

RE-EXPRESSION OF THROMBOSPONDIN-1 IN THE THALAMOCORTICAL WHISKER CIRCUIT AFTER
EXPERIMENTAL DIFFUSE TRAUMATIC BRAIN INJURY: POTENTIAL ROLE IN MEDIATING SYNAPTOGENESIS?

by

Sarah Ogle

Copyright © Sarah Ogle 2016

A Thesis Submitted to the Faculty of the

COLLEGE OF MEDICINE

In Partial Fulfillment of the Requirements

For the Degree of

MASTER OF SCIENCE
WITH A MAJOR IN
CLINICAL TRANSLATIONAL SCIENCE

In the Graduate College

THE UNIVERSITY OF ARIZONA

2016

STATEMENT BY AUTHOR

The thesis titled *Re-expression of Thrombospondin-1 in the Thalamocortical Whisker Circuit after Experimental Diffuse Traumatic Brain Injury: Potential Role in Mediating Synaptogenesis?* prepared by Sarah Ogle, D.O. has been submitted in partial fulfillment of requirements for a master's degree at the University of Arizona and is deposited in the University Library to be made available to borrowers under rules of the Library.

Brief quotations from this thesis are allowable without special permission, provided that an accurate acknowledgement of the source is made. Requests for permission for extended quotation from or reproduction of this manuscript in whole or in part may be granted by the copyright holder.

SIGNED: *Sarah Ogle, D.O.*

APPROVAL BY THESIS DIRECTOR

This thesis has been approved on the date shown below:

<hr style="width: 30%; margin: 0 auto;"/> <p><i>Dr. Ronald Hammer</i> <i>Professor Basic Medical Sciences</i> <i>Professor Pharmacology</i> <i>Clinical Translational Sciences Program director</i></p>	<p><u>5/9/2016</u> Date</p>
--	---------------------------------

<hr style="width: 30%; margin: 0 auto;"/> <p><i>Dr. Jonathan Lifshitz</i> <i>Professor Basic Medical Sciences</i> <i>Professor Neuroscience</i> <i>Translational Neurotrauma Research Program Director</i></p>	<p><u>5/9/2016</u> Date</p>
---	---------------------------------

Acknowledgments

For their mentorship, guidance, and patience, the author would like to Dr. Ronald Hammer, Dr. Jonathan Lifshitz and Dr. Theresa Currier Thomas. My experience and training over the past 2 years working in the Translational Neurotrauma Research Program has been phenomenal and I am grateful to have had the opportunity given by the Phoenix Integrated Surgical Residency program and Dr. Steven B. Johnson for allowing me to participate in dedicated research time and attend conferences to present my work. I cannot express enough gratitude to the members of the Translational Neurotrauma Research Program and Clinical Translational Science program for their guidance and wisdom while working on my Masters. For technical assistance, author would like to thank Hazel May, Aida Khodadad, Jack Reddaway, Daniel Griffiths, Ryan Hart, Amanda Lisembee, and Rachel Rowe.

Funding supported, in part by, ADHS14-00003606, NIH R03 NS-077098, NIH R01 NS-065052, Science Foundation Arizona and Phoenix Children's Hospital Mission Support Funds, Banner University Medical Center-Phoenix.

Table of contents

List of abbreviations, tables and figures.....	5
Abstract.....	6
1.0 Introduction: Traumatic brain injury.....	7
1.1 Epidemiology.....	7
1.2 Traumatic brain injury severity.....	7
1.3 Classification of traumatic brain injuries.....	7
1.4 Primary and secondary brain injuries.....	8
2.0 Brain injury outcomes.....	8
2.1 Glasgow outcome scale.....	8
2.2 Relationship to chronic neurological diseases.....	9
2.2.1 Chronic seizures.....	9
2.2.2 Dementia and Alzheimer's disease.....	9
2.2.3 Parkinson's disease.....	9
2.2.4 Chronic traumatic encephalopathy.....	9
2.2.5 Endocrinopathy.....	10
2.3 Sequela of mild traumatic brain injuries: post-concussive symptoms.....	10
3.0 Animal models of TBI.....	10
4.0 Whisker circuit.....	12
4.1 Whisker sensitivity and the Whisker Nuisance Task.....	12
5.0 Synaptogenesis.....	13
5.1 Synapses.....	13
5.2 Synaptogenesis.....	13
5.3 Secreted molecules critical to synaptogenesis.....	14
5.3.1 Brain derived neurotrophic factor.....	14
5.3.2 Hevin and SPARC.....	14
5.3.3 Glypicans.....	14
5.3.4 Thrombospondins.....	15
6.0 Evidence of circuit reorganization after dTBI.....	16
6.1 Glial response to TBI.....	20
7.0 Previous studies: TSP mediated synaptogenesis after neuronal insult.....	20
8.0 Scope and hypothesis.....	20
9.0 Methods.....	21
9.1 Subjects.....	21
9.2 Midline fluid percussion.....	21
9.3 Perfusion and dissection.....	21
9.4 RNA extraction.....	21
9.5 Quantitative PCR.....	21
9.6 Protein extraction.....	22
9.7 Automated capillary western.....	22
9.8 Statistical analysis.....	22
10.0 Results.....	25
10.1 Gene expression and Protein analysis of GAP-43 in the VPM and S1BF after dTBI.....	26
10.2 Gene expression and Protein analysis of SYNP in the VPM and S1BF after dTBI.....	27
10.3 Gene expression and Protein analysis of PSD-95 in the VPM and S1BF after dTBI.....	28
10.4 Protein analysis of PICK-1 in the VPM and S1BF after dTBI.....	29
10.5 Gene expression and Protein analysis of TSP-1 in the VPM and S1BF after dTBI.....	30
10.6 Gene expression and Protein analysis of TSP-2 in the VPM and S1BF after dTBI.....	31
10.7 Gene expression and Protein analysis of $\alpha 2\delta$ -1 in the VPM and S1BF after dTBI.....	32
11.0 Discussion.....	33
11.1 Summary of results.....	33
11.2 Clinical implications and future directions.....	35
References.....	37
Appendix A: Whisker Nuisance Behaviour Score Sheet.....	51
Appendix B: Technical guides for automated capillary western (Proteinsimple®).....	52

Abbreviation	Title
$\alpha 2\delta$ -1	$\alpha 2\delta$ -1 subunit of voltage-gated calcium channel
AD	Alzheimer's disease
AMPA	α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor
ApoER2	apolipoprotein E receptor 2
AUC	Area under the curve
BBB	Blood brain barrier
BDNF	Brain derived neurotrophic factor
CAM	Cell adhesion molecule
CTE	Chronic traumatic encephalopathy
CT	Computer tomography
dFPI	Days post-fluid percussion injury
DPI	Days post-injury
dTBI	Diffuse traumatic brain injury, concussion
FPI	Fluid percussion injury
GAP-43	Growth associated protein 43
GAPDH	Glyceraldehyde 3-phosphate dehydrogenase
GCS	Glasgow coma scale
GFAP	Glial fibrillary acid protein
GOS	Glasgow outcome scale
IBA1	Ionized calcium-binding adapter molecule 1
mFPI	Midline fluid percussion injury
MRI	Magnetic resonance imaging
NMDA	N-methyl-D-aspartate receptor
PCS	Post-concussive symptoms
PICK-1	Protein interacting with C-kinase 1
PR5	Principle trigeminal nucleus
PSD-95	Postsynaptic density 95
S1BF	Primary somatosensory barrel fields (cortex)
SEM	Standard error of mean
SYNP	Synaptophysin
TBI	Traumatic brain injury
TRK-B	Tropomyosin-related tyrosine kinase receptor B
TSP	Thrombospondin
TSP-1	Thrombospondin-1
TSP-2	Thrombospondin-2
VLDLR	Very low-density lipoprotein receptor
VPM	Ventral posteromedial nucleus of thalamus
WNT	Whisker Nuisance Task

Table number	Title	Page
1	Severity of brain injury stratification	7
2	Extended Glasgow coma outcome scale	9
3	Post-Concussive Symptoms	10
4	Known binding sites of Thrombospondin-1 in the CNS	15
5	Plate conditions for VPM samples	23
6	Plate conditions for S1BF samples	23
7	Example calculation for protein quantification	24

Figure number	Title	Page
1	FPI device and location of craniectomy	11
2	Whisker Barrel Circuit	12
3	Giemsa staining in the VPM	16
4	Silver staining in the VPM	16
5	Neuronal morphology VPM	18
6	Neuronal morphology S1BF	19
7	Example electropherogram	24
8	Glutamtergic synapse	25
9	GAP-43 gene expression and protein levels in the VPM and S1BF	26
10	SYNP gene expression and protein levels in the VPM and S1BF	27
11	PSD-95 gene expression and protein levels in the VPM and S1BF	28
12	PICK-1 protein levels in the VPM and S1BF	29
13	TSP-1 gene expression and protein levels in the VPM and S1BF	30
14	TSP-2 gene expression and protein levels in the VPM and S1BF	31
15	$\alpha 2\delta$ -1 gene expression and protein levels in the VPM and S1BF	32

ABSTRACT

RE-EXPRESSION OF THROMBOSPONDIN-1 IN THE THALAMOCORTICAL WHISKER CIRCUIT AFTER EXPERIMENTAL DIFFUSE TRAUMATIC BRAIN INJURY: POTENTIAL ROLE IN MEDIATING SYNAPTOGENESIS?

Introduction:

Annually, an estimated 2.5 million traumatic brain injuries (TBI) occur in the United States, of which, over 50,000 result in deaths. Currently, 5.3 million Americans are living with neurological dysfunction secondary to TBIs leading to a \$60 billion dollar cost in medical expenses and productivity losses. To date, there are limited treatments available to cure or ease the morbidity of TBI. Despite preventative efforts, traumatic brain injuries (TBI) occur at a staggering rate and it is estimated that 15-20% of survivors develop persistent post-traumatic neurological impairment. The purported source of neurological dysfunction is a result of circuit reorganization when the brain rebuilds itself.

After diffuse TBI, rodents have been shown to develop a late-onset, gain-of-function sensory sensitivity to whisker stimulation; similar to phonophobia and photophobia experienced by human TBI survivors. This morbidity coincides with evidence of post-TBI circuit reorganization, however the etiology of post-traumatic neurological impairment remains largely unknown.

Thrombospondin-1 (TSP-1) and thrombospondin-2 (TSP-2) are heavily expressed during pediatric neuronal synapse development. Expression of TSPs, however declines with age. Mechanistically during development, TSP mediates synaptogenesis via binding $\alpha 2\delta$ -1 subunit of the voltage-gated calcium channel receptor ($\alpha 2\delta$ -1). After neurological insult, re-expression of TSPs has been demonstrated and experimental modulation of the TSP/ $\alpha 2\delta$ -1 interaction has led to changes in morbidity. We therefore hypothesize that experimental diffuse TBI will result in re-expression of TSPs, which will be synchronous with increases in synaptic markers in the thalamocortical whisker circuit.

Methods: Adult male Sprague-Dawley rats underwent sham or moderate midline fluid percussion brain injury. At multiple time points over 2-months post-injury, expression of TSPs and synaptic markers were quantified from thalamocortical circuit (ventroposterior medial thalamus (VPM), primary somatosensory barrel fields (S1BF)) biopsies using qPCR and automated capillary westerns, respectively.

Results: TSP-1 gene expression and protein levels increase in the VPM during the first week after injury. Gene expression of TSP-1 did not significantly change over time in the S1BF, however, there was a significant increase in protein levels in the first and second weeks after injury. No significant changes were demonstrated in synaptic markers in the VPM over the time course. TSP-1 protein levels demonstrated a similar multimodal response to synaptic markers in the S1BF.

Conclusion: Re-expression of TSP-1 and synchronous changes in synaptic marker supports a role for TSP-1 mediated synaptogenesis after experimental diffuse TBI in the S1BF. These data positions us for future investigation of pharmacological inhibition of TSP-mediated synaptogenesis after TBI; which may represent a prophylactic strategy against circuit reorganization and neurological dysfunction after TBI.

1.0 Introduction: Traumatic Brain Injury

1.1 Epidemiology

Annually, 2.5 million Americans present to emergency department for traumatic brain injuries (TBI), of this, 50,000 result in death which represents one third of all injury related deaths^{29,30}. However, the true incidence of TBI is unknown as many, particularly mild TBI, patients may never seek medical attention or seek medical attention in outpatient settings (where incidence data is not readily available)³¹. Falls are the leading cause of TBIs overall and are the leading cause of death at the extremes of age, followed by motor vehicle collisions and assaults^{30,31}.

Survivors of traumatic brain injuries may have long lasting or permanent neurological dysfunction that impacts their lives, the lives of their families, and results in a \$60 billion economic burden including productivity losses. Return to work rates are correlated with severity of injury and are estimated between 12-70%³². TBIs alarming impact on society necessitates investigation into preventative measures, pathophysiology and development of potential therapies for survivors.

1.2 Traumatic brain injury severity

Clinical TBI severity is most commonly classified as either mild, moderate or severe using the Glasgow coma scale (GCS), imaging findings, loss of consciousness, and post-traumatic amnesia^{33,34}. Figure 1 describes the severity designations of TBIs. TBIs can also be further classified by the mechanism (e.g. blunt, penetrating, blast) or by parenchymal involvement (focal or diffuse). Seventy-five percent of the documented TBIs are classified as mild diffuse TBIs as a result of blunt trauma³⁵.

Mild	Moderate	Severe
Normal imaging	Normal or abnormal imaging	Normal or abnormal imaging
LOC <30 mins	LOC >30 minutes and <24 hours	LOC > 24 h hours
AMS <24 hours	AMS >24 hours	AMS >24 hours
PTA <24 hours	PTA >24 hours and <7 days	PTA >7 days
GCS = 13-15	GCS= 9-12	GCS= 3-8
LOC= loss of consciousness, AMS= altered mental status, PTA= post-traumatic amnesia, GCS= Glasgow coma scale		

Table 1. Severity of brain injury stratification.

1.3 Classification of traumatic brain injuries

The majority of the literature describes the damage from a TBI as focal or diffuse. However, there are a plethora of different TBI injury mechanisms and degrees of force. Therefore, rather than an injury being categorized as focal or diffuse, it should be recognized that TBI injuries are on a spectrum between focal and diffuse injury^{36,37}.

Focal injuries result from direct force to the cranium (coup) or parenchyma, in the case of penetrating injuries. Strong enough forces may also result a contra-coup injury if the brain is ricocheted off the opposing cranial wall. Focal damage may also be increased as a result from local mass effect of epi- or subdural hematomas³⁸. In focal injuries, the damage is more contained to a single area and is more likely to result in cell death and tissue volume loss³⁹.

Diffuse traumatic injuries result from acceleration/deceleration or rotational forces causing deformation of the brain tissue⁴⁰. Typically, the damage is located at gray-white interfaces as a result of sheering of the axons deafferentation of synapses⁴¹⁻⁴³. Delayed axotomy, which more likely occurs in mild TBIs, results from local damage leading to axonal swelling and cytoskeletal breakdown⁴⁴⁻⁴⁶. This is then followed by separation and Wallerian degradation and thus synaptic deafferentation⁴⁴⁻⁴⁶. Cell death is less likely to occur in diffuse injuries in comparison to focal damage and the damage may be scattered amongst healthy tissue⁴⁷⁻⁵¹. The severity of damage also depends on the direction of force, with lateral forces causing more axonal sheering than anterior-posterior forces⁴⁵.

Blast injuries, the leading cause of TBIs in the military, are similar to diffuse traumatic brain injuries. Blast injuries are diffuse in nature and may not demonstrate gross abnormalities on conventional imaging^{30,52}. However, blast injuries are unique as the ultrasonic waves elicit several concussive injuries from a single blast³⁵. Additionally, the victim may also suffer a second TBI from either being struck by objects thrown in the blast or by striking their head on an object or the ground as they are propelled due to the blast force⁵².

1.4 Primary and secondary brain injuries

Primary brain injury refers to the direct injury produced during the traumatic event. Following the traumatic injury, the physiological response to the injury may compound and worsen the initial injury, this being termed secondary brain injury. Primary brain injuries can be prevented or limited with the use of safety equipment and precautions, such as helmets, seat belts or with fall prevention measures.

Prevention and limitation of secondary brain injury is the goal of acute medical and/or surgical interventions after a TBI. Of the patients that initially survive, but later die while in the hospital, typically succumb to effects of secondary brain injuries⁵³. The pathophysiological response to brain injury begins with compromise of the blood-brain barrier, vasodilation leading to cerebral edema and increased intracranial pressure^{54,55}. Since the cranium is a fixed volume, in absence of a surgical decompressive craniectomy, a severe increase in intracranial pressure may lead to herniation and death.

Increased intracranial pressure leads to a decrease in cerebral blood flow and cerebral hypoxemia; which can be also worsened by polytrauma suffered by the patient^{53,56}. This then leads to increased calcium influx and potassium efflux leading to ionic imbalances, mitochondrial dysfunction, and membrane depolarization. This is followed by glutamate excitotoxicity, increased demand on the sodium-potassium pump to maintain membrane potentials, and increased metabolic glucose utilization^{35,46,55,57-60}.

Subsequent reperfusion to the tissue then leads to free radical production, inflammatory response, cytoskeletal and membrane degradation, axonal swelling with subsequent delayed axotomy, and possible cell death^{35,39,46,55,57-60}. Inflammatory mediators and immune cells assist with clearance of the damage, but may also contribute to subsequent morbidity as neurofibrillary tangles develop from dead neurons and tauopathy develops from the inflammatory amyloidiogenesis; hallmarks of several neurodegenerative diseases^{46,61}.

Axonal damage then leads to Wallerian degeneration of the distal aspect of the axon over a course of weeks to months after injury and may impact non-injured circuits causing amplification or spreading of neurological dysfunction to non-injured areas of the brain^{45,46,62}. It is postulated that axonal degeneration and synaptic deafferentation manifests as the neurological dysfunction seen in diffuse TBI⁶². In mild TBI, where cell death is less likely, there is evidence supporting regeneration of synaptic terminals which may assist in either recovery or the development of chronic neurological dysfunction⁶²⁻⁶⁴.

Secondary brain injury begins immediately after the primary injury, however secondary brain injury can continue for weeks to months after the initial injury⁶⁵. As the body of knowledge grows on TBI and its consequences, TBI has become considered more as a disease process, rather than an isolated event⁶⁶.

2.0 Brain injury outcomes

Outcomes and recovery from brain injury are variable depending on injury severity, underlying health status of the patient, age, location of injury. Prognosis has been shown to correlate to admission GCS, loss of consciousness and post-traumatic amnesia⁶². Sequela of mild traumatic brain injuries have recently come to the foresight of public health. Symptoms can often be subtle, but have significant impact on survivors' quality of life. Symptoms often go under reported and due to their insidious onset may not have their etiology linked to the TBI. The chronicity of symptoms that can be experienced by survivors warrants the investigation of the mechanism and underlying pathophysiology.

2.1 Glasgow outcome scale (GOS)

After the development of successful medical and surgical management techniques for stabilizing head injury patients, efforts were made in 1975 to describe the potential outcomes for severe TBI survivors⁶⁷. There are several disability scoring systems to describe patient outcomes, however the GOS is more commonly used by practitioners (Figure 2). King Jr. et. al (2005) found 3 month GOS, prolonged hypotension, pupillary, diffuse axonal injury, pupillary dilation reflex were predictors of 12 month outcomes⁶⁸.

Score	State	Abbreviation	Explanation
1	Dead	D	Dead
2	Vegetative state	VS	Vegetative state
3	Lower severe disability	SD-	Complete dependence on others
4	Upper severe disability	SD+	Partial dependence on others
5	Lower moderate disability	MD-	Inability to return to work or reduced participation in social activities
6	Upper moderate disability	MD+	Return to work with reduced capacity, able to participate in social activities
7	Lower good recovery	GR-	Good recovery with mild mental or social deficits
8	Upper good recovery	GR+	Good recovery without deficits

Table 2. Extended Glasgow Outcome Scale. The Glasgow coma scale is utilized as a predictor of prognosis after TBI.

2.2 Relationship to chronic neurological diseases: Chronic seizures, dementia & Alzheimer's disease, Parkinson's disease, chronic traumatic encephalopathy, endocrinopathy

TBI has been identified as an independent risk factor for a number of chronic neurological diseases. This link to neurodegeneration highlights TBI as being a disease process, rather than an isolated event. These long term consequences are only a brief collection of the current literature available.

2.2.1 Chronic seizures

Early post-TBI seizures begin within 7 days of the injury, whereas late post-TBI seizures begin 7 or more days after the injury^{69,70}. It is estimated that 20% of symptomatic epilepsy prevalence is attributed to TBIs⁶⁹. Seizures are more common after penetrating injuries than closed head injuries. Other risk factors include dural penetration with metal, bi-parietal contusions, multiple intracranial operations, multiple subdural contusions, subdural hematoma requiring evacuation, midline shift >5mm and severity of TBI⁷¹. Seizure risk is the highest in the first year after injury and then diminishes over time; however some individuals develop persistent seizures and continue to have seizures years after the initial injury⁶⁹.

2.2.2 Dementia and Alzheimer's Disease (AD)

The single strongest risk factor for developing of dementia is age, but studies have documented TBI increases risk of the development of dementia, including Alzheimer's disease^{59,69}. AD is characterized by progressive dementia and development of extracellular senile plaques of amyloid beta deposition and intracellular neurofibrillary tangles composed of tau proteins. Similar histopathological features can also be seen in acute brain injuries⁶¹. TBI studies have demonstrated post-traumatic dysregulation of several proteins implicated in the pathogenesis of neurodegenerative diseases⁶¹. Post-TBI hypoxia or poor cerebral perfusion is not uncommon. Hypoxia and poor cerebral perfusion have both been shown to accelerate beta-amyloid deposition, which are protein by-products indicative of neuronal degeneration and a hallmark of AD⁷²⁻⁷⁴.

2.2.3 Parkinson's disease

Parkinson's disease is a neurodegenerative disease of the dopamergic neurons in the substantia nigra, resulting in motor dysfunction and eventual dementia. Similar to Alzheimer's disease, Parkinson's disease presents the latter decades of life. Development of Parkinson's disease has been shown to be associated with TBI, including mild TBI with a loss of consciousness^{69,75,76}. Repetitive TBI increases the risk of developing Parkinson's disease⁷⁶.

2.2.4 Chronic traumatic encephalopathy (CTE)

Also known as dementia pugilistica or "punch-drunk" syndrome. CTE characteristic pathology involves tauopathy, axonal injury, microvascular injury, neurodegeneration, and chronic inflammation⁶⁵. CTE is associated repetitive TBIs over a chronic time period. At risk individuals include, athletes, military personal, and domestic violence victims⁷⁷. In athletes, symptoms typically develop 1-2 decades after retirement^{69,78-81}. CTE insidious onset begins with difficulties with concentration, attention and memory. Other symptoms such as headaches, dizziness or confusion may also occur. As CTE progresses, patients develop difficulties with insight, judgement and dementia. In the latter stages, patients will have motor difficulties, similar to Parkinson's disease. As their mental faculties decline, progressive memory loss, dementia and psychosis leads to social instability and behavioral disturbances⁴⁵. The development and degree of severity of the

disorder seems to be correlated with severity and number of TBIs. Recently, evidence of CTE like pathology has been in demonstrated in blast injury victims; whom are likely to receive multiple TBIs from a single blast as described above⁵².

2.2.5 Endocrinopathy

The pituitary and pituitary stalk are encased in the sella turcia within the floor of the cranium. As the remainder of the brain shifts within the cranium while undergoing accelerative, decelerative, or rotational forces there can be considerable amount of strain on the pituitary and pituitary stalk as it remains stationary. The pituitary and pituitary stalk play a critical role in the hypothalamic-pituitary axis, regulating hormonal release. Studies have described a number of dysregulatory responses after TBI involving growth hormone, somatotrophic, gonadotrophic, thyrotrophic, cortisol, and control of free water status⁸²⁻⁸⁶. It is estimated that ~20% of patients with severe TBI have endocrinopathies after TBI^{71,87}.

2.3 Sequela of mild traumatic brain injuries: post-concussive symptoms (PCS)

Sequela of TBI is understandably correlated with severity of injury⁸⁸. Recently, attention to the chronic sequela of mild TBI have gained the interest of researchers and practitioners with the realization of chronic neurological dysfunction of athletes and military veterans who have suffered multiple, mild TBI throughout their careers. It is estimated that ~17% of patients who suffered multiple concussions will develop CTE⁴⁵.

While most patients recover from mild traumatic brain injuries, 15-20% report having symptoms 1 month after injury; some who then go on to have persistent or permanent symptoms^{46,89,90}. The true incidence is unknown, again as many patients with mild TBI do not seek medical care or practitioners may not link the symptoms to the TBI. While the population of patients experiencing more than one symptom after one year is small (5%), such symptoms can cause considerable amount of distress, impact daily life and may prevent someone from returning to their normal activities⁴⁴.

Symptoms can be broadly categorized into cognitive, emotional or somatosensory symptoms. Table 3 lists post-concussive symptoms. The range of post-concussive symptoms reflects the diffuse nature of mild TBI (mTBI) impact on multiple brain regions and circuits⁹¹⁻⁹³. For example, thalamic damage after TBI has been associated in post-concussive headache, fatigue, cognitive deficits⁹⁴⁻⁹⁷. Thalamic susceptibility to damage after mTBI is thought to be due to high shear forces upon the relay axons and its multiple connections to other regions of the brain⁹⁸. These structural changes to axons have been suggested to contribute to the persistence in post-concussive symptoms⁹⁹.

At this time, it is unknown what severity or number of TBIs is required to alter mechanisms that normally lead to recovery towards a pathway of persistent PCS or CTE. Diagnosis of PCS has been controversial due to similar symptoms occurring in mental health disease, such as post-traumatic stress disorder or depression, post-traumatic endocrinopathies or other comorbidities. Common forms of imaging, such as computer tomography(CT) and magnetic resonance imaging (MRI), often do not demonstrate evidence of injury with mild diffuse traumatic brain injury^{95,100,101}. Specialized imaging studies, which may be more sensitive to pathophysiological changes after mTBI, such as diffusion-weighted imaging, diffusion tensor imaging, functional magnetic resonance imaging and spectroscopy have demonstrated changes in thalamic metabolism, decreased tissue volume, perfusion alterations, microstructural axonal injury, and changes in connectivity after TBI^{95,100-103}. Post-mortem histology, in comparison to non-injured controls, has demonstrated pathology supporting PCS having an intrinsic neurological etiology¹⁰⁴. The chronicity of symptoms that can be experienced by survivors warrants the investigation of the mechanism and underlying pathophysiology.

Cognitive	Emotional	Somatosensory
Concentration	Irritability	Headaches
Memory	Depression	Nausea/Vomiting
Executive Functioning	Anxiety	Balance/Dizziness
Learning		Sleep Disturbances
Confusion		Light/Sound Sensitivity

Table 3. Post-concussive symptoms

3.0 Animal models of TBI

Translational research requires the ability to replicate clinical conditions in a controlled setting. Currently there are several models of brain injury in use by the neurotrauma community. Historically, the majority of TBI research has focused on focal injuries and acute time points⁶⁵. With the rising recognition of the long-term consequences of mild traumatic brain injuries, interest has grown to investigate the mechanisms leading to chronic symptomatology and possible neurodegeneration over time. Additionally, investigation into repetitive TBI have been designed to investigate effects of multiple mild TBIs that are most commonly seen in athletes, domestic violence victims, and veterans.

Development of translational therapies, however, has been proven to be quite difficult based on data from animal models. This is likely because, in the clinical world, there is not an ability to control for the many variables that impact TBI outcomes. Variables including age, sex, number of TBIs, magnitude and direction of forces applied, presence of polytrauma in combination with TBI, and other comorbid conditions, including mental health diseases, have all been demonstrated to impact TBI outcomes⁶⁵. Moreover, evaluating symptoms such as headache, verbal/written communication skills, higher levels of executive functioning and some psychological effects of TBI are difficult to assess in animal models. Despite these differences a large effort has been made to develop clinically relevant models. Here we discuss the most widely used animal models.

Focal injuries have been most commonly modeled by weight drop methods and controlled cortical injury. Weight drop methods involve a weight dropped from a chosen height and may be delivered in either a closed or open head fashion¹⁰⁵. Controlled cortical injury uses a piston to deliver the force over exposed dura. Cortical control injuries are advantageous because they are more reproducible and severity of the injury can be more tightly controlled⁴⁰. Focal models are designed to apply a force to a designated area to produce a cerebral contusion or cavitation depending on the severity of the injury¹⁰⁶.

Penetrating injury models have also been developed to model open head injuries, such as a gunshot wound or shrapnel¹⁰⁶. These penetrating injuries can differ from other focal open injuries in that the damage is not only caused by the projectile alone, but also by the high energy transmitted. This high energy then causes shock waves through the brain parenchyma which may be strong enough produce a temporary deformation of the brain¹⁰⁷.

