis mediated by a caspase-dependent pathway. *J Neurosurg* 106**,** 667-676.

Kavalci, C., Pekdemir, M., Durukan, P., Ilhan, N., Yildiz, M., Serhatlioglu, S., and Seckin, D. (2007). The value of serum tau protein for the diagnosis of intracranial injury in minor head trauma. *Am J Emerg Med* 25**,** 391-395.

Kernie, S.G., Erwin, T.M., and Parada, L.F. (2001). Brain remodeling due to neuronal and astrocytic proliferation after controlled cortical injury in mice. *J Neurosci Res* 66**,** 317-326.

Kesler, S.R., Adams, H.F., Blasey, C.M., and Bigler, E.D. (2003). Premorbid intellectual functioning, education, and brain size in traumatic brain injury: an investigation of the cognitive reserve hypothesis. *Appl Neuropsychol* 10**,** 153-162.

Kessels, H.W., and Malinow, R. (2009). Synaptic AMPA receptor plasticity and behavior. *Neuron* 61**,** 340-350.

Keyvani, K., Sachser, N., Witte, O.W., and Paulus, W. (2004). Gene expression profiling in the intact and injured brain following environmental enrichment. *J Neuropathol Exp Neurol* 63**,** 598-609.

King, N.S., Crawford, S., Wenden, F.J., Caldwell, F.E., and Wade, D.T. (1999). Early prediction of persisting post-concussion symptoms following mild and moderate head injuries. *Br J Clin Psychol* 38 (Pt 1)**,** 15-25.

Kirino, T. (1982). Delayed neuronal death in the gerbil hippocampus following ischemia. *Brain Res* 239**,** 57-69.

Kirkegaard, M., Murai, N., Risling, M., Suneson, A., Jarlebark, L., and Ulfendahl, M. (2006). Differential gene expression in the rat cochlea after exposure to impulse noise. *Neuroscience* 142**,** 425-435.

Klein, R., Nanduri, V., Jing, S.A., Lamballe, F., Tapley, P., Bryant, S., Cordon-Cardo, C., Jones, K.R., Reichardt, L.F., and Barbacid, M. (1991). The trkB tyrosine protein kinase is a receptor for brain-derived neurotrophic factor and neurotrophin-3. *Cell* 66**,** 395-403.

Koenigs, M., Huey, E.D., Raymont, V., Cheon, B., Solomon, J., Wassermann, E.M., and Grafman, J. (2008). Focal brain damage protects against post-traumatic stress disorder in combat veterans. *Nat Neurosci* 11**,** 232-237.

Koski, C.L., Estep, A.E., Sawant-Mane, S., Shin, M.L., Highbarger, L., and Hansch, G.M. (1996). Complement regulatory molecules on human myelin and glial cells: differential expression affects the deposition of activated complement proteins. *J Neurochem* 66**,** 303-312.

Kovesdi, E., Luckl, J., Bukovics, P., Farkas, O., Pal, J., Czeiter, E., Szellar, D., Doczi, T., Komoly, S., and Buki, A. (2010). Update on protein biomarkers in traumatic brain injury with emphasis on clinical use in adults and pediatrics. *Acta Neurochir (Wien)* 152**,** 1-17.

Krueger, F., Pardini, M., Huey, E.D., Raymont, V., Solomon, J., Lipsky, R.H., Hodgkinson, C.A., Goldman, D., and Grafman, J. (2011). The role of the Met66 brain-derived neurotrophic factor allele in the recovery of executive functioning after combat-related traumatic brain injury. *J Neurosci* 31**,** 598-606.

Kubes, P., and Ward, P.A. (2000). Leukocyte recruitment and the acute inflammatory response. *Brain Pathol* 10**,** 127-135.

Kutner, K.C., Erlanger, D.M., Tsai, J., Jordan, B., and Relkin, N.R. (2000). Lower cognitive performance of older football players possessing apolipoprotein E epsilon4. *Neurosurgery* 47**,** 651-657; discussion 657-658.

Lacy, M., Jones, J., Whittemore, S.R., Haviland, D.L., Wetsel, R.A., and Barnum, S.R. (1995). Expression of the receptors for the C5a anaphylatoxin, interleukin-8 and FMLP by human astrocytes and microglia. *J Neuroimmunol* 61**,** 71-78.

Lau, A.A., Crawley, A.C., Hopwood, J.J., and Hemsley, K.M. (2008). Open field locomotor activity and anxiety-related behaviors in mucopolysaccharidosis type IIIA mice. *Behav Brain Res* 191**,** 130-136.

Lee, H., Whitfeld, P.L., and Mackay, C.R. (2008). Receptors for complement C5a. The importance of C5aR and the enigmatic role of C5L2. *Immunol Cell Biol* 86**,** 153-160.

Leibrock, J., Lottspeich, F., Hohn, A., Hofer, M., Hengerer, B., Masiakowski, P., Thoenen, H., and Barde, Y.A. (1989). Molecular cloning and expression of brain-derived neurotrophic factor. *Nature* 341**,** 149-152.

Leinhase, I., Holers, V.M., Thurman, J.M., Harhausen, D., Schmidt, O.I., Pietzcker, M., Taha, M.E., Rittirsch, D., Huber-Lang, M., Smith, W.R., Ward, P.A., and Stahel, P.F. (2006). Reduced neuronal cell death after experimental brain injury in mice lacking a functional alternative pathway of complement activation. *BMC Neurosci* 7**,** 55.

Levi-Montalcini, R., and Hamburger, V. (1951). Selective growth stimulating effects of mouse sarcoma on the sensory and sympathetic nervous system of the chick embryo. *J Exp Zool* 116**,** 321-361.

Lewis, S.B., Finnie, J.W., Blumbergs, P.C., Scott, G., Manavis, J., Brown, C., Reilly, P.L., Jones, N.R., and Mclean, A.J. (1996). A head impact model of early axonal injury in the sheep. *J Neurotrauma* 13**,** 505-514.

Liaquat, I., Dunn, L.T., Nicoll, J.A., Teasdale, G.M., and Norrie, J.D. (2002). Effect of apolipoprotein E genotype on hematoma volume after trauma. *J Neurosurg* 96**,** 90-96.

Liberman, J.N., Stewart, W.F., Wesnes, K., and Troncoso, J. (2002). Apolipoprotein E epsilon 4 and short-term recovery from predominantly mild brain injury. *Neurology* 58**,** 1038-1044.

Lichtman, S.W., Seliger, G., Tycko, B., and Marder, K. (2000). Apolipoprotein E and functional recovery from brain injury following postacute rehabilitation. *Neurology* 55**,** 1536-1539.

