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Post-traumatic seizures and changes in brain oxygen contribute to post-traumatic behavioural deficits.

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Post-traumatic seizures and changes in brain oxygen contribute to post-traumatic behavioural deficits.

by

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Abstract

Repetitive mild traumatic brain injuries (RmTBIs) are increasingly recognized to have long-term neurological sequelae in a significant proportion of patients. Individuals that have experienced RmTBIs exhibit a variety of physical, cognitive, or behavioural consequences that can negatively impact quality of life. Brain tissue oxygen levels are normally maintained through exquisite regulation of blood supply. However, during neurological events that result in alterations to brain tissue oxygen levels, neuronal dysfunction, brain damage (neuronal loss, astrocyte hypertrophy), and behavioural deficits have been observed, and are frequently related to poorer prognoses. The oxygenation response in the brain after mild TBIs or concussions have been poorly characterized, with most preliminary research limited to the cortex. Furthermore, the mechanisms by which traumatic brain injuries impact changes to brain oxygenation and vice versa remain unclear. In the current study we demonstrate that upon receiving RmTBIs, rats exhibit post-traumatic, electrographic, seizures that are accompanied by a long-lasting period of hippocampal hyperoxygenation. These seizures and the ensuing hyperoxic episodes are associated with deficits in working memory and motor coordination that are reversible through attenuation of the hyperoxia via administration of a calcium channel agonist, Bay K8644. We propose that the post-traumatic, postictal period of altered brain oxygenation is the basis for some of the common symptoms associated with mTBIs.

Keywords: Concussion, seizure, brain oxygenation, post-traumatic deficits, mTBI, symptoms, behaviour

Preface

Chapter 1 is a general introduction to mild traumatic brain injury, seizures, and brain oxygenation and blood flow, along with specific aims and hypotheses for the investigations described in the subsequent chapter.

Chapter 2 is an empirical data chapter exploring the electrographic sequelae after repetitive mild traumatic brain injury, oxygen trends following the injury, and the behavioural implications.

Chapter 3 is a discussion chapter that reviews the key findings of the research presented in this thesis and their implications on the field of neurotrauma and concussions.

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Dedication

I dedicate this work to myself.

Не попробуешь – не выиграешь. – If you won't try, you won't win.

Russian Proverb

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List of Abbreviations and Nomenclature

Symbol	Definition
ATP	Adenosine triphosphate
BBB	Blood-brain barrier
CBF	Cerebral blood flow
CCI	Controlled cortical impact
COX-2	Cyclooxygenase-2
CPP	Cerebral perfusion pressure
EAA	Excitatory amino acid
AMPA	D-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid
EEG	Electroencephalogram
FPI	Fluid percussion injury
LFPI	Lateral fluid percussion injury
GCS	Glasgow coma scale
HE	Hematoxylin-eosin
LOC	Loss of consciousness
NMDA	N-methyl-D-aspartate
PCS	Post-concussion syndrome
PIH	Postictal hypoxia
pO_2	Partial pressure of oxygen
PTA	Post-traumatic amnesia
PTE	Post-traumatic epilepsy
SPECT	Single-photon emission computed tomography
TBI	Traumatic brain injury
mTBI	Mild traumatic brain injury
RmTBI	Repetitive mild traumatic brain injury

Chapter 1: General Introduction

Mild Traumatic Brain Injuries and Concussions

Overview

Traumatic brain injures (TBIs) are a set of injuries that involve an insult to the brain through the application of an external mechanical force to the head or body that results in neuropathologic damage and dysfunction.[1] The US Department of Veterans Affairs and the Department of Defense's Clinical Practice Guideline For Management of Concussion/mild TBI (mTBI) defines this neuropathological dysfunction by the presence of any of the following clinical signs immediately following the event: any period of loss of or a decreased level of consciousness (LOC), any loss of memory for events immediately before or after the injury (post-traumatic amnesia, or PTA), any alteration in mental state at the time of the injury (confusion, disorientation, slowed thinking, etc.), neurologic deficits (weakness, loss of balance, change in vision, praxis, paresis/plegia, sensory loss, aphasia, etc.).[2] This range of symptomologies can be stratified using assessment scales such as the Glasgow Coma Scale (GCS) score to grade the severity of the traumatic brain injury as mild (GCS 13-15), moderate (GCS 9-13), or severe (3-8) (See Table 1).[3]

Table 1: Standard Glasgow Coma Scale

Eye Opening	**Best Verbal Response**	**Best Motor Response**
		6: obeys commands
	5: oriented	5: localizes
4: spontaneous	4: confused	4: withdraws
3: to speech	3: inappropriate words	3: abnormal flexion
2: to pain	2: incomprehensible sounds	2: extension
1: none	1: none	1: none
Total GCS Score: 3-15		

Of all severities of TBI, an estimated 75–85% are categorized as mild TBI (mTBI), which encompass concussions.[4] In the US, it is estimated that 1.6–3.8 million concussions occur annually, but the true frequency of concussion is likely far greater given that concussions are routinely under-recognized, underreported, and typically resolve spontaneously without medical care.[4]

Concussions are produced by linear or rotational accelerative and decelerative forces on the brain.[5] The application of these impulsive forces causes the brain to elongate and deform, placing stress on parts of the brain where tissues of different densities meet; that is, between gray and white matter.[6] The gray matter of the cortex is less dense than the white matter, so as the gray matter moves within the skull, the white matter lags behind, stretching individual neurons, glial cells, and blood vessels, subsequently altering membrane permeability.[6] While all cell compartments and blood vessels are affected by the injury, axons are especially susceptible to acceleration-deceleration injuries because of their viscoelastic nature, anisotropic arrangement in tracts, linear arrangement of microtubules and neurofilaments, and large surface to volume ratio of the axolemma to the axoplasm.[7] Due to the diffuse nature of the acceleration-deceleration forces, axonal fibres throughout the brain parenchyma are effected, often giving rise to a diverse array of impairments in cognitive, motor, and sensory function.[8] Concordantly, a hallmark of concussions as opposed to other forms of TBI is the absence of focal lesions or other macroscopic damage, leading many to call concussion a functional rather than structural injury.

Neurometabolic Cascade after TBI

Immediately after the biomechanical injury to the brain, axonal stretching and the disruption of neuronal membranes produce nonspecific depolarization, increases in membrane

conductance, and the opening of voltage-dependent K^+ channels, which leads to increased extracellular K^+.[9] Nonspecific depolarization results in an early, indiscriminate release of the excitatory amino acid (EAA) glutamate, which causes further depolarization and exacerbates the K^+ flux by activating kainate, NMDA, and D-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid (AMPA) receptors.[10] Normally, physiologic K^+ levels are maintained after mild perturbations through uptake of excess extracellular K^+ by surrounding glia. However, trauma and other larger insults can overwhelm this compensation and as extracellular K^+ increases, neuronal depolarization is triggered, leading to further release of EAAs, opening of EAA receptor channels (NMDA, AMPA, kainate), and still greater K^+ flux.[11] Activation of NDMA channels by EAAs also results in the influx of Ca^{2+}, and the resultant accumulation of Ca^{2+} is seen within hours of experimental mTBI and can persist for 2 – 4 days.[12] This excess intracellular Ca^{2+} may be sequestered in mitochondria, resulting in mitochondrial membrane potential depolarization and impaired oxidative metabolism.[13, 14]

Following this period of ionic flux, cellular sodium-potassium pumps (Na^C-K^C) are accelerated in effort to restore ionic homeostasis.[11] The energy-intensive nature of these pumps triggers a large increase in glucose use, wherein the abrupt increase in energy requirements is met by an increase in glycolysis due to an inability to increase ATP yield through oxidative metabolism (both because of the Ca^{2+} mediated impairment of mitochondrial function, and because cerebral oxidative metabolism typically runs near its maximum capacity).[11] Hyperglycolysis results in increased lactate production and accumulation which can result in neuronal dysfunction by inducing acidosis, membrane damage, altered blood brain barrier permeability, and leaves neurons more vulnerable to a secondary ischemic injury.[15-20]

Cerebral Blood Flow after TBI

Cerebral autoregulation (or more specifically, pressure autoregulation) is defined as the capacity of the brain to maintain constant cerebral blood flow (CBF) in the face of variations in systemic arterial pressure and is likely mediated by metabolic and myogenic factors.[21] Ultimately, blood flow is regulated through dilation and constriction of the cerebral vasculature in an activity-dependent manner.[22] Vasculature in brain areas with high metabolic demand will undergo vasodilation, allowing these areas to receive more blood flow and, consequently, more oxygen.[23]

CBF is highly sensitive to the effects of concussion and TBI: contrary to expectations, CBF and glucose metabolism, which are normally tightly coupled to neuronal activity, undergo an uncoupling after TBI wherein there is an acute period of increased CBF immediately followed by a large reduction.[24-30]. The extent and direction of changes in CBF seem to be related to injury severity: studies using animal models of moderate and severe TBI have seen ischemic reductions in CBF at the impact site, whereas less severe injury protocols have demonstrated increases in CBF immediately after TBI followed by a global decrease.[24, 26-32] Literature on CBF dynamics in mTBIs on the scale of concussions is limited.

In the clinical setting, this impairment or absence of the autoregulatory response is often reported in patients who have experienced a moderate or severe TBI.[33] However, heterogenous populations exist with respect to mTBI patients, wherein some patients autoregulate but others do not.[33] This disturbance in autoregulation has been correlated with unfavourable outcomes.[34] There is evidence suggesting that the lower shoulder of the autoregulatory curve is shifted to the right following moderate to severe TBI .[21, 35] If this is the case, it would indicate that a higher cerebral perfusion pressure is required for a TBI patient

to maintain normal CBF and that these patients would not be able to tolerate hypotension. This is particularly damaging because there have been reports of an immediate transient hypertensive peak followed by sustained systemic hypotension following TBI in animal models.[35, 36] Moreover, it has been hypothesized that this sudden rise in arterial blood pressure often observed post-moderate to severe TBI can break through cerebrovascular autoregulation and even damage the arteriolar endothelium and cause endothelial dysfunction.[37]

Maintenance of adequate perfusion is imperative: a decrease in cerebral perfusion pressure (CPP) may induce brain ischemia while an increase in CPP can provoke brain edema in injured tissue.[38-40] Consequently, when cerebral autoregulation is disturbed following TBI, the brain may be uniquely vulnerable to the effects of secondary insults and less capable of maintaining an adequate CBF and a correct metabolic balance.

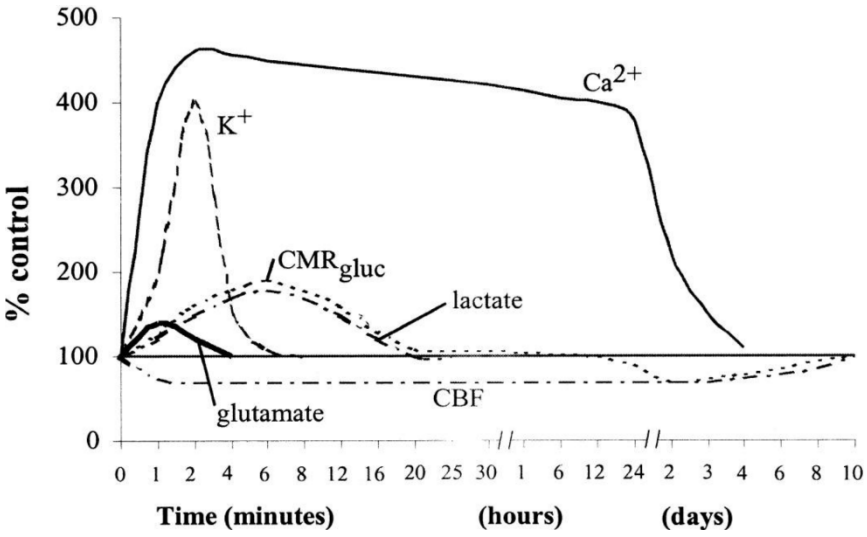

Figure 1: The neurometabolic cascade following experimental concussion. K+, potassium; Ca2+, calcium; CMRgluc, oxidative glucose metabolism; CBF, cerebral blood flow. Adapted from Hovda et al.[11]

Secondary Injury

Following the initial biomechanical injury and neurometabolic events that occur immediately following concussion, a variety of damaging processes occur in the subsequent hours and days that have been termed the “secondary injury”. The secondary injury is a delayed and a protracted period of further neurologic injury that is mediated by mechanisms such as excitotoxicity, oxidative stress, apoptosis, neuroinflammatory events, and further mitochondrial dysfunction (due to processes such as increased mitochondrial fission).[41, 42] The relationships among these diverse destructive mediators of the secondary injury enhance the neuronal damage and death processes that occur after TBI.[11] Thus, secondary injury cascades are responsible for the progression of pathophysiological processes after TBI.