Diffuse injuries are most often modeled by fluid percussion injuries (FPI), where a craniotomy is preformed and a fluid pulse is delivered, via pendulum drop, over the dura (Figure 1). Alternatively, a weight drop method can be used when the subject is placed on a flexible platform to allow for diffusion of energy after impact of the dropped weight. Placement of the craniotomy site for the delivery of the fluid pulse is critical to the type of injury that the investigator wants to elicit. Lateral fluid percussion creates a more focal injury, in comparison to parasagittal which creates both diffuse and focal damage. Midline fluid percussion creates the most evenly distributed injury, however slight variations in direction or angle of the force can create variable injury patterns. Fluid percussion models are advantageous as they too can be well replicated and the severity of the injury can be controlled by the degree of the pendulum delivering the fluid pulse⁴⁰.

Diffuse injuries can also be modeled by acceleration/inertia devices, designed to mimic the forces created in motor vehicle collisions. However, the devices required to perform these injuries may be limiting for researchers due to cost and technical operational skill level⁴⁰.

Blast injury models typically involve a blast delivery in an open-field or via a blast tube where pressurized shock wave is delivered by transmitting a blast force and rupturing a membrane separating the tube¹⁰⁶. This has become a clinically relevant model to demonstrate TBIs that are uniquely sustained in war theaters. The histopathology created by the blast models closely resembles the CTE like pathology and psychological manifestations seen in military veterans.

While these models best reproduce the physical forces of trauma, there are limitations to animal models to study TBI in humans. While TBI study in higher order animals could potentially provide more translational data, these studies are typically hindered by ethical, technical and financial limitations. To date, the majority of TBI research has been done in rodent models¹⁰⁷. Human craniums are geometrically different than most animal species used in TBI research, thereby impacting the biomechanics of the injury. Sulci and gyri that are present in the human brain and absent in some animal models may also impact the biomechanics of the injury and clinical pathology⁶⁵. And as previously stated, unlike animal models which have tightly controlled independent variables, the diversity in patients and TBI mechanism has made development of effective treatments difficult.

For the purpose of our study, we investigated the most common form of head injury, mild diffuse TBI (dTBI)¹⁰⁷. Methods involving focal trauma (e.g. controlled cortical impact, weight drop, lateral fluid percussion) would not be appropriate as there is more likely to be hematoma, gross tissue loss and neuronal cell death²⁹. Midline fluid percussion has been demonstrated to be the optimal choice for replication of an evenly distributed mild-moderate diffuse axonal injury, ease of use in rodents and is cost effective. Midline fluid percussion forces designed to replicate a diffuse TBI reproduce the

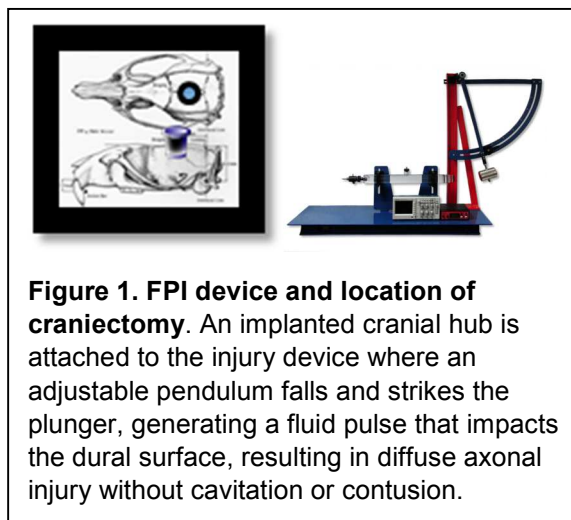


Figure 1. FPI device and location of craniotomy. An implanted cranial hub is attached to the injury device where an adjustable pendulum falls and strikes the plunger, generating a fluid pulse that impacts the dural surface, resulting in diffuse axonal injury without cavitation or contusion.

histopathology identified in autopsies, notably axonal damage, microvascular disruption, synaptic deafferentation and neuronal atrophy without overt cell death.

4.0 Whisker circuit

The thalamocortical circuit, or whisker circuit (Figure 2), in rodents is well understood and has been used as an *in vivo* model for neuronal plasticity and circuit activation due to its topographical organization¹⁰⁸. These features and its susceptibility to microvascular disruption, neuronal pathology, and glial activation make it an ideal region to study synaptic and circuitry changes after TBI.

Rodents, many whom are nocturnal and have poor eyesight, receive a large portion of sensory input from the environment from tactile input via their whiskers. Sensory input from mechanoreceptors on individual whiskers project to the trigeminal ganglion. The input then travels to the principal trigeminal nucleus (Pr5) of the hindbrain with topographic organization into barrelettes. The second order neurons then decussate via the lemniscal path and synapse in the ventral posteromedial nucleus of the thalamus (barrels). Third order neurons then project to ipsilateral barreloids in layer four of the primary somatosensory barrel fields in a topographic fashion¹⁰⁹⁻¹¹².

Information is then processed through the cortical layers in a columnar like fashion. Many intracortical synaptic connections co-exist in the cortical layers, suggesting redundant or amplification of incoming signaling. Layer four neurons connect to layers two, three and five; which then go on to synapse within the secondary somatosensory barrelfields and motor cortex¹¹³. Layer five and six neurons also give feedback to afferent neurons in the VPM¹¹⁴.

Synaptic connections are mainly excitatory glutamatergic synapses, although it should be noted that inhibitory interneurons do participate in the corticocortical connections. During development, environmental stimuli (e.g. whisking) is required for proper development of the whisker circuit. For proper topographic and synaptically active cortical circuit to form activation of post-synaptic NMDA and AMPA receptors is required, suggesting a Hebbian strengthening of circuitry¹¹⁴. This theory has been demonstrated in previous studies that have examined the effects of whisker stimuli deprivation¹¹⁴. Ablation of some whiskers induces expansion of neighboring “real estate” into the region which would have been occupied by the ablated whiskers. Ablation of all whiskers during development of the circuit leads to disorganized and arbitrary connections within the circuit, rather than the topographic formation of barrels, barreloids and barrelfields¹¹⁴.

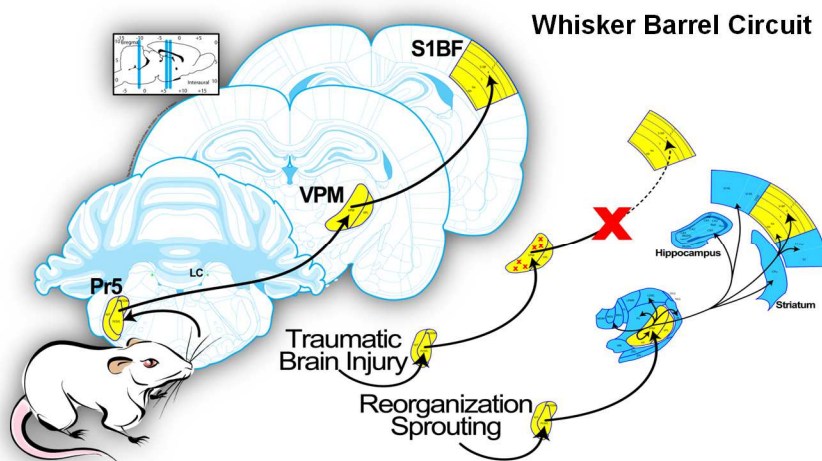


Figure 2. Whisker Barrel Circuit

Stimulation of the vibrissae (whiskers) activates a signal relay from the mystacial pad to trigeminal ganglion (Pr5) in the midbrain, to the contralateral barreloids in the ventral posterior medial nucleus (VPM) of the thalamus to layer IV of the barrel fields of the primary somatosensory cortex (S1BF). Axotomized neurons do not necessarily die, but can sprout and reorganize, forming new circuits.

4.1 Whisker sensitivity & the Whisker Nuisance Task (WNT)

Due to rodents' heavy reliance of environmental interpretation by whisker activity, it is not surprising that diffuse TBI (dTBI) somatosensory symptomatology manifests as whisker sensitivity. Whisker sensitivity is similar to phonophobia and photophobia experienced by human survivors, thus making it a useful model for the study of post-concussive symptoms¹¹⁵⁻¹¹⁷. What is curious about whisker sensitivity is that the symptom is not immediately present and does not manifest until 4-6 weeks post-TBI¹¹⁸. This delayed onset suggests that it is not the traumatic event itself, but the aftermath of trauma that contributes to the development of post-concussive symptoms.

This behavioral pathology is identified by the Whisker Nuisance Task (WNT)¹¹⁸. The WNT evaluates and quantifies rodent behavioral response to vigorous stimulation (Appendix B Whisker Nuisance Task Behavioral Point Criteria). Rodents are acclimated to an open field, after which three 5 minute intervals of whisker stimulation with a cotton-tip applicator. Both pre and post-TBI scores are obtained to evaluate for change in response over time and compared to uninjured animals.

The task scores behavioral components such as movement, respiratory rate, body position, evasive movements to identify distress due to whisker stimulation. This decreased tolerance to sensory stimulation, represented by agitation, is often seen in human survivors with post-concussive symptoms^{115,116}.

Whisker sensitivity and the understanding of *in vivo* structural and functional changes to the circuitry in the whisker circuit after TBI can potentially translate to human neural circuits contributing to post-TBI neuroplastic responses¹¹⁸. Delayed development of sensory sensitivity is not unique to rodents. Neuropathic pain from spinal cord injury also demonstrates delayed presentation and presents similarly at 4-6 weeks post-injury^{119,120}. It is postulated that delayed presentation is due to maladaptive circuit reorganization as the injured brain attempts to heal itself; which has been demonstrated to contribute to post-TBI epilepsy, central pain after stroke, and phantom limb pain or hyperalgesia following peripheral nerve lesions^{109,121-125}.

5.0 Synaptogenesis

5.1 Synapses

Synapses within the CNS are sites of neuronal to neuronal communication, consisting of a presynaptic neuron and a postsynaptic neuron. There are on average 10 billion neurons in the adult brain, each participating in roughly ten thousand synapses¹²⁶. The majority, ~80%, of synapses are excitatory and the remaining are inhibitory¹²⁶.

Following depolarization of the presynaptic membrane, release of neurotransmitters from presynaptic vesicles bind receptors on the postsynaptic neurons. Neurotransmitters are cleared from the synaptic cleft by either enzymatic degradation or reuptake by neuronal elements or glial cells. Binding of neurotransmitters at the postsynaptic neuron then leads to depolarization, hyperpolarization or activation of second messenger systems to continue the flow of communication.

Several neurological and psychiatric disorders have been linked to dysfunctional synapses, thus proper development and function of synapses is critical to neuropsychological health¹²⁷⁻¹³⁰.

5.2 Synaptogenesis

Induction of synaptogenesis can be stimulated by synaptogenic molecule secretion, neurotransmission or are activity-dependent¹³¹⁻¹³⁴. Varying lengths of time have been reported for the creation of a functional synapse from as little as 1-2 hours to several days¹³⁵. However, this complex process and subsequent maintenance or elimination of synapses is not fully understood. Many molecules have been implicated in synaptogenesis, however knockout models of many synaptogenic molecules have not fully halted the process¹²⁶. This review will focus on excitatory synaptogenesis in the CNS.

Initial formation of a synapse begins with outward growth of the presynaptic neuron and contact with its postsynaptic target (dendrite, muscle/organ, axon)^{126,136}. Outward growth of the presynaptic neuron is guided by the growth cone, located at the distal aspect of the growing dendrite, responding axonal guidance molecules secreted by postsynaptic neurons and glial cells^{132,137}. Growth associated protein 43 (GAP-43), a phosphoprotein located on the growth cone of presynaptic neurons, is indicative to developmental or plastic or regenerative axon growth¹³⁸⁻¹⁴². GAP-43 is maximally expressed during development and is generally down-regulated in adulthood. GAP-43 remains elevated in highly plastic areas of the brain and conditionally up-regulated in response to neuronal insult^{124,138-141,143}. GAP-43 regulates axonal growth, branching, axonal guidance, axonal morphology and synapse formation¹²⁴. GAP-43 begins to down-regulate once contact has been made with the postsynaptic neuron and synapse forms¹²⁴.

After contact, the connection is physically stabilized by binding of pre and postsynaptic cell adhesion molecules (CAMs)¹²⁶. Presynaptic and postsynaptic CAM binding promotes synaptogenesis through facilitation of presynaptic release and recruitment of postsynaptic receptor complex elements¹²⁶. A variety of classes of CAMs participate in synaptogenesis: integrins, cadherins, neuroligins/neuroexins, Ephrin-Bs/Ephrin. Neural cadherin (N-cadherin) is present in most synaptic sites and is particularly present during developmental synaptogenesis and activity-dependent synaptogenesis¹²⁶. Presynaptic neurexin and postsynaptic neuroligin binding have been implicated in synaptogenesis of inhibitory and excitatory synapses by facilitation presynaptic release and postsynaptic reception of neurotransmission^{126,144}. Neuroligin 1&3 have demonstrated to induce excitatory synapses and neuroligin 2 has been shown to induce inhibitory synapses¹⁴⁵⁻¹⁴⁸. Neuroligins have also demonstrated to bind to postsynaptic density elements, such as postsynaptic density 95 (PSD-95)¹²⁶. Postsynaptic density elements function as scaffolding proteins and bind postsynaptic receptors, such as N-methyl-D-aspartate (NMDA) receptors. Presynaptic Ephrin-Bs binding postsynaptic Ephrin receptors have been demonstrated to induce synaptogenesis, promote clustering of NMDA receptors, and neurotransmission¹⁴⁹⁻¹⁵⁶. Ephrin receptors have also demonstrated to recruit syndecan 2, a cell-surface heparan sulfate proteoglycan, which participates in maturation of dendritic spines¹²⁶.

Following initially pre and postsynaptic contact, the presynaptic terminal morphs from multiple filopodia into the classical bouton-like structures via postsynaptic secretion of Wnt¹⁵⁷⁻¹⁶⁰. Wnt signaling leads to cytoskeletal remodeling of the presynaptic terminal, regulates neurotransmission, and postsynaptic functioning¹⁶¹⁻¹⁶⁴. These morphological changes are associated with presynaptic and postsynaptic differentiation by the trafficking of presynaptic and postsynaptic scaffolding proteins and receptors at their given sites¹⁶⁵⁻¹⁶⁸. Minutes after contact, presynaptic Bassoon aggregates at the active zone to aid in neurotransmitter release¹³⁵. Accumulated presynaptic vesicles are then exocytosed at the active zone and bind postsynaptic receptors to strengthen the connection by neurotransmission¹⁶⁹. Synaptophysin (SYNP) is present on synaptic vesicles and is widely utilized as a marker for presynaptic terminals and for neuronal plasticity. It has been postulated that it also regulates synaptic vesicle exocytosis^{123,170}.

Postsynaptic terminal specialization begins with recruitment of numerous receptors and signaling components; such as, neurotransmitter receptors, NMDA receptors, postsynaptic densities, secondary messenger system molecules and glutamate receptors. Postsynaptic density 95 (PSD-95) is a well characterized subunit of a larger postsynaptic density structure. PSD-95 functions to anchor and organize NMDA receptors, as well as other postsynaptic density subunits. PSD-95 interaction with NMDA receptors have been demonstrated to play a role in synaptic plasticity^{171,172}. PSD-95 is elevated in development and then plateaus at the cessation of neurogenesis.

Following the recruitment of NMDA receptors, synapse maturation and stabilization occur. One indication of this is the recruitment of σ -amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) receptors, creating a functionally active synapse, and other non-NMDA glutamate receptors. Synapses lacking AMPA receptors are non-functional and are termed "silent synapses"¹⁷³. AMPA receptors are structurally made up of 4 GluR subunits. AMPA receptors participate in synaptic plasticity and are pivotal to long term potentiation and depression by promoting excitatory neurotransmission¹⁷⁴⁻¹⁷⁸. Activation of NMDA receptors and AMPA receptors also solidify synapses by stabilizing the bouton-like shape of the nerve terminals^{151,179-181}.

Trafficking of AMPA receptors is, in part, regulated by protein interacting with C-kinase (PICK-1)¹⁸². PICK-1 is a subunit of postsynaptic density element and is highly associated with AMPA receptors. PICK-1 binds to many ligands, including EphrinB, EphrinB2 receptors, glutamate receptors, and neuroligins¹⁸³. Over expression of PICK-1 results in increased AMPA receptors on postsynaptic terminals; thus, it can be inferred that PICK-1 plays a role in synaptogenesis⁵⁰.

5.3 Secreted molecules critical to synaptogenesis

5.3.1 Brain derived neurotrophic factor (BDNF)

BDNF is both secreted by and acts on pre and postsynaptic nerve terminals. Secreted BDNF has been demonstrated to induce both excitatory and inhibitory synapses¹⁸⁴⁻¹⁸⁶. BDNF acts by binding tropomyosin-related tyrosine kinase receptor B (TRK-B) on glutamatergic synapses¹⁸⁴⁻¹⁸⁶. BDNF facilitates neuronal regeneration, synapse formation, dendritic sprouting and enhances neurotransmission¹⁸⁴⁻¹⁸⁶. BDNF also enhances postsynaptic function by modulating response to NMDA and GABA receptors and upregulates AMPA receptors. BDNF has been demonstrated to regulate activity-dependent plasticity particularly in learning and memory¹⁸⁴⁻¹⁸⁶.

5.3.2 Hevin and SPARC

Hevin and SPARC are both astrocyte secreted molecules that are involved in the formation of excitatory synapse in the CNS¹⁸⁷. Both Hevin and SPARC are secreted during development and adulthood, unlike many other developmental synaptogenic molecules, therefore it has been postulated that Hevin and SPARC may be involved in synaptic maturation or maintenance¹⁸⁷. Hevin induces structurally normal, functionally silent synapses, and deletion of Hevin results in a decrease in the number of synapses formed in cell culture¹⁸⁷. SPARC acts to antagonize Hevin induced synaptogenesis via interfering with glutamate transmission and AMPA receptor trafficking¹⁸⁷.

5.3.3 Glypicans

Glypicans are heparin sulfate proteoglycans that can be found attached to neuronal membranes, via GPI anchoring, or secreted from astrocytes^{188,189}. The 5 glypican subtypes all have similar structures, but are expressed in different regions of the body¹⁹⁰. Glypicans 1, 2, 4, and 6 can be found in the CNS; whereas, glypicans 3 and 5 are found outside the nervous system¹⁹⁰. Glypicans in the CNS are highly expressed during development and but can also be found in the adult CNS. Glypican 1 has been demonstrated to be upregulated after focal brain injury and binds amyloid precursor protein resulting in inhibition of neurite outgrowth¹⁹¹. Other CNS glypicans have been demonstrated to act to guide growing axons and induce synaptogenesis to produce functionally active synapses.^{188,192} Glypicans 4 and 6 has also been shown to strengthen synaptic connections by aggregating AMPA receptors (GluA1)¹⁸⁸.

5.3.4 Thrombospondins (TSPs)

Thrombospondins (TSP) are group of 5 matricellular glycoproteins initially isolated in the 1970s grouped into 2 different subfamilies A&B. Subfamily A Thrombospondins are (TSP 1 & 2) are trimmers, whereas Subfamily B (TSP 3, 4, & 5) are pentamers. Thrombospondins are found throughout the body and have a variety of ligands^{193,194}. TSP-1, the first TSP to be discovered, is a 420 kDa protein of 3 polypeptides linked together by disulfide bonds. The protein has 6 different domains with areas of homology or repeats similar to properdin (a component of the complement cascade), epidermal growth factor, calmodulin's calcium-binding motif. TSP-1 was initially discovered in the alpha-granules in platelets and was released in response to thrombin and bound the surface of activated platelets, fibrinogen, fibronectin and von Willebrand factor^{193,195,196}. Clinically, TSP is generally involved in the modulation of cell- matrix interactions. Specifically, TSP has demonstrated roles in platelet function, angiogenesis, tumor progression, wound healing, vascular disease and recovery after neuronal insults¹⁹⁶.

TSP 1&2 are heavily expressed during developmental synaptogenesis and are then down regulated to minimal levels with age in the central nervous system. TSPs main roles are involved with creation and maintenance of neuronal synapses via aiding neuronal cell contact and axonal outgrowth^{2,197-200}. Christerpherson et al (2005) found astrocytes to released TSPs. TSPs has be found to be up-regulated in human brains in general, as opposed to other primates, which may account for greater neural plasticity²⁰¹.

TSPs have a number of roles in the CNS and have a variety of receptors (Table 4). TSPs act to promote migration of precursor cell, aid in the attachment and growth of neurons and induce synaptogenesis. Deficiency of TSPs in development have been shown to reduce the number of synapse formed; however this does not affect the progenitor cell population level²⁰². Overexpression of TSPs or the $\alpha 2\delta$ -1 subunit on a voltage-gated calcium channel ($\alpha 2\delta$ -1), results in increased synaptogenesis^{1,25,173}. Synapses created by overexpression of thrombospondins were noted to be morphologically normal and pre-synaptically active. Interestingly though, the newly created synapses were post-synaptically silent¹⁷³. Further investigation revealed that these post-synaptically inactive synapses secondary to lack of AMPA receptors¹⁷³.

The mechanism of action of TSPs on synaptogenesis after binding $\alpha 2\delta$ -1, a GPI anchored protein on pre and postsynaptic neurons, is the recruitment of cell adhesion and scaffolding proteins to future synaptic sites²⁰³. The downstream signaling is not yet entirely understood. TSP has been shown to act trough neuroligin-1 to accelerate synaptogenesis and has been shown to bind very-low-density-lipoprotein receptors causing activation of GTPase RhoA signaling leading to synaptic plasticity^{18,25,204,205}. The stimulus for the release of TSPs after injury appears to the result of ATP binding P2 receptors which are coupled with protein kinase signaling cascades involving mitogen- activated protein kinase (MAPKs) and Akt²⁰⁶.

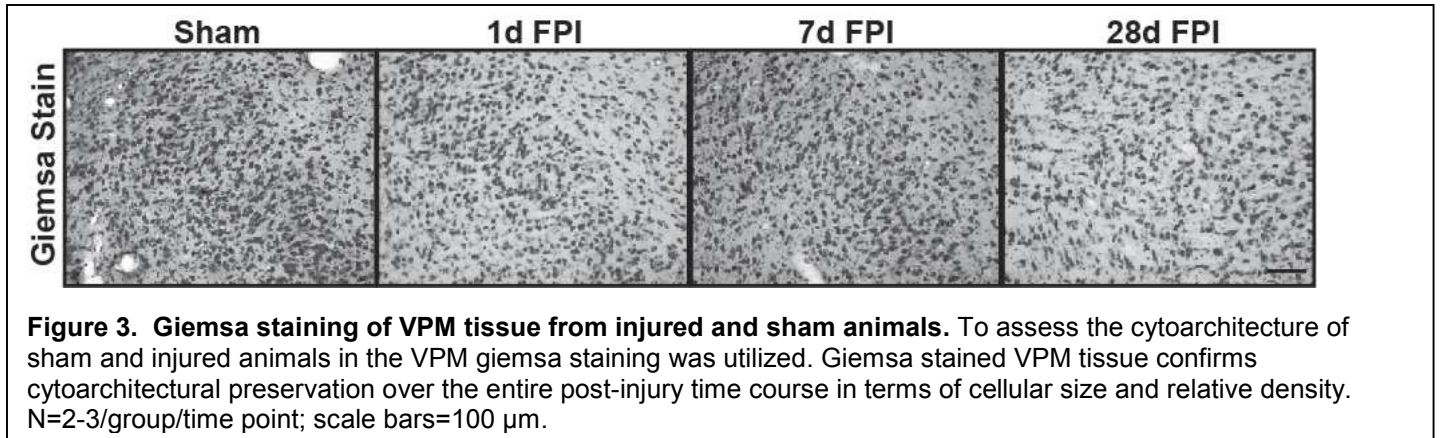
Receptor	TSP interaction site	CNS function	Citation
$\alpha 2\delta$ -1	Type 2 EGF-like repeats	Synapse formation	1
ApoER2	unknown	Reelin signaling; neuronal migration	2,3
CD36	Type 1 repeats	Microglial activation; brain lipid metabolism	4-6
CD47/IAP	C-terminal domain	Neurite development	7-9
Heparin	N-terminal domain	Cell-cell recognition and adhesion	10,11
HSPG	Type 1 repeats	Cell adhesion; astrocyte migration	12-14
Integrin	N-terminal domain Type 3 repeats	Neuronal migration; synapse architecture and function	15-17
Latent TGF- β	Type 1 repeats	Activiation of TGF- β ; Cytoskeletal stability; mobilization of synaptic machinery	18-20
LRP1/CRT	N-terminal domain	Endocytosis of MMPs , Notch signaling	21-24
Neuroligin	unknown	Synapse formation	25,26
Notch	unknown	Neural progenitor cell proliferation and differentiation; neuronal morphology	26,27
VLDLR	unknown	Reelin signaling; neuronal migration	2,3

Table 4. Known binding sites of Thrombospondin-1 in the Central Nervous System.

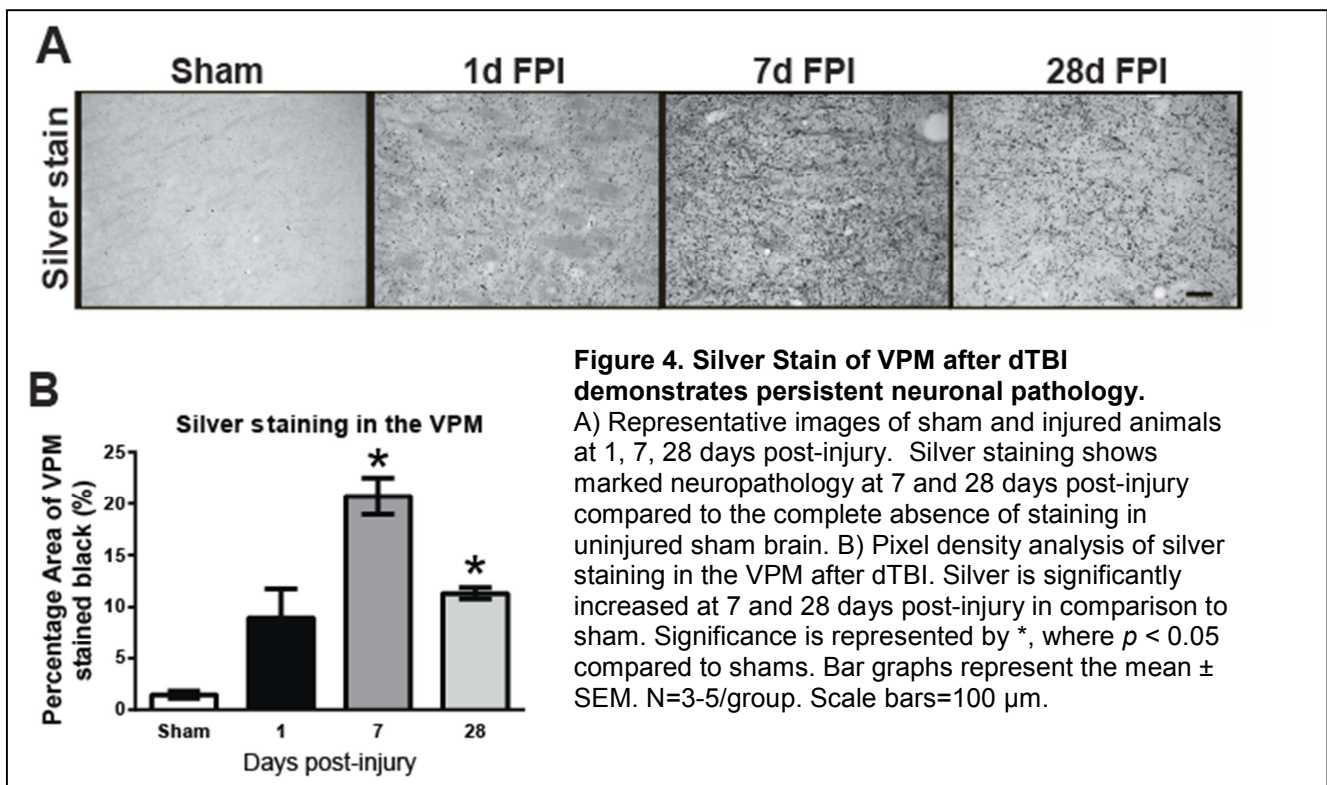
Adapted from Risher et. Al. 2012²⁸

6.0 Evidence of circuit reorganization after dTBI

Previous work in our lab has provided evidence of circuit reorganization in the whisker circuit in Sprague-Dawley rats utilizing mild fluid percussion to produce a mild diffuse TBI or concussion²⁰⁷. In this model, chronic histopathology has been demonstrated up to 4 weeks after injury. Initial investigation questioned whether or not the model produced overt neuronal cell death. Giemsa staining in the VPM (Figure 3) and S1BF demonstrates persevered cytoarchitecture without presence of hemorrhage, edema, or cavitations in the brain²⁰⁸. The lack of gross damage to the brain is consistent with what is seen in clinical cases where often mild TBI (mTBI) produces no signs of injury on conventional CT or MRI imaging^{95,100,101}.