Lie, D.C., Song, H., Colamarino, S.A., Ming, G.L., and Gage, F.H. (2004). Neurogenesis in the adult brain: new strategies for central nervous system diseases. *Annu Rev Pharmacol Toxicol* 44**,** 399-421.

Lighthall, J.W. (1988). Controlled cortical impact: a new experimental brain injury model. *J Neurotrauma* 5**,** 1-15.

Liliang, P.C., Liang, C.L., Lu, K., Wang, K.W., Weng, H.C., Hsieh, C.H., Tsai, Y.D., and Chen, H.J. (2010). Relationship between injury severity and serum tau protein levels in traumatic brain injured rats. *Resuscitation* 81**,** 1205-1208.

Lindgren, S., and Rinder, L. (1969). Production and distribution of intracranial and intraspinal pressure changes at sudden extradural fluid volume input in rabbits. *Acta Physiol Scand* 76**,** 340-351.

Ling, G.S., Marshall, S.A., and Moore, D.F. (2010). Diagnosis and management of traumatic brain injury. *Continuum (Minneapolis Minn)* 16**,** 27-40.

Lipsky, R.H., and Marini, A.M. (2007). Brain-derived neurotrophic factor in neuronal survival and behavior-related plasticity. *Ann N Y Acad Sci* 1122**,** 130-143.

Lipsky, R.H., Sparling, M.B., Ryan, L.M., Xu, K., Salazar, A.M., Goldman, D., and Warden, D.L. (2005). Association of COMT Val158Met genotype with executive functioning following traumatic brain injury. *J Neuropsychiatry Clin Neurosci* 17**,** 465-471.

Liu, M.C., Akle, V., Zheng, W., Kitlen, J., O'steen, B., Larner, S.F., Dave, J.R., Tortella, F.C., Hayes, R.L., and Wang, K.K. (2006). Extensive degradation of myelin basic protein isoforms by calpain following traumatic brain injury. *J Neurochem* 98, 700-712.

Long, J.B., Bentley, T.L., Wessner, K.A., Cerone, C., Sweeney, S., and Bauman, R.A. (2009). Blast overpressure in rats: recreating a battlefield injury in the laboratory. *J Neurotrauma* 26, 827-840.

Lu, B. (2003). Pro-region of neurotrophins: role in synaptic modulation. *Neuron* 39, 735-738.

Lu, J., Ng, K.C., Ling, G., Wu, J., Poon, D.J., Kan, E.M., Tan, M.H., Wu, Y.J., Li, P., Moochhala, S., Yap, E., Lee, L.K., Teo, M., Yeh, I.B., Sergio, D.M., Chua, F., Kumar, S.D., and Ling, E.A. (2012). Effect of blast exposure on the brain structure and cognition in Macaca fascicularis. *J Neurotrauma* 29, 1434-1454.

Luikart, B.W., and Parada, L.F. (2006). Receptor tyrosine kinase B-mediated excitatory synaptogenesis. *Prog Brain Res* 157, 15-24.

Maas, A.I., Stocchetti, N., and Bullock, R. (2008). Moderate and severe traumatic brain injury in adults. *Lancet Neurol* 7, 728-741.

Mac Donald, C.L., Dikranian, K., Song, S.K., Bayly, P.V., Holtzman, D.M., and Brody, D.L. (2007). Detection of traumatic axonal injury with diffusion tensor imaging in a mouse model of traumatic brain injury. *Exp Neurol* 205, 116-131.

Magavi, S.S., Leavitt, B.R., and Macklis, J.D. (2000). Induction of neurogenesis in the neocortex of adult mice. *Nature* 405, 951-955.

Magavi, S.S., and Macklis, J.D. (2001). Manipulation of neural precursors in situ: induction of neurogenesis in the neocortex of adult mice. *Neuropsychopharmacology* 25, 816-835.

Maguire, E.A., Gadian, D.G., Johnsrude, I.S., Good, C.D., Ashburner, J., Frackowiak, R.S., and Frith, C.D. (2000). Navigation-related structural change in the hippocampi of taxi drivers. *Proc Natl Acad Sci U S A* 97, 4398-4403.

Makale, M., Solomon, J., Patronas, N.J., Danek, A., Butman, J.A., and Grafman, J. (2002). Quantification of brain lesions using interactive automated software. *Behav Res Methods Instrum Comput* 34, 6-18.

Mandelkow, E.M., Stamer, K., Vogel, R., Thies, E., and Mandelkow, E. (2003). Clogging of axons by tau, inhibition of axonal traffic and starvation of synapses. *Neurobiol Aging* 24, 1079-1085.

Margulies, S.S., and Thibault, L.E. (1992). A proposed tolerance criterion for diffuse axonal injury in man. *J Biomech* 25, 917-923.

Margulies, S.S., Thibault, L.E., and Gennarelli, T.A. (1990). Physical model simulations of brain injury in the primate. *J Biomech* 23, 823-836.

Marklund, N., Blennow, K., Zetterberg, H., Ronne-Engstrom, E., Enblad, P., and Hillered, L. (2009). Monitoring of brain interstitial total tau and beta amyloid proteins by microdialysis in patients with traumatic brain injury. *J Neurosurg* 110, 1227-1237.

Marklund, N., Keck, C., Hoover, R., Soltesz, K., Millard, M., Lebold, D., Spangler, Z., Banning, A., Benson, J., and Mcintosh, T.K. (2005). Administration of monoclonal antibodies neutralizing the inflammatory mediators tumor necrosis factor alpha and interleukin -6 does not attenuate acute behavioral deficits following experimental traumatic brain injury in the rat. *Restor Neurol Neurosci* 23, 31-42.

Marklund, N., Salci, K., Ronquist, G., and Hillered, L. (2006). Energy metabolic changes in the early post-injury period following traumatic brain injury in rats. *Neurochem Res* 31, 1085-1093.

Marmarou, A., Foda, M.A., Van Den Brink, W., Campbell, J., Kita, H., and Demetriadou, K. (1994). A new model of diffuse brain injury in rats. Part I: Pathophysiology and biomechanics. *J Neurosurg* 80, 291-300.

Maruichi, K., Kuroda, S., Chiba, Y., Hokari, M., Shichinohe, H., Hida, K., and Iwasaki, Y. (2009). Graded model of diffuse axonal injury for studying head injury-induced cognitive dysfunction in rats. *Neuropathology* 29, 132-139.

Maurer, M.H., Berger, C., Wolf, M., Futterer, C.D., Feldmann, R.E., Jr., Schwab, S., and Kuschinsky, W. (2003). The proteome of human brain microdialysate. *Proteome Sci* 1, 7.