Repetitive mTBI

Individuals who undergo mTBIs experience a variety of physical, cognitive, and/or behavioural consequences. While post-traumatic cellular processes are usually self-limiting and normally resolve themselves over the course of several weeks resulting in full neurologic recovery after mTBI, 15–30% of subjects develop prolonged neurocognitive and behavioral changes.[3, 41] Repetitive mTBIs (RmTBIs) in particular are increasingly recognized to have worse prognoses and long-term neurological sequelae in a significant proportion of patients.[41, 43] Longitudinal measurements of patients that have experienced RmTBI showed poor executive function, depression scores, and cognitive changes that were related to the number of injuries received.[44] The reason for the increased proclivity of people who suffer multiple concussions to experience protracted or non-resolving symptoms may be due to the increased vulnerability of the brain to a secondary injury in the immediate post-traumatic state where cellular metabolism is stretched to its limits. For example, during the period of CBF-glucose metabolism uncoupling,

any further demand in energy (due to increased ionic flux) or reduction in energy (due to impaired blood flow or reduced ATP synthesis) may tip the scale in favor of irreversible neuronal injury.[11] Injured cells may be capable of recovering after an initial injury, but a second concussion during this energy crisis can lead to cell death.[11] Further, increased Ca^{2+} levels from a second concussion may impair mitochondrial metabolism at the time when the cell can least tolerate a reduction in ATP production. This additional Ca^{2+} influx may go on to activate proteases that initiate programmed cell death.[11]

Consequently, this enhanced vulnerability to secondary injury may result in the exacerbation and prolongation of symptoms for individuals suffering from RmTBI. If symptoms persist for more than 3 months, the condition is referred to as post-concussive syndrome (PCS). Signs and symptoms of PCS include sleep disturbances, motor impairments, cognitive impairments (ex. poor attention, concentration, and short term memory), somatic symptoms (ex. headaches, nausea), and/or emotional symptoms (ex. emotional lability, depression, anxiety).[45] Interestingly, many of the symptoms experienced after concussion and in PCS overlap with symptoms seen in people who suffer from seizures and seizure disorders.[45, 46]

Seizures

Behavioural, clinical, or electrographic seizures are the transient occurrences of abnormally excessive or hypersynchronous neuronal firing in the brain.[47] Most seizures last in the range of several seconds to 2 minutes, and approximately 8-10% of people will experience one or more seizures at some point in their life.[48] The current standard of seizure classification and organization divides seizures into two main categories based on how onset occurs: generalized and focal.[49] Generalized seizures rapidly engage large, bilateral portions of the brain whereas focal seizures originate in a particular area in one hemisphere of the brain and may

subsequently spread to other regions. Seizures that invade different brain areas produce different signs and symptoms: a seizure in the motor cortex may produce muscle movements whereas a seizure in the occipital lobe may result in visual auras such as colourful shapes and lights.[50] The areas of the brain affected by a seizure can be determined through technologies such as electroencephalography (EEG), single-photon emission computed tomography (SPECT), and by evaluating ictal behaviours.[51] Areas of the brain involved in a seizure exhibit epileptiform discharges which can be visualized through an EEG trace. Similarly, the location of a seizure in the brain can be inferred by evaluating behaviours are exhibited when epileptiform discharges occur in certain areas of the brain. While the best-known type of seizure is the generalized tonic-clonic seizure that presents as a convulsive event with loss of awareness and synchronous, involuntary movement of the extremities, not all seizures involve motor effects, and some are indiscernible by observation alone at all.

While seizures are commonly associated with epilepsy, the occurrence of a seizure is not necessarily indicative of epilepsy. A single seizure provoked by acute events such as reaction to medication, electrolyte imbalance, spiking fever, and traumatic brain injury is generally not classified as epilepsy.[52] Instead, a diagnosis of epilepsy requires the spontaneous generation of recurrent, unprovoked seizures at least 24 hours apart. In the case of post-traumatic epilepsy (PTE; a condition wherein epilepsy manifests as a complication of traumatic brain injury), seizures that occur in the first week after TBI are considered to be provoked by the head injury, and a diagnosis of PTE requires one or more unprovoked seizures occurring at least a week after TBI.[53]

Seizure Induced Hypoperfusion/ Hypoxia

Farrell et al. used implantable oxygen-measuring probes to record the partial pressure of oxygen in a small localized area within the CA1 region of the hippocampi of awake, freely-moving rodents before, during, and after elicited and self-generated seizures.[54] They discovered a small drop in hippocampal oxygenation at seizure onset followed by a subsequent increase in oxygenation during the seizure. However, they found that oxygen levels in the areas of the brain involved in the seizure fell precipitously to below the severe hypoxic level (pO2 < 10 mmHg) for approximately an hour following seizure termination in a phenomenon now termed postictal hypoxia (PIH).[54] See Figure 2. The severity of postictal hypoxia was positively correlated with the duration of the seizure. Farrell et al. also used arterial spin labelling to quantify cerebral blood flow in the postictal period in people with epilepsy: compared to an interictal baseline, cerebral blood flow was significantly reduced in the first postictal hour postictal in brain areas that underwent seizure activity.[54]

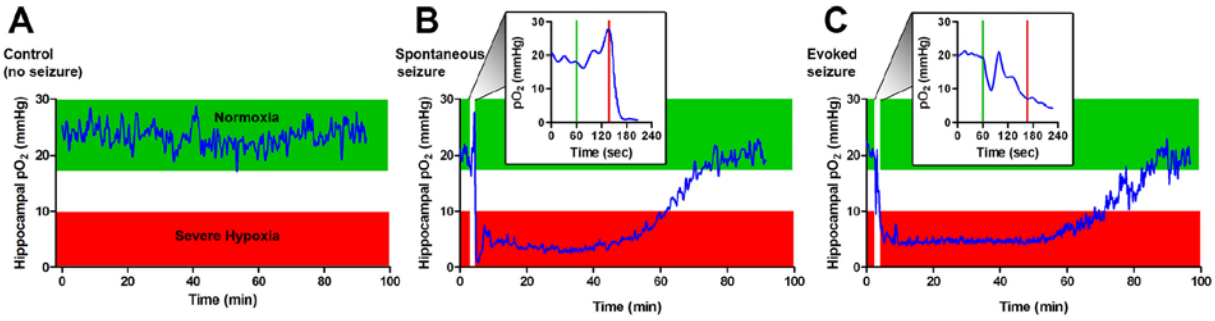

Figure 2: Seizures induce severe postictal hypoxia. (A) Local tissue oxygenation in the hippocampus of an awake, freely-moving rat (blue). Green denotes normoxia while red denotes severe hypoxia. (B) Representative oxygen profile before, during, and after a spontaneous seizure. The inset expands the time-scale during the seizure with the green and red lines denoting the beginning and end of an 80 s seizure. This inset corresponds to the white vertical block near

the beginning of the full oxygen recording. (C) Representative oxygen profile before, during, and after a 106 s electrically kindled seizure. Adapted from Farrell et al.[54]

The Mechanism of Postictal Hypoxia

The postictal hypoxic event discovered by Farrell et al. has been found to be mediated via local vasoconstriction and hypoperfusion. Blood flow was significantly reduced in the areas of the rat brain that exhibited postictal hypoxia, and this reduction was temporally linked to severe hypoxia. When hippocampal slices were stimulated to mimic the effects of a seizure, hippocampal arterioles underwent sustained constriction resulting in significant reductions in blood flow. This hypoperfusive/ hypoxic event was found to be mediated by cyclooxygenase-2 (COX-2) activity during seizures.[54] COX-2 is expressed post-synaptically by excitatory neurons and is responsible for the conversion of arachidonic acid, 2-arachidonoloylglycerol, and anandamide into prostaglandins. Prostaglandins in turn act on vasculature to produce vasoconstriction, thereby reducing blood flow.

Treatment prior to seizures with both selective (Celecoxib) and non-selective (Ibuprofen, Acetaminophen) COX-2 antagonists significantly attenuated postictal hypoxia, and mice with a genetic knockdown of COX-2 did not experience postictal hypoxia/hypoperfusion, despite having no alterations to seizure profiles in comparison to wildtype controls.[54] Consequently, it has been proposed that increased COX-2 activity during seizures leads to an increase in prostaglandin levels, resulting in arteriole constriction and subsequent localized hypoxia.[54]

Behavioural Consequences of Postictal Hypoxia

Following the termination of a seizure, many patients experience mild to severe sensory, cognitive, or motor deficits. Complex focal seizures can give rise to postictal confusion, amnesia, delirium, and motor deficits (for example, Todd’s paresis is focal weakness in a part of the body

after a seizure that is typically localized to either the left or right side of the body).[55-58] Postictal disturbances in mood are also common: 86% of patients experience postictal psychiatric symptoms which include feelings of hopelessness, anhedonia, frustration, irritability, and suicidal ideation.[59] Patients can experience postictal feelings of anxiety and fear, which include worrying, panicking, agoraphobia, and self-consciousness that can last for minutes to days after a seizure and post-ictal psychosis, which is characterized by delusions, paranoia, agitation, and an altered emotional state.[59] A large proportion of patients (82%) also experience postictal cognitive deficits.[59]

Until recently, it was thought that these postictal impairments were a direct result of the seizure itself. However, it has recently been proposed that these postictal deficits arise as a result of postictal hypoxia.[54] Neurons are generally the first cells in the brain to be affected by hypoxia, as they require extraordinary amounts of oxygen to maintain energetically expensive functions.[60] Neurons exposed to severe hypoxia for short periods of time will quickly be depleted of ATP, subsequently fail to maintain membrane ion gradients, and therefore lose the ability fire action potentials. Eventually, an influx of water, sodium ions, and calcium ions will cause the cell's membrane integrity to fail, following which the cell will undergo necrosis, a process that begins just minutes after the onset of hypoxia.[60]

Thus far, the role of PIH on two common postictal deficits have been interrogated in rats using kindled seizures and COX antagonists to extricate the effects of hypoperfusion/hypoxia from the effects of an afterdischarge.[54] Farrell et al. used the hanging bar test to assess motor weakness and the novel object recognition task to assay hippocampal dependent memory. Rats that had seizures (in the corpus callosum at the level of the forelimb area of the motor cortex) and experienced PIH displayed significantly worse motor facility after 40 minutes of being

hypoxic than rats that had seizures and had been pretreated with COX-2 blockers and therefore did not experience hypoxia.[54] Similarly, memory deficits exhibited by rats that were severely hypoxic following hippocampal seizures during the memory encoding phase of the novel object recognition task and were unable to form the memory, were prevented in rats that had seizures but had been had postictal hypoperfusion/ hypoxia blocked by the administration of an L-type calcium channel blocker that prevented vasoconstriction.[54] These two experiments seem to suggest that the severe hypoxia that follows a seizure, and not the seizure itself, is the underlying cause of postictal impairments.

Changes in Brain Oxygenation

Brain tissue oxygen levels are normally maintained through cerebral regulation of blood supply which ensures stable CBF across a large range of mean arterial pressures. Vascular smooth muscle can respond to changes in intravascular pressure and has a tendency to maintain vascular resistance and tone.[97] Energy supply and expenditures are also tightly coupled by neurovascular and neurometabolic factors.[97] Processes that expend energy, such as maintaining transmembrane ion gradients after action and postsynaptic potentials, neurotransmitter reuptake and recycling, and macromolecule synthesis, are neurovascularly coupled to blood flow.[97] Alterations in local perfusion arise as a result of/ in response to changes in neuronal activity either through local perfusion changes driven by the neurovascular unit (neurons, astrocytes, glia), or upstream endothelium dependent mechanisms.[98] Disruption of cerebral autoregulation resulting in a mismatch between the supply and demand of nutrients and oxygen resulting in either hypoxia or hyperoxia can have a plethora of detrimental effects.

Hypoxia

Under oxygenation of the brain in general gives rise to a myriad of problems: mild symptoms include difficulties with complex learning tasks and reductions in short-term memory. The work done by Farrell et al. discussed earlier highlights the deleterious effects that hypoxia has after seizures on functional outcomes.[54] Continued oxygen deprivation results in fainting, long-term loss of consciousness, coma, seizures, cessation of brain stem reflexes, and brain death. At a cellular level, the loss of oxygen causes depletion of energy stores leading to an inability of cells to maintain their ionic gradients, impairment of glutamate uptake, activation of degrading enzymes, and generation of free radicals and oxidative stress which ultimately lead to necrotic and apoptotic cell death and brain damage.[93]

Hyperoxia

While less often considered to be deleterious than hypoxia, there is mounting evidence from various clinical and experimental observations that hyperoxia, among other sources of oxidative stress, is an important trigger of brain injury. Hyperoxia-triggered mechanisms have been implicated in apoptosis, autophagy, changes in the expression of neurotrophins and growth factors, inflammation, and alterations in genes related to synaptic plasticity.[94] Moreover, hyperoxia can directly damage tissues via the production of reactive oxygen species (ROS) in excess of physiological antioxidant defence capabilities, leading to increased cell death by apoptosis and increased release of endogenous damage-associated molecular pattern molecules (DAMPs).[95]

Nifedipine and Bay K8644

Contraction of smooth muscle is initiated by a Ca^{2+}-mediated change in the thick filaments. In response to specific stimuli in smooth muscle, the intracellular concentration of

Ca^{2+} increases, and this activator Ca^{2+} combines with the acidic protein calmodulin. This complex activates myosin light chain kinase to phosphorylate the light chain of myosin, thereby causing muscular contraction and vasoconstriction in vascular smooth muscle. Cytosolic Ca^{2+} is increased through entry from the extracellular space through Ca^{2+} channels followed by Ca^{2+} release from intracellular stores (sarcoplasmic reticulum).[61]

Nifedipine

Nifedipine is a L-type calcium channel antagonist. It acts primarily on vascular smooth muscle cells by stabilizing voltage-gated L-type calcium channels in their inactive conformation.[62] By inhibiting the influx of calcium in smooth muscle cells, nifedipine prevents calcium-dependent myocyte contraction and vasoconstriction.[62]

Bay K8644

Bay K8644 is a L-type calcium channel agonist. It functions to increase the influx of Ca^{2+} through voltage-gated calcium channels by the opposite action as nifedipine, thereby enhancing calcium-dependent myocyte contraction and vasoconstriction.[63]

Animal Models of TBI

Findings using animal models are only as translationally meaningful as the relevance of the model to the human condition. More specifically, injury models must represent the etiological mechanisms (i.e., biomechanical forces) and pathophysiology of the injury. In the context of TBIs. A high velocity impact and head acceleration are key factors for injury generation and concussive-like outcomes.[64] To model this effectively, a simulation needs to impact a head that is permitted to move freely, and the impact must impart enough force to produce translational and rotational acceleration and deceleration of the head.[64, 65] Lastly, to

be therapeutically and pathophysiologically useful, the resulting symptomology must also be consistent with the symptoms reported by the clinical population, thereby recapitulating both the etiology and symptomology of the injury.

While several animal models of TBI have been developed, encapsulating a range of injury severities. Many of them produce injuries that are much more severe and often do not represent the etiological mechanisms or the pathophysiology of mild brain injury or concussions. Using a model that accurately replicates the conditions of mTBIs and concussions is imperative in unearthing a greater knowledge of the underlying mechanisms and neuropsychological outcomes of these common injuries.