Microscopic investigation of the whisker circuit aimed provide more information on the etiology of the development of behavioral morbidity. Amino-cupric silver staining (Figure 4), utilized to quantify neuronal degeneration, resides mainly in the axons and dendritic processes²⁰⁹. Previous work has identified significantly elevated neuronal pathology at 1, 7, and 28 days post- injury in the S1BF²⁰⁸. In the PR5 and VPM, similar results demonstrated increasing neuronal pathology at 7 days post-injury without complete resolution by 28 days post-injury²⁰⁹. Axonal damage and withering, as indicated by the silver staining, likely involves synapses which may have been perturbed too by secondary TBI pathophysiology. Partial resolution in the PR5 and VPM, may indicate a shift to the cessation of secondary TBI processes and return to normal neuronal homeostasis²⁰⁹. During this process, evidence of neuronal dendrite regrowth in a trophic environment leads to adaptive reorganization and recovery; however aberrant synaptogenesis could result in morbidity such as whisker sensitivity^{109,121,122,208-212}.



The presence of maladaptive circuit reorganization is further supported by dynamic changes in neuronal morphology throughout the time course in the whisker circuit. 3D reconstruction of Golgi stained neurons in the VPM indicated (Figure 5) an initial decrease in complexity over the first week after injury, signified by the decrease in branch points, branch ends and mean process length, suggests an initial injury effect and is supported by our findings of increased silver staining at 7 days post-injury. Subsequent regrowth is evident in the VPM, indicated by increased complexity by 28 days post-injury in comparison to 7 days post-injury, noted by the increase in process quantity, branch points, branch ends and mean process length. 3D reconstruction of Golgi stained neurons in the S1BF (Figure 6) resulted in a decrease in neuronal complexity 1 day post-injury indicated by a reduction in the number of dendrites, dendritic length, branch points and mean dendritic surface area. Dendrites returned to uninjured morphology by 7 days post-injury, supporting the presence of on-going reparative processes. Surprisingly though, there is a second reduction in complexity produced by 28 days post-injury, supporting the presence of ongoing neuronal pathology and circuit reorganization²¹³. Since structure begets function, it is possible that these morphological changes induce alterations in synaptic number or function which could participate in the development of whisker sensitivity^{42,118,122,214-216}.

Investigation into whisker circuit glutamate signaling revealed increased basal and potassium-evoked glutamate release in both the VPM and S1BF at 28 days post-injury in comparison to injured animals 7 days-post injury and uninjured animals¹²². What is more telling of the implications of post-traumatic changes in neuronal structure and circuit function is the linear relationship between the increase in evoked glutamate release and the increase whisker sensitivity scores during WNT^{118,121,122}. Taken together, the alterations in neuronal morphology and glutamate signaling supports the presence of circuit reorganization after diffuse TBI; which may then contribute to the development of whisker sensitivity.

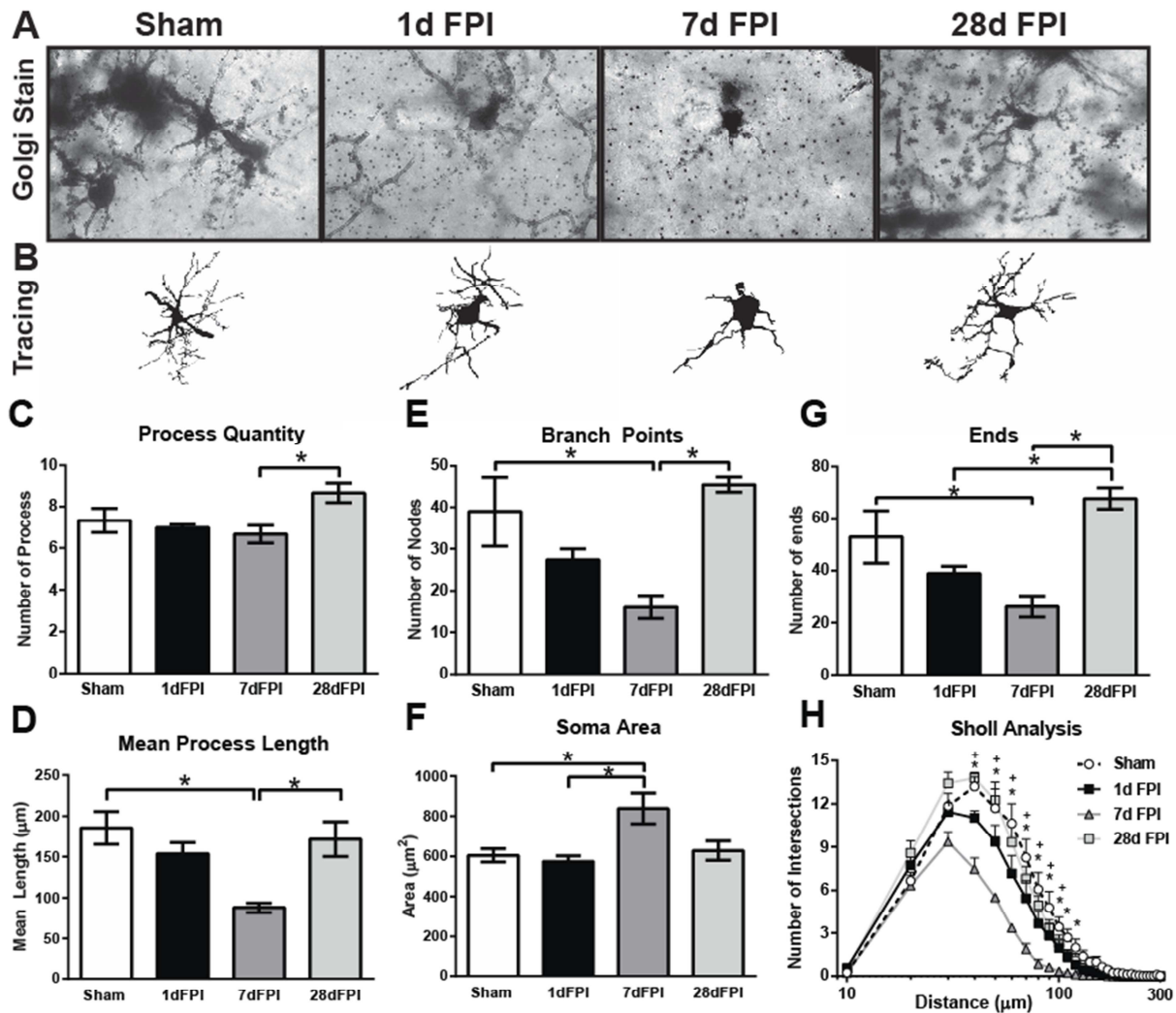


Figure 5. Morphology of VPM neurons changes as a function of time after traumatic brain injury.

A) Representative images of Golgi stained neurons at 20x magnification. B) 3D tracings of neurons represented in panel A. C) Changes in the process quantities over the time course, the number of processes were significantly increased at 28d FPI compared to 7d FPI, however remained similar at other time points $*P < 0.05$ via one-way ANOVA. D) Changes in the process length, there is a significant decrease at 7d FPI compared to shams and a recovery back to sham levels at 28d FPI, $*P < 0.05$ via one-way ANOVA. E) Changes in number of branch points, the places where a process splits into 2 or more branches, there is a significant reduction at 7d FPI compared to sham and 28d FPI, $*P < 0.05$ via one-way ANOVA. F) Changes in the soma area, showing a significant increase at 7d FPI compared to sham and 1d FPI, $*P < 0.05$ via one-way ANOVA. G) Changes in the number of ends, there is a significant decrease at 7d FPI compared to sham and 28d FPI as well as a significant increase between 1 and 28d FPI, $*P < 0.05$ via one-way ANOVA. H) Sholl analysis revealed fewer interactions at 7d FPI in comparison to sham between 50 and 70 μm from the soma as well as significant differences between 28d FPI and sham between 140 μm and 170 μm . Error bars represent the SEM and $*P < 0.05$

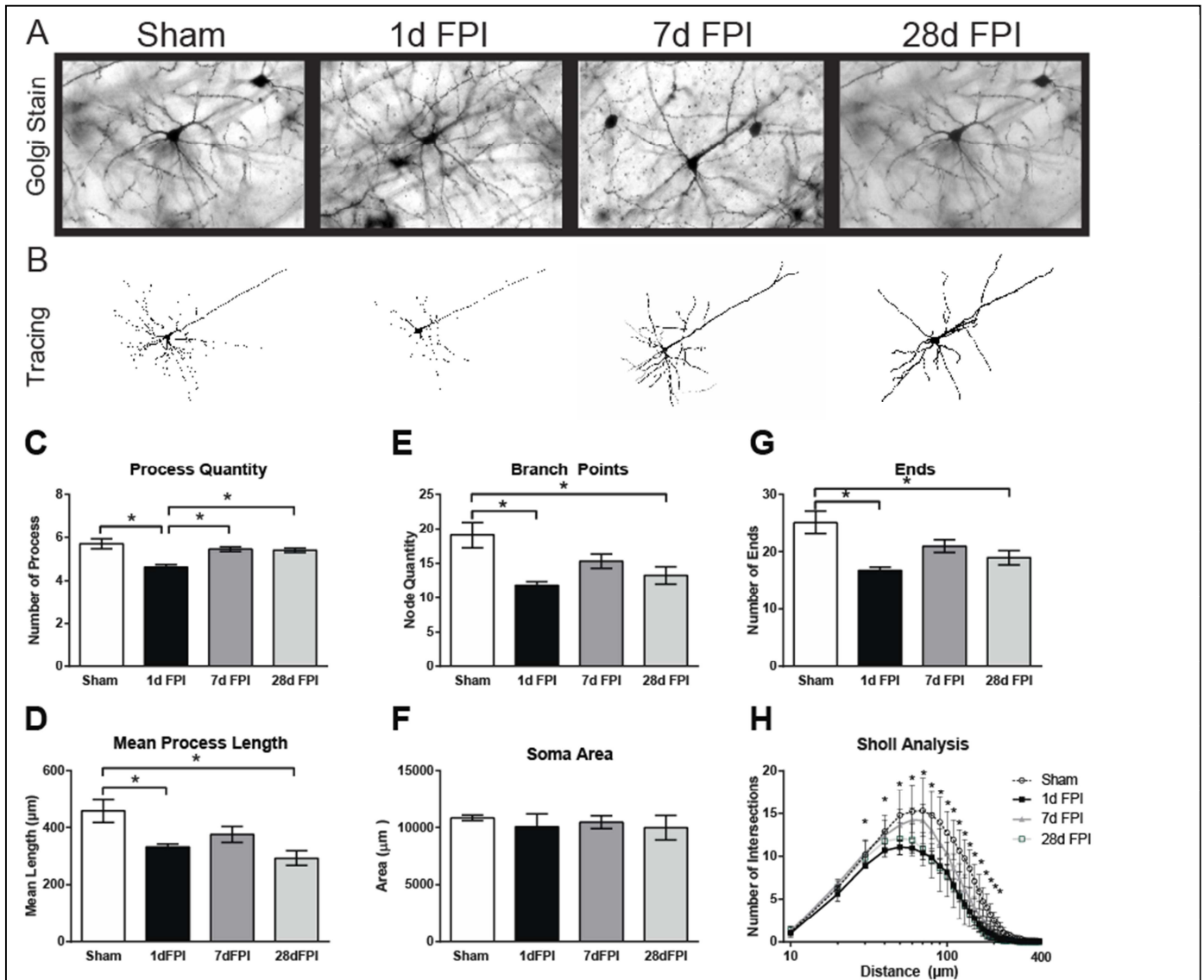


Figure 6. Morphology of S1BF neurons changes as a function of time after traumatic brain injury.

A) Representative neurons are displayed at 20x magnification. B) The 3D traces of the represented neurons in panel A. C) Process quantity was significantly decreased in dendrites at 1 day post-FPI compared to sham followed by an increase between 1 and 7 and 1 and 28 days post-FPI. * $P < 0.05$ via one-way ANOVA. D) The mean dendrite length was significantly shorter at 1 day and 28 days post-FPI compared to sham. * $P < 0.05$ via one-way ANOVA. E) The mean number of branch points are significantly decreased at 1 and 28 days post-FPI compared to sham. * $P < 0.05$ via one-way ANOVA. F) Soma volume did not change significantly over the post-injury time course ($F(3,12)=0.2221$; $p=0.8796$). G) Quantity of ends significantly decreased at 1 day and 28 days post-FPI compared to sham. * $P < 0.05$ via one-way ANOVA. H) Sholl analysis revealed significant differences in the number of dendritic intersections (10 μm concentric circles) between sham versus 1 day post-FPI at 40-190 μm ; sham versus 7 days post-injury at 80-180 μm ; sham versus 28 days post-FPI at 50-210 μm ; 1 day versus 7 days post-FPI at 50-120 μm and 7 days post-FPI versus 28 days post-FPI at 60-130 μm from the cell center ($F(3,672)=104.2$; $p<0.0001$).

6.1.1 Glial response to TBI

Alterations in neuronal morphology and synaptic functioning are not solely dependent on the neuronal pathology alone. Glial cells, the most abundant cells in the central nervous system, function to maintain neuronal homeostasis. Astrocytes serve a role in the maintenance of the blood-brain barrier, synapse formation and function, extracellular homeostasis, and neuronal maintenance^{201,217,218}. After the introduction of an insult to the CNS, astrocytes react, via calcium signaling cascades, and proliferate due to the presence of inflammatory mediators, indicators of cell injury, neurotransmitters, oxidative stress, ischemia, and metabolic toxins²¹⁷⁻²¹⁹. They function to protect neurons from injurious substances and aid in the repair of synapses and blood brain barrier²¹⁷. GFAP, a cytoskeletal filament, is upregulated in activate astrocytes and is the most commonly used indicator of astrogliosis in the literature^{219,220}. Their persistent activation to at least 28 days post-injury supports unresolved disruption in normal neuronal homeostasis. Furthermore, astrocytes involvement in synapse formation supports the potential for ongoing post-traumatic circuit reorganization²²¹⁻²²⁶.

In the healthy CNS, microglia function to constantly survey the environment for infection or neuronal damage. In the resting state, ramified microglia have thin long processes which aid to maintain homeostasis and phagocytose cellular debris. Microglia have also been implicated in synaptic plasticity, neurogenesis, synaptic pruning, and regulate neuronal cell death during development, and activity dependent learning. In the setting of trauma, microglia hypertrophy and become more amoeboid in shape, termed activated microglia^{227,228}. These activated microglia then travel to the site of trauma and release anti-inflammatory (M2) or pro-inflammatory (M1) factors depending on phenotypic differentiation triggered by local cytokines. While a mixed M1/M2 response appears initially after TBI, over time there seems to be a shift to an M1 predominant response^{228,229}. This chronic proinflammatory activation persists from weeks to months after injury, similar to astrocytes and is thought to contribute to poor outcomes after TBI²²⁹. Microglia can also form an elongated morphology, termed rod microglia, which have been demonstrated to parallel to neuronal elements and are thought to play a role in post-TBI synaptic plasticity²²⁷.

7.0 Previous studies: TSP mediated synaptogenesis after neuronal insult.

Thrombospondin levels are elevated during development, but decrease to minimal levels in adulthood^{13,173}. TSP expression can be induced by injury. Thrombospondins role in the CNS are thought to be involved in the development and maintenance of synapses TBIs, epilepsy, ischemia and spinal cord injury result in reactive astrogliosis and elevated levels of TSPs²⁰⁶. Xing et al (2014) demonstrated that insult to the brain caused the release of lipocalin-2. Lipocalin-2, initially known for its antibacterial properties after release from neutrophils, was also shown to be protective against ischemia in animal model kidneys²³⁰. Xing et al (2014) then found application of lipocalin-2 to astrocytes resulted in release of brain-derived neurotrophic factor and TSP-1; suggesting that its release may act as a vehicle for up-regulation in times of neuronal injury or insult²³⁰.

Recently, TSP-1 mediated synaptogenesis has been investigated after neuronal insult and its potential implications on circuit reorganization. Liauw et. al. (2008) found TSP-1 and TSP-2 to be up-regulated after stroke and were found to be beneficial in motor recovery in a rodent model²³¹. However, other investigators have found TSP-1 mediated synaptogenesis to be detrimental, resulting in epileptogenesis or neuropathic pain²³²⁻²⁴⁰. Excitingly, however, TSP-1 mediated synaptogenesis has been attenuated by TSP-antagonism with gabapentin. Eroglu et. al. (2009) confirmed gabapentin binding to the TSP receptor, $\alpha 5$ -1, responsible for inducing synaptogenesis. These studies support the presence of maladaptive circuit reorganization after neuronal insult.

8.0 Scope of project and Hypothesis

Previously, our lab has investigated the chronic consequences of mild TBI (mTBI) or concussion-like injuries using the translational rodent model of midline fluid percussion injury. As described, we have previously demonstrated chronic changes in histopathology with silver staining and changes in neuronal morphology in the VPM and S1BF^{208,213,241}. These structural changes are thought to induce functional changes in the thalamocortical whisker circuit. These functional changes have been demonstrated by increased cFOS immunohistochemical staining in the whisker circuit and increased basal and potassium-evoked glutamate signaling in the VPM and S1BF after dTBI^{122,123}. What is most intriguing about these findings is their relationship to the development of a rodent model of post-concussive symptoms; a late-onset, gain-of-function whisker sensitivity that develops 28 days after injury^{118,121}. This evidence of circuit reorganization and previous findings of elevated TSP-1 after neuronal insult by other investigators, including focal TBI models, leads us to the next logical question of the mechanism behind circuit reorganization and development of behavioral morbidity of whisker sensitivity in our model. We therefore hypothesize that experimental diffuse TBI will result in re-expression of TSPs, which will be synchronous with increases in synaptic markers in the thalamocortical whisker circuit.

9.0 Methods

9.1 Subjects

Adult male Sprague-Dawley rats (Harlan Laboratories, Inc., Indianapolis, IN) were used for all experiments. Rats were housed in a 12 h light/12h dark cycle (6:00/18:00) at a constant temperature ($23^{\circ}\text{C} \pm 2^{\circ}\text{C}$) with food and water available ad libitum according to the Association for Assessment and Accreditation of Laboratory Animal Care International. Rats were acclimated to their environment following shipment for at least 7 days prior to any experiments. After surgery, rats were evaluated daily for post-operative care by a physical examination and documentation of each animal's condition. The Institutional Animal Care and Use Committees at the University of Arizona (Phoenix, AZ) approved animal care.

9.2 Midline Fluid Percussion Injury (mFPI)

Rats (350-375g) were subjected to midline fluid percussion injury (FPI) consistent with methods described previously (Lifshitz, 2008 328 /id; Hosseini, 2009 22 /id; Lifshitz, 2007 258 /id). In total, 72 animals were used in this study. Briefly, animals were anesthetized with 5% isoflurane in 21% O_2 (compressed breathable air) and maintained at 2.5% via nose cone. During surgery, body temperature was maintained with a Deltaphase[®] isothermal heating pad (Braintree Scientific Inc., Braintree, MA). In a stereotactic frame (Kopf Instrument, Tujunga, CA), a midline scalp incision was created with 10 blade and the skull was then exposed via blunt dissection. A 4.8-mm circular craniotomy was performed (centered on the sagittal suture; midway between bregma and lambda) carefully ensuring the underlying dura or superior sagittal sinus were not disturbed. An injury cap was fabricated from the female portion of a 20 gauge Luer-Loc needle hub, which was cut and beveled and positioned in-lay with the craniotomy site. A skull screw was secured in a 1-mm hand-drilled hole into the right frontal bone. The injury hub was affixed over the craniotomy using cyanoacrylate gel and methyl-methacrylate (Hygenic Corp., Akron, OH). The incision was partially sutured closed with 4.0 Ethilon sutures and topical Lidocaine and antibiotic ointments were applied. Animals were returned to a warmed holding cage and monitored until ambulatory (approximately 60-90 min).

For injury induction, animals were re-anesthetized with 5% isoflurane 60-90 min after surgery. The dura was inspected through the injury-hub assembly to ensure a good seal between the hub and skull, no damage to the dura and dura/hub free of debris. The hub was then filled with normal saline and attached to the male end of the fluid percussion device (Custom Design and Fabrication, Virginia Commonwealth University, Richmond, VA). An injury of moderate severity (Averaged 2.1 atm) or sham injury was administered by releasing the pendulum onto the fluid-filled cylinder as reflexive responses returned. Animals were monitored for the presence of a forearm fencing response and the return of the righting reflex after injury²⁴². The fencing response is a tonic posturing characterized by extension and/or flexion of arms²⁴². The righting reflex time, the total time from the initial impact until the rat spontaneously rights itself from a supine position, is the primary indicator of injury severity. After injury, the injury hub assembly was removed *en bloc*, hemostasis and integrity of the dura was observed, and the incision was stapled closed. Moderate injuries were defined as having a righting reflex of 5-10 minutes ($7.1 \pm \text{SEM}$ minutes). Animals were then placed in a warmed holding cage before being returned to the vivarium. Adequate measures were taken to minimize pain or discomfort.

9.3 Perfusion and dissection

At 1, 3, 5, 7, 14, 21, 28 and 56 days post-injury, each animal was given a lethal dose of sodium pentobarbital 200 mg/kg (Euthasol[®], i.p.). Animals were transcardially perfused with ice-cold phosphate buffered saline (PBS) for 3 minutes. The brain was rapidly removed and rinsed with ice-cold PBS. Tissue biopsies (2 mm diameter) taken from the VPM were collected from 2 mm thick coronal sections made using a chilled rat brain matrix. Tissue biopsies from the right hemisphere were, stored in RNeasy[®] (Invitrogen catalog # AM7020), and kept at -20°C until mRNA was extracted for quantitative PCR. Tissue biopsies from the left hemisphere were flash frozen and stored in at -80°C until protein was extracted for automated capillary western analysis.

9.4 RNA extraction

Total mRNA was extracted from the VPM biopsies, previously stored in RNeasy[®], using the MagMAX[™]-96 Total RNA Isolation Kit (Invitrogen catalog; # AM1830). Following the manufacturer's protocol, biopsies were homogenized in TRI reagent[®] solution (Invitrogen; # AM9738) and extracted under acidic conditions. Total RNA was further purified using RNA binding beads. The ratio of the absorbance at 260 and 280 nm was used to assess RNA purity and quality (Nanodrop, Thermo-Scientific 2000). All RNA samples were within the established range for pure RNA (1.8-2.1), with an average A_{260}/A_{280} of 1.86 ± 0.09 standard deviation in the VPM and 1.976 ± 0.06 in the S1BF.

9.5 Quantitative PCR

Total RNA was converted to cDNA using the High Capacity RNA-to-cDNA Kit from Life Technologies[™] (catalog # 4387406). Optimization to identify the optimal concentration of cDNA indicated that either 10 ng (thrombospondin-1), 7.5 ng (thrombospondin-2) or 5 ng (for all other genes) be used in the preparation for quantitative real-time PCR (qPCR) using commercially-available gene expression assays. The Applied Biosystems TaqMan[®] Gene Expression Assay for post-synaptic density 95kb (PSD-95; Rn00571479_m1), synaptophysin (SYP; Rn01528256_m1), growth associated

protein 43kb (GAP43; Rn00567901_m1), thrombospondin 1 (TSP1; Rn01513697_m1), thrombospondin 2 (TSP2; Rn02111874_S1) and alpha2delta-1 ($\alpha 2\delta$ -1)(Rn01442580_m1).

Gene assays were optimized to run under universal thermal cycling conditions, with amplification efficiencies of 100% by the manufacturer. Within each animal, relative gene expression was normalized to the 18S rRNA endogenous control and then to gene expression levels in the sham group using the $2^{-\Delta\Delta CT}$ method²⁴³, which relates gene expression to the PCR cycle number at which the fluorescence signals exceed a threshold above baseline. Samples were run in triplicate and 18s was run in duplicate according to manufacturer's instructions.

9.6 Protein extraction

Total protein was extracted from the VPM previously stored at -80°C . Tissues were homogenized in 250 μl of ice-cold extraction buffer (pH 8.0) containing 0.24 M Tris, 0.74 M NaCl, 100 μl TritonX100 with a protease inhibitor cocktail (complete, Roche Diagnostics; #11836153001). Tissue from VPM was homogenized with the Precellys@24 machine (Bertin Technologies, Montigny le Bretonneux, France) for 40 seconds bouts until solution was completely clear (being chilled on ice for 2 minutes between bouts). Samples were then centrifuged at $3,000 \times g$ for 15 minutes and the supernatant stored in 10-20 μl aliquots in the -80°C for analysis. Protein concentrations were determined using the Bicinchoninic acid assay (BCA) using manufactures instructions (Pierce, Rockford, IL).

9.7 Automated capillary western –ProteinSimple (Wes)

Protein expression was evaluated using automated capillary western (ProteinSimple® Wes™). For this process, primary antibody information can be found in tables 5 and 6. Secondary antibodies, streptavidin HRP, dithiothreitol (DTT), molecular weight fluorescent standards (internal control), luminol, hydrogen peroxide, sample buffer, antibody diluent, running buffer, wash buffer, capillaries and plates (stacking matrix, separation matrix, wash buffer, matrix removal buffer) were purchased from ProteinSimple (Santa Clara, CA). The instrument utilized was The Simple Western™ and was also purchased from ProteinSimple (Santa Clara, CA). Technical properties were described by Rustandi et al and Loughney et al^{244,245}.

After protein extractions, samples were prepared according to the manufacturer's recommendations. Samples were combined with sample buffer and master-mix (40mM DTT, 0.1x ProteinSimple Sample Buffer, and 1x Fluorescent Standards) to achieve the desired concentration. Samples were then denatured via heating block at the optimized temperature (see tables 5 and 6). The ladder, samples, primary and HRP-conjugated secondary antibody, wash buffer, antibody diluent and chemiluminescent were then placed in the designated wells per experimental design. Each plate was centrifuged at 2500 RPM for 5 minutes and placed into the automated capillary western machine where proteins were separated by size (electrophoresis), immobilized and immunoprobed in individual capillaries. Once loaded into the instrument, the following metrics were utilized for separation and detection: Separation matrix loading time 200 sec, stacking matrix loading time 15 sec, sample loading time 9 sec, separation time 25 min at 375V. After separation, samples are the exposed to UV light for 4sec and capillaries are immobilized for 200 sec to allow for cross-linking between the sample and wall of the capillary. The matrix is removed with matrix removal buffer and 3 consecutive wash buffer cycles. Sample were then blocked with antibody diluent for 5 min followed by primary antibody (incubation times indicated in tables 5 and 6). Secondary antibodies were incubated for 30 min followed by detection with chemiluminescence (luminol/ hydrogen peroxide solution). Capillaries were then imaged at the following times: 5, 15, 30, 60, 120, 240 and 480 seconds.

The associated software, Compass (ProteinSimple®), generates an electropherogram (figure 9) with peaks corresponding to the expression of proteins of interest and calculates the area under the curve (AUC) for each peak. To calculate relative protein expression, the AUC for the protein of interest was then divided by the AUC for the housekeeping protein. Animals were run in duplicates; therefore, the ratios were averaged for each animal. Animals of a given plate were then normalized to shams on the same plate. An example calculation is provided in table 5.

Prior to running all samples, each protein of interested was optimized for primary antibody (from different manufactures), antibody concentration, protein concentration, multiplexing with housekeeping protein (actin/GAPDH), denaturing process, loading conditions, and exposure times. For experimental samples, days post-injury were randomized between plates, where the same 3 shams were ran on all plates to ensure continuity between plates.

9.8 Statistical analyses

Changes in relative gene and protein expression obtained at each time point were compared over the time course and to sham animals. A one-way ANOVA with Fischer LSD post-hoc analysis was performed with significance set at $p < 0.05$. All statistical analyses were performed using Prism 6 (Graph Pad, La Jolla, CA). Data are presented as the mean \pm the standard error from the mean (SEM).

Table 5. Plate conditions for VPM samples

Antibody	Species	Expected band	Actual Band	Ig company, catalog #, primary antibody time	Denaturing Temp (celcius)	[Protein] Ug/ul	[Ig]	Biological control
TSP-1	Rabbit	124	~155	Abcam (ab85762) 60 mins	70°C X 10 mins	2	1/50	Actin-mouse
PSD-95	Mouse	95	~95	Pierce (MA1-045) 60 mins	95* X5 mins	1	1/100	Actin-mouse
SYNP	Rabbit	34	~63	Abcam (ab52636) 60 mins	95* X5 mins	1	1/100	Actin-mouse
A2d1	Mouse	150	~180s	Abcam (ab2864) 30 mins	70°C X 10 mins	0.5	1/2000	Gapdh
Gap-43	Rabbit	43	~80s	Millipore (ab5220) 30 mins	70°C X 10 mins	0.5	1/2000	Gapdh
TSP-2	rabbit	58, 125, 135, 180	~low 60s	Novus (224420002) 30 mins	70°C X 10 mins	0.5	1/50	Gapdh
GAPDH	Rabbit	38	39-41	Abcam (ab8245)			1/1000	
Actin	Rabbit	47	~47	Sigma (a2066)			1/100	
Actin	Mouse	47	~47	Sigma (A5441)			1/2500	

Table 6. Plate conditions for S1BF samples.