Mccullagh, S., Oucherlony, D., Protzner, A., Blair, N., and Feinstein, A. (2001). Prediction of neuropsychiatric outcome following mild trauma brain injury: an examination of the Glasgow Coma Scale. *Brain Inj* 15, 489-497.

Mcintosh, T.K., Noble, L., Andrews, B., and Faden, A.I. (1987). Traumatic brain injury in the rat: characterization of a midline fluid-percussion model. *Cent Nerv Syst Trauma* 4, 119-134.

Mckenzie, K.J., Mclellan, D.R., Gentleman, S.M., Maxwell, W.L., Gennarelli, T.A., and Graham, D.I. (1996). Is beta-APP a marker of axonal damage in short-surviving head injury? *Acta Neuropathol* 92**,** 608-613.

Mckinney, R.A., Debanne, D., Gahwiler, B.H., and Thompson, S.M. (1997). Lesion-induced axonal sprouting and hyperexcitability in the hippocampus in vitro: implications for the genesis of posttraumatic epilepsy. *Nat Med* 3**,** 990-996.

Meyer, K., Helmick, K., Doncevic, S., and Park, R. (2008). Severe and penetrating traumatic brain injury in the context of war. *J Trauma Nurs* 15**,** 185-189; quiz 190-181.

Middlemas, D.S., Lindberg, R.A., and Hunter, T. (1991). trkB, a neural receptor protein-tyrosine kinase: evidence for a full-length and two truncated receptors. *Mol Cell Biol* 11**,** 143-153.

Moore, B.W. (1965). A soluble protein characteristic of the nervous system. *Biochem Biophys Res Commun* 19**,** 739-744.

Moore, D.F., and Jaffee, M.S. (2010). Military traumatic brain injury and blast. *NeuroRehabilitation* 26**,** 179-181.

Morales, D.M., Marklund, N., Lebold, D., Thompson, H.J., Pitkanen, A., Maxwell, W.L., Longhi, L., Laurer, H., Maegele, M., Neugebauer, E., Graham, D.I., Stocchetti, N., and Mcintosh, T.K. (2005). Experimental models of traumatic brain injury: do we really need to build a better mousetrap? *Neuroscience* 136**,** 971-989.

Morgan, B.P., Gasque, P., Singhrao, S., and Piddlesden, S.J. (1997). The role of complement in disorders of the nervous system. *Immunopharmacology* 38**,** 43-50.

Morgan, J.I., and Curran, T. (1988). Calcium as a modulator of the immediate-early gene cascade in neurons. *Cell Calcium* 9**,** 303-311.

Morganti-Kossman, M.C., Lenzlinger, P.M., Hans, V., Stahel, P., Csuka, E., Ammann, E., Stocker, R., Trentz, O., and Kossmann, T. (1997). Production of cytokines following brain injury: beneficial and deleterious for the damaged tissue. *Mol Psychiatry* 2**,** 133-136.

Morganti-Kossmann, M.C., Rancan, M., Otto, V.I., Stahel, P.F., and Kossmann, T. (2001). Role of cerebral inflammation after traumatic brain injury: a revisited concept. *Shock* 16**,** 165-177.

Mudo, G., Persson, H., Timmusk, T., Funakoshi, H., Bindoni, M., and Belluardo, N. (1993). Increased expression of trkB and trkC messenger RNAs in the rat forebrain after focal mechanical injury. *Neuroscience* 57**,** 901-912.

Narayan, R.K., Michel, M.E., Ansell, B., Baethmann, A., Biegon, A., Bracken, M.B., Bullock, M.R., Choi, S.C., Clifton, G.L., Contant, C.F., Coplin, W.M., Dietrich, W.D., Ghajar, J., Grady, S.M., Grossman, R.G., Hall, E.D., Heetderks, W., Hovda, D.A., Jallo, J., Katz, R.L., Knoller, N., Kochanek, P.M., Maas, A.I., Majde, J., Marion, D.W., Marmarou, A., Marshall, L.F., Mcintosh, T.K., Miller, E., Mohberg, N., Muizelaar, J.P., Pitts, L.H., Quinn, P., Riesenfeld, G., Robertson, C.S., Strauss, K.I., Teasdale, G., Temkin, N., Tuma, R., Wade, C., Walker, M.D., Weinrich, M., Whyte, J., Wilberger, J., Young, A.B., and Yurkewicz, L. (2002). Clinical trials in head injury. *J Neurotrauma* 19**,** 503-557.

Nataf, S., Levison, S.W., and Barnum, S.R. (2001). Expression of the anaphylatoxin C5a receptor in the oligodendrocyte lineage. *Brain Res* 894**,** 321-326.

Nathoo, N., Chetty, R., Van Dellen, J.R., and Barnett, G.H. (2003). Genetic vulnerability following traumatic brain injury: the role of apolipoprotein E. *Mol Pathol* 56**,** 132-136.

Naumann, T., Casademunt, E., Hollerbach, E., Hofmann, J., Dechant, G., Frotscher, M., and Barde, Y.A. (2002). Complete deletion of the neurotrophin receptor p75NTR leads to long-lasting increases in the number of basal forebrain cholinergic neurons. *J Neurosci* 22**,** 2409-2418.

Nelson, D.W., Thornquist, B., Maccallum, R.M., Nystrom, H., Holst, A., Rudehill, A., Wanecek, M., Bellander, B.M., and Weitzberg, E. (2011). Analyses of cerebral microdialysis in patients with traumatic brain injury: relations to intracranial pressure, cerebral perfusion pressure and catheter placement. *BMC Med* 9**,** 21.

Neves-Pereira, M., Mundo, E., Muglia, P., King, N., Macciardi, F., and Kennedy, J.L. (2002). The brain-derived neurotrophic factor gene confers susceptibility to bipolar disorder: evidence from a family-based association study. *Am J Hum Genet* 71**,** 651-655.

Ng, H.K., Mahaliyana, R.D., and Poon, W.S. (1994). The pathological spectrum of diffuse axonal injury in blunt head trauma: assessment with axon and myelin strains. *Clin Neurol Neurosurg* 96**,** 24-31.

Nilsson, P., Hillered, L., Ponten, U., and Ungerstedt, U. (1990). Changes in cortical extracellular levels of energy-related metabolites and amino acids following concussive brain injury in rats. *J Cereb Blood Flow Metab* 10**,** 631-637.

Nudo, R.J., and Milliken, G.W. (1996). Reorganization of movement representations in primary motor cortex following focal ischemic infarcts in adult squirrel monkeys. *J Neurophysiol* 75**,** 2144-2149.

O'barr, S.A., Caguioa, J., Gruol, D., Perkins, G., Ember, J.A., Hugli, T., and Cooper, N.R. (2001). Neuronal expression of a functional receptor for the C5a complement activation fragment. *J Immunol* 166**,** 4154-4162.