Fluid Percussion Injury

In fluid percussion injury (FPI) models, the traumatic brain injury is delivered by a pendulum striking the piston of a reservoir of fluid to generate a fluid pressure pulse to the intact dura through a craniotomy, which is made either centrally around the midline, or laterally (LFPI) over the parietal bone, between bregma and lambda. The percussion produces a brief displacement and deformation of brain tissue, and the severity of injury depends on the strength of the pressure pulse.[66] The LFPI model is one of the most widely used TBI animal models. In rats, LFPI produces a combination of focal cortical contusion and diffuse subcortical (such as hippocampus and thalamus) neuronal injury, which occurs within minutes of the impact, progresses to a loss of neurons by 12 h, and does not markedly expand into other brain regions by 7 days post injury (figure 3).[67] The contused cortex beneath the injury site enlarges over weeks to become a cavity lined with glia and continues to expand up to one year post-injury due to ongoing cell death. LFPI produces neurobehavioural and cognitive deficits such as difficulties with movement and memory that are commonly seen in patients with TBI.[66] Cognitive

dysfunction and neurological impairments persist for more than a year following severe LFPI.[66] Recent advancements in the FPI apparatus has allowed for increased biomechanical control of the insult, with impact pressure and dwell time precisely controlled through a microprocessor.[66] However, FPI models have high mortality compared with other models, probably due to the brainstem-compromised prolonged apnea.

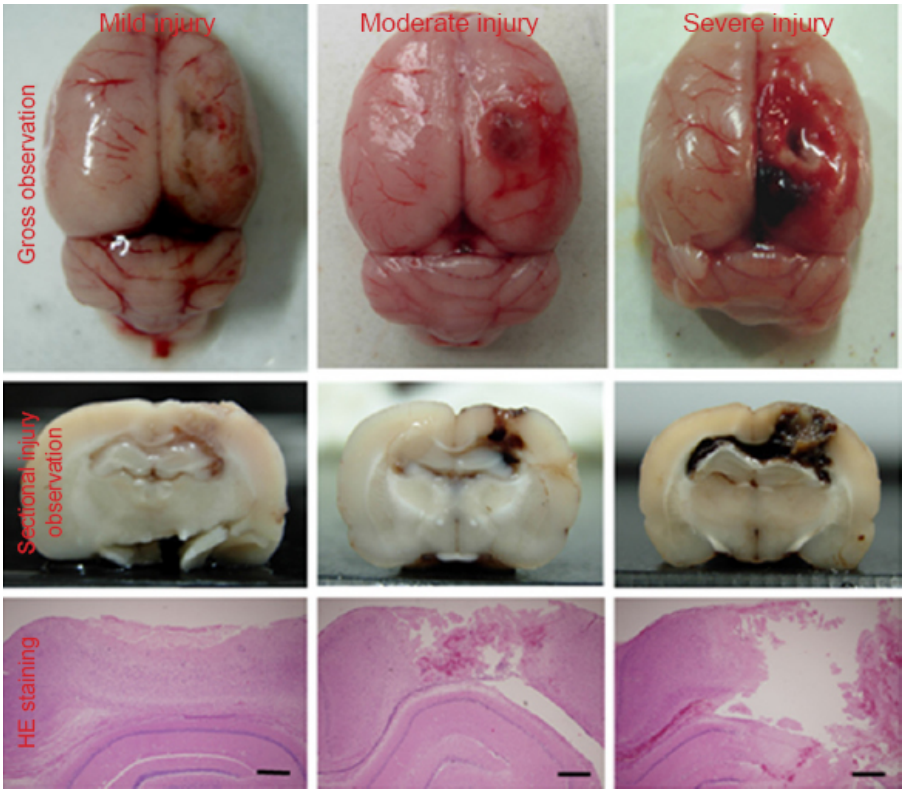

Figure 3: Pathomorphological changes in the rat brain following different degrees of traumatic brain injury induced by fluid percussion. Forty-eight hours after the fluid percussion experiment, gross observation, sectional injury observation, and hematoxylin-eosin (HE) staining displayed obvious differences in the degree of injury between the different experimental groups. Scale bars: 500 μm. Adapted from Lin et al.[67]

Controlled Cortical Impact

The controlled cortical impact (CCI) model uses a pneumatic or electromagnetic impact device to drive a rigid impactor onto the exposed intact dura, and mimics cortical tissue loss, acute subdural hematoma, axonal injury, concussion, blood–brain barrier (BBB) dysfunction and even coma. The controlled impact is delivered to the intact dura through a unilateral craniotomy most often between bregma and lambda, causing deformation of the underlying cortex. Neuropathological evaluations of the CCI TBI model has reported that the associated damage can be widespread, including acute cortical, hippocampal and thalamic degeneration. The advantage of this injury model over other TBI models is the ease at which mechanical factors, such as time, velocity and depth of impact, can be controlled.

Weight Drop and Lateral Impactor

Marmarou et al. developed one of the only models of DAI — Marmarou's impact acceleration model — that mimic human diffuse mTBI caused by falls or motor vehicle accidents.[68] The trauma device consists of a weight set that falls freely from a designated height through a Plexiglas tube. In anaesthetized rats with skull exposure made by a midline incision, a stainless steel disc is mounted with glue to the skull midline between lambda and bregma to prevent skull fracture. The rats are then placed on a foam bed and subjected to the impact by dropping the weight onto the stainless steel disc.[68] The Marmarou model causes widespread and bilateral damage of neurons, axons, dendrites, and microvasculature as well as extensive DAI, particularly in the corpus callosum, internal capsule, optic tracts, cerebral and cerebellar peduncules, and the long tracts in the brainstem.[68] It also induces motor and cognitive deficits such as difficulties with beam walking and memory.[68] However, a key

disadvantage of weight-drop models is the relatively high variability in injury severity and injury location.

A recent innovative modification to the weight drop model – the lateral impactor – involves placing a lightly anesthetized rat in the prone position on a Teflon® board with the left side of the head facing the lateral impactor device. A cylindrical weight is propelled towards the head using a pneumatic air compressed barrel. The weight makes impact with a small aluminum plate placed against the rat's head. The purpose of this plate is to reduce the risk of bone or skull damage while still ensuring that the injury is produced via the application of rotational, acceleration, and deceleration forces to the rat's head.[69]

Summary

Although the FPI and CCI models have been successful in delivering controlled, highly reproducible injuries, the need for a craniotomy and the focal nature of the lesions they generate limit their relevance when studying mTBIs. We chose to use the lateral impact model because of the etiological similarity between the injury generated from the impact delivered by the lateral impactor and those seen in the human condition. Just as human concussions and mTBIs most often occur when the head and body are unrestrained, the skull remains intact, and there is minimal loss of consciousness, the lateral impactor delivers an injury when an animal's head and body are permitted to move freely, the skull is intact and unexposed, and the animal is only lightly anesthetized.[5] Further, the lateral location of the impact allows for the pre-injury implantation of probes on the dorsal surface of the rat's head without impeding the delivery of the injury.

Aims and Hypotheses

Concussions and PCS are characterized by headaches, dizziness, balance or motor disturbances, decreased concentration, memory problems, depression, anxiety, deficits of executive function, and sleep disturbances.[65] Most of these symptoms share a significant overlap with those displayed by people with epilepsy after they have a seizure.[46] Recently, these postictal deficits have been traced to a seizure-triggered vascular event that causes dramatic reductions in brain oxygenation.[54] We aimed to explore if the shared symptomology between TBIs and postictal events arise as a result of shared pathophysiology.

Aim 1: Investigating electrographic phenomena in the acute period after mTBI

The unique characteristics of the lateral impact model of mTBIs – namely the closed head, light anesthesia, and lateral impact location – allow for the pre-traumatic implantation of depth electrodes into the brain. This permits us to investigate post-traumatic EEG in the immediate and acute phase of the injury, which has not previously been accomplished. We hypothesize that a portion of mTBIs will be proceeded by electrographic events in the immediate to acute post-traumatic phase. These events will have not previously been recorded because of the difficulty in obtaining EEG signals that early after trauma in other animal or human investigations.

Aim 2: Investigating changes in hippocampal oxygen after mTBI

The same advantages of the lateral impact model outlined in Aim 1 also permit for the implantation of oxygen sensing probes that will allow us to measure hippocampal oxygen from immediately after to hours after trauma. Changes in brain oxygenation have been recorded in the hours to days time period after a variety of other insults such as strokes and severe TBIs, but this phenomena has only sporadically been reported in TBI patients.[33, 70] We hypothesize that a

subset of animals who undergo a TBI will exhibit long lasting changes in brain oxygen that will become exacerbated as the number of injuries accumulates. Further we hypothesize that the animals that display alterations in brain oxygenation will be the same subset of animals that demonstrate EEG abnormalities.

Aim 3: Characterize the behavioural sequelae of EEG and brain oxygenation abnormalities

Previous studies have demonstrated that changes, both increases and decreases, in brain oxygenation are deleterious for behavioural function, including learning and memory, and plasticity, and have been extensively attributed to neurodegeneration pathology (Farrell, Watts).[54, 71] Similarly, the existing EEG literature is replete with reports of abnormalities in association with different neuropsychiatric disorders, and even in individuals with epilepsy, interictal (that is, between seizures) EEG abnormalities may be associated with neuropsychological impairments.[72, 73] Consequently, we hypothesize that rats that demonstrate EEG and brain oxygen changes will demonstrate behavioural deficits consistent with those reported by people who suffer from concussions.

Chapter 2: Post-traumatic seizures and changes in brain oxygen contribute to post-traumatic behavioural deficits.

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Abstract

Repetitive mild traumatic brain injuries (RmTBIs) are increasingly recognized to have long-term neurological sequelae in a significant proportion of patients. Individuals that have had RmTBIs exhibit a variety of physical, cognitive, or behavioural consequences that can negatively impact quality of life. Brain tissue oxygen levels are normally maintained through exquisite regulation of blood supply. However, during neurological events that result in alterations to brain tissue oxygen levels, neuronal dysfunction, brain damage (neuronal loss, astrocyte hypertrophy), and behavioural deficits have been observed, and are frequently related to poorer prognoses. The oxygenation response in the brain aftermild TBIs or concussions have been poorly characterized, with most preliminary research limited to the cortex. Furthermore, the mechanisms by which traumatic brain injuries impact changes to brain oxygenation and vice versa remain unclear. In the current study we demonstrate that upon receiving RmTBIs, rats exhibit post-traumatic, electrographic seizures that are accompanied by a long-lasting period of hippocampal hyperoxygenation. These seizures and the ensuing hyperoxic episodes are associated with deficits in working memory and motor coordination that are reversible through attenuation of the hyperoxia via administration of a calcium channel agonist, Bay K8644. We propose that the post-traumatic, postictal period of altered brain oxygenation is the basis for some of the common symptoms associated with mTBIs.

Introduction

Traumatic brain injures (TBIs) are a set of injuries that involve an insult to the brain through the application of an external mechanical force to the head or body that result in neuropathologic damage and dysfunction.[1] Of all severities of TBI, an estimated 75–85% are categorized as mild TBI (mTBI), which includes concussions.[2] mTBI differs from moderate or severe TBI in that no gross structural damage (such as focal lesions or macroscopic damage) can be identified when using conventional brain imaging techniques, leading many to call concussion a functional rather than structural injury. In the US, it is estimated that 1.6–3.8 million concussions occur annually, but the true frequency of concussion is likely far greater given that concussions are routinely under-recognized, underreported, and typically resolve spontaneously without medical care.[2]

Individuals who undergo mTBIs experience a variety of physical, cognitive, or behavioural consequences, with RmTBIs generally being related to worse prognoses.[42] Longitudinal measurements of patients that have had RmTBIs showed poor executive function, depression scores, and cognitive changes that were related to the number of injuries received.[3] While mTBI-induced post-traumatic cellular processes are usually self-limiting and normally resolve themselves over the course of several weeks, 15–30% of individuals develop prolonged neurocognitive and behavioral changes referred to as post-concussive syndrome (PCS).[4, 5] RmTBIs in particular are increasingly recognized to increase risk for PCS and have long-term neurological sequelae in a significant proportion of patients.[5, 6]

Determining the pathophysiological underpinnings of the symptoms of concussion is a major focus of mTBI research. While animal models of mTBI have elucidated a metabolic cascade of events in the acute phase following injury, it remains unclear how these metabolic

events cause the behavioural deficits identified after injury.[7] This lack of understanding regarding the etiology of these deficits means that we also currently lack prognostic tools to effectively distinguish between individuals that will recover without incident and those that will go on to suffer from lingering symptomology in PCS.

Although there have been several studies assessing electrical brain activity in the subacute to late period after a mTBI, very few studies have investigated brain activity immediately after such an injury.[8] While there are no published studies of human EEG during the actual injury event, Dow et al. recorded EEG as soon as 10 – 15 minutes after a closed head injury and found that some individuals had diffuse slowing that resolved quickly (ex. within an hour), especially in those recordings made the soonest after injury.[8] To our knowledge, no studies have looked at correlations between abnormal EEG and neurologic exam scores in the acute phase following a mTBI. However, examination of EEG abnormalities in the initial months following a mTBI demonstrated that, while relatively few (23%) abnormal EEGs were accompanied by abnormal neurologic exam signs, most patients (86%) with abnormal neurologic exam signs had an abnormal EEG.[9]

Considering that most symptoms of mTBI resolve in the months following the injury, it is interesting that many symptomatic patients exhibit abnormal EEG patterns at later time points. This may indicate that underlying changes in brain activity that manifest as EEG abnormalities may be related to mTBI symptomology. The lack of observation of brain activity and simultaneous assessment of symptomology in the acute phase of mTBI presents a gap in the literature that may shed more light on the relationship between electrophysiologic abnormalities and behavioral dysfunction after mTBI.

It has previously been hypothesized that the loss of consciousness and other functional impairments that follow mTBIs are due to a brief episode of vascular dysfunction or dysregulation.[41] However, the mechanisms triggering this vascular event are uncertain. Previous studies have shown region dependent changes in cerebral blood flow following mTBI.[10, 11] Therefore, post-traumatic changes in brain activity could drive changes in vascular supply to the brain through the complex connections between neurons, astrocytes, endothelial cells of the blood brain barrier, and the other cells that comprise the neurovascular unit. Consequently, we sought to examine EEG characteristics throughout the delivery of RmTBI and evaluate corresponding changes in brain oxygenation and behavioural function in the acute phase after the injury.

Male Wistar rats were chronically implanted with a bipolar electrode and an oxygen sensing probe (optrode) in the ventral and dorsal hippocampus, respectfully. The rats then received 3 mTBIs or sham injuries, over 5 days using our lateral impact device.[69] Immediately following induction of the third and final injury (15 minutes post-injury), behavioral outcomes were measured using behavioral tasks designed to examine motor and cognitive symptoms consistent with clinical signs of mTBI. If the post-traumatic hyperoxia was due to a vascular event, then altering calcium conductance should change vascular diameter and alter the oxygen status of rats post-trauma. BAY K8644, an L-type calcium channel agonist, was used to induce vasoconstriction in effort to attenuate the post-traumatic hyperoxic period.[13] These experiments were used to evaluate the relationship between post-traumatic oxygen status and behavioural function.