Antibody	Species	Expected band	Actual Band	Ig company, catalog #, antibody time	Denaturing Temp (celcius)	[Protein] Ug/ul	[Ig]	Biological control
TSP-1	Rabbit	124	~155	Abcam (ab85762) 60 mins	37°C X 30 min	0.5	1/25	Actin
PSD-95	Mouse	95	~95	Pierce (MA1-045) 60 mins	95* X5 min	0.5	1/100	Actin
Synp	Rabbit	34	~63	Abcam (ab52636) 60 mins	95* X5 min	0.5	1/100	Actin
A2d1	Mouse	150	~180s	Abcam (ab2864) 30 mins	95* X5 min	0.25	1/1500	Gapdh
Gap-43	Rabbit	43	~80s	Millipore (ab5220) 30 mins	95* X5 min	0.25	1/2000	Gapdh
TSP-2	Rabbit	58, 125, 135, 180	~low 60s	Novus (224420002) 30 mins	95* X5 min	0.25	1/50	Gapdh
Pick-1	Mouse	50s	Mid 50s	Abcam (ab85762) 60 mins	95* X5 min	0.25	1/1500	Gapdh
Gapdh	Rabbit	38	39-41	Abcam (ab8245)			1/1000	
Actin	Rabbit	47	~47	Sigma (a2066)			1/250	
Actin	Mouse	47	~47	Sigma (A5441)			1/1000	

Figure 7. Example electropherogram.

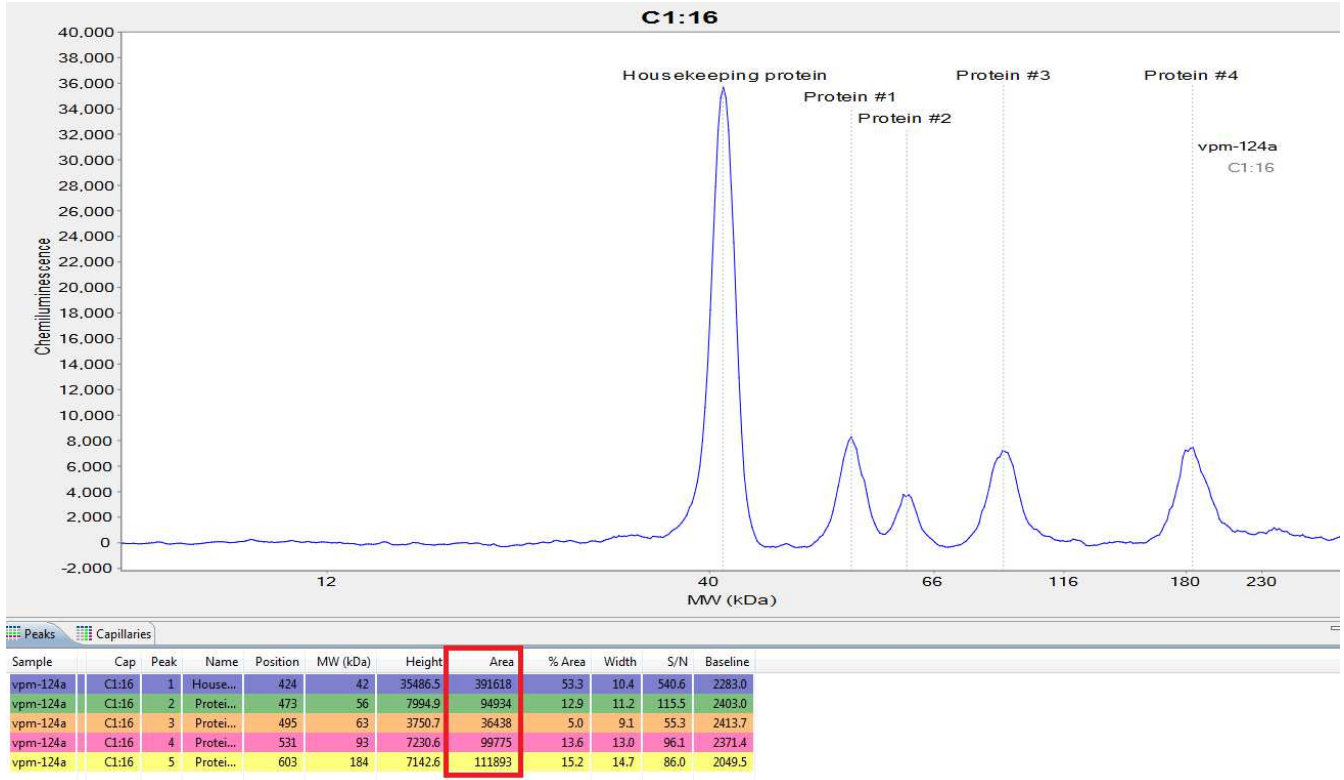


Table 7. Example calculation for protein quantification.

Sample	Cappillary well#	protein	AUC	AUC protein: AUC housekeeping protein	average for each animal	average sham ratio
sham #1	C1:4	housekeeping	125014	126650/125014=	1.013086534	0.962342007
sham #1	C1:4	target protein	126650			
sham #1	C1:5	housekeeping	91106	0.91159748	0.688936522	0.739741191
sham #1	C1:5	target protein	83052			
sham #2	C1:6	housekeeping	64497	0.705164581	0.567945044	0.957756855
sham #2	C1:6	target protein	45481			
sham #2	C1:7	housekeeping	74426	0.672708462	0.708492196	0.957756855
sham #2	C1:7	target protein	50067			
sham #3	C1:25	housekeeping	228545	0.468861712	0.567945044	0.957756855
sham #3	C1:24	target protein	107156			
sham #3	C1:25	housekeeping	228545	0.667028375	0.708492196	0.957756855
sham #3	C1:25	target protein	152446			
injured #1	C1:8	housekeeping	112705	0.767277406	0.708492196	0.957756855
injured #1	C1:8	target protein	86476			
injured #1	C1:9	housekeeping	99825	0.649706987	0.708492196	0.957756855
injured #1	C1:9	target protein	64857			

10.0 Results

While previous studies have evaluated changes in gene and protein expression of TSP mediated synaptogenesis after various brain insults, diffuse traumatic brain injury (dTBI) induced by midline fluid percussion is a novel model^{230,231,235}.

In this study, male Sprague-Dawley rats (330-350g) underwent sham or moderate midline fluid percussion brain injury (mFPI; 12.1atm; 6-10min righting reflex). At multiple time points over 56 days post-injury (DPI) (e.g 1, 3, 5, 7, 14, 21, 28, 56 DPI), gene and protein expression of purported injury-induced astrocyte-secreted mediators of synaptogenesis and other markers of synaptogenesis were quantified from tissue biopsies of the ventral posteromedial nucleus of the thalamus (VPM) and primary somatosensory barrel fields of the cortex (S1BF).

Potential mediators of synaptogenesis identified for these experiments include Thrombospondin-1 (TSP-1), Thrombospondin-2 (TSP-2), and the purported TSP-1 receptor, $\alpha 2\delta$ -1 subunit of the L-type calcium channel ($\alpha 2\delta$ -1)^{1,173,231}. Markers of synapse would include synaptophysin (SYNP), Growth associated protein (GAP-43), and Post-synaptic density 95 (PSD-95) and Protein interacting with C-kinase 1 (PICK-1). Gene expression was quantified using quantitative real-time polymerase chain reaction (qPCR) and protein expression for thrombospondin-1 (TSP-1), thrombospondin-2 (TSP-2), $\alpha 2\delta$ -1, SYNP, GAP -43, Protein expression for SYNP, GAP-43 and PSD-95 were evaluated using protein quantification by automated capillary western (Proteinsimple). Day 21 was excluded from the gene analysis of the S1BF as there were only 2 samples useable for qPCR procedures.

For statistical analysis, a One-way ANOVA statistical analysis with a Fischer's LSD post-hoc was then performed to evaluate for significant changes in expression over time to compare injured animals to sham.

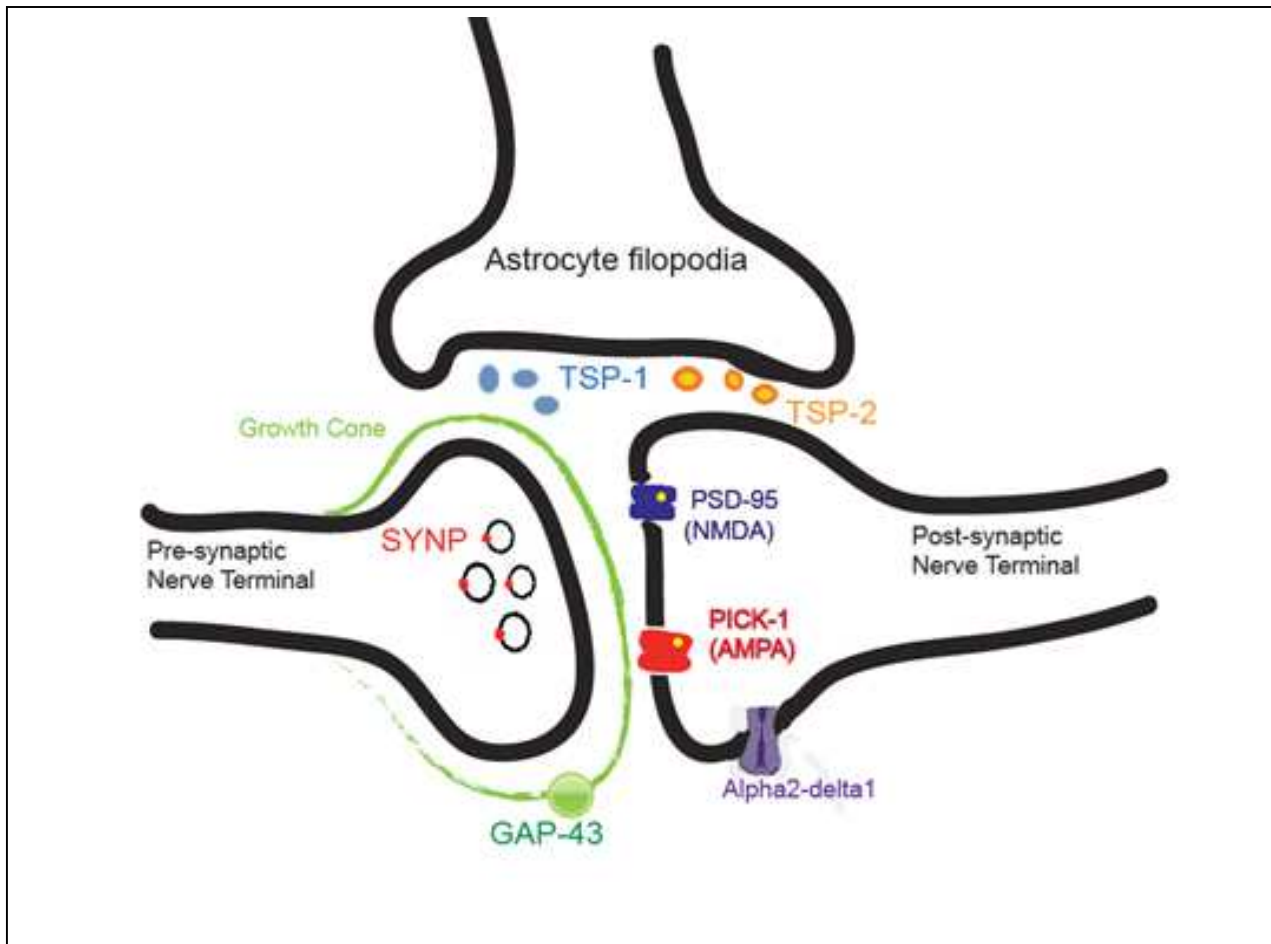


Figure 8. Glutamatergic Synapse.

TSP-1: Thrombospondin 1
 $\alpha 2\delta$ -1: $\alpha 2\delta$ -1 subunit of voltage-gated calcium channel
SYNP: Synaptophysin

TSP-2: Thrombospondin 2
GAP-43: Growth associate protein 43
PSD-95: Post-synaptic density 95
PICK-1: Protein interacting with C-kinase 1

10.1 Gene expression of Growth Associated Protein 43 increases in the S1BF after diffuse TBI.

Growth associated protein 43 (GAP-43) is associated with neuronal growth and is located within the neuronal growth cone^{246,247}. While lower levels exist in adulthood in comparison to development, GAP-43 can still be observed in areas of synaptic plasticity^{246,248,249}. Similarly, studies evaluating various focal and diffuse brain insults have demonstrated an increase in GAP-43 expression^{246,249-253}. In these studies, we hypothesized to see an increase in GAP-43 gene and protein expression in the VPM and S1BF after dTBI.

In the VPM, there were no significant changes over the time course in gene expression ($F(8,41)=1.602$, $p=0.1543$, $N=3-9$ /time point) or protein levels ($F(8, 43)=1.982$, $p=0.072$, $N=3-9$ / time point). In the S1BF, there was a significant change in gene expression over time ($F(7,41)=2.353$, $p=0.0407$, $N=3-12$ / time point), with post-hoc analysis indicating increased expression at 7DPI. Protein levels in the S1BF did not change significantly over time ($F(8,28)=0.9868$, $p=0.467$, $N=3-6$ / time point).

Results from the VPM gene and protein analysis and the S1BF protein analysis did not support our hypothesis. Gene analysis in the S1BF did support our hypothesis of increased expression after dTBI.

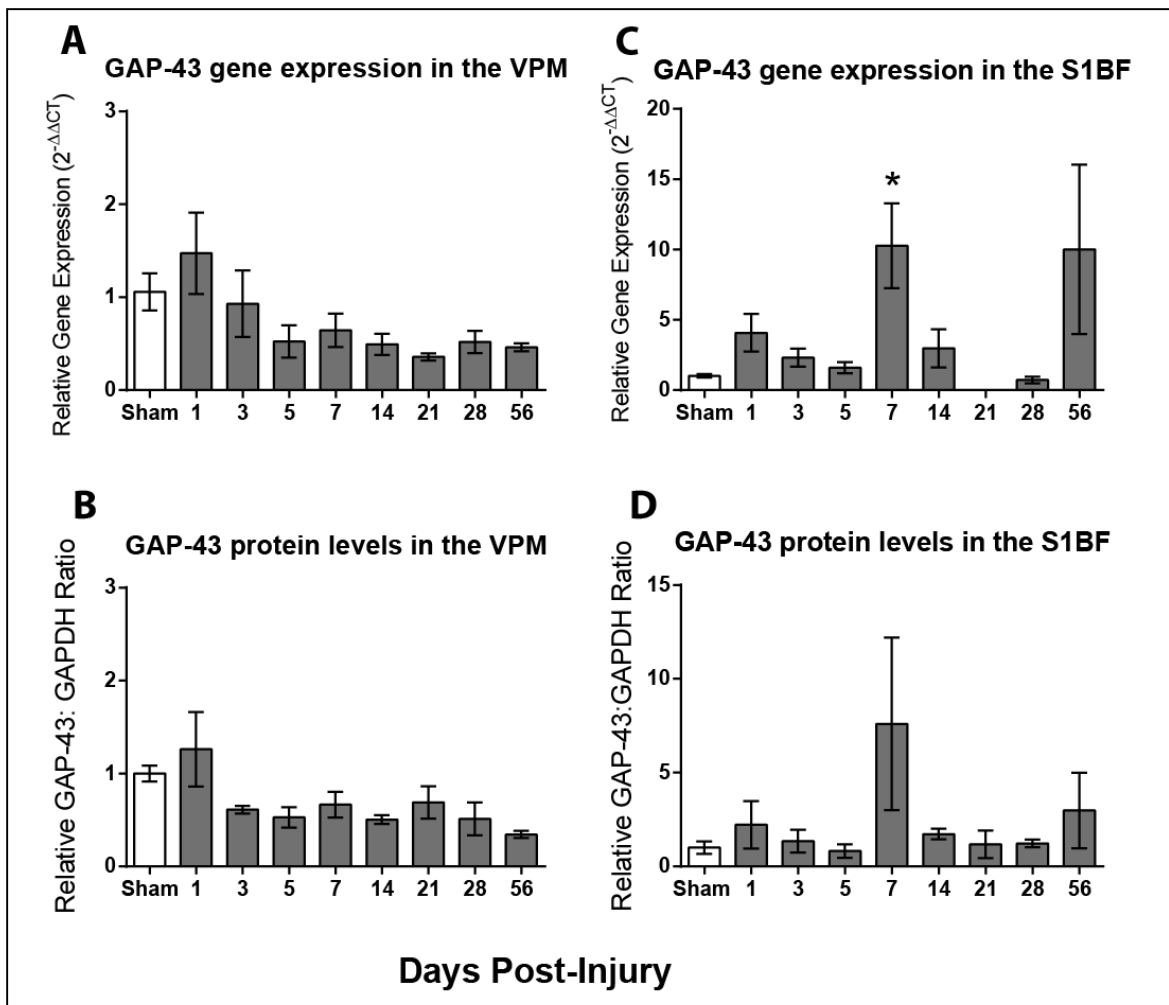


Figure 9. GAP-43 gene expression and protein levels in the VPM and S1BF over time after dTBI.

A) Gene expression of GAP-43 in the VPM was assessed via qPCR. No significant changes in gene expression of GAP-43 occurred after dTBI in comparison to sham in the VPM. $N=3-9$ /time point. B) Protein levels of GAP-43 in the VPM were assessed via automated capillary western blot analysis. No significant changes in protein levels of GAP-43 occurred after dTBI in comparison to sham in the VPM. $N=3-9$ / time point. C) Gene expression of GAP-43 in the S1BF was assessed via qPCR. Gene expression of GAP-43 significantly increased at 7 days post-injury in comparison to sham in the S1BF. $N=3-12$ / time point. D) Protein levels of GAP-43 in the S1BF were assessed via automated capillary western blot analysis. No significant changes in protein levels of GAP-43 occurred after dTBI in comparison to sham in the S1BF. $N=3-6$ / time point. The data are presented as the mean \pm SEM. * $p < 0.05$ in comparison to sham.

10.2 Gene expression of synaptophysin (SYNP) increases in the S1BF after dTBI.

SYNP is a presynaptic marker that is ubiquitously expressed throughout the brain. Increases can be indicative of synaptogenesis^{254,255}. In these studies, we hypothesized to see an initial decrease in SYNP, associated with decreased synapses secondary to trauma, followed by an increase in gene expression and protein levels associated with re-growth after dTBI.

In the VPM, there were no significant changes over the time course in gene expression ($F(7, 33)=2.2.241$, $p=0.0557$, $N=3-7$ / time point) or protein levels ($F(8, 34)=0.6175$, $p=0.757$, $N=3-6$ /time point). Day 21 was excluded from the gene analysis as there were only 2 samples useable for qPCR procedures. In the S1BF, there was a significant change in gene expression over time ($F(7,43)=4.08$, $p=0.0016$, $N=3-10$ / time point). Post-hoc analysis of gene expression demonstrated increased gene expression at 1 DPI. Protein levels of SYNP were multimodal in character and significantly changed over time ($F(8, 31)=3.186$, $p=0.0094$, $N= 3-6$ /time point).

Results from the VPM gene and protein analysis and the S1BF protein analysis did not support our hypothesis. Gene expression and protein level analysis in the S1BF did support our hypothesis of increased expression after dTBI. Of note, while not significant, the multimodal levels of SYNP over the time course is suggestive of an initial decrease followed by increase, which would be consistent with our hypothesis.

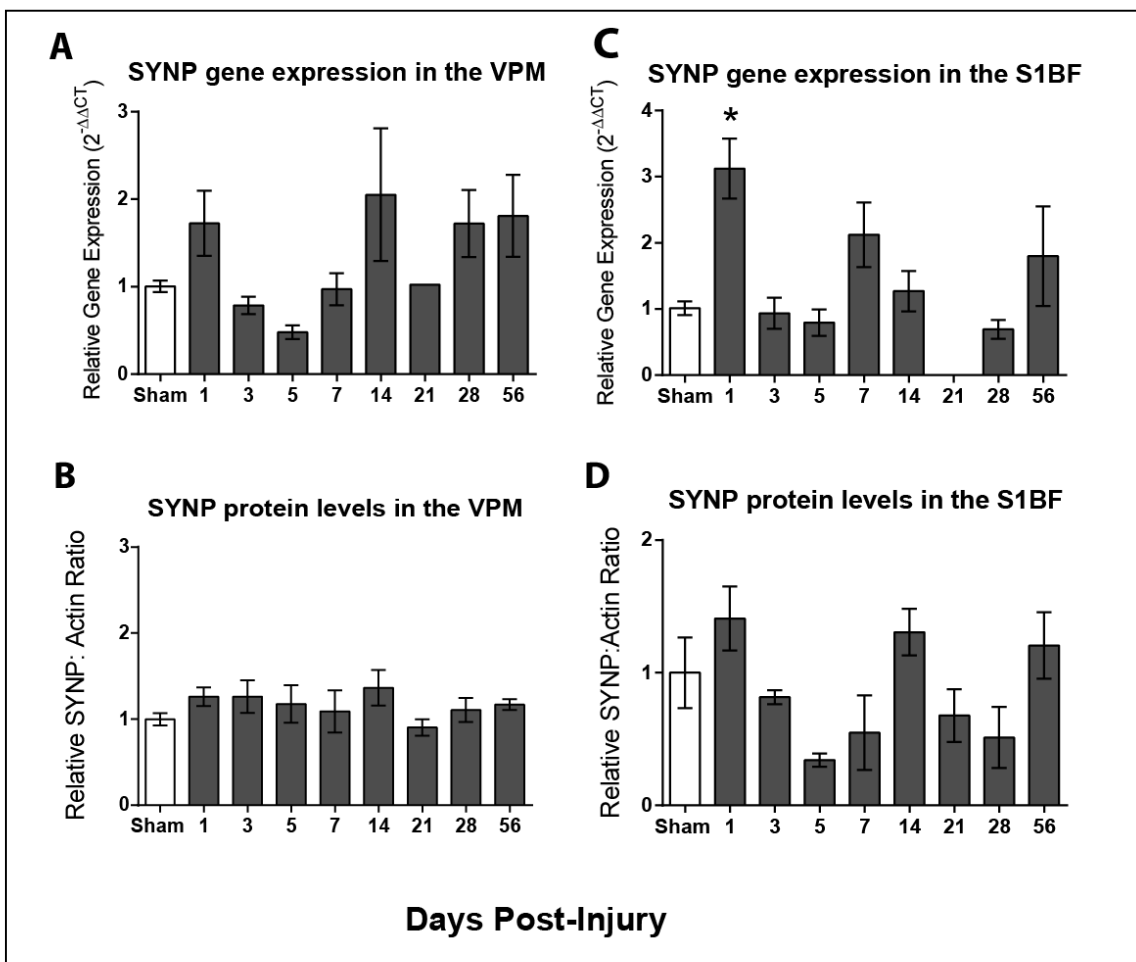


Figure 11. SYNP gene expression and protein levels in the VPM and S1BF over time after dTBI.

A) Gene expression of SYNP was assessed in the VPM after dTBI via qPCR. In the VPM, there were no significant changes over the time course in gene expression in comparison to sham. $N=3-7$ / time point. B) Protein levels of SYNP in the VPM over time were assessed via automated capillary western blot analysis. There were no significant changes in SYNP protein levels in the VPM after dTBI in comparison to sham. $N=3-6$ /time point. C) In the S1BF, there was a significant increase in gene expression in SYNP at 1 day post-injury in comparison to sham. $N=3-10$ / time point. D) Protein levels of SYNP were multimodal in character and significantly changed over time in comparison to sham. $N= 3-6$ /time point. The data are presented as the mean \pm SEM. * $p < 0.05$ in comparison to sham.

10.3 Post-synaptic density 95 (PSD-95) demonstrates decreased gene expression in the VPM and multimodal gene and protein levels in the S1BF.

PSD-95 is neuronal post-synaptic density marker that plays a role in synapse maturation²⁵⁶⁻²⁵⁸. Protein levels of PSD-95 has been shown to decrease in the subacute phase after neuronal insult without successful recovery 1-2 weeks after injury^{259,260}. Given this, we hypothesize there will be an initial decrease in PSD-95 protein levels in the immediate days after mFPI, followed by an increase in gene expression and protein levels signifying recovery efforts in the whisker circuit.

In the VPM, there is a significant change in gene expression of PDS-95 after dTBI ($F(8,29)=3.304$, $p=0.0084$, $N=3-8/$ time point). Post-hoc analysis signified decreased gene expression at 1, 5, 7, 21 DPI. Protein levels of PSD-95 in the VPM did not significantly change over the time course ($F(8, 82)=0.41$, $p=0.9085$, $N=3-8/time$ point). In the S1BF, PSD-95 gene expression, significantly changed over time ($F(7, 43)=4.149$, $p=0.0014$, $N=3-11/time$ point). Post-hoc analysis demonstrated increased gene expression at 1DPI. Protein levels in the S1BF of PSD-95 demonstrated significant changes over the time course ($F(8, 29)=2.435$, $p=0.0378$, $N=3-6/time$ point). Post-hoc analysis signified decreased protein levels at 5 and 28 DPI.

In these studies, gene and protein analysis of PSD-95 in the VPM did not support our hypothesis. Similarly, protein levels of PSD-95 in the S1BF also did not support our hypothesis of an initial decrease in levels, followed by increased protein levels during the recovery phase. Gene expression of PSD-95 in the S1BF did however did support our hypothesis with increased expression after dTBI.

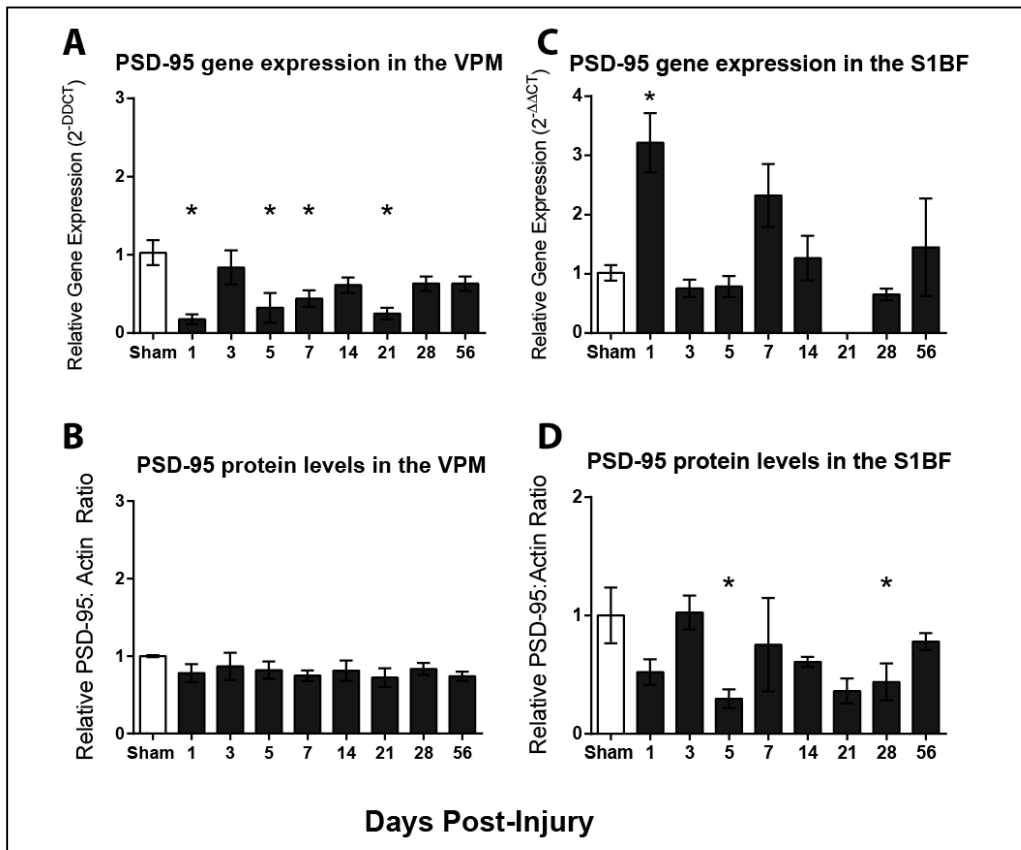


Figure 11. PSD-95 gene expression and protein levels in the VPM and S1BF over time after dTBI.

A) Gene expression of PSD-95 was assessed in the VPM via qPCR. Gene expression significantly decreased at 1, 5, 7, 14 days post-injury in comparison to sham. $N=3-8/$ time point. B) Protein levels of PSD-95 were assessed in the VPM via automated capillary western blot analysis. Protein levels of PSD-95 in the VPM did not significantly change over the time course in comparison to sham. $N=3-8/time$ point. C) Gene expression of PSD-95 was assessed in the S1BF via qPCR. Gene expression of PSD-95 in the S1BF after dTBI did not significantly change over time in comparison to sham. $N=3-11/time$ point. D) Protein levels of PSD-95 were assessed in the S1BF via automated capillary western blot analysis. Protein levels of PSD-95 in the S1BF after dTBI significantly decreased in comparison to sham at 5 and 28 days post-injury. $N=3-6/time$ point. The data are presented as the mean \pm SEM. * $p < 0.05$ in comparison to sham.