Oderfeld-Nowak, B., Orzylowska-Sliwinska, O., Soltys, Z., Zaremba, M., Januszewski, S., Janeczko, K., and Mossakowski, M. (2003). Concomitant up-regulation of astroglial high and low affinity nerve growth factor receptors in the CA1 hippocampal area following global transient cerebral ischemia in rat. *Neuroscience* 120**,** 31-40.

Okada, T., Hashimoto, R., Numakawa, T., Iijima, Y., Kosuga, A., Tatsumi, M., Kamijima, K., Kato, T., and Kunugi, H. (2006). A complex polymorphic region in the brain-derived neurotrophic factor (BDNF) gene confers susceptibility to bipolar disorder and affects transcriptional activity. *Mol Psychiatry* 11**,** 695-703.

Okie, S. (2006). Reconstructing lives--a tale of two soldiers. *N Engl J Med* 355**,** 2609-2615.

Ommaya, A.K., Grubb, R.L., Jr., and Naumann, R.A. (1971). Coup and contre-coup injury: observations on the mechanics of visible brain injuries in the rhesus monkey. *J Neurosurg* 35**,** 503-516.

Ono, K., Kikuchi, A., Nakamura, M., Kobayashi, H., and Nakamura, N. (1980). "Human head tolerance to sagittal impact reliable estimation deduced from experimental head injury using subhuman primates and human cadaver skulls.", in: *Proc. of the 24th STAPP Car Crash Conference.*).

Parent, J.M. (2003). Injury-induced neurogenesis in the adult mammalian brain. *Neuroscientist* 9**,** 261-272.

Pascual-Leone, A., Amedi, A., Fregni, F., and Merabet, L.B. (2005). The plastic human brain cortex. *Annu Rev Neurosci* 28**,** 377-401.

Peloso, P.M., Carroll, L.J., Cassidy, J.D., Borg, J., Von Holst, H., Holm, L., and Yates, D. (2004). Critical evaluation of the existing guidelines on mild traumatic brain injury. *J Rehabil Med***,** 106-112.

Penkowa, M., Camats, J., Hadberg, H., Quintana, A., Rojas, S., Giralt, M., Molinero, A., Campbell, I.L., and Hidalgo, J. (2003). Astrocyte-targeted expression of interleukin-6 protects the central nervous system during neuroglial degeneration induced by 6-aminonicotinamide. *J Neurosci Res* 73**,** 481-496.

Penkowa, M., Giralt, M., Carrasco, J., Hadberg, H., and Hidalgo, J. (2000). Impaired inflammatory response and increased oxidative stress and neurodegeneration after brain injury in interleukin-6-deficient mice. *Glia* 32**,** 271-285.

Pezawas, L., Verchinski, B.A., Mattay, V.S., Callicott, J.H., Kolachana, B.S., Straub, R.E., Egan, M.F., Meyer-Lindenberg, A., and Weinberger, D.R. (2004). The brain-derived neurotrophic factor val66met polymorphism and variation in human cortical morphology. *J Neurosci* 24**,** 10099-10102.

Phillips, H.S., Hains, J.M., Laramee, G.R., Rosenthal, A., and Winslow, J.W. (1990). Widespread expression of BDNF but not NT3 by target areas of basal forebrain cholinergic neurons. *Science* 250**,** 290-294.

Pierce, J.E., Smith, D.H., Trojanowski, J.Q., and Mcintosh, T.K. (1998). Enduring cognitive, neurobehavioral and histopathological changes persist for up to one year following severe experimental brain injury in rats. *Neuroscience* 87**,** 359-369.

Plantman, S., Ng, K.C., Lu, J., Davidsson, J., and Risling, M. (2011). Characterization of a novel rat model of penetrating traumatic brain injury. *J Neurotrauma*.

Plantman, S., Ng, K.C., Lu, J., Davidsson, J., and Risling, M. (2012). Characterization of a novel rat model of penetrating traumatic brain injury. *J Neurotrauma* 29**,** 1219-1232.

Pleines, U.E., Morganti-Kossmann, M.C., Rancan, M., Joller, H., Trentz, O., and Kossmann, T. (2001). S-100 beta reflects the extent of injury and outcome, whereas neuronal specific enolase is a better indicator of neuroinflammation in patients with severe traumatic brain injury. *J Neurotrauma* 18**,** 491-498.

Poo, M.M. (2001). Neurotrophins as synaptic modulators. *Nat Rev Neurosci* 2**,** 24-32.

Povlishock, J.T. (1992). Traumatically induced axonal injury: pathogenesis and pathobiological implications. *Brain Pathol* 2**,** 1-12.

Povlishock, J.T., Becker, D.P., Cheng, C.L., and Vaughan, G.W. (1983). Axonal change in minor head injury. *J Neuropathol Exp Neurol* 42**,** 225-242.

Povlishock, J.T., and Christman, C.W. (1995). The pathobiology of traumatically induced axonal injury in animals and humans: a review of current thoughts. *J Neurotrauma* 12**,** 555-564.

Povlishock, J.T., and Jenkins, L.W. (1995). Are the pathobiological changes evoked by traumatic brain injury immediate and irreversible? *Brain Pathol* 5**,** 415-426.

Povlishock, J.T., and Pettus, E.H. (1996). Traumatically induced axonal damage: evidence for enduring changes in axolemmal permeability with associated cytoskeletal change. *Acta Neurochir Suppl* 66**,** 81-86.

Pozzo-Miller, L.D., Gottschalk, W., Zhang, L., Mcdermott, K., Du, J., Gopalakrishnan, R., Oho, C., Sheng, Z.H., and Lu, B. (1999). Impairments in high-frequency transmission, synaptic vesicle docking, and synaptic protein distribution in the hippocampus of BDNF knockout mice. *J Neurosci* 19**,** 4972-4983.

Pruunsild, P., Kazantseva, A., Aid, T., Palm, K., and Timmusk, T. (2007). Dissecting the human BDNF locus: bidirectional transcription, complex splicing, and multiple promoters. *Genomics* 90**,** 397-406.

Pulsinelli, W.A., Brierley, J.B., and Plum, F. (1982). Temporal profile of neuronal damage in a model of transient forebrain ischemia. *Ann Neurol* 11**,** 491-498.

Raabe, A., Grolms, C., Sorge, O., Zimmermann, M., and Seifert, V. (1999). Serum S-100B protein in severe head injury. *Neurosurgery* 45**,** 477-483.

Raabe, A., and Seifert, V. (2000). Protein S-100B as a serum marker of brain damage in severe head injury: preliminary results. *Neurosurg Rev* 23**,** 136-138.