For the purposes of this paper, hippocampal "hyperoxia" was defined as oxygen levels exceeding 3 standard deviations above the mean hippocampal pO_2 of an awake, freely moving rat. This was approximately 30 mmHg, which we have termed the "hyperoxic threshold".

Methods

Animals

Young adult male Wistar rats weighing between 250 – 350 g at the start of experimentation were used in this study (Charles River, Canada). Rats were housed individually in clear plastic cages and were maintained on a 12:12 hr light/dark cycle lights on at 07:00 hr. Food and water were available *ad libitum*. All experimental procedures occurred during the light phase. The experiments and procedures were approved by the University of Calgary Conjoint Facilities Research Ethics Board and conducted in accordance with the Canadian Council of Animal Care.

Implant procedure

Electrodes were constructed from Teflon-coated, stainless steel wire, 178 µm in diameter (A-M Systems, Sequim, WA). Wire ends were stripped of Teflon and connected to gold-plated male amphenol pins. Rats were anaesthetized with a 5% isoflurane, and maintained between 1% and 2%. Lidocaine (2%) was administered subcutaneously at the incision site. One bipolar electrode was chronically implanted under stereotaxic control in the ventral hippocampus.[74]

Oxygen recordings were obtained using an implantable fiber-optic oxygen-sensing device. Light pulses (525 nm) through a fiber-optic cable excite a platinum fluorophore at the tip of the probe, inducing fluorescence (measured at 650 nm) that is quenched by oxygen within a local area (~500 µm3). Fluorescence decay time can then be used to derive the partial pressure of

O_2 (pO_2).[96] pO_2 is significant because it is directly related to how much oxygen is present in tissue or blood, thereby representing their oxygenation levels. The technology (Oxylite, Oxford Optronics, United Kingdom) does not consume oxygen while measuring absolute pO_2 values. The manufacturer individually calibrates each biologically inert probe, called an optrode. The implantable system was designed by Jeff F. Dunn in collaboration with Oxford Optronics. The implant was chronically implanted under stereotaxic control in the dorsal hippocampus. We allowed five days between implantation and initiation of measurements to ensure that the effects of acute trauma were minimized. pO_2 measurements at 1 Hz were then be made by connecting the implant to the Oxylite using an extension fiber optic lead. The probe provides accurate and continuous measurements of local pO_2 levels in brain tissue in awake, freely moving animals.

The implants were adhered and anchored to the skull using dental cement and four stainless steel screws. One of the four screws served as a ground electrode. Subsequent experimental procedures commenced no earlier than five days following surgery.

Table 2: Stereotaxic coordinates for surgical implantation.

Sensor	Location	Anterior (+)/ Posterior (-)	Lateral Right (+)/ Left (-)	Ventral (from brain surface)
Electrode	Rat ventral hippocampus	-5.6 mm	+5.2 mm	-7 mm
Optrode	Rat dorsal hippocampus	-3.5 mm	+3.5 mm	-3.5 mm

RmTBI procedure

RmTBIs were administered using our lateral impact device as described previously.[65] Rats were subjected to three injury or sham procedures spaced one day apart. On injury days, rats were connected to the EEG and oxygen sensing system and allowed 15 minutes to adjust before taking a 10 minute baseline recording of both metrics. They were then anesthetized using

inhalant isoflurane gas until they were no longer responsive to a toe pinch (~30 s) before being placed in flexible plastic restraint bag. Next, they were placed in the prone position on a Teflon® board with the left side of the head facing the lateral impactor device. A 50-g cylindrical weight was propelled towards the head at approximately 12 m/s using a pneumatic air compressed barrel resulting in linear acceleration of the rat's head of 125 Gs. [75] The weight made impact with a small aluminum plate placed against the rat's head. The purpose of this plate was to reduce the risk of bone or skull damage while still ensuring the production of rotational, acceleration, and deceleration forces. While previous studies using this RmTBI protocol have involved lesser impactor velocities (~7-8 m/s), the animals used in those experiments were younger and therefore smaller (~postnatal day 40, and 120g) at the time of the injury. We increased the speed of the impactor due to the increased size and weight of the animals used in this study. The larger sized animals need a greater force imparted during the impact to generate the same extent of acceleration ($\vec{F} = m\vec{a}$, or rearranged, $\vec{a} = \vec{F}/_{m}$). Furthermore, increases in skull thickness as the animals aged mitigated concerns of skull fracture at the increased impact velocity. Immediately after delivery of the injury, rats were reconnected to the EEG and oxygen sensing system, and recorded for a further 30 minutes.

Drug Delivery

Rats implanted with ventral hippocampal electrodes and dorsal hippocampal optrodes were used in this study. Bay K8644 was obtained from Sigma-Aldrich and dissolved in 100% DMSO. Drug administration (1 mg/ kg) was done intraperitoneally immediately before or after delivery of the third traumatic brain injury.

Behavioural Testing

Behavioural tests were chosen to model two symptoms commonly associated with mTBI. Fifteen minutes following delivery of the final mTBI, rats completed the novel context mismatch task to assess short-term working memory deficits, immediately followed by the beam-walk task to measure motor deficits, namely loss of balance and coordination.

Novel Context Mismatch (NCM)

The NCM has been used a measure of short-term working memory.[76] On PID 3-4 rats were placed in two different contexts (Context A and B) for 5 min each, one context immediately preceding the other. Context A was a hexagonal, black bin in a dark room containing two identical objects. Context B was a square, white bin in a brightly lit room containing a different pair of identical objects. On PID5, 15 minutes after delivery of the third injury, rats were exposed to a probe trial; Context A (5 min) → Context B (5 min) → home-cage (5 min) → Novel Context (5 min). The novel context consisted of a modified Context A, containing one object from context A and one object from context B (the novel object). Exploration of the novel context was videotaped, and a research associate recorded the amount of time each rat spent investigating the novel object and the old object. All of the objects and context containers were cleaned with Virkon® between each testing session.

Beamwalk

Following completion of the novel context mismatch task, rats were placed in their home cage for 5 minutes before undertaking the beamwalk task. This procedure was designed to assess the balance and motor function impairments that are often seen following concussion.[77] Rats were placed at one end of a 165 cm long tapered beam with the wide end at the start and the narrower end placed in their home cage. The beam was suspended between two platforms

approximately 1 m off the ground and had 2 cm ledges that catch the hind legs if the rat slipped off the central portion of the beam. Each rat underwent 1 unscored pre-training trial. The following 4 trials were videotaped and scored. Hind leg foot slips were scored every time the rat used the safety ledge with the rear foot while moving across the beam. The beam was cleaned with Virkon® between each rat.

Statistics

EEG power was computed using Spike 2 version 7 from Cambridge Electronic Design Limited. Statistical analysis of EEG power was computed using one way ANOVAs with seizure status as the investigated factor. Statistical analysis for oxygen curves was completed using one-way ANOVAs with seizure status as the investigated factor. Statistical analysis for behavioural analysis was completed using three-way ANOVAs with injury (RmTBI; sham), seizure (seizure; no-seizure) and Bay K8644 (Bay K8644; no Bay K8644) as factors. Post hoc follow-up pairwise comparisons were conducted where applicable. All statistical analyses were conducted using SPSS 20.0 for Mac and considered significant if $p < 0.05$. For all graphs means are displayed ± standard error. As there were no significant differences between rats that received Bay K8644 immediately before or immediately after the injury, the groups were combined for analyses.

Results

Implant Location

To ensure that the implantation of the electrode was done correctly, animals were sacrificed, and their brains extracted. A razor was used to coronally slice the brain along the electrode tract (figure 4). Gross observation of the tract reveals that the electrode tip was placed in the ventral hippocampus.

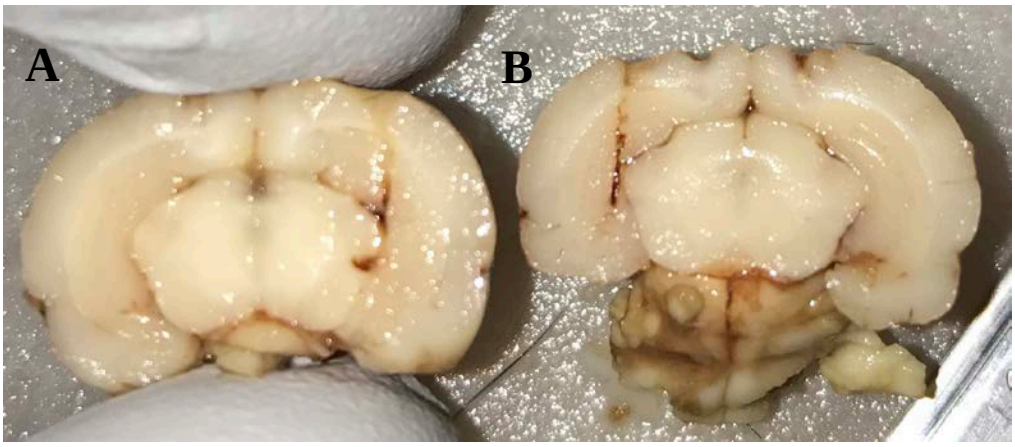


Figure 4: Coronal slice of a brain along the plane of the electrode tract. A) Section of the brain anterior to the electrode tract. B) Section of the brain posterior to the electrode tract.

Post-Traumatic EEG

To evaluate hippocampal activity in the acute period immediately following RmTBI, we recorded EEG from the ventral hippocampus before, immediately after, and 60 minutes following the injuries. After delivery of each mTBI, a proportion of rats demonstrated non-convulsive, electrographic seizures (figure 5B) within the first 30 seconds post-trauma, that we have termed immediate, post-traumatic seizures. These seizures lasted between 15 to 45 seconds in length. As the number of injuries accumulated in the RmTBI protocol, the proportion of rats that demonstrated these immediate, post-traumatic seizures increased (figure 6). In the hour following the trauma, some rats also exhibited interspersed bouts of seizure activity not immediately following the trauma, which we have termed delayed seizures (figure 5C). Following the trauma, EEG activity recorded from the hippocampus seemed to decrease in amplitude (figure 5D). We conducted a power analysis of the EEG signal before and 3 minutes after trauma, which appeared to show a dramatic reduction in power across the evaluated spectrum in rats that exhibited a seizure after trauma (figure 7). Decomposition and quantification of the EEG signal into the functionally distinct frequency domains, delta (0–4 Hz),

theta (4–8 Hz), alpha (8–12 Hz), beta (12–30 Hz), and gamma (30–100 Hz), confirmed a significant post-traumatic reduction of band power across the theta, alpha, beta, and gamma domains in rats that exhibited a seizure after trauma (figure 7C). While it appears that rats that did not have a seizure also showed a reduction, although less dramatic than that did display a seizure, of EEG power, the difference was not significant (figure 7C).

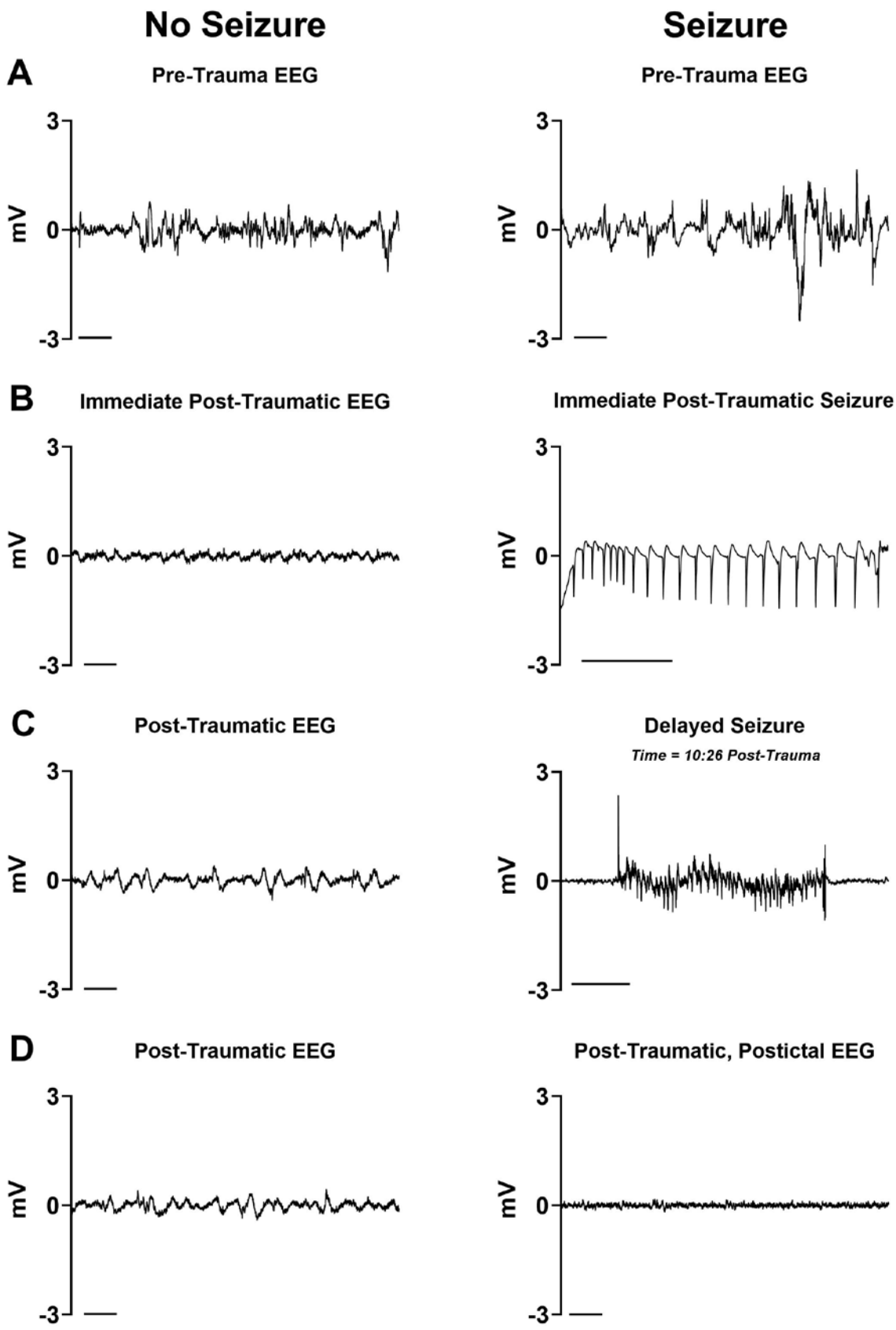

No Seizure
Seizure
A
Pre-Trauma EEG
Pre-Trauma EEG
B
Immediate Post-Traumatic EEG
Immediate Post-Traumatic Seizure
C
Post-Traumatic EEG
Delayed Seizure
Time = 10:26 Post-Trauma
D
Post-Traumatic EEG
Post-Traumatic, Postictal EEG
3
0
-3
mV

Figure 5: Trauma induced seizures and subsequent EEG quiescence. A) Representative EEG tracings from naïve rats before delivery of any traumatic brain injuries. B) Some rats demonstrate non-behavioural, electrographic, high amplitude, low frequency seizures within the first 30 seconds after trauma that last for approximately 15-45 seconds. C) In the 24 hours following trauma spontaneous epileptiform activity arose in rats that demonstrated immediate post-traumatic seizures, which we have termed delayed seizures. D) While all rats demonstrate reduced EEG activity post-trauma, rats that demonstrate immediate post-traumatic seizures also show increased quiescence in the post-traumatic period. All scale bars represent 5 seconds.