10.4 Protein interacting with C-kinase 1 (PICK-1) protein levels do not change overtime after dTBI.

PICK-1 is post-synaptic density protein that is associated with synapse maturation via trafficking AMPA receptors, thereby creating functional synapses^{261,262}. We therefore, hypothesize there will be an initial decrease in protein levels in the immediate days after mFPI, followed by an increase in gene and protein expression signifying recovery efforts in the whisker circuit.

In these studies, protein expression of PICK-1 did not demonstrate significant changes in protein levels over the time course in the VPM ($F(8, 47)=2.138$, $p=0.0505$, $N=4-9$ /time point) or the S1BF ($F(8, 28)=1.647$, $p=0.1638$, $N=3-4$ /time point).

These studies did not support our hypothesis of an initial decrease in protein levels in the immediate days after mFPI, followed by an increase in gene and protein expression signifying recovery efforts in the whisker circuit.

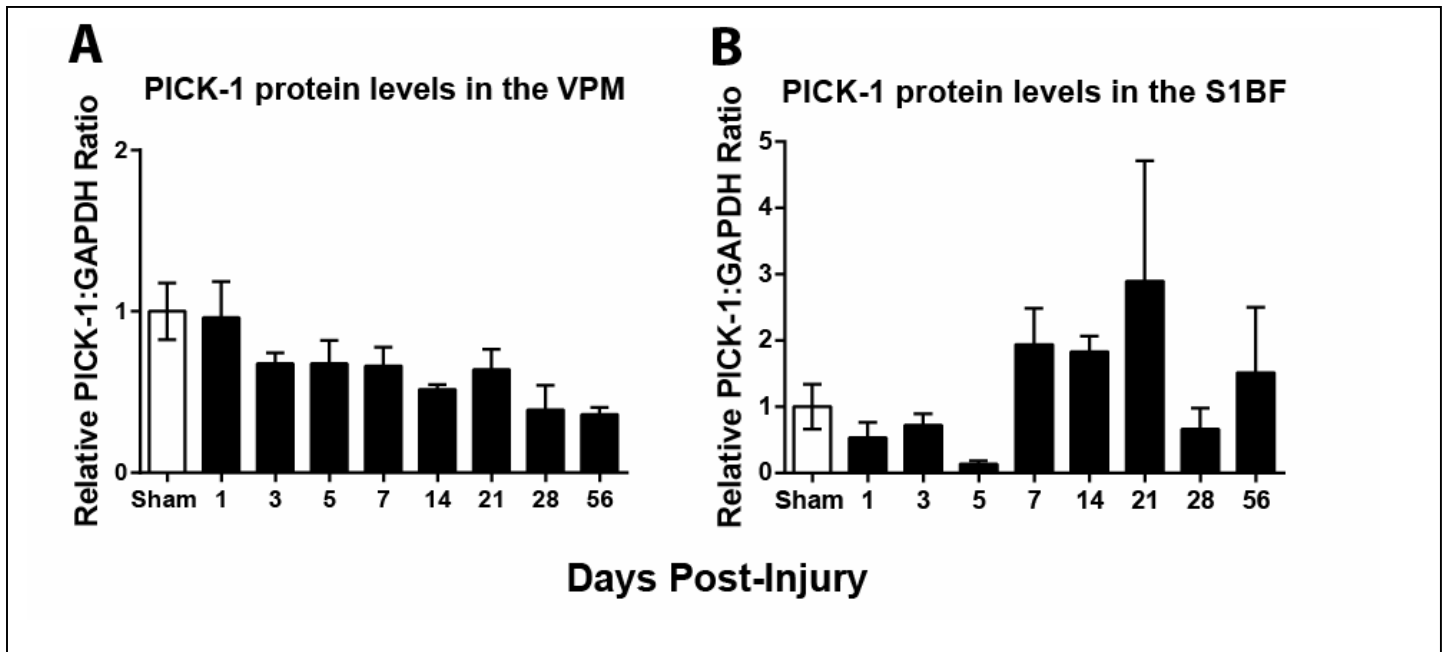


Figure 12. PICK-1 protein levels in the VPM and S1BF over time after dTBI.

A) Protein levels of PICK-1 were assessed in the VPM via automated capillary western blot analysis. Protein levels of PSD-95 in the VPM did not significantly change over the time course in comparison to sham. $N=4-9$ /time point.

B) Protein levels of PICK were assessed in the S1BF via automated capillary western blot analysis. Protein levels of PSD-95 in the S1BF did not significantly change over the time course in comparison to sham. $N=3-4$ /time point. The data are presented as the mean \pm SEM. * $p < 0.05$ in comparison to sham.

10.5 Thrombospondin-1 (TSP-1) protein expression increases in the VPM and S1BF after dTBI.

TSP-1 is known to be expressed during embryonic development and aid in synaptogenesis; however, levels steadily decline to negligible amounts after post-natal day 20¹⁷³. Recently, it has been found that TSP-1 are conditionally expressed after various neuronal insult model^{1,194,231,232,235}. We hypothesize TSP-1 will increase in the sub-acute period after dTBI, with subsequent decline over the time course in the whisker circuit.

In these studies, TSP-1 gene expression ($F(8, 48)=19.68, p < 0.0001, N=3-11/\text{time point}$) and protein levels ($F(8, 32)=5.384, p=0.0002, N=4-6/\text{time point}$) significantly changed over the time course in the VPM. Post-hoc analysis identified increased gene expression at 1 and 5 DPI and increased protein levels at 7 DPI. TSP-1 gene expression ($F(7,47)=2.107, p=0.727, N=5-12/\text{time point}$) did not significantly change in the S1BF over time. TSP-1 protein levels ($F(8,28)=4.128, p=0.0023, N=3-5/\text{time point}$) significantly changed over the time course with post-hoc analysis identifying increased TSP-1 protein levels at 1,3, and 14 DPI.

These studies validated our hypothesis of increased gene expression in the VPM and increased protein expression in the VPM and S1BF, however gene expression of TSP-1 in the S1BF did not support our hypothesis.

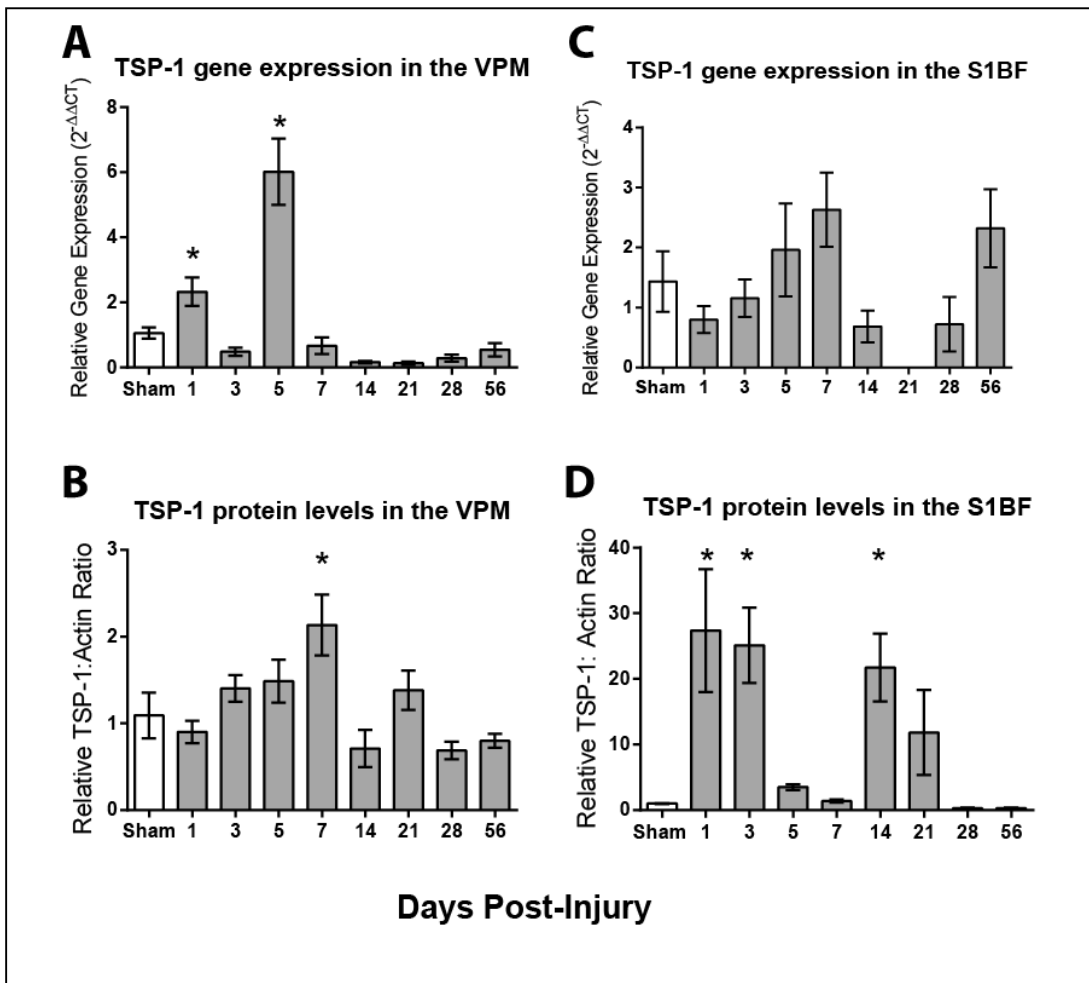


Figure 13. TSP-1 gene expression and protein levels in the VPM and S1BF over time after dTBI.

A) Gene expression of TSP-1 was assessed in the VPM after dTBI via qPCR. TSP-1 gene expression significantly increased at 1 and 5 days post-injury in comparison to sham after dTBI in the VPM. $N=3-11/\text{time point}$. B) Protein levels of TSP-1 in the VPM were assessed via automated capillary western blot analysis. Protein levels of TSP-1 in the VPM significantly increased at 7 days post-injury in comparison to sham after dTBI. $N= 4-6/\text{time point}$. C) Gene expression of TSP-1 was assessed in the S1BF after dTBI via qPCR. TSP-1 gene expression did not significantly change in comparison to sham after dTBI in the S1BF. $N=5-12/\text{time point}$ D) Protein levels of TSP-1 in the S1BF were assessed via automated capillary western blot analysis. Protein levels of TSP-1 in the S1BF increased significantly at 1, 3, 14 days post injury in comparison to sham after dTBI. $N=3-5/\text{time point}$. The data are presented as the mean \pm SEM. * $p < 0.05$ in comparison to sham.

10.6 Thrombospondin-2 (TSP-2) gene expression in the VPM decreases after dTBI

Similar to TSP-1, TSP-2 is an astrocyte secreted molecule that aids in development but is largely unexpressed in the adult CNS¹⁷³. Previous studies have reported increased in TSP-2 mRNA expression between 7-14 days after insult, suggesting a maintenance role in synaptogenesis²³¹. We hypothesized that will be an increase in TSP-2 after dTBI.

In these studies. TSP-2 gene expression in the VPM significantly changed over the time course ($F(8, 60)=2.899$, $p=0.0084$, $N=3-13$ / time point). Post-hoc analysis demonstrated decreased gene expression at 3 days post- injury. Protein levels of TSP-2 in the VPM did not significantly change over time in the VPM ($F(8, 43)=2.908$, $p=0.011$, $N=3-9$ / time point). In the S1BF, neither gene expression ($F(6, 40)=1.742$, $p=0.164$, $N=4-11$ /time point) or protein levels ($F(8, 23)=1.439$, $p=0.2334$, $N=3-5$ /time point) of TSP-2 significantly changed over time. Day 28 was excluded from S1BF gene analysis as there were only 2 samples available for qPCR procedures. These data do no support our hypothesis of increased gene and protein expression in the whisker circuit after dTBI.

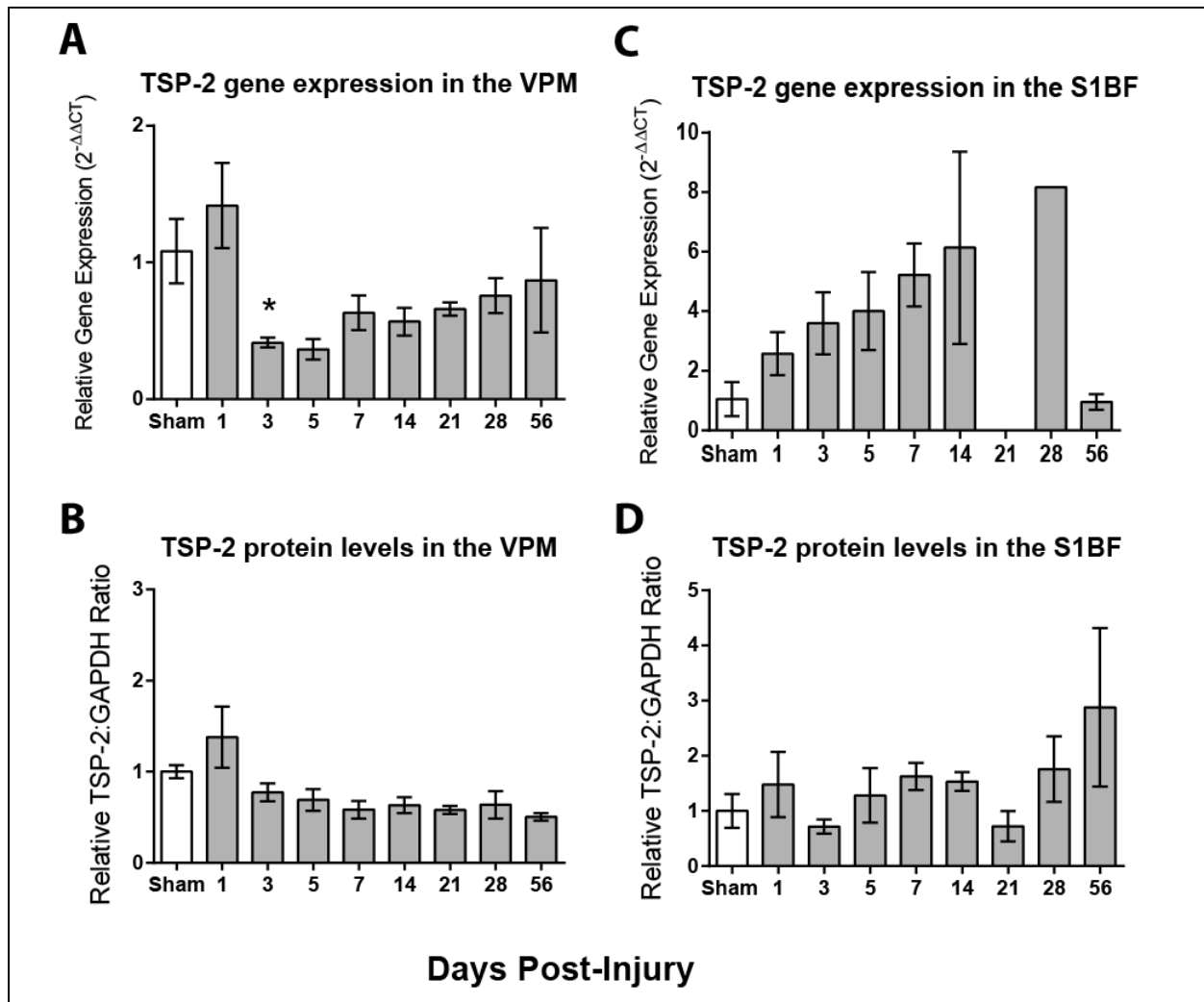


Figure 14. TSP-2 gene expression and protein levels in the VPM and S1BF over time after dTBI.

A) Gene expression of TSP-2 was assessed in the VPM after dTBI via qPCR. TSP-2 gene expression significantly decreased at 3 days post-injury in comparison to sham after dTBI in the VPM. $N=3-11$ /time point. B) Protein levels of TSP-2 in the VPM were assessed via automated capillary western blot analysis. Protein levels of TSP-2 in the VPM did not significantly change in comparison to sham after dTBI. $N=3-9$ / time point. C) Gene expression of TSP-2 was assessed in the S1BF after dTBI via qPCR. TSP-2 gene expression did not significantly change in comparison to sham after dTBI in the S1BF. $N=3-9$ / time point. D) Protein levels of TSP-2 in the S1BF were assessed via automated capillary western blot analysis. Protein levels of TSP-2 did not significantly change in comparison to sham after dTBI in the S1BF. $N=3-5$ /time point. The data are presented as the mean \pm SEM. * $p < 0.05$ in comparison to sham.

10.7 $\alpha 2\delta$ -1 subunit L-type calcium channel receptor $\alpha 2\delta$ -1

$\alpha 2\delta$ -1 is ubiquitously expressed in the healthy adult brain and has been demonstrated to be the receptor for TSP-1¹. Studies in peripheral nervous tissue have shown up-regulation of the $\alpha 2\delta$ -1 subunit after injury and involvement in neuropathic pain syndromes^{233,236,263-267}. We therefore hypothesize to observe an increase in $\alpha 2\delta$ -1 in the VPM and S1BF after dTBI.

In these studies, $\alpha 2\delta$ -1 gene expression ($F(8, 61)=6.91$, $p<0.0001$, $N=4-17$ /time point) and protein levels ($F(8, 43)=4.77$, $p=0.0003$, $N=3-8$ /time point) in the VPM significantly changed over time. Post-hoc analysis identified decreased gene expression at 3, 5, 14, 21, 56 DPI and decreased protein expression at 5, 14-56 DPI. $\alpha 2\delta$ -1 gene expression ($F(7,63)=2.06$, $p=0.612$, $N=4-14$ /time point) and $\alpha 2\delta$ -1 protein levels ($F(8, 23)=1.051$, $P=0.429$, $N=3-5$ /time point) in the S1BF, however, did not significantly change over time. These data do not support our hypothesis of increased gene expression and protein levels in the VPM and S1BF after dTBI.

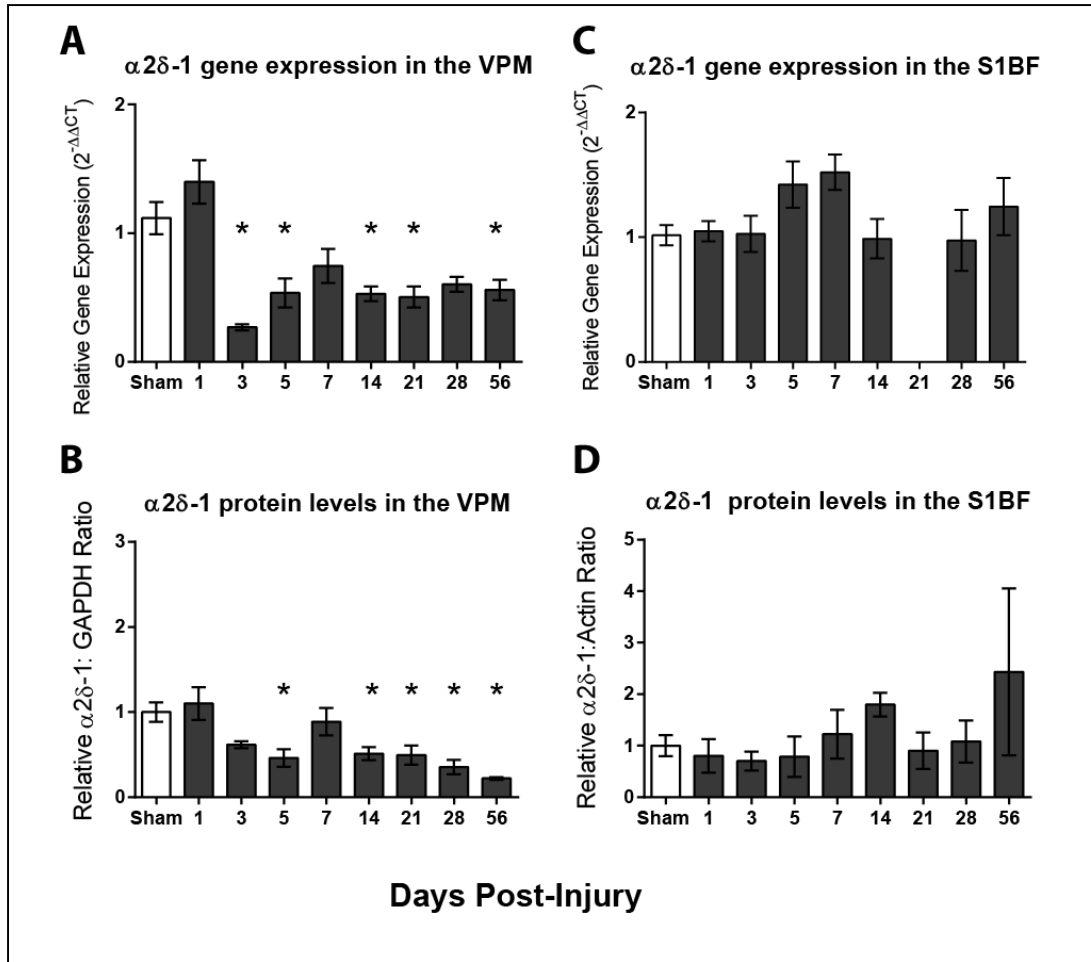


Figure 15. $\alpha 2\delta$ -1 gene expression and protein levels in the VPM and S1BF over time after dTBI.

A) Gene expression of $\alpha 2\delta$ -1 was assessed in the VPM after dTBI via qPCR. $\alpha 2\delta$ -1 gene expression significantly decreased at 3, 5, 14-56 days post-injury in comparison to sham after dTBI in the VPM. $N=4-17$ /time point. B) Protein levels of $\alpha 2\delta$ -1 in the VPM were assessed via automated capillary western blot analysis. Protein levels of $\alpha 2\delta$ -1 in the VPM significantly decreased at 5, 14-56 days post injury in comparison to sham after dTBI. $N=3-8$ /time point. C) Gene expression of $\alpha 2\delta$ -1 was assessed in the S1BF after dTBI via qPCR. $\alpha 2\delta$ -1 gene expression did not significantly change in comparison to sham after dTBI in the S1BF. $N=4-14$ /time point. D) Protein levels of $\alpha 2\delta$ -1 in the S1BF were assessed via automated capillary western blot analysis. Protein levels of $\alpha 2\delta$ -1 did not significantly change in comparison to sham after dTBI in the S1BF. $N=3-5$ /time point. The data are presented as the mean \pm SEM. * $p < 0.05$ in comparison to sham.

11.0 Discussion

11.1 Summary of results

These data support our hypothesis that TSP-1 would increase following experimental dTBI. Gene and protein expression of TSP-1 significantly increased in the VPM after dTBI, however, gene expression and protein levels of synaptic markers (GAP-43, SYNP, PSD, PICK-1 and $\alpha\delta$ -1) in VPM did not indicate TSP-1 mediated synaptogenesis. TSP-1 gene expression in the S1BF did not significantly change over the time course, however TSP-1 protein levels demonstrated 20-30X increase after dTBI. Synaptic markers demonstrated multimodal expression throughout the time course suggesting varying rates of synaptogenesis and pruning of synapses. Taken together, the significant increase of TSP-1 protein levels at 1, 3, and 14 days post-injury and multimodal expression of synaptic markers throughout the time course suggests that TSP-1 may have a role in mediating post-TBI synaptogenesis.

Midline FPI has previously been shown to result in neuronal degeneration and altered neuronal morphology without producing overt cell death over 28 days post-injury²⁴¹. Thus following injury, it was hypothesized to see an upregulation in GAP-43 in response to injury and indicate neuronal growth and repair of synapses²⁴⁹. GAP-43 has been demonstrated to up-regulate after neuronal insult and injury^{142,143}. Previously, we have demonstrated a significant decrease in GAP-43 gene expression at 7 days post-injury with recovery back to sham levels in the VPM in the same model¹²³. In these studies, there were no significant changes in gene expression or protein levels of GAP-43 in the VPM, thus our data did not reproduce previous experiments. In the S1BF, we previously demonstrated no significant changes in gene expression of GAP-43 at 7 or 28 days post-injury; however in these studies, the S1BF demonstrated increased gene expression at 7 days post-injury¹²³. A similar trend was observed in the protein levels, although significance was not reached.

The dissimilar response in GAP-43 gene expression may either reflect regionally specific responses or susceptibility to dTBI. Alternatively, it may be possible that the window of elevated GAP-43 gene expression or protein levels have been missed by the selected time course. Christman et. al (1997) demonstrated elevated GAP-43 protein levels at 28 days after mFPI, indicating that diffuse trauma may lead to a prolonged regenerative phase²¹⁵. While growth is demonstrated in the neuronal morphology with increased number of dendrites, silver staining in both regions remains elevated at 28 days post-injury^{208,241}. Given this, one could hypothesize that if the balance between neuronal growth and degeneration was skewed more towards degeneration at 28 days-post injury, based on silver staining in the VPM and S1BF, it is possible the subsequent shift to regrowth was not captured during the 4 week lag before the final time point of 56 days post-injury²⁶⁸.

Similar to GAP-43, SYNP has been utilized as a marker of regrowth after neuronal insult. Our previous studies demonstrated decreased SYNP gene expression at 7 days post-injury with recovery by 28 days post-injury in the VPM and no significant changes in the S1BF over time after dTBI¹²³. Here we found no significant differences in gene expression or protein levels in the VPM. In the S1BF, gene expression of SYNP was significantly increased at 1 day post-injury and demonstrated multimodal peaks and troughs throughout the time course. Protein levels of SYNP, while not significantly different over time in the S1BF, paralleled the gene expression with multimodal peaks and troughs throughout the time course. These data did not support our hypothesis of acute loss of SYNP protein levels after dTBI, signifying loss of synaptic terminals due to primary and secondary brain injury, followed by up-regulation of gene expression and protein levels supporting regrowth after injury. Shoji et. al (2006) demonstrated similar findings, in a fluid percussion model, with no significant changes in protein levels in SYNP at 2, 15 or 30 days post-injury²⁶⁹. Shoji et. al. (2006) concluded that since immunohistochemical staining of SYNP demonstrated increased staining at sites of injury, without overt neuronal cell loss in their model, that SYNP may be concentrated to sites of injury rather than total protein levels of SYNP being up-regulated²⁶⁹. Redistribution of SYNP to sites of injury could potentially explain the findings of these data, particularly considering our model has previously demonstrated to not cause gross neuronal cell loss; however even in the presence of cytoarchitectural preservation, a loss of synapses due to deafferentation from the primary and secondary brain injury is still likely present and thus one would expect to see at least a temporary decrease in synaptic vesicles and SYNP^{208,241}.

PSD-95 and PICK-1 were hypothesized to follow a similar pattern as GAP-43 and SYNP demonstrating injury effect followed by restoration of synaptic connections; although since PICK-1 is associated with synapse maturation, one would hypothesize increased PICK-1 levels to temporarily lag behind trends in PSD-95^{182,261,262}. In regards to PSD-95, we observed a significant decrease in PSD-95 gene expression in the VPM at 1, 5, 7, 21 days post-injury, but without any significant changes in protein levels over the time course. These data in the VPM do not support the ongoing hypothesis of an acute loss of PSD-95 levels due to synaptic followed by recovery. Gene expression of PSD-95 in the S1BF demonstrated a significant increase at 1 day post-injury, but with paradoxical decreases in protein levels at days 5 and 28 days post-injury. The early decrease in PSD-95 in protein levels at 5 days post-injury in the S1BF may represent delayed synaptic deafferentation and Wallerian degeneration during the pathogenesis of secondary brain injury^{35,39,46,55,57-60}. The delayed decrease in protein levels of PSD-95 in the S1BF could potentially represent synaptic pruning after sprouting of new synapses. Synaptic pruning is a part of the normal maintenance of synapses performed by glial cells²⁷⁰. During normal development and plasticity, synaptic terminals that never find targets or synapses that do not obtain the necessary strengthening signals are pruned and eliminated; however, excessive pruning has been implicated in neurological

diseases such as Alzheimer's disease and Schizophrenia^{270,271}. Future studies, therefore, may look to evaluate the synapse quantification in the context of behavioral outcomes and symptomatology post-TBI.

PICK-1 did not demonstrate any significant changes in protein levels over the time course in either the VPM or the S1BF. Although significant conclusions cannot be drawn from the data, VPM protein level trends steadily declined over the time course and in the S1BF demonstrated a rise in protein levels at the 7, 14 and 21 days post-injury. The steady decline in the protein levels in the VPM, could reflect an inability to reestablish synaptic connections. The ongoing neurodegeneration and reactive environment, as demonstrated by the ongoing glial response and morphological changes in the neurons, may hinder successful synaptic connections^{208,227,241}. Severe TBI can lead to the formation of glial scars, which then can preclude axonal growth, molecular signaling and synaptic formation²¹⁷. The trend of PICK-1 protein levels in the S1BF, eluded to low levels of PICK-1 acutely, followed by a possible increase in protein levels from days 7-21 post-injury and then a return to sham levels at 4 and 8 weeks post-injury. These data are somewhat similar to the data found with other synaptic markers in each region that may provide some insight as to what could potentially be occurring after injury.