Rahpeymai, Y., Hietala, M.A., Wilhelmsson, U., Fotheringham, A., Davies, I., Nilsson, A.K., Zwirner, J., Wetsel, R.A., Gerard, C., Pekny, M., and Pekna, M. (2006). Complement: a novel factor in basal and ischemia-induced neurogenesis. *EMBO J* 25**,** 1364-1374.

Raymont, V., and Grafman, J. (2006). Cognitive neural plasticity during learning and recovery from brain damage. *Prog Brain Res* 157**,** 199-206.

Raymont, V., Greathouse, A., Reding, K., Lipsky, R., Salazar, A., and Grafman, J. (2008). Demographic, structural and genetic predictors of late cognitive decline after penetrating head injury. *Brain* 131**,** 543-558.

Raymont, V., Salazar, A.M., Krueger, F., and Grafman, J. (2011). "Studying injured minds" - the Vietnam head injury study and 40 years of brain injury research. *Frontiers in neurology (Neurotrauma)* 2 (15)**,** 1-11.

Raymont, V., Salazar, A.M., Lipsky, R., Goldman, D., Tasick, G., and Grafman, J. (2010). Correlates of posttraumatic epilepsy 35 years following combat brain injury. *Neurology* 75**,** 224-229.

Reilly, P.L., Graham, D.I., Adams, J.H., and Jennett, B. (1975). Patients with head injury who talk and die. *Lancet* 2**,** 375-377.

Ribases, M., Gratacos, M., Armengol, L., De Cid, R., Badia, A., Jimenez, L., Solano, R., Vallejo, J., Fernandez, F., and Estivill, X. (2003). Met66 in the brain-derived neurotrophic factor (BDNF) precursor is associated with anorexia nervosa restrictive type. *Mol Psychiatry* 8**,** 745-751.

Richardson, R.M., Sun, D., and Bullock, M.R. (2007). Neurogenesis after traumatic brain injury. *Neurosurg Clin N Am* 18**,** 169-181, xi.

Richmond, D.R., Damon, E.G., Bowen, I.G., Fletcher, E.R., and White, C.S. (1967). Air-blast studies with eight species of mammals. Techn Progr Rep DASA 1854. *Fission Prod Inhal Proj***,** 1-44.

Risling, M., and Davidsson, J. (2012). Experimental animal models for studies on the mechanisms of blast-induced neurotrauma. *Front Neurol* 3**,** 30.

Risling, M., Fried, K., Linda, H., Cullheim, S., and Meier, M. (1992). Changes in nerve growth factor receptor-like immunoreactivity in the spinal cord after ventral funiculus lesion in adult cats. *J Neurocytol* 21**,** 79-93.

Risling, M., Plantman, S., Angeria, M., Rostami, E., Bellander, B.M., Kirkegaard, M., Arborelius, U., and Davidsson, J. (2011). Mechanisms of blast induced brain injuries, experimental studies in rats. *Neuroimage* 54 Suppl 1**,** S89-97.

Riva-Depaty, I., Fardeau, C., Mariani, J., Bouchaud, C., and Delhaye-Bouchaud, N. (1994). Contribution of peripheral macrophages and microglia to the cellular reaction after mechanical or neurotoxin-induced lesions of the rat brain. *Exp Neurol* 128**,** 77-87.

Rodriguez-Tebar, A., Dechant, G., and Barde, Y.A. (1990). Binding of brain-derived neurotrophic factor to the nerve growth factor receptor. *Neuron* 4**,** 487-492.

Rogers, J., Cooper, N.R., Webster, S., Schultz, J., Mcgeer, P.L., Styren, S.D., Civin, W.H., Brachova, L., Bradt, B., and Ward, P. (1992). Complement activation by beta-amyloid in Alzheimer disease. *Proc Natl Acad Sci U S A* 89**,** 10016-10020.

Romner, B., Ingebrigtsen, T., Kongstad, P., and Borgesen, S.E. (2000). Traumatic brain damage: serum S-100 protein measurements related to neuroradiological findings. *J Neurotrauma* 17**,** 641-647.

Rose, C.R., Blum, R., Pichler, B., Lepier, A., Kafitz, K.W., and Konnerth, A. (2003). Truncated TrkB-T1 mediates neurotrophin-evoked calcium signalling in glia cells. *Nature* 426, 74-78.

Ross, D.T., Meaney, D.F., Sabol, M.K., Smith, D.H., and Gennarelli, T.A. (1994). Distribution of forebrain diffuse axonal injury following inertial closed head injury in miniature swine. *Exp Neurol* 126, 291-299.

Rostami, E. (2011). Detection of biomarkers in serum after experimental diffuse and focal traumatic brain injury. *ScienceMED* 2.

Rostami, E., Krueger, F., Zoubak, S., Dal Monte, O., Raymont, V., Pardini, M., Hodgkinson, C.A., Goldman, D., Risling, M., and Grafman, J. (2011). BDNF polymorphism predicts general intelligence after penetrating traumatic brain injury. *PLoS One* 6, e27389.

Rothermundt, M., Peters, M., Prehn, J.H., and Arolt, V. (2003). S100B in brain damage and neurodegeneration. *Microsc Res Tech* 60, 614-632.

Rothoerl, R.D., Woertgen, C., and Brawanski, A. (2000). S-100 serum levels and outcome after severe head injury. *Acta Neurochir Suppl* 76, 97-100.

Rothwell, N.J. (1999). Annual review prize lecture cytokines - killers in the brain? *J Physiol* 514 (Pt 1), 3-17.

Ruttan, L., Martin, K., Liu, A., Colella, B., and Green, R.E. (2008). Long-term cognitive outcome in moderate to severe traumatic brain injury: a meta-analysis examining timed and untimed tests at 1 and 4.5 or more years after injury. *Arch Phys Med Rehabil* 89, S69-76.

Saatman, K.E., Duhaime, A.C., Bullock, R., Maas, A.I., Valadka, A., and Manley, G.T. (2008). Classification of traumatic brain injury for targeted therapies. *J Neurotrauma* 25, 719-738.

Saljo, A., Arrhen, F., Bolouri, H., Mayorga, M., and Hamberger, A. (2008). Neuropathology and pressure in the pig brain resulting from low-impulse noise exposure. *J Neurotrauma* 25, 1397-1406.

Saljo, A., Bao, F., Haglid, K.G., and Hansson, H.A. (2000). Blast exposure causes redistribution of phosphorylated neurofilament subunits in neurons of the adult rat brain. *J Neurotrauma* 17, 719-726.

Schallert, T., Leasure, J.L., and Kolb, B. (2000). Experience-associated structural events, subependymal cellular proliferative activity, and functional recovery after injury to the central nervous system. *J Cereb Blood Flow Metab* 20, 1513-1528.