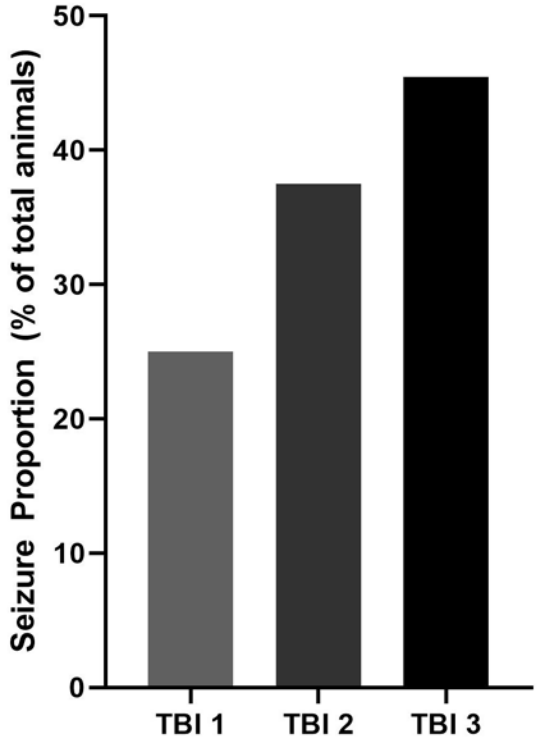


Figure 6: As the number of traumas accumulates, the proportion of rats that demonstrate the type of seizure shown in Figure 4B also increases.

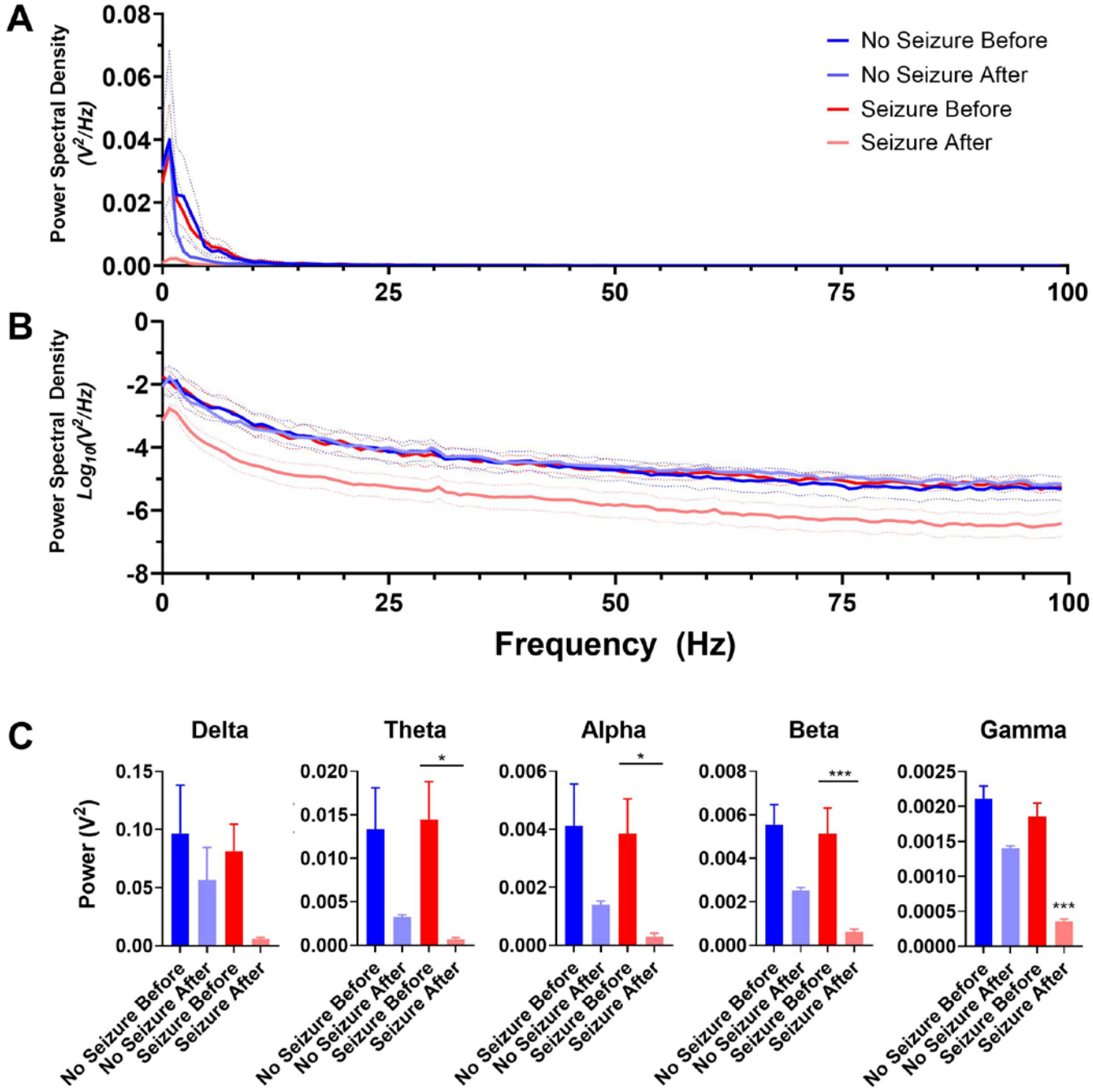


Figure 7: Immediate post-traumatic seizures result in decreased post-traumatic EEG power. A) Signal power distribution along the range of evaluated frequencies before and after trauma. The "no seizure before" curve is the analysis of pre-traumatic EEG from rats that did not go on to

have an immediate post-traumatic seizure. The “no seizure after” curve is the analysis post-traumatic EEG from those same rats. Similarly, the “seizure before” group is the analysis of pre-traumatic EEG from rats that did have an immediate post-traumatic seizure, and the “seizure after” is the post-traumatic EEG analysis of those same rats after they’ve undergone an mTBI and had a seizure. B) Log transformation of 3A. C) The power spectrum was separated and quantified in bins corresponding to brain wave frequencies: delta (0–4 Hz), theta (4–8 Hz), alpha (8–12 Hz), beta (12–28 Hz), and gamma (28–100 Hz). Error bars represent ± standard error. *p<0.05 **p<0.01 ***p<0.001. No Seizure Before (n=5), No Seizure After (n=5); Seizure Before (n=7), Seizure After (n=7).

Table 3: Summary of significant statistical results from the one-way ANOVAs of EEG power.

Frequency Domain	**Effect of Group**	***Post Hoc: Seizure Before***	***Post Hoc: Seizure After***
	F(p)	**(p)**	**(p)**
Delta	3.36(<.05)	*Seizure After* (.096)	*Seizure Before* (.096)
		No Seizure Before (.971)	*No Seizure Before* (.049)
		No Seizure After (.906)	*No Seizure After* (.469)
Theta	5.54(<.001)	*Seizure After* (.013)	*Seizure Before* (.013)
		No Seizure Before (.994)	*No Seizure Before* (.034)
		No Seizure After (.122)	*No Seizure After* (.939)
Alpha	5.34(<.001)	*Seizure After* (.0276)	*Seizure Before* (.028)
		No Seizure Before (.996)	*No Seizure Before* (.016)
		No Seizure After (.293)	*No Seizure After* (.803)

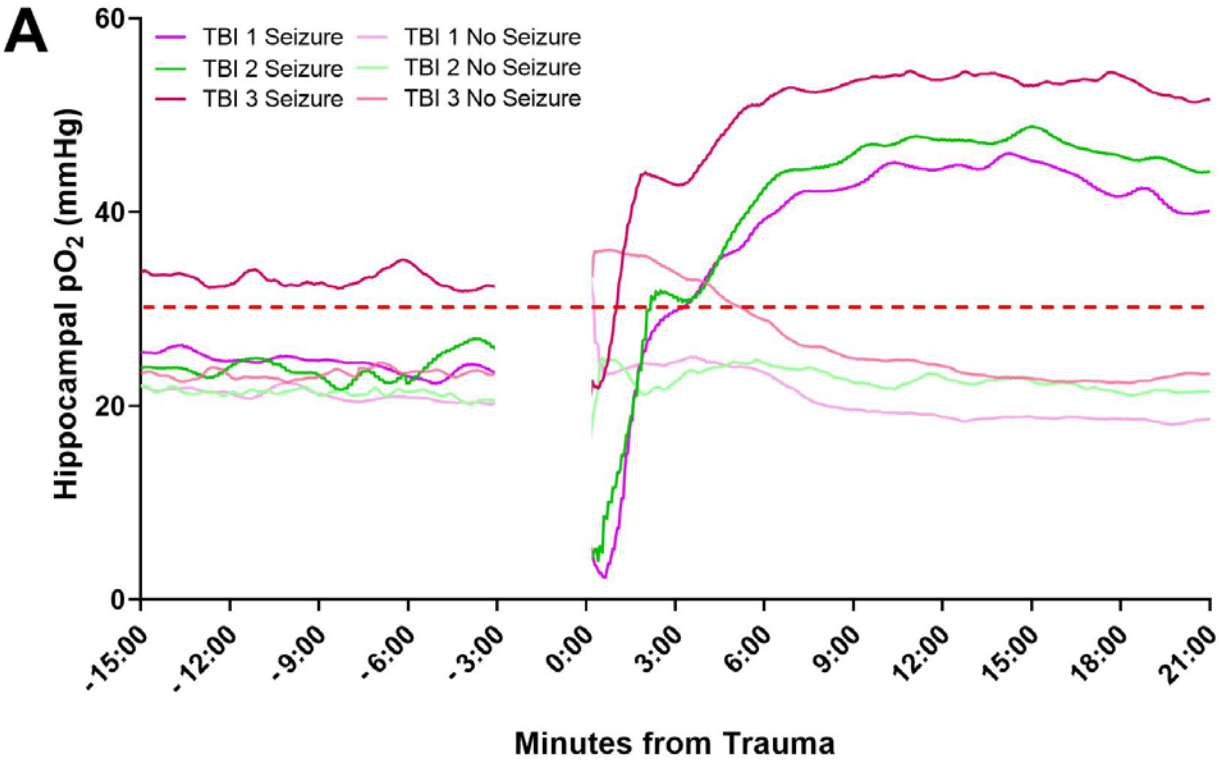
Beta	13.29(<.001)	*Seizure After* (.001)	*Seizure Before* (.001)
		No Seizure Before (.975)	*No Seizure Before* (<.001)
		No Seizure After (.106)	*No Seizure After* (.240)
Gamma	47.41(<0.001)	*Seizure After* (<.001)	*Seizure Before* (<0.001)
		No Seizure Before (.516)	*No Seizure Before* (<.001)
		No Seizure After (.133)	*No Seizure After* (<.001)

**No Seizure Before (n=5), No Seizure After (n=5); Seizure Before (n=7), Seizure After (n=7).*

Post-Traumatic Oxygen

Oxygen was recorded from the dorsal hippocampus simultaneously with EEG over the course of the RmTBI protocol. A pre-trauma baseline was recorded before delivery of the trauma and acute post-traumatic oxygen monitoring. After trauma, rats who did not have a seizure exhibited a slight, acute elevation in brain oxygen before returning to below the hyperoxic threshold. Rats who did have a seizure demonstrated an acute period of reduced hippocampal oxygenation immediately after delivery of the mTBI that seemed to temporally correspond to the seizure followed by a subacute period of hyperoxia (figure 8). Oxygen recordings were then divided into 1-minute long bins (figure 8B). The difference between hippocampal oxygenation between seizure and no seizures groups became statistically significant after approximately 6 minutes post-trauma for all TBIs. The hyperoxic period seemed to resolve after the first trauma (on day 1) over the course of the following 48 hours, resulting in a return to baseline by the time the second trauma was due to be delivered (on day 3) (figure 8B). However, following delivery of the second trauma, hippocampal oxygenation remained elevated above the hyperoxic

threshold until the baseline for the third mTBI was recorded 48 hours following the second mTBI (figure 8B).