Despite the synaptic marker data not reaching significance to reflect the previously demonstrated injury effects on neuronal morphology, the data did support an increase in TSP-1 protein levels in both the VPM and S1BF. Increased TSP-1 would provide a source for the induction of synaptogenesis after injury. Histologically, there is an increase in astrocytosis at days 7 and 28 post-injury that would supply the source of TSP-1; however, since TSP-1 alone only induces the formation of silent synapse, additional signaling would be required to create functional synapses to restore connectivity. TSP-1 and TSP-2 have a similar structure and therefore are thought to bind the same receptors and perform similar functions, though TSP-1 has been much more extensively studied in the CNS^{173,194}.

Contrary to our hypothesis, TSP-2 did not significantly increase in gene expression or protein levels in either VPM or S1BF. Whether or not an absence in elevated TSP-2 gene expression and protein levels after dTBI has a beneficial or detrimental effect on post-TBI synaptogenesis remains unknown. Lin et al (2003) noted a difference in the temporal expression of TSP-1 and TSP-2 after cerebral ischemia, supporting the possibility of TSP-1 and TSP-2 having somewhat different functions¹⁹⁴. While both TSP-1 and TSP-2 have been demonstrated to induce synaptogenesis, studies looking at wound healing and angiogenesis have also supported possible differences in function. TSP-2 null mice have been demonstrated to have accelerated wound healing and angiogenesis; however the TSP-2 has been shown to be critical for repairing the integrity of the blood brain barrier (BBB) and preventing BBB leakiness after foreign body insertion in the CNS^{194,272-275}. The multiple functions of TSP-1 and TSP-2 on angiogenesis and extracellular matrix components, or the lack there of, could potentially have significant implications on restoration of function or the development of morbidity after TBI and future studies would be necessary to further define each the role of TSP-1 and TSP-2 in these areas. For the purpose of this discussion we will focus on the potential implications of TSP-1 on synaptogenesis and the development of circuit reorganization after TBI.

TSP-1 binds multiple receptors in the CNS. Table 4 lists known binding sites and actions of TSP-1 in the CNS. More recently TSP-1 has been found to bind the $\alpha 2\delta$ -1 subunit of the voltage-gated calcium channel receptor ($\alpha 2\delta$ -1) to induce synaptogenesis¹. This interaction has since spurred various studies investigating role of TSP-1/ $\alpha 2\delta$ -1 on various neuronal insult models. Studies in the peripheral nervous system have investigated the role of $\alpha 2\delta$ -1 in pain and have demonstrated up-regulation of $\alpha 2\delta$ -1 after peripheral nerve injury sites^{233,237,251,265}. Here we demonstrate a decrease in $\alpha 2\delta$ -1 gene expression and protein levels in the VPM and no significant changes in gene expression and protein levels in the S1BF; these data did not support our hypothesis of increased $\alpha 2\delta$ -1 after dTBI in either region. Given the whisker sensitivity that is found ~28 days after injury in this model, it would have been intriguing to observe increased $\alpha 2\delta$ -1 protein levels in the VPM or S1BF²⁴¹. Gabapentin and pregabalin (gabapentinoids) are FDA approved drugs that are used for a variety of conditions, such as epilepsy and neuropathic pain. Gabapentinoids binds $\alpha 2\delta$ -1 and have been demonstrated to decrease calcium influx into nerve terminals and neurotransmission leading to decreased central pain symptoms after stroke in rodent models²³⁴.

Evidence from rodent models of central pain syndrome after stroke have suggested that the etiology is secondary to thalamic hyperactivity and circuit reorganization of corticothalamic relays^{120,276}. Though central pain after stroke can be present immediately after the incident, the symptoms develop over weeks to months. Similarly, 3-4 weeks after a mild-moderate midline fluid percussion injury (FPI) rodents develop a late-onset gain-of-function sensory, which persists to at least 56 days post-FPI¹¹⁸. This sensory sensitivity is similar to photophobia and phonophobia experienced by human TBI survivors²⁷⁷⁻²⁸⁰. Sensory sensitivity to whisker stimulation is mediated through a glutamatergic circuit in rats that connects the VPM thalamic nucleus to the barrel fields of the S1BF and corresponds with the onset of hypersensitive presynaptic glutamate release in the thalamus and can be exacerbated by elevated intracranial pressures after injury^{111,118,121,122,208,210,214,281-283}. Lack of gross structural damage in the whisker circuit as indicated by giemsa staining, we looked to microscopic observations of chronic pathology in the VPM and S1BF to explain the behavioral changes^{118,208,241}.

Increased cFOS staining in the VPM and S1BF, signified the increase of circuit activation; which could potentially have implications of somatosensation in the form of whisker sensitivity¹²³. Thomas et. al. (2012) later supported this by demonstrating increase basal and potassium-evoked glutamate signaling in the VPM and S1BF at 28 days after injury; paralleling the onset of whisker sensitivity¹²². Given these alterations in behavior and circuit function, we next look for histological findings could help to explain these differences from uninjured animals.

Neuronal morphology was assessed and quantified via Golgi staining and 3D reconstruction. Neuronal morphology in the VPM and the S1BF demonstrated to be quite dynamic and regionally unique. The VPM demonstrated decreased in complexity from 1 day post-injury to 7 days post-injury, with later resolution back to sham morphology by 28 days post-injury²⁴¹. In the S1BF, we observed an initial injury effect seen with a decrease in complexity at 1 day after injury and resolution by 7 days post-injury; however, by 28 days post-injury there was a second decrease in complexity. These changes in neuronal structure over time could potentially have significant implications on neuronal function and circuitry, and behavioral morbidity.^{42,118,122,214-216}

The purpose of this study was to initiate an investigation in to the potential molecular mechanism for our previous findings. By uncovering the mechanism by which circuit reorganization occurs and behavioral morbidity develops (e.g. whisker sensitivity), translational studies could then be conducted to begin development for potential therapies for treatment or the prophylaxis of post-concussive symptoms.

Although, our current findings in the gene expression and protein levels of synaptic markers did not support our hypothesis demonstrating an injury effect, followed by increases indicating recovery efforts and restoration of synapses, we did find significant increases in protein levels of TSP-1 in both the VPM and S1BF after dTBI. Our outcomes in synaptic markers in the VPM and S1BF could indicate that TSP-1 does not mediate post-TBI synaptogenesis in our model, due to the non-synchronous trends in our results. Alternatively, the difference in synaptic marker gene expression and protein levels in the VPM and the S1BF after dTBI could be a reflection of a dose-dependent response. TSP-1 protein levels increased 2X in the VPM, whereas TSP-1 protein levels the S1BF increased 20-30X. Christopherson et. al (2005) demonstrated increasing synaptic puncta, via colocalization of presynaptic synaptotagmin and postsynaptic PSD-95, with the application of increasing concentrations of TSP-1 in retinal ganglion cultures. However, Christopherson et. al (2005) also found that the number of synaptic puncta eventually plateaued with increasing concentrations of TSP-1 and did not impact the length of neuronal axons, dendrite number or protein levels of synaptic markers, indicating that TSP-1 function may be more involved with protein localization, rather than synthesis. Thus, the lack of significant increases in synaptic marker protein levels in the VPM may not entirely rule out the presence of TSP-1 mediated synaptogenesis in the VPM after dTBI. Future studies would need to employ immunohistochemistry to confirm or refute this mechanism.

12.0 Clinical implications and future directions

12.1 Clinical implications

Here we investigated the molecular mechanism behind a translational model of post-concussive symptoms. Post-concussive symptoms can be broadly described as being cognitive (fatigue, foggy, memory, concentration, cognitive slowing), emotional (depression, irritability, anxiety) and somatosensory (headaches, visual disturbances, dizziness, photo/phono phobia, nausea, vomiting, balance disturbances, numbness/tingling, sleep disturbances)²⁸⁴⁻²⁸⁸. Those at risk for developing post-concussive symptoms are more likely to be female, extremes of age, history of multiple TBI, history of mental health diagnoses^{289,290}. While most who suffer from a mild TBI recover without complications, an estimated 15% of adult patients and 15-30% of pediatric patients have post-concussive symptoms at 1 month after injury and some symptoms may not develop until weeks after the injury^{284,291-293}. However the true incidence of post-concussive symptoms is unknown as many mild TBI patients do not seek care for their injury^{31,294}.

The range of post-concussive symptoms reflects the diffuse nature of concussion and the injury impact on multiple brain regions⁹¹⁻⁹³. However, it is also possible that damage to one area of the brain could have impact other regions of the

brain. As there are many intercortical and subcortical connections throughout the brain, damage resulting in improper signaling, could spread a message of dysfunction to the brain globally. For example, the thalamus plays an important role as a relay for multiple cortical pathways and thalamic damage may have diffuse consequences. Thalamic damage after TBI has been associated in post-concussive headache, fatigue, cognitive deficits and central pain⁹⁴⁻⁹⁷. Thalamic susceptibility to damage after mTBI is thought to be due to high sheer forces upon the relay axons⁹⁸. These structural changes to axons have been suggested to contribute to the persistence in post-concussive symptoms⁹⁹.

Common forms of imaging, such as computer tomography and magnetic resonance imaging, often do not demonstrate evidence of injury with mild traumatic brain injury^{95,100,101}. Specialized imaging studies, which may be more sensitive to pathophysiological changes after mTBI, such as diffusion-weighted imaging, diffusion tensor imaging, functional magnetic resonance imaging, and spectroscopy have demonstrated changes in thalamic metabolism, decreased tissue volume, perfusion alterations, microstructural axonal injury, and changes in connectivity after TBI^{95,100-103}. These imaging modalities may help identify patients with or at risk for post-concussive symptoms in the future as these techniques become more rapid to perform and readily available.

Alternatively, TSP-1 could potentially be used a serum biomarker for TBI patients and serve as an indicator to physicians when to deliver therapies. Wang et al (2015) looked at serum TSP-1 after TBI and found elevated TSP-1 levels to be an independent risk factor for 1 week mortality, 6 month mortality, and poor 6 month outcomes. This further supports the possibility that TSP-1 leads to neurologic dysfunction after TBI, perhaps secondary to circuit reorganization. If future studies indicate TSP-1 to be causative, rather than correlative, of circuit reorganization and poor outcomes, gabapentin could then be used prophylactically to prevent morbidity from ever developing. This would then improve the lives of countless survivors in the future.

1.1 Future directions

First, the findings of TSP-1 in the whisker circuit after dTBI warrants the investigation of effect of blocking potential mediate post-TBI synaptogenesis on whisker sensitivity. Given that TSP-1 is increased in the VPM and S1BF during the first and second weeks after injury, one could hypothesize that treatment with gabapentin for the duration of TSP-1 elevation after TBI could be prophylactic to preventing circuit reorganization and behavioral morbidity. If those studies found positive results, they could then be followed up with evaluating the ideal dose, length of therapy and if therapy could initiated after the rise in TSPs had started.

Another future direction would be to investigate TSPs role in TBI and circuit reorganization in the pediatric population. Given that TSP is normally elevated during development, it would be beneficial to determine if TSP in this population is detrimental or beneficial. If gabapentin therapy proved to be beneficial in an adult population, it would also be useful to know if would be beneficial or if preventing synaptogenesis in the pediatric population after TBI would have deleterious effects. Although there have been a few juvenile animal studies that have demonstrated that gabapentin therapy, does not interfere with normal developmental synaptogenesis in the setting of reactive post-insult TSP mediate synaptogenesis²⁹⁵.

Finally, future directions for these findings will also investigate the role of TSPs in other brain regions associated with post-concussive symptoms. Areas of the brain of interest would be ones associated with cognition and emotions. Pursuing the impact of TSP-1 and circuit reorganization in the brain after dTBI in these areas could then aid to decipher whether or not similar therapies (e.g. gabapentin) could be applied and aid in the development of precision medicine for TBI survivors.

References

- 1 Eroglu, C. *et al.* Gabapentin receptor alpha2delta-1 is a neuronal thrombospondin receptor responsible for excitatory CNS synaptogenesis. *Cell* **139**, 380-392, doi:10.1016/j.cell.2009.09.025 (2009).
- 2 Blake, S. M. *et al.* Thrombospondin-1 binds to ApoER2 and VLDL receptor and functions in postnatal neuronal migration. *The EMBO journal* **27**, 3069-3080, doi:10.1038/emboj.2008.223 (2008).
- 3 Herz, J. & Chen, Y. Reelin, lipoprotein receptors and synaptic plasticity. *Nature reviews. Neuroscience* **7**, 850-859, doi:10.1038/nrn2009 (2006).
- 4 Asch, A. S., Silbiger, S., Heimer, E. & Nachman, R. L. Thrombospondin sequence motif (CSVTGG) is responsible for CD36 binding. *Biochemical and biophysical research communications* **182**, 1208-1217 (1992).
- 5 Guo, N., Krutzsch, H. C., Inman, J. K. & Roberts, D. D. Thrombospondin 1 and type I repeat peptides of thrombospondin 1 specifically induce apoptosis of endothelial cells. *Cancer research* **57**, 1735-1742 (1997).
- 6 Abumrad, N. A., Ajmal, M., Pothakos, K. & Robinson, J. K. CD36 expression and brain function: does CD36 deficiency impact learning ability? *Prostaglandins & other lipid mediators* **77**, 77-83, doi:10.1016/j.prostaglandins.2004.09.012 (2005).
- 7 Gao, A. G., Lindberg, F. P., Dimitry, J. M., Brown, E. J. & Frazier, W. A. Thrombospondin modulates alpha v beta 3 function through integrin-associated protein. *J Cell Biol* **135**, 533-544 (1996).
- 8 Kanda, S., Shono, T., Tomasini-Johansson, B., Klint, P. & Saito, Y. Role of thrombospondin-1-derived peptide, 4N1K, in FGF-2-induced angiogenesis. *Experimental cell research* **252**, 262-272, doi:10.1006/excr.1999.4622 (1999).
- 9 Ohnishi, H. *et al.* Differential localization of Src homology 2 domain-containing protein tyrosine phosphatase substrate-1 and CD47 and its molecular mechanisms in cultured hippocampal neurons. *The Journal of neuroscience : the official journal of the Society for Neuroscience* **25**, 2702-2711, doi:10.1523/JNEUROSCI.5173-04.2005 (2005).
- 10 Lawler, J., McHenry, K., Duquette, M. & Derick, L. Characterization of human thrombospondin-4. *J Biol Chem* **270**, 2809-2814 (1995).
- 11 Cole, G. J., Loewy, A. & Glaser, L. Neuronal cell-cell adhesion depends on interactions of N-CAM with heparin-like molecules. *Nature* **320**, 445-447, doi:10.1038/320445a0 (1986).
- 12 Sun, X., Mosher, D. F. & Rapraeger, A. Heparan sulfate-mediated binding of epithelial cell surface proteoglycan to thrombospondin. *J Biol Chem* **264**, 2885-2889 (1989).
- 13 Iruela-Arispe, M. L., Liska, D. J., Sage, E. H. & Bornstein, P. Differential expression of thrombospondin 1, 2, and 3 during murine development. *Developmental dynamics : an official publication of the American Association of Anatomists* **197**, 40-56, doi:10.1002/aja.1001970105 (1993).
- 14 Faber-Elman, A., Lavie, V., Schwartz, I., Shaltiel, S. & Schwartz, M. Vitronectin overrides a negative effect of TNF-alpha on astrocyte migration. *FASEB journal : official publication of the Federation of American Societies for Experimental Biology* **9**, 1605-1613 (1995).
- 15 Lawler, J. & Hynes, R. O. An integrin receptor on normal and thrombasthenic platelets that binds thrombospondin. *Blood* **74**, 2022-2027 (1989).
- 16 Bentley, A. A. & Adams, J. C. The evolution of thrombospondins and their ligand-binding activities. *Molecular biology and evolution* **27**, 2187-2197, doi:10.1093/molbev/msq107 (2010).
- 17 Beumer, K., Matthies, H. J., Bradshaw, A. & Broadie, K. Integrins regulate DLG/FAS2 via a CaM kinase II-dependent pathway to mediate synapse elaboration and stabilization during postembryonic development. *Development* **129**, 3381-3391 (2002).
- 18 Murphy, L. J., Gong, Y. & Murphy, L. C. Regulation of transforming growth factor gene expression in human endometrial adenocarcinoma cells. *The Journal of steroid biochemistry and molecular biology* **41**, 309-314 (1992).
- 19 Schultz-Cherry, S., Lawler, J. & Murphy-Ullrich, J. E. The type 1 repeats of thrombospondin 1 activate latent transforming growth factor-beta. *J Biol Chem* **269**, 26783-26788 (1994).
- 20 Packard, M., Mathew, D. & Budnik, V. Wnts and TGF beta in synaptogenesis: old friends signalling at new places. *Nature reviews. Neuroscience* **4**, 113-120, doi:10.1038/nrn1036 (2003).

- 21 Mikhailenko, I., Kounnas, M. Z. & Strickland, D. K. Low density lipoprotein receptor-related protein/alpha 2-macroglobulin receptor mediates the cellular internalization and degradation of thrombospondin. A process facilitated by cell-surface proteoglycans. *J Biol Chem* **270**, 9543-9549 (1995).
- 22 Goicoechea, S., Orr, A. W., Pallero, M. A., Eggleton, P. & Murphy-Ullrich, J. E. Thrombospondin mediates focal adhesion disassembly through interactions with cell surface calreticulin. *J Biol Chem* **275**, 36358-36368, doi:10.1074/jbc.M005951200 (2000).
- 23 Emonard, H. *et al.* Low density lipoprotein receptor-related protein mediates endocytic clearance of pro-MMP-2-TIMP-2 complex through a thrombospondin-independent mechanism. *J Biol Chem* **279**, 54944-54951, doi:10.1074/jbc.M406792200 (2004).
- 24 Kinoshita, A., Shah, T., Tangredi, M. M., Strickland, D. K. & Hyman, B. T. The intracellular domain of the low density lipoprotein receptor-related protein modulates transactivation mediated by amyloid precursor protein and Fe65. *J Biol Chem* **278**, 41182-41188, doi:10.1074/jbc.M306403200 (2003).
- 25 Xu, J., Xiao, N. & Xia, J. Thrombospondin 1 accelerates synaptogenesis in hippocampal neurons through neuroligin 1. *Nat Neurosci* **13**, 22-24, doi:10.1038/nn.2459 (2010).
- 26 Graf, E. R., Zhang, X., Jin, S. X., Linhoff, M. W. & Craig, A. M. Neurexins induce differentiation of GABA and glutamate postsynaptic specializations via neuroligins. *Cell* **119**, 1013-1026, doi:10.1016/j.cell.2004.11.035 (2004).
- 27 Meng, H., Zhang, X., Hankenson, K. D. & Wang, M. M. Thrombospondin 2 potentiates notch3/jagged1 signaling. *J Biol Chem* **284**, 7866-7874, doi:10.1074/jbc.M803650200 (2009).
- 28 Risher, W. C. & Eroglu, C. Thrombospondins as key regulators of synaptogenesis in the central nervous system. *Matrix biology : journal of the International Society for Matrix Biology* **31**, 170-177, doi:10.1016/j.matbio.2012.01.004 (2012).
- 29 Angoa-Perez, M. *et al.* Animal models of sports-related head injury: bridging the gap between pre-clinical research and clinical reality. *Journal of neurochemistry* **129**, 916-931, doi:10.1111/jnc.12690 (2014).
- 30 Control, C. f. D. TBI: Get the Facts. (2011).
- 31 Langlois, J. A., Rutland-Brown, W. & Wald, M. M. The epidemiology and impact of traumatic brain injury: a brief overview. *The Journal of head trauma rehabilitation* **21**, 375-378 (2006).
- 32 Shames, J., Treger, I., Ring, H. & Giaquinto, S. Return to work following traumatic brain injury: trends and challenges. *Disability and rehabilitation* **29**, 1387-1395, doi:10.1080/09638280701315011 (2007).
- 33 Schwab, K. A., Gudmundsson, L. S. & Lew, H. L. Long-term functional outcomes of traumatic brain injury. *Handbook of clinical neurology* **128**, 649-659, doi:10.1016/B978-0-444-63521-1.00040-6 (2015).
- 34 Perrin, P. B. *et al.* Measures of injury severity and prediction of acute traumatic brain injury outcomes. *The Journal of head trauma rehabilitation* **30**, 136-142, doi:10.1097/HTR.000000000000026 (2015).
- 35 McKee, A. C. & Daneshvar, D. H. The neuropathology of traumatic brain injury. *Handbook of clinical neurology* **127**, 45-66, doi:10.1016/B978-0-444-52892-6.00004-0 (2015).
- 36 Povlishock, J. T. & Katz, D. I. Update of neuropathology and neurological recovery after traumatic brain injury. *J.Head Trauma Rehabil.* **20**, 76-94 (2005).
- 37 Andriessen, T. M., Jacobs, B. & Vos, P. E. Clinical characteristics and pathophysiological mechanisms of focal and diffuse traumatic brain injury. *Journal of cellular and molecular medicine* **14**, 2381-2392, doi:10.1111/j.1582-4934.2010.01164.x (2010).
- 38 Smith, D. H., Meaney, D.F. Axonal damage in Traumatic Brain Injury. *Progress in Clinical Neuroscience* **6**, 483-495 (2000).
- 39 Taber, K. H. & Hurley, R. A. Update on mild traumatic brain injury: neuropathology and structural imaging. *The Journal of neuropsychiatry and clinical neurosciences* **25**, 1-5, doi:10.1176/appi.neuropsych.12120401 (2013).
- 40 O'Connor, W. T., Smyth, A. & Gilchrist, M. D. Animal models of traumatic brain injury: a critical evaluation. *Pharmacology & therapeutics* **130**, 106-113, doi:10.1016/j.pharmthera.2011.01.001 (2011).
- 41 Bandak, F. A., Ling, G., Bandak, A. & De Lanerolle, N. C. Injury biomechanics, neuropathology, and simplified physics of explosive blast and impact mild traumatic brain injury. *Handbook of clinical neurology* **127**, 89-104, doi:10.1016/B978-0-444-52892-6.00006-4 (2015).
- 42 Povlishock, J. T., Erb, D. E. & Astruc, J. Axonal response to traumatic brain injury: reactive axonal change, deafferentation, and neuroplasticity. *J.Neurotrauma* **9 Suppl 1**, S189-S200 (1992).

- 43 Wang, J., Fox, M. A. & Povlishock, J. T. Diffuse traumatic axonal injury in the optic nerve does not elicit retinal ganglion cell loss. *Journal of neuropathology and experimental neurology* **72**, 768-781, doi:10.1097/NEN.0b013e31829d8d9d (2013).
- 44 Iverson, G. L. Outcome from mild traumatic brain injury. *Current opinion in psychiatry* **18**, 301-317, doi:10.1097/01.yco.0000165601.29047.ae (2005).
- 45 McKee, A. C. *et al.* Chronic traumatic encephalopathy in athletes: progressive tauopathy after repetitive head injury. *Journal of neuropathology and experimental neurology* **68**, 709-735, doi:10.1097/NEN.0b013e3181a9d503 (2009).
- 46 Kiraly, M. & Kiraly, S. J. Traumatic brain injury and delayed sequelae: a review--traumatic brain injury and mild traumatic brain injury (concussion) are precursors to later-onset brain disorders, including early-onset dementia. *TheScientificWorldJournal* **7**, 1768-1776, doi:10.1100/tsw.2007.269 (2007).
- 47 Farkas, O. & Povlishock, J. T. Cellular and subcellular change evoked by diffuse traumatic brain injury: a complex web of change extending far beyond focal damage. *Prog. Brain Res.* **161**, 43-59 (2007).
- 48 Curia, G., Longo, D., Biagini, G., Jones, R. S. & Avoli, M. The pilocarpine model of temporal lobe epilepsy. *Journal of neuroscience methods* **172**, 143-157, doi:10.1016/j.jneumeth.2008.04.019 (2008).
- 49 Jin, X., Huguenard, J. R. & Prince, D. A. Reorganization of inhibitory synaptic circuits in rodent chronically injured epileptogenic neocortex. *Cerebral cortex* **21**, 1094-1104, doi:10.1093/cercor/bhq181 (2011).
- 50 Jin, X., Prince, D. A. & Huguenard, J. R. Enhanced excitatory synaptic connectivity in layer v pyramidal neurons of chronically injured epileptogenic neocortex in rats. *The Journal of neuroscience : the official journal of the Society for Neuroscience* **26**, 4891-4900, doi:10.1523/JNEUROSCI.4361-05.2006 (2006).
- 51 Singleton, R. H., Zhu, J., Stone, J. R. & Povlishock, J. T. Traumatically induced axotomy adjacent to the soma does not result in acute neuronal death. *The Journal of neuroscience : the official journal of the Society for Neuroscience* **22**, 791-802 (2002).
- 52 Kovacs, S. K., Leonessa, F. & Ling, G. S. Blast TBI Models, Neuropathology, and Implications for Seizure Risk. *Frontiers in neurology* **5**, 47, doi:10.3389/fneur.2014.00047 (2014).
- 53 Ghajar, J. Traumatic brain injury. *Lancet* **356**, 923-929, doi:10.1016/S0140-6736(00)02689-1 (2000).
- 54 Karve, I. P., Taylor, J. M. & Crack, P. J. The contribution of astrocytes and microglia to traumatic brain injury. *British journal of pharmacology* **173**, 692-702, doi:10.1111/bph.13125 (2016).
- 55 Golding, E. M. Sequelae following traumatic brain injury. The cerebrovascular perspective. *Brain research. Brain research reviews* **38**, 377-388 (2002).
- 56 Giza, C. C. & Hovda, D. A. The Neurometabolic Cascade of Concussion. *Journal of athletic training* **36**, 228-235 (2001).
- 57 McKee, A. C., Daneshvar, D. H., Alvarez, V. E. & Stein, T. D. The neuropathology of sport. *Acta neuropathologica* **127**, 29-51, doi:10.1007/s00401-013-1230-6 (2014).
- 58 Siesjo, B. K. & Siesjo, P. Mechanisms of secondary brain injury. *European journal of anaesthesiology* **13**, 247-268 (1996).
- 59 Johnson, V. E., Stewart, W. & Smith, D. H. Axonal pathology in traumatic brain injury. *Experimental neurology* **246**, 35-43, doi:10.1016/j.expneurol.2012.01.013 (2013).
- 60 Park, E., Bell, J. D. & Baker, A. J. Traumatic brain injury: can the consequences be stopped? *CMAJ : Canadian Medical Association journal = journal de l'Association medicale canadienne* **178**, 1163-1170, doi:10.1503/cmaj.080282 (2008).
- 61 Sivanandam, T. M. & Thakur, M. K. Traumatic brain injury: a risk factor for Alzheimer's disease. *Neuroscience and biobehavioral reviews* **36**, 1376-1381, doi:10.1016/j.neubiorev.2012.02.013 (2012).
- 62 Povlishock, J. T. & Katz, D. I. Update of neuropathology and neurological recovery after traumatic brain injury. *The Journal of head trauma rehabilitation* **20**, 76-94 (2005).
- 63 Phillips, L. L. & Reeves, T. M. Interactive pathology following traumatic brain injury modifies hippocampal plasticity. *Restorative neurology and neuroscience* **19**, 213-235 (2001).
- 64 Phillips, L. L., Lyeth, B. G., Hamm, R. J. & Povlishock, J. T. Combined fluid percussion brain injury and entorhinal cortical lesion: a model for assessing the interaction between neuroexcitation and deafferentation. *Journal of neurotrauma* **11**, 641-656, doi:10.1089/neu.1994.11.641 (1994).