Schmidt, O.I., Heyde, C.E., Ertel, W., and Stahel, P.F. (2005). Closed head injury--an inflammatory disease? *Brain Res Brain Res Rev* 48, 388-399.

Schneider, A., Biernat, J., Von Bergen, M., Mandelkow, E., and Mandelkow, E.M. (1999). Phosphorylation that detaches tau protein from microtubules (Ser262, Ser214) also protects it against aggregation into Alzheimer paired helical filaments. *Biochemistry* 38, 3549-3558.

Seitz, R.J., Kleiser, R., and Butefisch, C.M. (2005). Reorganization of cerebral circuits in human brain lesion. *Acta Neurochir Suppl* 93, 65-70.

Selkoe, D.J. (2002). Alzheimer's disease is a synaptic failure. *Science* 298, 789-791.

Sharma, R., and Laskowitz, D.T. (2012). Biomarkers in Traumatic Brain Injury. *Curr Neurol Neurosci Rep*.

Sharp, D.J., and Ham, T.E. (2011). Investigating white matter injury after mild traumatic brain injury. *Curr Opin Neurol* 24, 558-563.

Shaw, G., Yang, C., Ellis, R., Anderson, K., Parker Mickle, J., Scheff, S., Pike, B., Anderson, D.K., and Howland, D.R. (2005). Hyperphosphorylated neurofilament NF-H is a serum biomarker of axonal injury. *Biochem Biophys Res Commun* 336, 1268-1277.

Shaw, G.J., Jauch, E.C., and Zemlan, F.P. (2002). Serum cleaved tau protein levels and clinical outcome in adult patients with closed head injury. *Ann Emerg Med* 39, 254-257.

Shulga, A., Magalhaes, A.C., Autio, H., Plantman, S., Di Lieto, A., Nykjaer, A., Carlstedt, T., Risling, M., Arumae, U., Castren, E., and Rivera, C. (2012). The loop diuretic bumetanide blocks posttraumatic p75NTR upregulation and rescues injured neurons. *J Neurosci* 32, 1757-1770.

Shulga, A., Thomas-Crusells, J., Sigl, T., Blaesse, A., Mestres, P., Meyer, M., Yan, Q., Kaila, K., Saarma, M., Rivera, C., and Giehl, K.M. (2008). Posttraumatic GABA(A)-mediated [Ca2+]i increase is essential for the induction of brain-derived neurotrophic factor-dependent survival of mature central neurons. *J Neurosci* 28, 6996-7005.

Siesjo, B.K. (1978). *Brain Energy Metabolism*. New York: John Wiley.

Siesjo, B.K., Hu, B., and Kristian, T. (1999). Is the cell death pathway triggered by the mitochondrion or the endoplasmic reticulum? *J Cereb Blood Flow Metab* 19, 19-26.

Siesjo, B.K., and Siesjo, P. (1996). Mechanisms of secondary brain injury. *Eur J Anaesthesiol* 13, 247-268.

Singleton, R.H., Zhu, J., Stone, J.R., and Povlishock, J.T. (2002). Traumatically induced axotomy adjacent to the soma does not result in acute neuronal death. *J Neurosci* 22, 791-802.

Sklar, P., Gabriel, S.B., Mcinnis, M.G., Bennett, P., Lim, Y.M., Tsan, G., Schaffner, S., Kirov, G., Jones, I., Owen, M., Craddock, N., Depaulo, J.R., and Lander, E.S. (2002). Family-based association study of 76 candidate genes in bipolar disorder: BDNF is a potential risk locus. Brain-derived neutrophic factor. *Mol Psychiatry* 7, 579-593.

Smith, D.H., Chen, X.H., Xu, B.N., Mcintosh, T.K., Gennarelli, T.A., and Meaney, D.F. (1997). Characterization of diffuse axonal pathology and selective hippocampal damage following inertial brain trauma in the pig. *J Neuropathol Exp Neurol* 56, 822-834.

Solomon, J., Raymont, V., Braun, A., Butman, J.A., and Grafman, J. (2007). User-friendly software for the analysis of brain lesions (ABLe). *Comput Methods Programs Biomed* 86, 245-254.

Spurrier, B., Ramalingam, S., and Nishizuka, S. (2008). Reverse-phase protein lysate microarrays for cell signaling analysis. *Nat Protoc* 3, 1796-1808.

Stahel, P.F., Flierl, M.A., Morgan, B.P., Persigehl, I., Stoll, C., Conrad, C., Touban, B.M., Smith, W.R., Beauchamp, K., Schmidt, O.I., Ertel, W., and Leinhase, I. (2009). Absence of the complement regulatory molecule CD59a leads to exacerbated neuropathology after traumatic brain injury in mice. *J Neuroinflammation* 6, 2.

Stahel, P.F., Kossmann, T., Morganti-Kossmann, M.C., Hans, V.H., and Barnum, S.R. (1997). Experimental diffuse axonal injury induces enhanced neuronal C5a receptor mRNA expression in rats. *Brain Res Mol Brain Res* 50, 205-212.

Stahel, P.F., Morganti-Kossmann, M.C., Perez, D., Redaelli, C., Gloor, B., Trentz, O., and Kossmann, T. (2001). Intrathecal levels of complement-derived soluble membrane attack complex (sC5b-9) correlate with blood-brain barrier dysfunction in patients with traumatic brain injury. *J Neurotrauma* 18, 773-781.

Stahnisch, F.W., and Nitsch, R. (2002). Santiago Ramon y Cajal's concept of neuronal plasticity: the ambiguity lives on. *Trends Neurosci* 25, 589-591.

Starmark, J.E., Stalhammar, D., Holmgren, E., and Rosander, B. (1988). A comparison of the Glasgow Coma Scale and the Reaction Level Scale (RLS85). *J Neurosurg* 69, 699-706.

Stephens, M., Smith, N.J., and Donnelly, P. (2001). A new statistical method for haplotype reconstruction from population data. *Am J Hum Genet* 68, 978-989.

Stevens, B., Allen, N.J., Vazquez, L.E., Howell, G.R., Christopherson, K.S., Nouri, N., Micheva, K.D., Mehalow, A.K., Huberman, A.D., Stafford, B., Sher, A., Litke, A.M., Lambris, J.D., Smith, S.J., John, S.W., and Barres, B.A. (2007). The classical complement cascade mediates CNS synapse elimination. *Cell* 131, 1164-1178.

Stone, J.R., Singleton, R.H., and Povlishock, J.T. (2001). Intra-axonal neurofilament compaction does not evoke local axonal swelling in all traumatically injured axons. *Exp Neurol* 172, 320-331.