A
TBI 1 Seizure
TBI 1 No Seizure
TBI 2 Seizure
TBI 2 No Seizure
TBI 3 Seizure
TBI 3 No Seizure
Hippocampal pO2 (mmHg)
60
40
20
0
-15:00
-12:00
-9:00
-6:00
-3:00
0:00
3:00
6:00
9:00
12:00
15:00
18:00
21:00
Minutes from Trauma

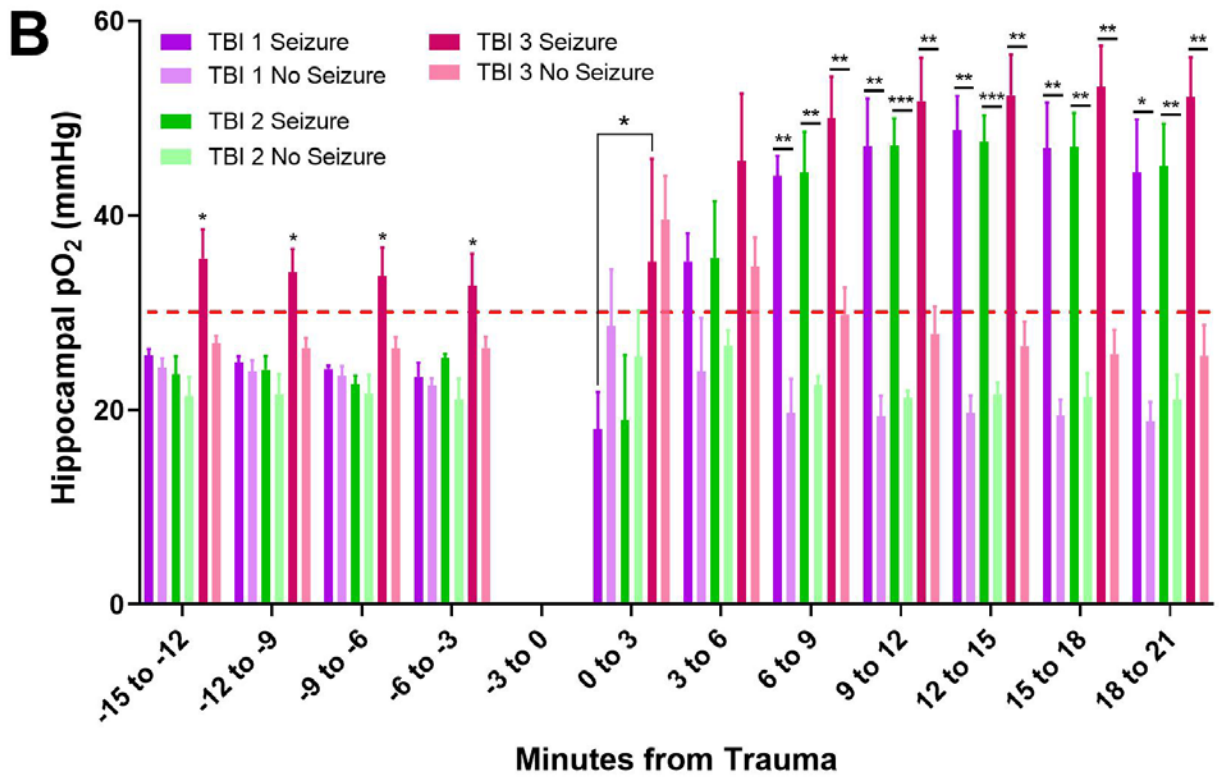

B
TBI 1 Seizure
TBI 1 No Seizure
TBI 2 Seizure
TBI 2 No Seizure
TBI 3 Seizure
TBI 3 No Seizure
Hippocampal pO2 (mmHg)
60
40
20
0
-15 to -12
-12 to -9
-9 to -6
-6 to -3
-3 to 0
0 to 3
3 to 6
6 to 9
9 to 12
12 to 15
15 to 18
18 to 21
Minutes from Trauma

Figure 8: Rats who have post-traumatic seizures demonstrate a different brain oxygen trajectory than rats who undergo a traumatic brain injury but do not have a seizure. A) Hippocampal brain oxygen through each of the mTBIs in the injury protocol. B) Each of the oxygen traces in figure 4A sorted and quantified into 3 minute long bins. Error bars represent ± standard error. The dashed red line at 30 mmHg represents the hyperoxic threshold. *p<0.05 **p<0.01 ***p<0.001. TBI 1 Seizure (n=5), TBI 1 No Seizure (n=6); TBI 2 Seizure (n=5), TBI 2 No Seizure (n=6); TBI 3 Seizure (n=6), TBI 3 No Seizure (n=5)

Table 4A: Summary of significant statistical results from the one-way ANOVAs of seizure groups between TBIs

Time Bin	**-15 to -12**	**-12 to -9**	**-9 to -6**	**-6 to -3**
Comparison				
F(p)	9.75(<0.05)	11.49(<0.05)	11.61(<0.05)	5.74(<0.05)
***Post Hoc* (p)**				
	TBI 1 x TBI 2: (0.80)	*TBI 1 x TBI 2:* (0.94)	*TBI 1 x TBI 2:* (0.81)	*TBI 1 x TBI 2:* (0.79)
	TBI 1 x TBI 3: (0.03)	*TBI 1 vs TBI 3:* (0.02)	*TBI 1 x TBI 3:* (0.02)	*TBI 1 x TBI 3:* (0.04)
	TBI 2 x TBI 3: (0.01)	*TBI 2 x TBI 3:* (0.01)	*TBI 2 x TBI 3:* (0.01)	*TBI 2 x TBI 3:* (0.10)

**TBI 1 Seizure (n=5), TBI 1 No Seizure (n=6); TBI 2 Seizure (n=5), TBI 2 No Seizure (n=6); TBI 3 Seizure (n=6), TBI 3 No Seizure (n=5)*

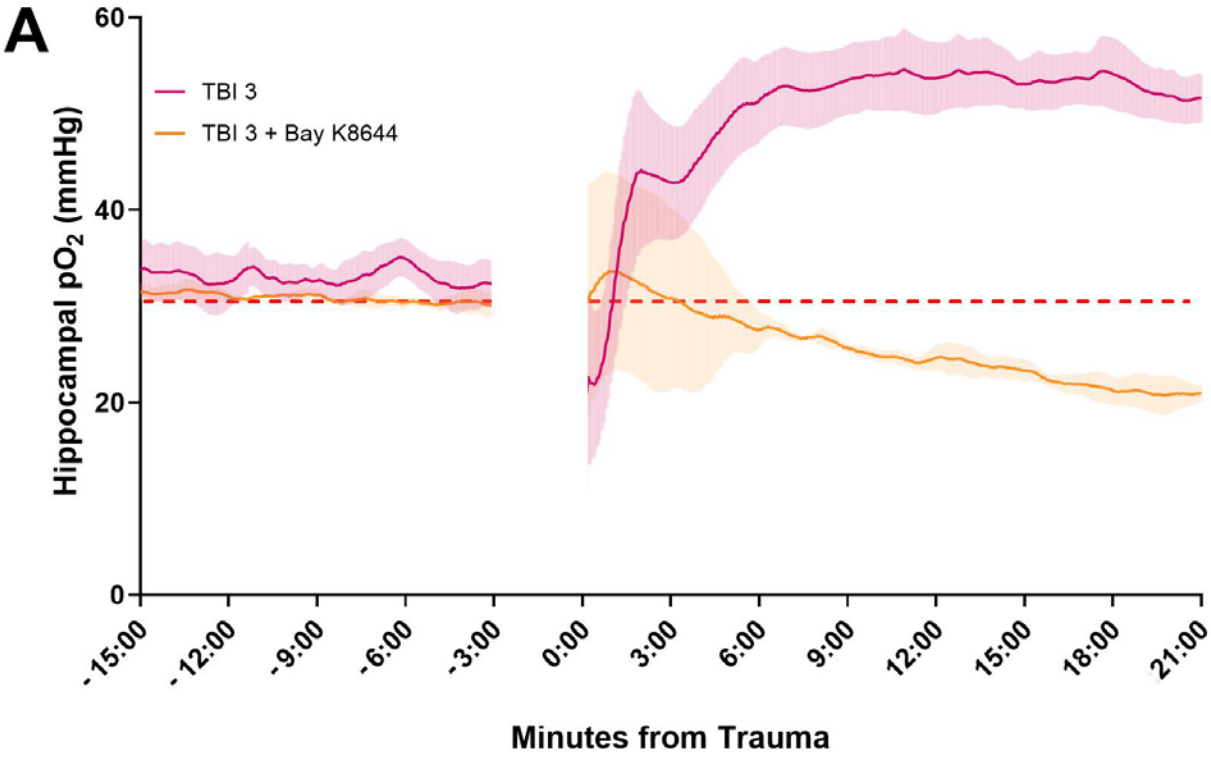
Table 4B: Summary of significant statistical results from the one-way ANOVAs between seizure and non-seizure groups within TBIs.

Time Bin	6 to 9	9 to 12	12 to 15	15 to 18	18 to 21
Comparison					
TBI 1: Seizure x No Seizure F(p)	36.69(<0.01)	27.22(<0.01)	52.51(<0.01)	30.09(<0.01)	19.48(<0.05)
TBI 2: Seizure x No Seizure F(p)	26.90(<0.01)	82.63(<0.001)	75.34(0.001)	35.90(<0.01)	22.66(<0.01)
TBI 3: Seizure x No Seizure F(p)	17.33(<0.01)	23.16(<0.01)	23.49(<0.01)	35.71(<0.01)	27.65(<0.01)

**TBI 1 Seizure (n=5), TBI 1 No Seizure (n=6); TBI 2 Seizure (n=5), TBI 2 No Seizure (n=6); TBI 3 Seizure (n=6), TBI 3 No Seizure (n=5)*

Attenuating Post-Traumatic Hyperoxia with Bay K8644

We further investigated the post-traumatic hyperoxia by using an L-type calcium channel agonist, Bay K8644. Rats went through mTBIs 1 and 2 as per the aforementioned experimental paradigm and were monitored for whether or not they had a seizure. Rats that have a post-traumatic seizure once tend to continue to have post-traumatic seizures after subsequent TBIs Consequently, rats that exhibited a seizure by TBI 2 were predicted to also have a seizure after TBI 3 and Bay K8644 was administered either immediately before or immediately after the third TBI. Results were then only collected from rats who received Bay K8644 and also had a seizure after TBI 3. Oxygen traces from rats that had a seizure after TBIs 1 and 2 followed the same trajectory as previously described in figure 4 (figure 9). Administration of Bay K8644 before TBI resulted in an initial period of variable oxygenation, followed by a reduction to slightly below baseline levels (figure 9).


A
TBI 3
TBI 3 + Bay K8644
Hippocampal pO2 (mmHg)
60
40
20
0
-15:00
-12:00
-9:00
-6:00
-3:00
0:00
3:00
6:00
9:00
12:00
15:00
18:00
21:00
Minutes from Trauma

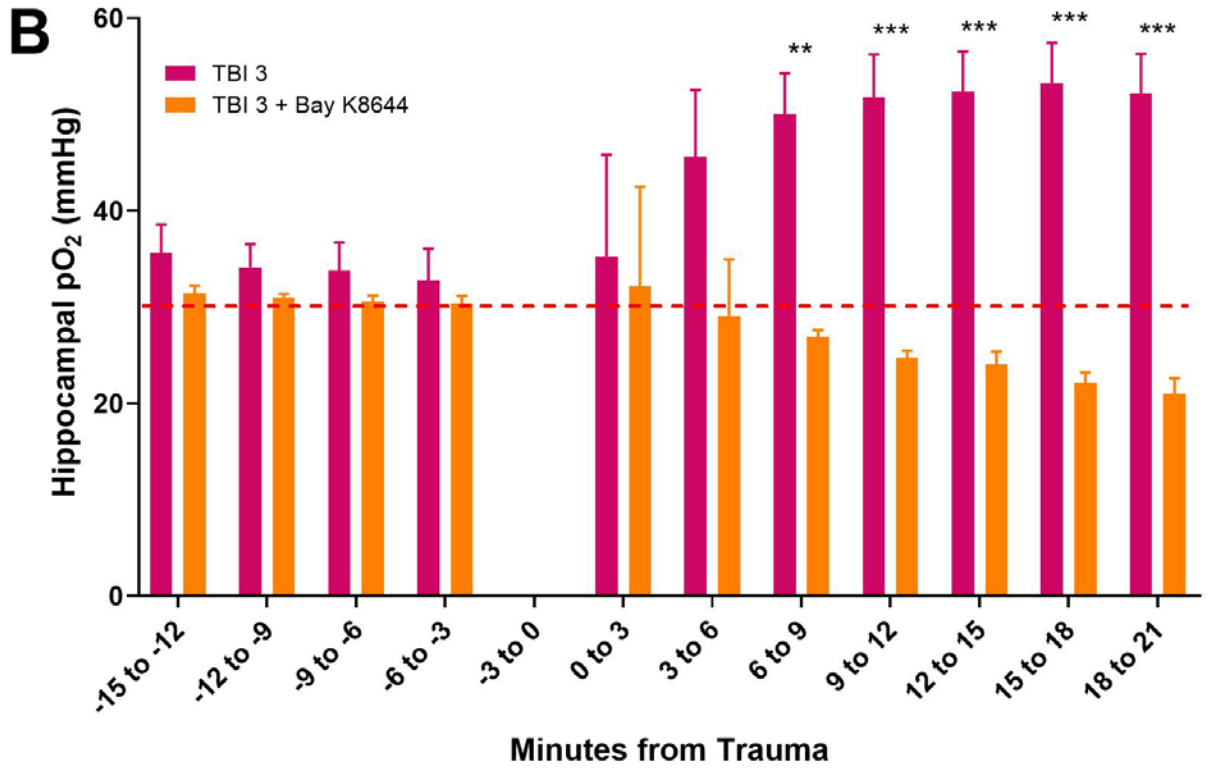

B
TBI 3
TBI 3 + Bay K8644
Hippocampal pO2 (mmHg)
60
40
20
0
**
***
***
***
***
-15 to -12
-12 to -9
-9 to -6
-6 to -3
-3 to 0
0 to 3
3 to 6
6 to 9
9 to 12
12 to 15
15 to 18
18 to 21
Minutes from Trauma

Figure 9: Pre-traumatic administration of Bay K8644 before the third trauma to rats that have exhibited a history of seizures in the previous two TBIs. A) Hippocampal oxygenation through TBI 3 (data from figure 8) and TBI 3 with the administration of Bay K8644. B) Each of the oxygen traces in figure 8A sorted and quantified into 3 minute long bins. Error bars and shaded areas represent ± standard error. The dashed red line at 30 mmHg represents the hyperoxic threshold. *p<0.05 **p<0.01 ***p<0.001. TBI 3 (n=6), TBI 3 + Bay K8644 (n=6).