- 65 Xiong, Y., Mahmood, A. & Chopp, M. Animal models of traumatic brain injury. *Nature reviews. Neuroscience* **14**, 128-142, doi:10.1038/nrn3407 (2013).
- 66 Stahel, P. F. *et al.* Absence of the complement regulatory molecule CD59a leads to exacerbated neuropathology after traumatic brain injury in mice. *Journal of neuroinflammation* **6**, 2, doi:10.1186/1742-2094-6-2 (2009).
- 67 Jeannett, B. Development of Glasgow Coma and Outcome Scales. *Nepal Journal of Neuroscience* **2**, 24-28 (2004).
- 68 King, J. T., Jr., Carlier, P. M. & Marion, D. W. Early Glasgow Outcome Scale scores predict long-term functional outcome in patients with severe traumatic brain injury. *Journal of neurotrauma* **22**, 947-954, doi:10.1089/neu.2005.22.947 (2005).
- 69 Bazarian, J. J., Cernak, I., Noble-Haeusslein, L., Potolicchio, S. & Temkin, N. Long-term neurologic outcomes after traumatic brain injury. *The Journal of head trauma rehabilitation* **24**, 439-451, doi:10.1097/HTR.0b013e3181c15600 (2009).
- 70 Garga, N. & Lowenstein, D. H. Posttraumatic epilepsy: a major problem in desperate need of major advances. *Epilepsy currents / American Epilepsy Society* **6**, 1-5, doi:10.1111/j.1535-7511.2005.00083.x (2006).
- 71 Masel, B. E. & DeWitt, D. S. Traumatic brain injury: a disease process, not an event. *Journal of neurotrauma* **27**, 1529-1540, doi:10.1089/neu.2010.1358 (2010).
- 72 Sun, X. *et al.* Hypoxia facilitates Alzheimer's disease pathogenesis by up-regulating BACE1 gene expression. *Proceedings of the National Academy of Sciences of the United States of America* **103**, 18727-18732, doi:10.1073/pnas.0606298103 (2006).
- 73 Mayeux, R. *et al.* Plasma A[β]40 and A[β]42 and Alzheimer's disease: relation to age, mortality, and risk. *Neurology* **61**, 1185-1190 (2003).
- 74 Tesco, G. New advances in Alzheimer's disease: from biology to therapy. *Current genomics* **8**, 484-485, doi:10.2174/138920207783769558 (2007).
- 75 Bower, J. H. *et al.* Head trauma preceding PD: a case-control study. *Neurology* **60**, 1610-1615 (2003).
- 76 Goldman, S. M. *et al.* Head injury and Parkinson's disease risk in twins. *Annals of neurology* **60**, 65-72, doi:10.1002/ana.20882 (2006).
- 77 Bieniek, K. F. *et al.* Chronic traumatic encephalopathy pathology in a neurodegenerative disorders brain bank. *Acta neuropathologica* **130**, 877-889, doi:10.1007/s00401-015-1502-4 (2015).
- 78 Porter, M. D. A 9-year controlled prospective neuropsychologic assessment of amateur boxing. *Clinical journal of sport medicine : official journal of the Canadian Academy of Sport Medicine* **13**, 339-352 (2003).
- 79 Porter, M. D. & Fricker, P. A. Controlled prospective neuropsychological assessment of active experienced amateur boxers. *Clinical journal of sport medicine : official journal of the Canadian Academy of Sport Medicine* **6**, 90-96 (1996).
- 80 Drew, R. H., Templer, D. I., Schuyler, B. A., Newell, T. G. & Cannon, W. G. Neuropsychological deficits in active licensed professional boxers. *Journal of clinical psychology* **42**, 520-525 (1986).
- 81 Erb, D. E. & Povlishock, J. T. Axonal damage in severe traumatic brain injury: an experimental study in cat. *Acta neuropathologica* **76**, 347-358 (1988).
- 82 Agha, A., Phillips, J., O'Kelly, P., Tormey, W. & Thompson, C. J. The natural history of post-traumatic hypopituitarism: implications for assessment and treatment. *The American journal of medicine* **118**, 1416, doi:10.1016/j.amjmed.2005.02.042 (2005).
- 83 Agha, A. *et al.* Anterior pituitary dysfunction in survivors of traumatic brain injury. *The Journal of clinical endocrinology and metabolism* **89**, 4929-4936, doi:10.1210/jc.2004-0511 (2004).
- 84 Schneider, H. J. *et al.* Prevalence of anterior pituitary insufficiency 3 and 12 months after traumatic brain injury. *European journal of endocrinology / European Federation of Endocrine Societies* **154**, 259-265, doi:10.1530/eje.1.02071 (2006).
- 85 Klose, M. *et al.* Prevalence and predictive factors of post-traumatic hypopituitarism. *Clinical endocrinology* **67**, 193-201, doi:10.1111/j.1365-2265.2007.02860.x (2007).
- 86 Klose, M. *et al.* Acute and long-term pituitary insufficiency in traumatic brain injury: a prospective single-centre study. *Clinical endocrinology* **67**, 598-606, doi:10.1111/j.1365-2265.2007.02931.x (2007).
- 87 Masel, B. E. & Urban, R. Chronic Endocrinopathies in Traumatic Brain Injury Disease. *Journal of neurotrauma* **32**, 1902-1910, doi:10.1089/neu.2014.3526 (2015).

- 88 Masson, F. *et al.* Prevalence of impairments 5 years after a head injury, and their relationship with disabilities and outcome. *Brain injury* **10**, 487-497 (1996).
- 89 Ruff, R. Two decades of advances in understanding of mild traumatic brain injury. *The Journal of head trauma rehabilitation* **20**, 5-18 (2005).
- 90 McLean, A., Jr., Dikmen, S. S. & Temkin, N. R. Psychosocial recovery after head injury. *Archives of physical medicine and rehabilitation* **74**, 1041-1046 (1993).
- 91 Christodoulou, C. *et al.* Functional magnetic resonance imaging of working memory impairment after traumatic brain injury. *J.Neurol.Neurosurg.Psychiatry* **71**, 161-168 (2001).
- 92 Levine, B. *et al.* Functional reorganisation of memory after traumatic brain injury: a study with H(2)(15)O positron emission tomography. *J.Neurol.Neurosurg.Psychiatry* **73**, 173-181 (2002).
- 93 Hillary, F. G. *et al.* Changes in resting connectivity during recovery from severe traumatic brain injury. *Int J Psychophysiol*, doi:10.1016/j.ijpsycho.2011.03.011 (2011).
- 94 Tang, L. *et al.* Thalamic resting-state functional networks: disruption in patients with mild traumatic brain injury. *Radiology* **260**, 831-840, doi:10.1148/radiol.11110014 (2011).
- 95 Grossman, E. J. *et al.* Thalamus and cognitive impairment in mild traumatic brain injury: a diffusional kurtosis imaging study. *Journal of neurotrauma* **29**, 2318-2327, doi:10.1089/neu.2011.1763 (2012).
- 96 Little, D. M. *et al.* Thalamic integrity underlies executive dysfunction in traumatic brain injury. *Neurology* **74**, 558-564, doi:10.1212/WNL.0b013e3181c5d5 (2010).
- 97 van der Horn, H. J. *et al.* Post-concussive complaints after mild traumatic brain injury associated with altered brain networks during working memory performance. *Brain imaging and behavior*, doi:10.1007/s11682-015-9489-y (2015).
- 98 Sayed, T. E., Mota, A. Fraternali, F., Ortiz, M. . Biomechanics of traumatic brain injury. *Comput. Methods Appl. Mech. Engrg* **197**, 4692-4701 (2008).
- 99 Kulkarni, P. *et al.* Use of Anisotropy, 3D Segmented Atlas, and Computational Analysis to Identify Gray Matter Subcortical Lesions Common to Concussive Injury from Different Sites on the Cortex. *PloS one* **10**, e0125748, doi:10.1371/journal.pone.0125748 (2015).
- 100 Grossman, E. J., Inglese, M. & Bammer, R. Mild traumatic brain injury: is diffusion imaging ready for primetime in forensic medicine? *Topics in magnetic resonance imaging : TMRI* **21**, 379-386, doi:10.1097/RMR.0b013e31823e65b8 (2010).
- 101 Shenton, M. E. *et al.* A review of magnetic resonance imaging and diffusion tensor imaging findings in mild traumatic brain injury. *Brain imaging and behavior* **6**, 137-192, doi:10.1007/s11682-012-9156-5 (2012).
- 102 Abdel-Dayem, H. M. *et al.* SPECT brain perfusion abnormalities in mild or moderate traumatic brain injury. *Clinical nuclear medicine* **23**, 309-317 (1998).
- 103 Anderson, C. V., Wood, D. M., Bigler, E. D. & Blatter, D. D. Lesion volume, injury severity, and thalamic integrity following head injury. *Journal of neurotrauma* **13**, 59-65 (1996).
- 104 King, N. S. Post-concussion syndrome: clarity amid the controversy? *The British journal of psychiatry : the journal of mental science* **183**, 276-278 (2003).
- 105 Albert-Weissenberger, C. & Siren, A. L. Experimental traumatic brain injury. *Experimental & translational stroke medicine* **2**, 16, doi:10.1186/2040-7378-2-16 (2010).
- 106 Zhang, Y. P. *et al.* Traumatic brain injury using mouse models. *Translational stroke research* **5**, 454-471, doi:10.1007/s12975-014-0327-0 (2014).
- 107 Cernak, I. Animal models of head trauma. *NeuroRx : the journal of the American Society for Experimental NeuroTherapeutics* **2**, 410-422, doi:10.1602/neurorx.2.3.410 (2005).
- 108 Wakimoto, M. *et al.* Classic Cadherins Mediate Selective Intracortical Circuit Formation in the Mouse Neocortex. *Cerebral cortex* **25**, 3535-3546, doi:10.1093/cercor/bhu197 (2015).
- 109 Khodadad, A., Adelson, P. D., Lifshitz, J. & Thomas, T. C. The time course of activity-regulated cytoskeletal (ARC) gene and protein expression in the whisker-barrel circuit using two paradigms of whisker stimulation. *Behavioural brain research* **284**, 249-256, doi:10.1016/j.bbr.2015.01.032 (2015).
- 110 Bechara, A. *et al.* Hoxa2 Selects Barrelette Neuron Identity and Connectivity in the Mouse Somatosensory Brainstem. *Cell reports* **13**, 783-797, doi:10.1016/j.celrep.2015.09.031 (2015).

- 111 Woolsey, T. A. & Van der Loos, H. The structural organization of layer IV in the somatosensory region (SI) of mouse cerebral cortex. The description of a cortical field composed of discrete cytoarchitectonic units. *Brain Res.* **17**, 205-242 (1970).
- 112 O'Leary, D. D., Ruff, N. L. & Dyck, R. H. Development, critical period plasticity, and adult reorganizations of mammalian somatosensory systems. *Current opinion in neurobiology* **4**, 535-544 (1994).
- 113 Giaume, C., Maravall, M., Welker, E. & Bonvento, G. The Barrel Cortex as a Model to Study Dynamic Neuroglial Interaction. *Neuroscientist* **15**, 351-366, doi:10.1177/1073858409336092 (2009).
- 114 Vitali, I. & Jabaudon, D. Synaptic biology of barrel cortex circuit assembly. *Seminars in cell & developmental biology* **35**, 156-164, doi:10.1016/j.semcd.2014.07.009 (2014).
- 115 Bohnen, N., Twijnstra, A., Wijnen, G. & Jolles, J. Tolerance for light and sound of patients with persistent post-concussional symptoms 6 months after mild head injury. *Journal of neurology* **238**, 443-446 (1991).
- 116 Waddell, P. A. & Gronwall, D. M. Sensitivity to light and sound following minor head injury. *Acta neurologica Scandinavica* **69**, 270-276 (1984).
- 117 Alwis, D. S., Yan, E. B., Morganti-Kossmann, M. C. & Rajan, R. Sensory cortex underpinnings of traumatic brain injury deficits. *PLoS one* **7**, e52169, doi:10.1371/journal.pone.0052169 (2012).
- 118 McNamara, K. C., Lisembee, A. M. & Lifshitz, J. The whisker nuisance task identifies a late-onset, persistent sensory sensitivity in diffuse brain-injured rats. *J.Neurotrauma* **27**, 695-706 (2010).
- 119 Nestic, O. *et al.* Transcriptional profiling of spinal cord injury-induced central neuropathic pain. *Journal of neurochemistry* **95**, 998-1014, doi:10.1111/j.1471-4159.2005.03462.x (2005).
- 120 Wang, G. & Thompson, S. M. Maladaptive homeostatic plasticity in a rodent model of central pain syndrome: thalamic hyperexcitability after spinothalamic tract lesions. *The Journal of neuroscience : the official journal of the Society for Neuroscience* **28**, 11959-11969, doi:10.1523/JNEUROSCI.3296-08.2008 (2008).
- 121 Thomas, T. C., Spaulding, T. P., Smith, L. E. & Lifshitz, J. Diffuse Brain Injury Alters Synaptogenesis over a Time Course That Corresponds to Late-Onset Behavioral Morbidity. *Journal of neurotrauma* **29**, A13-A14 (2012).
- 122 Thomas, T. C., Hinzman, J. M., Gerhardt, G. A. & Lifshitz, J. Hypersensitive glutamate signaling correlates with the development of late-onset behavioral morbidity in diffuse brain-injured circuitry. *Journal of neurotrauma* **29**, 187-200, doi:10.1089/neu.2011.2091 (2012).
- 123 Hall, K. D. & Lifshitz, J. Diffuse traumatic brain injury initially attenuates and later expands activation of the rat somatosensory whisker circuit concomitant with neuroplastic responses. *Brain research* **1323**, 161-173, doi:10.1016/j.brainres.2010.01.067 (2010).
- 124 Emery, D. L., Royo, N. C., Fischer, I., Saatman, K. E. & McIntosh, T. K. Plasticity following injury to the adult central nervous system: is recapitulation of a developmental state worth promoting? *Journal of neurotrauma* **20**, 1271-1292, doi:10.1089/089771503322686085 (2003).
- 125 Parent, J. M., Tada, E., Fike, J. R. & Lowenstein, D. H. Inhibition of dentate granule cell neurogenesis with brain irradiation does not prevent seizure-induced mossy fiber synaptic reorganization in the rat. *The Journal of neuroscience : the official journal of the Society for Neuroscience* **19**, 4508-4519 (1999).
- 126 Garner, C. C., Zhai, R. G., Gundelfinger, E. D. & Ziv, N. E. Molecular mechanisms of CNS synaptogenesis. *Trends in neurosciences* **25**, 243-251 (2002).
- 127 Gutierrez, R. C. *et al.* Altered synchrony and connectivity in neuronal networks expressing an autism-related mutation of neuroligin 3. *Neuroscience* **162**, 208-221, doi:10.1016/j.neuroscience.2009.04.062 (2009).
- 128 Lee, K. *et al.* AMPA Receptors as Therapeutic Targets for Neurological Disorders. *Advances in protein chemistry and structural biology* **103**, 203-261, doi:10.1016/bs.apcsb.2015.10.004 (2016).
- 129 Noga, J. T., Hyde, T. M., Bachus, S. E., Herman, M. M. & Kleinman, J. E. AMPA receptor binding in the dorsolateral prefrontal cortex of schizophrenics and controls. *Schizophrenia research* **48**, 361-363 (2001).
- 130 O'Neill, M. J., Bleakman, D., Zimmerman, D. M. & Nisenbaum, E. S. AMPA receptor potentiators for the treatment of CNS disorders. *Current drug targets. CNS and neurological disorders* **3**, 181-194 (2004).
- 131 Tessier-Lavigne, M. Wiring the brain: the logic and molecular mechanisms of axon guidance and regeneration. *Harvey lectures* **98**, 103-143 (2002).
- 132 Tessier-Lavigne, M. & Goodman, C. S. The molecular biology of axon guidance. *Science* **274**, 1123-1133 (1996).

- 133 Varoqueaux, F. *et al.* Total arrest of spontaneous and evoked synaptic transmission but normal synaptogenesis in the absence of Munc13-mediated vesicle priming. *Proceedings of the National Academy of Sciences of the United States of America* **99**, 9037-9042, doi:10.1073/pnas.122623799 (2002).
- 134 Verhage, M. *et al.* Synaptic assembly of the brain in the absence of neurotransmitter secretion. *Science* **287**, 864-869 (2000).
- 135 Friedman, H. V., Bresler, T., Garner, C. C. & Ziv, N. E. Assembly of new individual excitatory synapses: time course and temporal order of synaptic molecule recruitment. *Neuron* **27**, 57-69 (2000).
- 136 Juttner, R. & Rathjen, F. G. Molecular analysis of axonal target specificity and synapse formation. *Cellular and molecular life sciences : CMLS* **62**, 2811-2827, doi:10.1007/s00018-005-5299-5 (2005).
- 137 Dickson, B. J. Molecular mechanisms of axon guidance. *Science* **298**, 1959-1964, doi:10.1126/science.1072165 (2002).
- 138 Luque, J. M., Puig, N., Martínez, J. M. a., González-García, C. & Ceña, V. n. Glutamate N-methyl-d-aspartate receptor blockade prevents induction of GAP-43 after focal ischemia in rats. *Neuroscience Letters* **305**, 87-90, doi:[http://dx.doi.org/10.1016/S0304-3940\(01\)01833-X](http://dx.doi.org/10.1016/S0304-3940(01)01833-X) (2001).
- 139 Oestreicher, A. B., De Graan, P. N. E., Gispen, W. H., Verhaagen, J. & Schrama, L. H. B-50, the growth associated protein-43: modulation of cell morphology and communication in the nervous system. *Progress in Neurobiology* **53**, 627-686, doi:[http://dx.doi.org/10.1016/S0301-0082\(97\)00043-9](http://dx.doi.org/10.1016/S0301-0082(97)00043-9) (1997).
- 140 Aigner, L. *et al.* Overexpression of the neural growth-associated protein GAP-43 induces nerve sprouting in the adult nervous system of transgenic mice. *Cell* **83**, 269-278, doi:[http://dx.doi.org/10.1016/0092-8674\(95\)90168-X](http://dx.doi.org/10.1016/0092-8674(95)90168-X) (1995).
- 141 Benowitz, L. I. & Routtenberg, A. GAP-43: an intrinsic determinant of neuronal development and plasticity. *Trends in neurosciences* **20**, 84-91, doi:[http://dx.doi.org/10.1016/S0166-2236\(96\)10072-2](http://dx.doi.org/10.1016/S0166-2236(96)10072-2) (1997).
- 142 Hulsebosch, C. E., DeWitt, D. S., Jenkins, L. W. & Prough, D. S. Traumatic brain injury in rats results in increased expression of Gap-43 that correlates with behavioral recovery. *Neuroscience Letters* **255**, 83-86, doi:[http://dx.doi.org/10.1016/S0304-3940\(98\)00712-5](http://dx.doi.org/10.1016/S0304-3940(98)00712-5) (1998).
- 143 Grasselli, G. & Strata, P. Structural plasticity of climbing fibers and the growth-associated protein GAP-43. *Frontiers in neural circuits* **7**, 25, doi:10.3389/fncir.2013.00025 (2013).
- 144 Craig, A. M. & Kang, Y. Neurexin-neuroigin signaling in synapse development. *Current opinion in neurobiology* **17**, 43-52, doi:10.1016/j.conb.2007.01.011 (2007).
- 145 Chih, B., Engelman, H. & Scheiffele, P. Control of excitatory and inhibitory synapse formation by neuroligins. *Science* **307**, 1324-1328, doi:10.1126/science.1107470 (2005).
- 146 Scheiffele, P., Fan, J., Choih, J., Fetter, R. & Serafini, T. Neuroligin expressed in nonneuronal cells triggers presynaptic development in contacting axons. *Cell* **101**, 657-669 (2000).
- 147 Song, J. Y., Ichtchenko, K., Sudhof, T. C. & Brose, N. Neuroligin 1 is a postsynaptic cell-adhesion molecule of excitatory synapses. *Proceedings of the National Academy of Sciences of the United States of America* **96**, 1100-1105 (1999).
- 148 Varoqueaux, F., Jamain, S. & Brose, N. Neuroligin 2 is exclusively localized to inhibitory synapses. *European journal of cell biology* **83**, 449-456, doi:10.1078/0171-9335-00410 (2004).
- 149 Ethell, I. M. *et al.* EphB/syndecan-2 signaling in dendritic spine morphogenesis. *Neuron* **31**, 1001-1013 (2001).
- 150 (!!! INVALID CITATION !!! {}).
- 151 Henderson, J. T. *et al.* The receptor tyrosine kinase EphB2 regulates NMDA-dependent synaptic function. *Neuron* **32**, 1041-1056 (2001).
- 152 Henkemeyer, M., Itkis, O. S., Ngo, M., Hickmott, P. W. & Ethell, I. M. Multiple EphB receptor tyrosine kinases shape dendritic spines in the hippocampus. *J Cell Biol* **163**, 1313-1326, doi:10.1083/jcb.200306033 (2003).
- 153 Irie, M. *et al.* Binding of neuroligins to PSD-95. *Science* **277**, 1511-1515 (1997).
- 154 Irie, F. & Yamaguchi, Y. EphB receptors regulate dendritic spine development via intersectin, Cdc42 and N-WASP. *Nat Neurosci* **5**, 1117-1118, doi:10.1038/nn964 (2002).
- 155 Moeller, M. L., Shi, Y., Reichardt, L. F. & Ethell, I. M. EphB receptors regulate dendritic spine morphogenesis through the recruitment/phosphorylation of focal adhesion kinase and RhoA activation. *J Biol Chem* **281**, 1587-1598, doi:10.1074/jbc.M511756200 (2006).

- 156 Penzes, P. *et al.* Rapid induction of dendritic spine morphogenesis by trans-synaptic ephrinB-EphB receptor activation of the Rho-GEF kalirin. *Neuron* **37**, 263-274 (2003).
- 157 Dailey, M. E., Buchanan, J., Bergles, D. E. & Smith, S. J. Mossy fiber growth and synaptogenesis in rat hippocampal slices in vitro. *The Journal of neuroscience : the official journal of the Society for Neuroscience* **14**, 1060-1078 (1994).
- 158 Fiala, J. C., Feinberg, M., Popov, V. & Harris, K. M. Synaptogenesis via dendritic filopodia in developing hippocampal area CA1. *The Journal of neuroscience : the official journal of the Society for Neuroscience* **18**, 8900-8911 (1998).
- 159 Jontes, J. D., Buchanan, J. & Smith, S. J. Growth cone and dendrite dynamics in zebrafish embryos: early events in synaptogenesis imaged in vivo. *Nat Neurosci* **3**, 231-237, doi:10.1038/72936 (2000).
- 160 Hinds, J. W. & Hinds, P. L. Synapse formation in the mouse olfactory bulb. II. Morphogenesis. *The Journal of comparative neurology* **169**, 41-61, doi:10.1002/cne.901690104 (1976).
- 161 Goold, R. G., Owen, R. & Gordon-Weeks, P. R. Glycogen synthase kinase 3beta phosphorylation of microtubule-associated protein 1B regulates the stability of microtubules in growth cones. *J Cell Sci* **112 (Pt 19)**, 3373-3384 (1999).
- 162 Lucas, J. Z. & Sherman, I. W. Plasmodium falciparum: thrombospondin mediates parasitized erythrocyte band 3-related adhesin binding. *Experimental parasitology* **89**, 78-85, doi:10.1006/expr.1998.4257 (1998).
- 163 Salinas, P. C. Retrograde signalling at the synapse: a role for Wnt proteins. *Biochemical Society transactions* **33**, 1295-1298, doi:10.1042/BST20051295 (2005).
- 164 Ahmad-Annuar, A. *et al.* Signaling across the synapse: a role for Wnt and Dishevelled in presynaptic assembly and neurotransmitter release. *J Cell Biol* **174**, 127-139, doi:10.1083/jcb.200511054 (2006).
- 165 Boeckers, T. M. The postsynaptic density. *Cell Tissue Res* **326**, 409-422, doi:10.1007/s00441-006-0274-5 (2006).
- 166 Montgomery, J. M., Zamorano, P. L. & Garner, C. C. MAGUKs in synapse assembly and function: an emerging view. *Cellular and molecular life sciences : CMLS* **61**, 911-929, doi:10.1007/s00018-003-3364-5 (2004).
- 167 Shen, L., Liang, F., Walensky, L. D. & Huganir, R. L. Regulation of AMPA receptor GluR1 subunit surface expression by a 4. 1N-linked actin cytoskeletal association. *The Journal of neuroscience : the official journal of the Society for Neuroscience* **20**, 7932-7940 (2000).
- 168 Zhai, R. *et al.* Temporal appearance of the presynaptic cytomatrix protein bassoon during synaptogenesis. *Molecular and cellular neurosciences* **15**, 417-428, doi:10.1006/mcne.2000.0839 (2000).
- 169 Harris, K. M. & Landis, D. M. Membrane structure at synaptic junctions in area CA1 of the rat hippocampus. *Neuroscience* **19**, 857-872 (1986).
- 170 Alder, J., Kanki, H., Valtorta, F., Greengard, P. & Poo, M. M. Overexpression of synaptophysin enhances neurotransmitter secretion at Xenopus neuromuscular synapses. *The Journal of neuroscience : the official journal of the Society for Neuroscience* **15**, 511-519 (1995).
- 171 Glantz, L. A., Gilmore, J. H., Hamer, R. M., Lieberman, J. A. & Jarskog, L. F. Synaptophysin and postsynaptic density protein 95 in the human prefrontal cortex from mid-gestation into early adulthood. *Neuroscience* **149**, 582-591, doi:10.1016/j.neuroscience.2007.06.036 (2007).
- 172 Hata, Y. & Takai, Y. Roles of postsynaptic density-95/synapse-associated protein 90 and its interacting proteins in the organization of synapses. *Cellular and molecular life sciences : CMLS* **56**, 461-472 (1999).
- 173 Christopherson, K. S. *et al.* Thrombospondins are astrocyte-secreted proteins that promote CNS synaptogenesis. *Cell* **120**, 421-433, doi:10.1016/j.cell.2004.12.020 (2005).
- 174 Muller, D., Joly, M. & Lynch, G. Contributions of quisqualate and NMDA receptors to the induction and expression of LTP. *Science* **242**, 1694-1697 (1988).
- 175 Kano, M. & Kato, M. Quisqualate receptors are specifically involved in cerebellar synaptic plasticity. *Nature* **325**, 276-279, doi:10.1038/325276a0 (1987).
- 176 Kessels, H. W. & Malinow, R. Synaptic AMPA receptor plasticity and behavior. *Neuron* **61**, 340-350, doi:10.1016/j.neuron.2009.01.015 (2009).
- 177 Malinow, R. AMPA receptor trafficking and long-term potentiation. *Philosophical transactions of the Royal Society of London. Series B, Biological sciences* **358**, 707-714, doi:10.1098/rstb.2002.1233 (2003).
- 178 Song, I. & Huganir, R. L. Regulation of AMPA receptors during synaptic plasticity. *Trends in neurosciences* **25**, 578-588 (2002).

- 179 Fischer, M., Kaech, S., Wagner, U., Brinkhaus, H. & Matus, A. Glutamate receptors regulate actin-based plasticity
in dendritic spines. *Nat Neurosci* **3**, 887-894, doi:10.1038/78791 (2000).
- 180 Matsuzaki, M., Honkura, N., Ellis-Davies, G. C. & Kasai, H. Structural basis of long-term potentiation in single
dendritic spines. *Nature* **429**, 761-766, doi:10.1038/nature02617 (2004).
- 181 Okamoto, K., Nagai, T., Miyawaki, A. & Hayashi, Y. Rapid and persistent modulation of actin dynamics regulates
postsynaptic reorganization underlying bidirectional plasticity. *Nat Neurosci* **7**, 1104-1112, doi:10.1038/nn1311
(2004).
- 182 Lu, W. & Ziff, E. B. PICK1 interacts with ABP/GRIP to regulate AMPA receptor trafficking. *Neuron* **47**, 407-421,
doi:10.1016/j.neuron.2005.07.006 (2005).
- 183 Torres, R. *et al.* PDZ proteins bind, cluster, and synaptically colocalize with Eph receptors and their ephrin
ligands. *Neuron* **21**, 1453-1463 (1998).
- 184 Webster, M. J., Herman, M. M., Kleinman, J. E. & Shannon Weickert, C. BDNF and trkB mRNA expression in the
hippocampus and temporal cortex during the human lifespan. *Gene expression patterns : GEP* **6**, 941-951,
doi:10.1016/j.modgep.2006.03.009 (2006).
- 185 Ohira, K. & Hayashi, M. A new aspect of the TrkB signaling pathway in neural plasticity. *Current*
neuropharmacology **7**, 276-285, doi:10.2174/157015909790031210 (2009).
- 186 Kaplan, G. B., Vasterling, J. J. & Vedak, P. C. Brain-derived neurotrophic factor in traumatic brain injury, post-
traumatic stress disorder, and their comorbid conditions: role in pathogenesis and treatment. *Behavioural*
pharmacology **21**, 427-437, doi:10.1097/FBP.0b013e32833d8bc9 (2010).
- 187 Kucukdereli, H. *et al.* Control of excitatory CNS synaptogenesis by astrocyte-secreted proteins Hevin and SPARC.
Proceedings of the National Academy of Sciences of the United States of America **108**, E440-449,
doi:10.1073/pnas.1104977108 (2011).
- 188 Allen, N. J. *et al.* Astrocyte glypicans 4 and 6 promote formation of excitatory synapses via GluA1 AMPA
receptors. *Nature* **486**, 410-414, doi:10.1038/nature11059 (2012).
- 189 Luxardi, G. *et al.* Glypicans are differentially expressed during patterning and neurogenesis of early mouse brain.
Biochemical and biophysical research communications **352**, 55-60, doi:10.1016/j.bbrc.2006.10.185 (2007).
- 190 Song, H. H. & Filmus, J. The role of glypicans in mammalian development. *Biochimica et biophysica acta* **1573**,
241-246 (2002).
- 191 Hagino, S. *et al.* Expression pattern of glypican-1 mRNA after brain injury in mice. *Neurosci Lett* **349**, 29-32
(2003).
- 192 Blanchette, C. R., Perrat, P. N., Thackeray, A. & Benard, C. Y. Glypican Is a Modulator of Netrin-Mediated Axon
Guidance. *PLoS biology* **13**, e1002183, doi:10.1371/journal.pbio.1002183 (2015).
- 193 Nowokere Esemuede, M. D. a., Taeseung Lee, M.D., Ph.D.a, Daphne Pierre-Paul, M.D.a, Bauer E. Sumpio, M.D.,
Ph.D.a, Vivian Gahtan, M.D.a. The role of thrombospondin-1 in human disease. *Journal of Surgical Research* **122**,
135-142 (2004).
- 194 Lin, T. N. *et al.* Differential regulation of thrombospondin-1 and thrombospondin-2 after focal cerebral
ischemia/reperfusion. *Stroke; a journal of cerebral circulation* **34**, 177-186 (2003).
- 195 Andrea Resovia, D. P., Giovanna Chiorinob, Giulia Taraboletta,. Current understanding of the thrombospondin-1
interactome. *Matrix Biology* (2014).
- 196 JC, A. Thrombospondin-1. *Int J Biochem Cell Biol.* **29**, 861-865 (1997).
- 197 Chamak, B., Morandi, V. & Mallat, M. Brain macrophages stimulate neurite growth and regeneration by
secreting thrombospondin. *Journal of neuroscience research* **38**, 221-233, doi:10.1002/jnr.490380213 (1994).
- 198 Neugebauer, K. M., Emmett, C. J., Venstrom, K. A. & Reichardt, L. F. Vitronectin and thrombospondin promote
retinal neurite outgrowth: developmental regulation and role of integrins. *Neuron* **6**, 345-358 (1991).
- 199 O'Shea, K. S., Liu, L. H. & Dixit, V. M. Thrombospondin and a 140 kd fragment promote adhesion and neurite
outgrowth from embryonic central and peripheral neurons and from PC12 cells. *Neuron* **7**, 231-237 (1991).
- 200 Osterhout, D. J., Frazier, W. A. & Higgins, D. Thrombospondin promotes process outgrowth in neurons from the
peripheral and central nervous systems. *Developmental biology* **150**, 256-265 (1992).
- 201 Barres, B. A. The mystery and magic of glia: a perspective on their roles in health and disease. *Neuron* **60**, 430-
440, doi:10.1016/j.neuron.2008.10.013 (2008).