Strich, S.J. (1961). Shearing of nerve fibres as a cause of brain damage due to head injury: a pathological study of twenty cases. *The Lancet* 2, 443-448.

Suehiro, E., Singleton, R.H., Stone, J.R., and Povlishock, J.T. (2001). The immunophilin ligand FK506 attenuates the axonal damage associated with rapid rewarming following posttraumatic hypothermia. *Exp Neurol* 172, 199-210.

Sullivan, H.G., Martinez, J., Becker, D.P., Miller, J.D., Griffith, R., and Wist, A.O. (1976). Fluid-percussion model of mechanical brain injury in the cat. *J Neurosurg* 45, 521-534.

Sun, Q., and Gamblin, T.C. (2009). Pseudohyperphosphorylation causing AD-like changes in tau has significant effects on its polymerization. *Biochemistry* 48, 6002-6011.

Sundstrom, A., Marklund, P., Nilsson, L.G., Cruts, M., Adolfsson, R., Van Broeckhoven, C., and Nyberg, L. (2004). APOE influences on neuropsychological function after mild head injury: within-person comparisons. *Neurology* 62, 1963-1966.

Szeszko, P.R., Lipsky, R., Mentschel, C., Robinson, D., Gunduz-Bruce, H., Sevy, S., Ashtari, M., Napolitano, B., Bilder, R.M., Kane, J.M., Goldman, D., and Malhotra, A.K. (2005). Brain-derived neurotrophic factor val66met polymorphism and volume of the hippocampal formation. *Mol Psychiatry* 10, 631-636.

Tan, Y., Zhou, S., Liu, Y., and Li, Z. (1998). A gross and microscopic study of cerebral injuries accompanying maxillofacial high-velocity projectile wounding in dogs. *J Oral Maxillofac Surg* 56, 345-348.

Taniuchi, M., Clark, H.B., Schweitzer, J.B., and Johnson, E.M., Jr. (1988). Expression of nerve growth factor receptors by Schwann cells of axotomized peripheral nerves: ultrastructural location, suppression by axonal contact, and binding properties. *J Neurosci* 8, 664-681.

Taupin, V., Toulmond, S., Serrano, A., Benavides, J., and Zavala, F. (1993). Increase in IL-6, IL-1 and TNF levels in rat brain following traumatic lesion. Influence of pre- and post-traumatic treatment with Ro5 4864, a peripheral-type (p site) benzodiazepine ligand. *J Neuroimmunol* 42**,** 177-185.

Teasdale, G., and Jennett, B. (1974). Assessment of coma and impaired consciousness. A practical scale. *Lancet* 2**,** 81-84.

Teasdale, G.M., Nicoll, J.A., Murray, G., and Fiddes, M. (1997). Association of apolipoprotein E polymorphism with outcome after head injury. *Lancet* 350**,** 1069-1071.

Thoenen, H. (1995). Neurotrophins and neuronal plasticity. *Science* 270**,** 593-598.

Thomas, D.G., Palfreyman, J.W., and Ratcliffe, J.G. (1978). Serum-myelin-basic-protein assay in diagnosis and prognosis of patients with head injury. *Lancet* 1**,** 113-115.

Thompson, H.J., Lifshitz, J., Marklund, N., Grady, M.S., Graham, D.I., Hovda, D.A., and Mcintosh, T.K. (2005). Lateral fluid percussion brain injury: a 15-year review and evaluation. *J Neurotrauma* 22**,** 42-75.

Timofeev, I., Carpenter, K.L., Nortje, J., Al-Rawi, P.G., O'connell, M.T., Czosnyka, M., Smielewski, P., Pickard, J.D., Menon, D.K., Kirkpatrick, P.J., Gupta, A.K., and Hutchinson, P.J. (2011). Cerebral extracellular chemistry and outcome following traumatic brain injury: a microdialysis study of 223 patients. *Brain* 134**,** 484-494.

Trouw, L.A., Blom, A.M., and Gasque, P. (2008). Role of complement and complement regulators in the removal of apoptotic cells. *Mol Immunol* 45**,** 1199-1207.

Tyler, W.J., and Pozzo-Miller, L.D. (2001). BDNF enhances quantal neurotransmitter release and increases the number of docked vesicles at the active zones of hippocampal excitatory synapses. *J Neurosci* 21**,** 4249-4258.

Tzourio-Mazoyer, N., Landeau, B., Papathanassiou, D., Crivello, F., Etard, O., Delcroix, N., Mazoyer, B., and Joliot, M. (2002). Automated anatomical labeling of activations in SPM using a macroscopic anatomical parcellation of the MNI MRI single-subject brain. *Neuroimage* 15**,** 273-289.

Unden, J., Astrand, R., Waterloo, K., Ingebrigtsen, T., Bellner, J., Reinstrup, P., Andsberg, G., and Romner, B. (2007). Clinical significance of serum S100B levels in neurointensive care. *Neurocrit Care* 6**,** 94-99.

Underwood, C.K., and Coulson, E.J. (2008). The p75 neurotrophin receptor. *Int J Biochem Cell Biol* 40**,** 1664-1668.

Ungerstedt, U., and Rostami, E. (2004). Microdialysis in neurointensive care. *Curr Pharm Des* 10**,** 2145-2152.

Van Beek, J., Elward, K., and Gasque, P. (2003). Activation of complement in the central nervous system: roles in neurodegeneration and neuroprotection. *Ann N Y Acad Sci* 992**,** 56-71.

Vargas, M.E., and Barres, B.A. (2007). Why is Wallerian degeneration in the CNS so slow? *Annu Rev Neurosci* 30**,** 153-179.

Verweij, B.H., Muizelaar, J.P., Vinas, F.C., Peterson, P.L., Xiong, Y., and Lee, C.P. (2000). Impaired cerebral mitochondrial function after traumatic brain injury in humans. *J Neurosurg* 93**,** 815-820.

Vespa, P., Bergsneider, M., Hattori, N., Wu, H.M., Huang, S.C., Martin, N.A., Glenn, T.C., Mcarthur, D.L., and Hovda, D.A. (2005). Metabolic crisis without brain ischemia is common after traumatic brain injury: a combined microdialysis and positron emission tomography study. *J Cereb Blood Flow Metab* 25**,** 763-774.

Vos, P.E., Jacobs, B., Andriessen, T.M., Lamers, K.J., Borm, G.F., Beems, T., Edwards, M., Rosmalen, C.F., and Vissers, J.L. (2010). GFAP and S100B are biomarkers of traumatic brain injury: an observational cohort study. *Neurology* 75**,** 1786-1793.

Walf, A.A., and Frye, C.A. (2007). The use of the elevated plus maze as an assay of anxiety-related behavior in rodents. *Nat Protoc* 2**,** 322-328.