Table 5: Summary of significant statistical results from the one-way ANOVAs for oxygen curves from TBI 3 and TBI 3 with the administration of Bay K8644 after trauma

Time Bin	**6 to 9**	**9 to 12**	**12 to 15**	**15 to 18**	**18 to 21**
F(p)	29.04 (<0.01)	36.27 (<0.01)	42.16 (<0.01)	51.40(<0.01)	50.17(<0.01)

**TBI 3 (n=6), TBI 3 + Bay K8644 (n=6)*

Behavioural Analysis

Rats in the RmTBI + seizure group exhibited significant impairments in in all behaviour measures evaluated. RmTBI – seizure rats performed almost identically in the novel context task and did not significantly differ in time to cross the beam nor in footslips in the beamwalk task (figure 10). Rats who received Bay K8644 (the RmTBI + seizure + Bay K8644 group) did not significantly differ from sham nor RmTBI – seizure groups, although they did trend towards significance in the novel object task. Table 6 lists all statistical results from the one-way ANOVA for the behavioural test battery. In summary, significant behavioural impairments only existed in the post-traumatic seizures (RmTBI + seizure) where rats demonstrated poorer short term working memory in NCM task (spent less time investigating the novel object), and showed

worse motor coordination in the beamwalk task (took longer to cross the beam and made more foot placement errors).

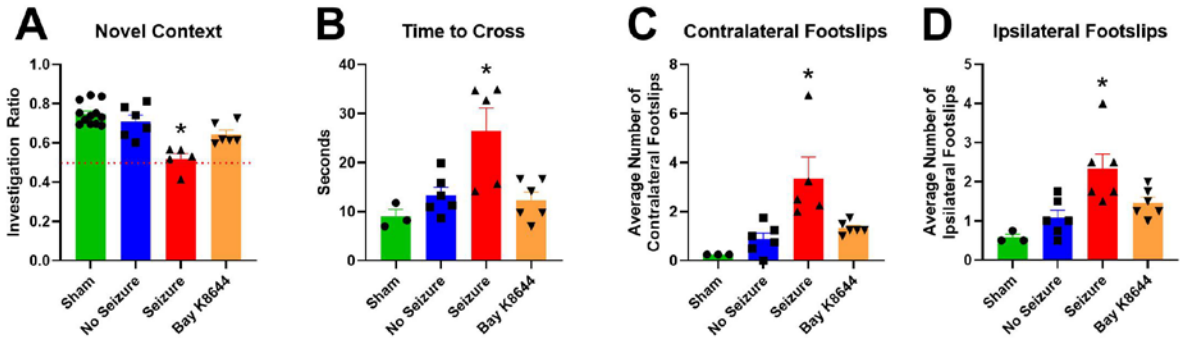


Figure 10: Behavioural deficits between experimental groups. A) Rats who received RmTBI and had a seizure (RmTBI + seizure, n=5) performed significantly worse than both sham (n=12) and injury but no seizure (RmTBI – seizure, n=6) rats in the novel context task. B) Rats that received RmTBI and had a seizure (RmTBI + seizure, n=5) took significantly longer to cross the beam in the beamwalk task than RmTBI - seizure (n=6) rats, and rats that had a seizure, and received Bay K8644 (RmTBI + seizure + Bay K8644, n=6), and sham rats (n=3). C) RmTBI + seizure rats (n=5) showed significantly greater contralateral hindleg footslips in the beamwalk task than RmTBI – seizure rats (n=6), RmTBI + seizure + BayK8644 rats (n=6), and sham rats (n=3). D) RmTBI + seizure rats (n=5) showed significantly greater ipsilateral hindleg footslips in the beamwalk task than RmTBI – seizure rats (n=6), RmTBI + seizure + BayK8644 rats (n=6), and sham rats (n=3). Data are depicted as mean with error bars representing standard error. * $p<0.05$

Table 6: Summary of statistical results from the one-way ANOVAs for behavioral measures.

Behavioural Test	**Effect of Group** **F(p)**	***Post hoc: Sham*** **(p)**	***Post hoc: RmTBI + Seizure*** **(p)**
Novel Context Mismatch	11.86 (<.001)	*RmTBI - Seizure* (.999)	*Sham* (.000)

Sham (n=12) *RmTBI – seizure (n=6)* *RmTBI + seizure (n=5)* *RmTBI + seizure + Bay K8644 (n=6)*		*RmTBI + Seizure* (.000) *RmTBI + Seizure + Bay K8644* (.075)	*RmTBI - Seizure* (.001) *RmTBI + Seizure + Bay K8644* (.031)
Beamwalk: Time to Cross *Sham (n=3)* *RmTBI – seizure (n=6)* *RmTBI + seizure (n=5)* *RmTBI + seizure + Bay K8644 (n=6)*	9.95 (.001)	*RmTBI – Seizure* (.61) *RmTBI + Seizure* (.002) *RmTBI + Seizure + Bay K8644* (.840)	*Sham* (.002) *RmTBI – Seizure* (.011) *RmTBI + Seizure + Bay K8644* (.002)
Beamwalk: Contralateral Footslips *Sham (n=3)* *RmTBI – seizure (n=6)* *RmTBI + seizure (n=5)* *RmTBI + seizure + Bay K8644 (n=6)*	7.05 (<.01)	*RmTBI – Seizure* (.910) *RmTBI + Seizure* (.008) *RmTBI + Seizure + Bay K8644* (.810)	*Sham* (.008) *RmTBI – Seizure* (.016) *RmTBI + Seizure + Bay K8644* (.012)
Beamwalk: Ipsilateral Footslips ***Sham (n=3)*** ***RmTBI – seizure (n=6)*** ***RmTBI + seizure (n=5)***	7.29 (<.01)	*RmTBI – Seizure* (.642) *RmTBI + Seizure* (.003) *RmTBI + Seizure + Bay K8644* (.199)	*Sham* (.003) *RmTBI – Seizure* (.010) *RmTBI + Seizure + Bay K8644* (.008)

RmTBI + seizure + Bay K8644 (n=6)			

Chapter 3: Discussion

We've demonstrated that immediate, post-traumatic seizures that are behaviourally undetectable manifest even after mild traumatic brain injuries, and the generation of these seizures increase in probability as the number of mTBIs accumulate. These events are proceeded by a prolonged period of increased oxygenation that seems to increase in duration as subsequent traumas and seizures accrue. Post-traumatic seizures and hyperoxia produce behavioural deficits in short term working memory and motor coordination that are reversible by attenuation of the hyperoxic event.

Electrographic findings in the immediate phase after mTBI

Using *in vivo* techniques in awake, freely moving rodents, we detected localized, electrographic, hippocampal seizures that immediately follow the delivery of mTBIs. It is possible that these events are triggered by the synchronized stretching of axons as the brain accelerates and decelerates during the injury process. This axonal stretching and membrane disruption produces coordinated, nonspecific depolarization and increases in membrane conductance, which may be responsible for the changes in excitability and the resultant seizure activity that we have observed after mTBI.[16] While this is the first report on the *immediate* electrographic undercurrents of *mild* TBIs, subacute studies on severe TBI have observed changes in excitability and circuit function.[18] In turn, changes in excitability have been found to have an effect a variety of molecular pathways, such as calcium regulation and thus on molecular markers of plasticity such as neurotrophins, as well as on the regulation of the hypothalamic pituitary adrenal (HPA) axis, which is necessary for neuronal vitality and function.[18, 19] Consequently, post-injury changes in brain activation have been postulated to manifest phenotypically as deleterious changes to cognitive and affective well-being.[20] It is

therefore possible, that the seizure activity we observed following mTBI may be an indication of the same types of changes to neuronal excitability as previously identified in more severe TBIs. Consequently, this phenomenon may be a key tenet of the pathophysiological underpinnings of the behavioural deficits experienced by people who have undergone an mTBI.

Power analysis of pre- and post-traumatic EEG of the subsets of rats that did and did not have a seizure revealed a profound dampening of the EEG signal in rats that had a seizure after mTBI. It could be possible that the metabolic demand of a seizure may result in a period of quiescence as a function of the metabolic fatigue left in its wake.[17] Interestingly the most significant dampening of the EEG signal occurred in the beta and gamma frequency domains, with a statistically significant difference between post-trauma seizure and post-trauma no seizure groups. In humans, beta and gamma waves are thought to be involved in cognitive functioning, learning, memory, and information processing. Suppression of these waves has been found in Attention Deficit Hyperactivity Disorder (ADHD), depression, and in learning disabilities.[78, 79] It could be possible that the postictal dampening of these brain waves may be the fingerprint that post-traumatic seizures leave on brain activity as evidence of their contribution to post-traumatic deficits. That is, disorders that result in the chronic manifestation of concentration, memory, executive function, and mood dysfunction display suppression of the EEG signal in the same domains as that observed after post-traumatic seizures. Coincidentally, post-traumatic seizures occur after an event that also is associated with many of those same behavioural dysfunctionalities. This commonality may hint at a shared mechanism behind the symptomology between the chronic deficits of some neurological disorders and the acute/ subacute deficits after concussions. In the case of concussions, it may be possible that the events that trigger this mechanism are post-traumatic seizures.

Just as there is individual heterogeneity in mTBI experience, where not every mTBI produces the same severity of symptomology across populations; not all mTBIs produce post-traumatic seizures. Comparing the proportion of rats that had a post-traumatic seizure (figure 5) to data from the NFL characterizing concussion (as verified from clinical information from athletic medical staff) probability across a range of accelerations (figure 11), reveals a dissimilarity between post-traumatic seizure incidence (25% after the first trauma and 45.45% after three traumas) and concussion probability at the acceleration delivered in this study (93.20% at 125 Gs).[75, 80] However, this disparity is likely because the NFL data were heavily weighted toward injurious impacts and were not representative of all impacts that occurred, including many impacts that did not result in a concussion.[81] Further, the NFL data does not characterize how many impacts the players had received preceding the recorded and characterized impact seen in figure 10. Players in the NFL data could have had dozens of impacts preceding the one documented by Pellman et al. Post-impact and post-concussive vulnerability to further injury, particularly in cases where a second concussive injury is sustained within days of the first, has been widely reported, both in rat models and in humans, to lead to a higher chance of developing symptomology and more severe deficits.[44] Similarly, we demonstrated that the proportion of experimental rats that exhibited post-traumatic seizures increased as the number of successive injuries accumulated (figure 6), but not all rodents were susceptible.

There may be a relationship between having a concussion and having a seizure after a head impact: they happen because of the same types of impacts, they occur at similar rates, and they result in similar behavioural outcomes (figure 10). It could be possible that individuals with concussions and the worst symptomologies after mTBI are, in fact, the individuals that

experienced post-traumatic seizures. Thus, the reason why individuals are more vulnerable to worse symptoms after RmTBIs may be because successive injuries place one at greater risk for the generation of post-traumatic seizures than isolated ones (figure 6). While this type of abductive reasoning cannot prove the relationship between the two, “if it looks like a duck, swims like a duck, and quacks like a duck, then it probably is a duck”.

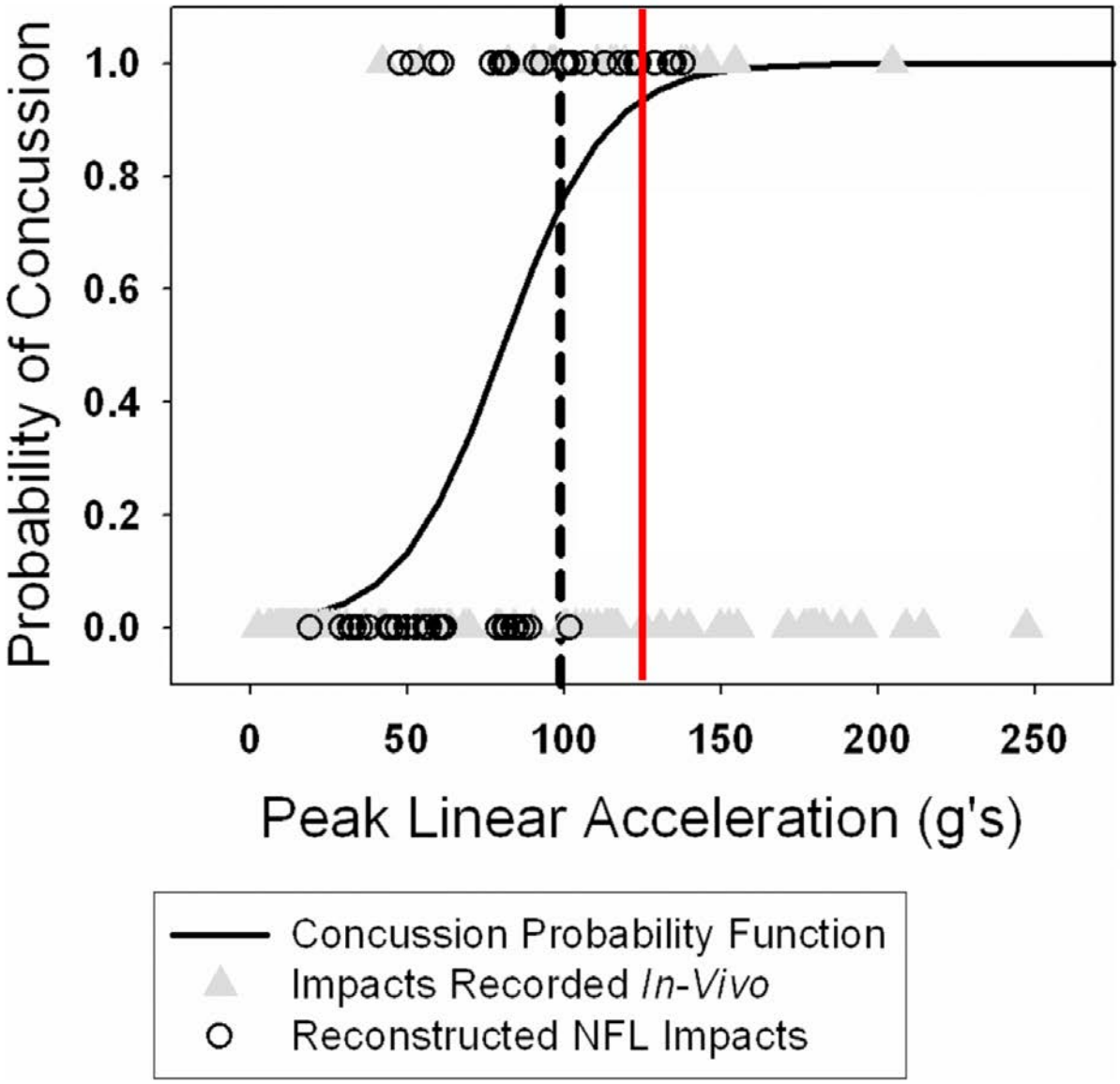


Figure 11: Linear acceleration concussion probability function generated from NFL impacts reconstructed in the laboratory, as in Pellman.[80] The dashed black line represents the acceleration at which there is a 75% probability of concussion (98.9 Gs). The red line representa the acceleration of the mTBIs delivered through the lateral impact device in this study. [75] Adapted from Greenwald.[81]

Moreover, it has been reported, anecdotally and more recently in peer reviewed literature, that there is an association between reported number of previous concussions and likelihood of incident concussion.[82] The etiology underlying this association may be explained in the context of our present findings. The initial manifestation of a seizure results in the priming of neuronal networks for synchronicity – that is, the generation of a "locus" for the propagation of further seizure activity. Consequently, after an initial seizure, the threshold required to produce another one is lowered, and it becomes easier to experience subsequent seizures.[83] The epidemiological findings that a single concussion increases risk for future concussions may support our findings that seizures are an important aspect of mTBI pathophysiology.