- 202 Lu, Z. & Kipnis, J. Thrombospondin 1--a key astrocyte-derived neurogenic factor. *FASEB journal : official publication of the Federation of American Societies for Experimental Biology* **24**, 1925-1934, doi:10.1096/fj.09-150573 (2010).
- 203 Bolton, M. M. & Eroglu, C. Look who is weaving the neural web: glial control of synapse formation. *Current opinion in neurobiology* **19**, 491-497, doi:10.1016/j.conb.2009.09.007 (2009).
- 204 Murphy-Ullrich, J. E. The de-adhesive activity of matricellular proteins: is intermediate cell adhesion an adaptive state? *The Journal of clinical investigation* **107**, 785-790, doi:10.1172/JCI12609 (2001).
- 205 Murakoshi, H., Wang, H. & Yasuda, R. Local, persistent activation of Rho GTPases during plasticity of single dendritic spines. *Nature* **472**, 100-104, doi:10.1038/nature09823 (2011).
- 206 Tran, M. D., Furones-Alonso, O., Sanchez-Molano, J. & Bramlett, H. M. Trauma-induced expression of astrocytic thrombospondin-1 is regulated by P2 receptors coupled to protein kinase cascades. *Neuroreport* **23**, 721-726, doi:10.1097/WNR.0b013e32835688fe (2012).
- 207 Lifshitz, J. in *Animal Models of Acute Neurological Injuries* (eds J. Chen, Z. Xu, X. M. Xu, & J. Zhang) (The Humana Press, Inc., 2008).
- 208 Lifshitz, J. & Lisembee, A. M. Neurodegeneration in the somatosensory cortex after experimental diffuse brain injury. *Brain Struct Funct*, doi:10.1007/s00429-011-0323-z (2011).
- 209 Miremami, J. D., Talauliker, P. M., Harrison, J. L. & Lifshitz, J. Neuropathology in sensory, but not motor, brainstem nuclei of the rat whisker circuit after diffuse brain injury. *Somatosens Mot Res* **31**, 127-135, doi:10.3109/08990220.2014.897602 (2014).
- 210 Lifshitz, J., Kelley, B. J. & Povlishock, J. T. Perisomatic thalamic axotomy after diffuse traumatic brain injury is associated with atrophy rather than cell death. *J. Neuropathol. Exp. Neurol.* **66**, 218-229 (2007).
- 211 Gold, B. G., Mobley, W. C. & Matheson, S. F. Regulation of Axonal Caliber, Neurofilament Content, and Nuclear-Localization in Mature Sensory Neurons by Nerve Growth-Factor. *Journal of Neuroscience* **11**, 943-955 (1991).
- 212 Rich, K. M., Yip, H. K., Osborne, P. A., Schmidt, R. E. & Johnson, E. M. Role of Nerve Growth-Factor in the Adult Dorsal-Root Ganglia Neuron and Its Response to Injury. *Journal of Comparative Neurology* **230**, 110-118, doi:DOI 10.1002/cne.902300110 (1984).
- 213 Lifshitz, J. & Lisembee, A. M. Neurodegeneration in the somatosensory cortex after experimental diffuse brain injury. *Brain Struct Funct* **217**, 49-61, doi:10.1007/s00429-011-0323-z (2012).
- 214 Learoyd, A. & Lifshitz, J. Comparison of Rat Sensory Behavioral Tests to Detect Somatosensory Morbidity after Diffuse Brain Injury. *Journal of neurotrauma* **28**, A88-A88 (2011).
- 215 Christman, C. W., Salvant, J. B., Jr., Walker, S. A. & Povlishock, J. T. Characterization of a prolonged regenerative attempt by diffusely injured axons following traumatic brain injury in adult cat: a light and electron microscopic immunocytochemical study. *Acta Neuropathol.* **94**, 329-337 (1997).
- 216 Hulsebosch, C. E., DeWitt, D. S., Jenkins, L. W. & Prough, D. S. Traumatic brain injury in rats results in increased expression of Gap-43 that correlates with behavioral recovery. *Neurosci.Lett.* **255**, 83-86 (1998).
- 217 Sofroniew, M. V. Molecular dissection of reactive astrogliosis and glial scar formation. *Trends in neurosciences* **32**, 638-647, doi:10.1016/j.tins.2009.08.002 (2009).
- 218 Moraga-Amaro, R., Jerez-Baraona, J. M., Simon, F. & Stehberg, J. Role of astrocytes in memory and psychiatric disorders. *Journal of physiology, Paris* **108**, 240-251, doi:10.1016/j.jphysparis.2014.08.005 (2014).
- 219 Choudhury, G. R. & Ding, S. Reactive astrocytes and therapeutic potential in focal ischemic stroke. *Neurobiology of disease* **85**, 234-244, doi:10.1016/j.nbd.2015.05.003 (2016).
- 220 Anderson, M. A., Ao, Y. & Sofroniew, M. V. Heterogeneity of reactive astrocytes. *Neurosci Lett* **565**, 23-29, doi:10.1016/j.neulet.2013.12.030 (2014).
- 221 Barker, A. J. & Ullian, E. M. Astrocytes and synaptic plasticity. *Neuroscientist* **16**, 40-50, doi:10.1177/1073858409339215 (2010).
- 222 Ullian, E. M., Christopherson, K. S. & Barres, B. A. Role for glia in synaptogenesis. *Glia* **47**, 209-216, doi:10.1002/glia.20082 (2004).
- 223 Slezak, M. & Pfrieger, F. W. New roles for astrocytes: regulation of CNS synaptogenesis. *Trends in neurosciences* **26**, 531-535, doi:10.1016/j.tins.2003.08.005 (2003).
- 224 Allen, N. J. Role of glia in developmental synapse formation. *Current opinion in neurobiology* **23**, 1027-1033, doi:10.1016/j.conb.2013.06.004 (2013).

- 225 Verkhatsky, A. & Nedergaard, M. Astroglial cradle in the life of the synapse. *Philosophical transactions of the Royal Society of London. Series B, Biological sciences* **369**, 20130595, doi:10.1098/rstb.2013.0595 (2014).
- 226 Molofsky, A. V. *et al.* Astrocytes and disease: a neurodevelopmental perspective. *Genes & development* **26**, 891-907, doi:10.1101/gad.188326.112 (2012).
- 227 Ziebell, J. M., Adelson, P. D. & Lifshitz, J. Microglia: dismantling and rebuilding circuits after acute neurological injury. *Metabolic brain disease* **30**, 393-400, doi:10.1007/s11011-014-9539-y (2015).
- 228 Loane, D. J. & Kumar, A. Microglia in the TBI brain: The good, the bad, and the dysregulated. *Experimental neurology* **275 Pt 3**, 316-327, doi:10.1016/j.expneurol.2015.08.018 (2016).
- 229 Loane, D. J. & Byrnes, K. R. Role of microglia in neurotrauma. *Neurotherapeutics : the journal of the American Society for Experimental NeuroTherapeutics* **7**, 366-377, doi:10.1016/j.nurt.2010.07.002 (2010).
- 230 Xing, C. *et al.* Neuronal production of lipocalin-2 as a help-me signal for glial activation. *Stroke; a journal of cerebral circulation* **45**, 2085-2092, doi:10.1161/STROKEAHA.114.005733 (2014).
- 231 Liauw, J. *et al.* Thrombospondins 1 and 2 are necessary for synaptic plasticity and functional recovery after stroke. *Journal of cerebral blood flow and metabolism : official journal of the International Society of Cerebral Blood Flow and Metabolism* **28**, 1722-1732, doi:10.1038/jcbfm.2008.65 (2008).
- 232 Andresen, L. *et al.* Gabapentin attenuates hyperexcitability in the freeze-lesion model of developmental cortical malformation. *Neurobiology of disease* **71**, 305-316, doi:10.1016/j.nbd.2014.08.022 (2014).
- 233 Boroujerdi, A. *et al.* Calcium channel alpha-2-delta-1 protein upregulation in dorsal spinal cord mediates spinal cord injury-induced neuropathic pain states. *Pain* **152**, 649-655, doi:10.1016/j.pain.2010.12.014 (2011).
- 234 Castel, A. & Vachon, P. Gabapentin reverses central pain sensitization following a collagenase-induced intrathalamic hemorrhage in rats. *Journal of pain research* **7**, 5-12, doi:10.2147/JPR.S55201 (2013).
- 235 Li, H. *et al.* Gabapentin decreases epileptiform discharges in a chronic model of neocortical trauma. *Neurobiology of disease* **48**, 429-438, doi:10.1016/j.nbd.2012.06.019 (2012).
- 236 Luo, Z. D. *et al.* Injury type-specific calcium channel alpha 2 delta-1 subunit up-regulation in rat neuropathic pain models correlates with antiallodynic effects of gabapentin. *The Journal of pharmacology and experimental therapeutics* **303**, 1199-1205, doi:10.1124/jpet.102.041574 (2002).
- 237 Patel, R. & Dickenson, A. H. Mechanisms of the gabapentinoids and alpha 2 delta-1 calcium channel subunit in neuropathic pain. *Pharmacology research & perspectives* **4**, e00205, doi:10.1002/prp2.205 (2016).
- 238 Rossi, A. R., Angelo, M. F., Villarreal, A., Lukin, J. & Ramos, A. J. Gabapentin administration reduces reactive gliosis and neurodegeneration after pilocarpine-induced status epilepticus. *PLoS one* **8**, e78516, doi:10.1371/journal.pone.0078516 (2013).
- 239 Yang, Y. *et al.* Gabapentinoid Insensitivity after Repeated Administration is Associated with Down-Regulation of the alpha2delta-1 Subunit in Rats with Central Post-Stroke Pain Hypersensitivity. *Neuroscience bulletin* **32**, 41-50, doi:10.1007/s12264-015-0008-3 (2016).
- 240 Zhang, J. L. *et al.* Gabapentin reduces allodynia and hyperalgesia in painful diabetic neuropathy rats by decreasing expression level of Nav1.7 and p-ERK1/2 in DRG neurons. *Brain research* **1493**, 13-18, doi:10.1016/j.brainres.2012.11.032 (2013).
- 241 S.B. Ogle, H. G. M., R.K. Rowe, B. Rumney, S.B. Johnson, P.D. Adelson, J. Lifshitz, T.C. Thomas. Diffuse Brain Injury Causes Circuit Reorganization that Coincides with Thrombospondin-1 Expression. *Oral Presentation Academic Surgical Congress Jacksonville, FL February 2016* (2015).
- 242 Hosseini, A. H. & Lifshitz, J. Brain injury forces of moderate magnitude elicit the fencing response. *Medicine and science in sports and exercise* **41**, 1687-1697, doi:10.1249/MSS.0b013e31819fcd1b (2009).
- 243 Livak, K. J. & Schmittgen, T. D. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. *Methods* **25**, 402-408, doi:10.1006/meth.2001.1262 (2001).
- 244 Rustandi, R. R. *et al.* Qualitative and quantitative evaluation of Simon, a new CE-based automated Western blot system as applied to vaccine development. *Electrophoresis* **33**, 2790-2797, doi:10.1002/elps.201200095 (2012).
- 245 Loughney, J. W., Lancaster, C., Ha, S. & Rustandi, R. R. Residual bovine serum albumin (BSA) quantitation in vaccines using automated Capillary Western technology. *Analytical biochemistry* **461**, 49-56, doi:10.1016/j.ab.2014.05.004 (2014).

- 246 Simmons, A. M., Tanyu, L. H., Horowitz, S. S., Chapman, J. A. & Brown, R. A. Developmental and regional patterns of GAP-43 immunoreactivity in a metamorphosing brain. *Brain, behavior and evolution* **71**, 247-262, doi:10.1159/000127045 (2008).
- 247 McGuire, C. B., Snipes, G. J. & Norden, J. J. Light-microscopic immunolocalization of the growth- and plasticity-associated protein GAP-43 in the developing rat brain. *Brain research* **469**, 277-291 (1988).
- 248 Neve, R. L., Finch, E. A., Bird, E. D. & Benowitz, L. I. Growth-associated protein GAP-43 is expressed selectively in associative regions of the adult human brain. *Proceedings of the National Academy of Sciences of the United States of America* **85**, 3638-3642 (1988).
- 249 Hulsebosch, C. E., DeWitt, D. S., Jenkins, L. W. & Prough, D. S. Traumatic brain injury in rats results in increased expression of Gap-43 that correlates with behavioral recovery. *Neurosci Lett* **255**, 83-86 (1998).
- 250 Higo, N. *et al.* Increased expression of the growth-associated protein 43 gene in the sensorimotor cortex of the macaque monkey after lesioning the lateral corticospinal tract. *The Journal of comparative neurology* **516**, 493-506, doi:10.1002/cne.22121 (2009).
- 251 Van der Zee, C. E. *et al.* Expression of growth-associated protein B-50 (GAP43) in dorsal root ganglia and sciatic nerve during regenerative sprouting. *The Journal of neuroscience : the official journal of the Society for Neuroscience* **9**, 3505-3512 (1989).
- 252 Marklund, N. *et al.* Cognitive outcome following brain injury and treatment with an inhibitor of Nogo-A in association with an attenuated downregulation of hippocampal growth-associated protein-43 expression. *Journal of neurosurgery* **107**, 844-853, doi:10.3171/JNS-07/10/0844 (2007).
- 253 Perovic, M., Mladenovic, A., Rakic, L., Ruzdijic, S. & Kanazir, S. Increase of GAP-43 in the rat cerebellum following unilateral striatal 6-OHDA lesion. *Synapse* **56**, 170-174, doi:10.1002/syn.20142 (2005).
- 254 Bergmann, M., Post, A., Rittel, I., Bechmann, I. & Nitsch, R. Expression of synaptophysin in sprouting neurons after entorhinal lesion in the rat. *Experimental brain research* **117**, 80-86 (1997).
- 255 Knaus, P., Betz, H. & Rehm, H. Expression of synaptophysin during postnatal development of the mouse brain. *Journal of neurochemistry* **47**, 1302-1304 (1986).
- 256 Elias, G. M., Elias, L. A., Apostolides, P. F., Kriegstein, A. R. & Nicoll, R. A. Differential trafficking of AMPA and NMDA receptors by SAP102 and PSD-95 underlies synapse development. *Proceedings of the National Academy of Sciences of the United States of America* **105**, 20953-20958, doi:10.1073/pnas.0811025106 (2008).
- 257 Ehrlich, I., Klein, M., Rumpel, S. & Malinow, R. PSD-95 is required for activity-driven synapse stabilization. *Proceedings of the National Academy of Sciences of the United States of America* **104**, 4176-4181, doi:10.1073/pnas.0609307104 (2007).
- 258 Prange, O., Wong, T. P., Gerrow, K., Wang, Y. T. & El-Husseini, A. A balance between excitatory and inhibitory synapses is controlled by PSD-95 and neuroligin. *Proceedings of the National Academy of Sciences of the United States of America* **101**, 13915-13920, doi:10.1073/pnas.0405939101 (2004).
- 259 Yan, B. C. *et al.* Postsynaptic density protein (PSD)-95 expression is markedly decreased in the hippocampal CA1 region after experimental ischemia-reperfusion injury. *Journal of the neurological sciences* **330**, 111-116, doi:10.1016/j.jns.2013.04.023 (2013).
- 260 Gao, S. *et al.* Spatiotemporal expression of PSD-95 and nNOS after rat sciatic nerve injury. *Neurochemical research* **33**, 1090-1100, doi:10.1007/s11064-007-9555-y (2008).
- 261 Hanley, J. G. PICK1: A multi-talented modulator of AMPA receptor trafficking. *Pharmacology & therapeutics* **118**, 152-160, doi:10.1016/j.pharmthera.2008.02.002 (2008).
- 262 Xu, J. Y., Kam, C. E., Luo, J. H. & Xia, J. PICK1 Mediates Synaptic Recruitment of AMPA Receptors at Neurexin-Induced Postsynaptic Sites. *Journal of Neuroscience* **34**, 15415-15424, doi:10.1523/Jneurosci.0296-14.2014 (2014).
- 263 Bauer, C. S. *et al.* The increased trafficking of the calcium channel subunit alpha2delta-1 to presynaptic terminals in neuropathic pain is inhibited by the alpha2delta ligand pregabalin. *The Journal of neuroscience : the official journal of the Society for Neuroscience* **29**, 4076-4088, doi:10.1523/JNEUROSCI.0356-09.2009 (2009).
- 264 Bauer, C. S., Tran-Van-Minh, A., Kadurin, I. & Dolphin, A. C. A new look at calcium channel alpha2delta subunits. *Current opinion in neurobiology* **20**, 563-571, doi:10.1016/j.conb.2010.05.007 (2010).
- 265 Lana, B. *et al.* Differential upregulation in DRG neurons of an alpha2delta-1 splice variant with a lower affinity for gabapentin after peripheral sensory nerve injury. *Pain* **155**, 522-533, doi:10.1016/j.pain.2013.12.001 (2014).

- 266 Xiao, W., Boroujerdi, A., Bennett, G. J. & Luo, Z. D. Chemotherapy-evoked painful peripheral neuropathy: analgesic effects of gabapentin and effects on expression of the alpha-2-delta type-1 calcium channel subunit. *Neuroscience* **144**, 714-720, doi:10.1016/j.neuroscience.2006.09.044 (2007).
- 267 Boroujerdi, A. *et al.* Injury discharges regulate calcium channel alpha-2-delta-1 subunit upregulation in the dorsal horn that contributes to initiation of neuropathic pain. *Pain* **139**, 358-366, doi:10.1016/j.pain.2008.05.004 (2008).
- 268 Huttenlocher, P. R. & Dabholkar, A. S. Regional differences in synaptogenesis in human cerebral cortex. *The Journal of comparative neurology* **387**, 167-178 (1997).
- 269 Shojo, H. & Kibayashi, K. Changes in localization of synaptophysin following fluid percussion injury in the rat brain. *Brain research* **1078**, 198-211, doi:10.1016/j.brainres.2006.01.063 (2006).
- 270 Hong, S., Dissing-Olesen, L. & Stevens, B. New insights on the role of microglia in synaptic pruning in health and disease. *Current opinion in neurobiology* **36**, 128-134, doi:10.1016/j.conb.2015.12.004 (2016).
- 271 Eroglu, C. & Barres, B. A. Regulation of synaptic connectivity by glia. *Nature* **468**, 223-231, doi:10.1038/nature09612 (2010).
- 272 Krady, M. M. *et al.* Thrombospondin-2 modulates extracellular matrix remodeling during physiological angiogenesis. *The American journal of pathology* **173**, 879-891, doi:10.2353/ajpath.2008.080128 (2008).
- 273 Tian, W., Sawyer, A., Kocaoglu, F. B. & Kyriakides, T. R. Astrocyte-derived thrombospondin-2 is critical for the repair of the blood-brain barrier. *The American journal of pathology* **179**, 860-868, doi:10.1016/j.ajpath.2011.05.002 (2011).
- 274 Agah, A., Kyriakides, T. R., Lawler, J. & Bornstein, P. The lack of thrombospondin-1 (TSP1) dictates the course of wound healing in double-TSP1/TSP2-null mice. *The American journal of pathology* **161**, 831-839, doi:10.1016/S0002-9440(10)64243-5 (2002).
- 275 Kyriakides, T. R., Tam, J. W. & Bornstein, P. Accelerated wound healing in mice with a disruption of the thrombospondin 2 gene. *The Journal of investigative dermatology* **113**, 782-787, doi:10.1046/j.1523-1747.1999.00755.x (1999).
- 276 Canavero, S. & Bonicalzi, V. Central pain syndrome: elucidation of genesis and treatment. *Expert review of neurotherapeutics* **7**, 1485-1497, doi:10.1586/14737175.7.11.1485 (2007).
- 277 Galvin, J., Froude, E. H. & Imms, C. Sensory processing abilities of children who have sustained traumatic brain injuries. *The American journal of occupational therapy : official publication of the American Occupational Therapy Association* **63**, 701-709 (2009).
- 278 Basford, J. R. *et al.* An assessment of gait and balance deficits after traumatic brain injury. *Archives of physical medicine and rehabilitation* **84**, 343-349, doi:10.1053/apmr.2003.50034 (2003).
- 279 Bergemalm, P. O. & Lyxell, B. Appearances are deceptive? Long-term cognitive and central auditory sequelae from closed head injury. *International journal of audiology* **44**, 39-49 (2005).
- 280 Chang, T. T., Ciuffreda, K. J. & Kapoor, N. Critical flicker frequency and related symptoms in mild traumatic brain injury. *Brain injury* **21**, 1055-1062, doi:10.1080/02699050701591437 (2007).
- 281 Robertson, C. L. *et al.* Increased adrenomedullin in cerebrospinal fluid after traumatic brain injury in infants and children. *Journal of neurotrauma* **18**, 861-868, doi:10.1089/089771501750451785 (2001).
- 282 Land, P. W., Buffer, S. A., Jr. & Yaskosky, J. D. Barreloids in adult rat thalamus: three-dimensional architecture and relationship to somatosensory cortical barrels. *J.Comp Neurol.* **355**, 573-588 (1995).
- 283 Henderson, T. A. & Jacquin, M. F. in *Cerebral cortex* (eds E. G. Jones & I. T. Diamond) 123-198 (Plenum, 1995).
- 284 McAllister, T. W. Neuropsychiatric sequelae of head injuries. *The Psychiatric clinics of North America* **15**, 395-413 (1992).
- 285 Broshek, D. K., De Marco, A. P. & Freeman, J. R. A review of post-concussion syndrome and psychological factors associated with concussion. *Brain injury* **29**, 228-237, doi:10.3109/02699052.2014.974674 (2015).
- 286 Blume, H. & Hawash, K. Subacute concussion-related symptoms and postconcussion syndrome in pediatrics. *Current opinion in pediatrics* **24**, 724-730, doi:10.1097/MOP.0b013e328359e4cc (2012).
- 287 Barlow, K. M. *et al.* Epidemiology of postconcussion syndrome in pediatric mild traumatic brain injury. *Pediatrics* **126**, e374-381, doi:10.1542/peds.2009-0925 (2010).

- 288 Ayr, L. K., Yeates, K. O., Taylor, H. G. & Browne, M. Dimensions of postconcussive symptoms in children with mild traumatic brain injuries. *Journal of the International Neuropsychological Society : JINS* **15**, 19-30, doi:10.1017/S1355617708090188 (2009).
- 289 Stein, M. B. *et al.* Prognostic indicators of persistent post-concussive symptoms after deployment-related mild traumatic brain injury: A prospective longitudinal study in U.S. Army soldiers. *Journal of neurotrauma*, doi:10.1089/neu.2015.4320 (2016).
- 290 Rabinowitz, A. R. *et al.* Prevalence and Predictors of Poor Recovery from Mild Traumatic Brain Injury. *Journal of neurotrauma* **32**, 1488-1496, doi:10.1089/neu.2014.3555 (2015).
- 291 Ragnarsson, K. T. Results of the NIH consensus conference on "rehabilitation of persons with traumatic brain injury". *Restorative neurology and neuroscience* **20**, 103-108 (2002).
- 292 McAllister, T. W. *et al.* Differential working memory load effects after mild traumatic brain injury. *NeuroImage* **14**, 1004-1012, doi:10.1006/nimg.2001.0899 (2001).
- 293 Alves, W., Macciocchi, S., Barth, J. Postconcussive symptoms after uncomplicated mild head injury. *Journal of Head Trauma and Rehabilitation* **8**, 48-59 (1993).
- 294 Control, N. C. f. I. P. a. Report to Congress on Mild Traumatic Brain Injury in the United States: Steps to Prevent a Serious Public Health Problem. <http://www.cdc.gov/traumaticbraininjury/pdf/mtbireport-a.pdf> (2003).
- 295 Lo, F. S., Zhao, S. & Erzurumlu, R. S. Astrocytes promote peripheral nerve injury-induced reactive synaptogenesis in the neonatal CNS. *Journal of neurophysiology* **106**, 2876-2887, doi:10.1152/jn.00312.2011 (2011).

Appendix A

Whisker Nuisance Behavioral Point Criteria

Animal ID#: _____

0 = Normal Behavior

Date: _____

1 = Partial Exhibition of **Meaningful** Abnormal/Responsive Behavior

Time: _____

2 = Total Exhibition of **Meaningful** Abnormal/Responsive Behavior

Observer's Initials: _____

Stim initials: _____

Note: Maximum number of points per criterion is 2.

Behavior Criteria

	<i>Stimulation Present</i>				<i>Absent</i>
	P0	P1	P2	P3	P4
Movement					
• Walking around, exhibits curious behavior	0	0	0	0	0
• Slow <u>OR</u> stationary, limited curiosity, cautious	1	1	1	1	1
• Freezing, defensive and fearful	2	2	2	2	2
Stance and Body Position					
• Relaxed, looking skyward, forepaws under body	0	0	0	0	0
• Cowering, guarded, grounded forepaws	2	2	2	2	2
Breathing					
• Normal range	0	0	0	0	0
• Forced, gasping	2	2	2	2	2
Whisker Position					
• Fully Protracted (both sides)	0	0	0	0	0
• Protraction and Retraction	1	1	1	1	1
• Fully Retracted (both sides)	2	2	2	2	2
Whisking Response					
• Standard whisking, normal movement	0	0	0	0	0
• Tremors, twitching	1	1	1	1	1
• None, stopped	2	2	2	2	2
Evading Stimulation					
• No evasive behavior	0	0	0	0	--
• Escape behavior or directed movement to avoid/protect whiskers	2	2	2	2	--
Response to Stick Presentation					
• Ambivalence <u>OR</u> curiosity of stick	0	0	0	0	--
• Avoiding and anxiety <u>OR</u> biting and attacking <u>OR</u> freezing	2	2	2	2	--
Grooming					
• No, minimal or normal grooming	0	0	0	0	0
• Irritated scratching/rubbing/pulling	2	2	2	2	2
Score Total	<u>12</u>	<u>16</u>	<u>16</u>	<u>16</u>	<u>12</u>

Notes: _____

Appendix B

Links to additional Proteinsimple Simple Western Automated Capillary Western technical information.

1. Wes User Guide

http://www.proteinsimple.com/documents/Wes_User_Guide.pdf

2. Wes 25-capillary Total Protein Master Kit Product Insert

http://www.proteinsimple.com/documents/043-026_RevA_Wes_TP01-TP02_master_kit_PI.pdf

3. Simple Western Size Troubleshooting Guide

http://www.proteinsimple.com/documents/Simple_Western_Size_Troubleshooting_Guide.pdf

4. Compass Software User Guide for Wes, Sally Sue and Peggy Sue

http://www.proteinsimple.com/documents/Compass_Software_User_Guide_for_Wes,_Sally_Sue_and_Peggy_Sue.pdf

5. Simple Western Size Assay Development Guide

http://www.proteinsimple.com/documents/042-889_Rev1_Size_Assay_Development_Guide.pdf