Wang, K.C., Kim, J.A., Sivasankaran, R., Segal, R., and He, Z. (2002). P75 interacts with the Nogo receptor as a co-receptor for Nogo, MAG and OMgp. *Nature* 420**,** 74-78.

Wang, X., and Michaelis, E.K. (2010). Selective neuronal vulnerability to oxidative stress in the brain. *Front Aging Neurosci* 2**,** 12.

Warden, D.L., French, L.M., Shupenko, L., Fargus, J., Riedy, G., Erickson, M.E., Jaffee, M.S., and Moore, D.F. (2009). Case report of a soldier with primary blast brain injury. *Neuroimage* 47 Suppl 2**,** T152-153.

Warrington, E.K., James, M., and Maciejewski, C. (1986). The WAIS as a lateralizing and localizing diagnostic instrument: a study of 656 patients with unilateral cerebral lesions. *Neuropsychologia* 24, 223-239.

Waterloo, K., Ingebrigtsen, T., and Romner, B. (1997). Neuropsychological function in patients with increased serum levels of protein S-100 after minor head injury. *Acta Neurochir (Wien)* 139, 26-31; discussion 31-22.

Wechsler, D. (1997). Wechsler Memory Scale - III. *New York:* Psychological Corporation.

Weinshilboum, R.M., Otterness, D.M., and Szumlanski, C.L. (1999). Methylation pharmacogenetics: catechol O-methyltransferase, thiopurine methyltransferase, and histamine N-methyltransferase. *Annu Rev Pharmacol Toxicol* 39, 19-52.

Whalen, M.J., Carlos, T.M., Kochanek, P.M., Wisniewski, S.R., Bell, M.J., Clark, R.S., Dekosky, S.T., Marion, D.W., and Adelson, P.D. (2000). Interleukin-8 is increased in cerebrospinal fluid of children with severe head injury. *Crit Care Med* 28, 929-934.

White, C.S., Bowen, I.G., and Richmond, D.R. (1965). Biological tolerance to air blast and related biomedical criteria. CEX-65.4. *CEX Rep Civ Eff Exerc*, 1-239.

Will, B., Galani, R., Kelche, C., and Rosenzweig, M.R. (2004). Recovery from brain injury in animals: relative efficacy of environmental enrichment, physical exercise or formal training (1990-2002). *Prog Neurobiol* 72, 167-182.

Williams, A.J., Hartings, J.A., Lu, X.C., Rolli, M.L., Dave, J.R., and Tortella, F.C. (2005). Characterization of a new rat model of penetrating ballistic brain injury. *J Neurotrauma* 22, 313-331.

Witte, O.W. (1998). Lesion-induced plasticity as a potential mechanism for recovery and rehabilitative training. *Curr Opin Neurol* 11, 655-662.

Woods, R.P., Mazziotta, J.C., and Cherry, S.R. (1993). MRI-PET registration with automated algorithm. *J Comput Assist Tomogr* 17, 536-546.

Xerri, C., Merzenich, M.M., Peterson, B.E., and Jenkins, W. (1998). Plasticity of primary somatosensory cortex paralleling sensorimotor skill recovery from stroke in adult monkeys. *J Neurophysiol* 79, 2119-2148.

Xiao-Sheng, H., Sheng-Yu, Y., Xiang, Z., Zhou, F., and Jian-Ning, Z. (2000). Diffuse axonal injury due to lateral head rotation in a rat model. *J Neurosurg* 93, 626-633.

Yamazaki, Y., Yada, K., Morii, S., Kitahara, T., and Ohwada, T. (1995). Diagnostic significance of serum neuron-specific enolase and myelin basic protein assay in patients with acute head injury. *Surg Neurol* 43, 267-270; discussion 270-261.

Yang, K., Perez-Polo, J.R., Mu, X.S., Yan, H.Q., Xue, J.J., Iwamoto, Y., Liu, S.J., Dixon, C.E., and Hayes, R.L. (1996). Increased expression of brain-derived neurotrophic factor but not neurotrophin-3 mRNA in rat brain after cortical impact injury. *J Neurosci Res* 44, 157-164.

Yao, J., Harvath, L., Gilbert, D.L., and Colton, C.A. (1990). Chemotaxis by a CNS macrophage, the microglia. *J Neurosci Res* 27, 36-42.

Yasuda, M., Takeuchi, K., Hiruma, M., Iida, H., Tahara, A., Itagane, H., Toda, I., Akioka, K., Teragaki, M., Oku, H., and Et Al. (1990). The complement system in ischemic heart disease. *Circulation* 81, 156-163.

Yu, S., Kaneko, Y., Bae, E., Stahl, C.E., Wang, Y., Van Loveren, H., Sanberg, P.R., and Borlongan, C.V. (2009). Severity of controlled cortical impact traumatic brain injury in rats and mice dictates degree of behavioral deficits. *Brain Res* 1287, 157-163.

Zaccaro, M.C., Ivanisevic, L., Perez, P., Meakin, S.O., and Saragovi, H.U. (2001). p75 Co-receptors regulate ligand-dependent and ligand-independent Trk receptor activation, in part by altering Trk docking subdomains. *J Biol Chem* 276, 31023-31029.

Zemlan, F.P., Jauch, E.C., Mulchahey, J.J., Gabbita, S.P., Rosenberg, W.S., Speciale, S.G., and Zuccarello, M. (2002). C-tau biomarker of neuronal damage in severe brain injured patients: association with elevated intracranial pressure and clinical outcome. *Brain Res* 947, 131-139.

Zemlan, F.P., Rosenberg, W.S., Luebbe, P.A., Campbell, T.A., Dean, G.E., Weiner, N.E., Cohen, J.A., Rudick, R.A., and Woo, D. (1999). Quantification of axonal damage in traumatic brain injury: affinity purification and characterization of cerebrospinal fluid tau proteins. *J Neurochem* 72, 741-750.

Ziebell, J.M., and Morganti-Kossmann, M.C. (2010). Involvement of pro- and anti-inflammatory cytokines and chemokines in the pathophysiology of traumatic brain injury. *Neurotherapeutics* 7, 22-30.

Zimmer, D.B., Cornwall, E.H., Landar, A., and Song, W. (1995). The S100 protein family: history, function, and expression. *Brain Res Bull* 37**,** 417-429.

Zweckberger, K., Hackenberg, K., Jung, C.S., Hertle, D.N., Kiening, K.L., Unterberg, A.W., and Sakowitz, O.W. (2011). Cerebral metabolism after early decompression craniotomy following controlled cortical impact injury in rats. *Neurol Res* 33**,** 875-880.