Seizures Versus a Mild Injury

The presence of seizures is most commonly associated with moderate to severe TBIs and not mTBIs. It may then be tempting to classify the injury delivered in this study along those lines. However, when classifying a TBI, we must do so using etiological, structural, and symptomology criteria. A mild brain injury is often caused by a blunt head trauma without penetration resulting in acceleration or deceleration forces to the head.[5, 84] Although 95 Gs of acceleration has been defined as the concussion threshold in many sports, players can develop sports-related concussion from both high and low-impact hits.[75, 85] Following the injury, an mTBI generally results in diffuse axonal injury, axonal atrophy, and other pathophysiologic

responses (see Chapter 1: Mild Traumatic Brain Injuries and Concussions). However, a mild injury doesn't seem to involve rampant necrotic and apoptotic cell death, nor macroscopic legions seen in moderate and severe TBIs.[84] Lastly, mTBI is characterized by a GCS score of 13-15, with the primary symptomology being transient confusion, disorientation, memory deficits, and other neurologic and neuropsychological dysfunctions (see Chapter 1: Mild Traumatic Brain Injuries and Concussions).[3] In contrast, moderate to severe TBIs involve GCS scores from <9-12 including LOC for minutes to days, and more severe and long term behavioural deficits.[84]

The injury delivered in this study using the lateral impactor delivers a blunt force trauma that doesn't cause a penetrative brain injury nor a skull fracture, and results in acceleration of the head at 125 Gs.[69, 75] This falls above the concussion threshold, but remains well within the range of accelerations that result in concussions seen in professional sports and even at the high school and collegiate level.[75, 80, 81, 86] In fact, one study on college football players found that the mean acceleration of reported concussions was 102.8 Gs with a range from 60.51 – 168.71 Gs, well encompassing the accelerations generated in this study.[87] Secondly, anatomical and histological explorations of the rat brain following an injury delivered through the lateral impactor do not result in macroscopic damage, gross anatomical deformities, nor widespread cell death.[65, 69, 75] Lastly, LOC as reflected by time-to-right after an injury delivered through the lateral impactor is generally <1 minute, and behavioural deficits are mild, heterogenous, and spontaneously resolving.[65, 69, 75] Similarly, while it would be difficult to try and interpret the GCS for rats, if one were to do so, it would be difficult to come up with an interpretation yielding a GCS of <12 after the injury delivered in this study.

Although the presence of seizures may tempt one to classify the injury delivered in this study as moderate to severe, the etiological, structural, and symptomological evidence says otherwise. The reason that seizures are almost always used to diagnose more severe injuries is because they are used as such in humans, where a seizure, especially after a trauma, is typically observed by virtue of convulsions and other behavioural phenomena. The seizures reported in this study are almost entirely electrographic, with the only behavioural correlate during the actual seizure being behavioural arrest during the event. If this phenomenon holds true in humans, it would be incredibly easy to dismiss the 15-45 seconds of post-trauma ictal behavioural arrest as just confusion or to miss the event entirely. Consequently, while it may be true that behavioural or convulsive seizures are typical of moderate to severe TBIs, electrographic seizures likely are not.

Brain oxygenation after mTBI

It has been contended that concussion/mTBI are metabolic rather than structural injuries, wherein a metabolic mismatch between energy demand and energy supply results in cellular vulnerability that is particularly susceptible to further injury.[25] This theory is consistent with our electrographic findings: a seizure involves a period of heightened metabolic demand and this period of metabolic mismatch has been related to behavioural impairments consistent with a failure to meet the oxygen demands of the brain. Investigations on oxygen and blood flow in the postictal period of regions of the brain that have exhibited brief seizures have shown a period of severe hypoxia, that, when reversed, prevents the behavioural impairments that follow seizures.[54]

Similarly, vascular dysfunction or dysregulation has been theorized to contribute to post-traumatic symptomology.[41] A number of studies have examined cerebral blood flow in the

subacute period following moderate to severe TBIs, with mixed results.[18] However, most severe TBI studies demonstrate a triphasic blood flow response, beginning with an injury severity dependent reduction in cerebral blood flow immediately following the insult on day 0, followed by hyperperfusion on post-injury days 1–3, and vasospasm on post-injury days 4–15.[41, 43]

To our knowledge, this is the first report on the oxygen status of deeper brain structures immediately after an mTBI. We observed an increase in brain oxygen in the hippocampus immediately after the injury and post-traumatic seizures, culminating in a period of hyperoxia wherein oxygen levels were above 1.5 fold baseline levels that gradually resolved in the following 24hrs. Interestingly, rats that received an mTBI but did not demonstrate post-traumatic seizures did not exhibit this phenomenon of post-traumatic, postictal hyperoxia, leading us to believe that the changes in oxygenation were a result of the post-traumatic seizures, and not the mTBI itself.

This change in oxygenation is surprising because it does not follow the typical trajectory of brain tissue that has undergone a seizure, despite the fact that we observed post-traumatic seizures in the same structures we recorded oxygen from.[54] Moreover, it seems that post-mTBI oxygenation in the hippocampus follows the opposite trend as that identified in the cortex following severe TBI. That is, while after severe TBI, the cortex experiences hypoxia that gradually resolves over the first day, the hippocampus after mTBI experiences hyperoxia that resolves over the same period.[54]

It is possible that both these irregularities are related. That is, perhaps one of two things could be occurring: blood flow could be increased in some areas of the brain (through vasodilation of local blood vessels) without increases in overall blood supply to the brain,

resulting in other areas receiving less blood supply. Or blood flow could be reduced in some areas of the brain (through localized vasoconstriction) without decreases in overall blood supply to the brain, resulting in increased perfusion pressure to other areas not experiencing the same vasoconstrictive drive. In the context of the current findings, post-traumatic seizures could result in the same vascular events as seizures in tissue such as the cortex that has previously been found to become hypoxic upon injury, resulting in shunting of blood flow to other tissues, such as the hippocampus, where we observed hyperoxia.

Alternatively, however, it could be possible that that mTBIs are simply fundamentally different from severe TBIs in their pathophysiology and therefore have different vascular and oxygenation implications. It has previously been discussed at length in this paper, but the diffuse and non-macroscopic damage sustained in mTBIs may result in the activation of different mechanisms and pathways than the much more destructive pathology seen in severe TBIs. Similarly, it could also be possible that different mechanisms of eliciting seizures result in different physiological outcomes as well. There have been reports of seizures elicited by soman (O-pinacolyl methylphosphonofluoridate) administration that result in a hyperoxic post-seizure hippocampal oxygenation state, and there is some data indicating that febrile seizures result in postictal hyperoxia as well.[88]

Regardless, these changes in blood supply and therefore brain oxygenation could have deleterious effects by virtue of the Goldilocks effect: just as too little oxygen supply in some areas of the brain could have negative effects resulting in dysfunction, so too could too much oxygen supply. Hyperoxia can directly damage tissues via the production of reactive oxygen species (ROS) in excess of physiological antioxidant defence capabilities, leading to increased cell death by apoptosis and increased release of endogenous damage-associated molecular

pattern molecules (DAMPs) that stimulate an inflammatory response.[89, 90] In a retrospective study of more than 3000 patients with TBIs, both hypoxia and hyperoxia on admission were associated with worse outcomes.[91] Similar findings were reported by a retrospective study of 1547 TBI patients with both low and high admission PaO2 levels independently associated with worse outcomes.[92]

The effect of changes in oxygenation on immediate behavioural outcomes

Rats that demonstrated post-traumatic seizures and deviations in post-traumatic brain oxygenation demonstrated marked deficits in short term working memory and motor function compared to sham rats and rats that received a mTBI but did not demonstrate post-traumatic seizures or hyperoxia. In fact, rats that received the injury but did not have seizures or have substantial changes in brain oxygenation performed similarly to rats that did not receive any injury at all. It is therefore possible that the true culprit, at least with respect to the behavioural measures tested in this study, is not the physical trauma itself, but the ensuing electrical and vascular events that follow. This is consistent with recent assertions in the field claiming that concussions and other mTBIs are metabolic and not structural injuries.[7]

If it is these deviations in blood supply and oxygenation in the brain that were the cause of the post-traumatic behavioural deficits, blocking this abnormal pathophysiological response should attenuate the deficits. Indeed, treatment with a vasoconstrictive agent, Bay K8644, to restore vascular tone reduced the extent of hyperoxia after injury and rescued many of the deficits demonstrated by rats after RmTBI. This suggests that restoring oxygen to normoxic levels may be a viable treatment for post-traumatic deficits. In broader terms, it seems that dysregulation of physiologic parameters such as oxygenation are a key aspect of the behavioural deficits commonly observed to follow mTBI. Consequently, it may be useful to investigate ways

with which to restore the autoregulatory capacity of the brain as a method to combat the severity and length of post-traumatic deficits.

The fact that rats treated with Bay K8644 did not perform as well as rats that did not have seizures or demonstrate hyperoxia at all can be explained by the fact that, while some regions of the brain, such as the hippocampus, were hyperoxic, there were other regions that may have been hypoxic or under-oxygenated. Treatment with a vasoconstrictive agent may have exacerbated that condition. Alternatively, even if there were no post-traumatically under-oxygenated areas of the brain, it is very unlikely that they entire brain was hyperoxic. As a result, treatment with Bay K8644 would likely have reduced oxygenation in normally oxygenated or unaffected tissues to below normal levels. Both possibilities are consistent with our Goldilocks hypothesis, that both hyperoxia and hypoxia may be deleterious to function.

This may also present a rationale for why there is such a plethora of different symptomologies for different mTBI patients. It is already known that different impact locations result in the manifestation of different deficits.[69] Different regions in the brain may experience perturbations in oxygenation depending on trauma impact location and other injury dynamics, resulting in different deficits based on which brain structures are affected. Thus, restoration of normal autoregulatory function in the brain is paramount to overcoming post-traumatic deficits. Moreover, it could also be possible that post-traumatic, postictal hyperoxia is not the only contributor to post-traumatic deficits. Post-traumatic seizures may result in a plethora of downstream cellular events beyond changes in vascular tone that may be partly responsible neurological dysfunction and behavioural deficits.

Mild traumatic brain injuries versus concussions

Why is it that very similar traumas can result in very different symptoms between and even within the same people? Two people in the same car during a symmetric collision can walk away with profoundly different consequences: one may walk away relatively unharmed, while the other may suffer from a plethora of debilitating symptoms. Most of the literature in the field of neurotrauma uses the terms mTBI and concussion interchangeably. While the biomechanical forces behind these phenomena may be the same, we propose that there is a fundamental difference between an mTBI and a concussion. We've demonstrated that there are two electrographic, vascular, and behaviourally distinct subsets within the mTBI population. One group that undergoes a biomechanically event typical of an mTBI and doesn't suffer many other physiological complications (the person that walked away from the collision relatively unscathed), and another that generates post-traumatic seizures and post-traumatic, postictal hyperoxia (the person who was dizzy and couldn't remember the date). Consequently, a case could be made that an mTBI is the broader term encompassing all injuries that involve the application of linear or rotational accelerative and decelerative forces on the brain, and concussions are a smaller, more specific subset of these injuries that give rise to the post-traumatic seizures and changes in brain oxygenation that manifest more severe behavioural deficits.

Taking this idea one step further, it could also be possible that the asymptomatic group experienced a trauma that did not develop into an injury, and the group that was symptomatic was the group that had a trauma that generated an actual traumatic brain injury. The person who walked away unscathed did not have an injury at all, and it was the person who was symptomatic who was injured. In this paper, and indeed in much of the field, it is assumed that every animal

that undergoes an mTBI injury protocol, whether it be the lateral impact, the weight drop, or the plethora of other variations of these models, has an injury by virtue of receiving an impact. However, considering the findings of this study it is likely that the same impact does not generate an mTBI in every case. Intuitively, this seems to make sense: the two people in the car crash had two different outcomes: one had an injury and the other didn't, just as in this paper different rats did and did not have seizures and behavioural deficits. This difference in outcome from the same injury is likely caused by individual variation between animals (in the context of this paper) and people. Heterogeneity within populations means that different individuals are either more or less likely to be injured given an impact, independent of the parameters of the impact.

Limitations

Concussions and mTBIs involve the movement of the brain inside the skull. The relatively stiff, stainless-steel electrode deep inside the brain may provide a stabilizing presence anchoring the brain to the skull, thereby preventing movement of the brain in relation to the skull. Indeed, the electrode tracts observed in this study (figure 4) do not differ macroscopically from the electrode tracts seen in studies that don't involve mTBIs indicating that the brain is not moving significantly around the electrode (which would have been observed as a much larger tract than what was seen). While this may have prevented the brain from contacting the skull during the coup and counter-coup movement of the head after an impact, the brain still experienced acceleration and deceleration. Consequently, although the electrode may have prevented some of the injury from occurring, the brain still likely underwent the shearing and stretching that characterizes the bulk of the damage seen in mTBIs and concussions.

The observer effect is the idea that observation of a phenomenon inevitably changes that phenomenon, which is often the result of instruments that, by necessity, alter the state of what they measure in some manner. It cannot be ignored that the implantation of probes into the brain is a relatively damaging technique. The implantation procedure compromises the blood brain barrier by puncturing the dura and exposing the brain to not only non-CNS cells, but also to the external environment and the materials used to form the head cap. Moreover, as the electrode is lowered into the brain, it causes brain damage on the way down. The presence of a tract visible to the naked eye (figure 4) is evidence of the non-inconsequential damage inflicted by the positioning of the implant. It could be possible that this damage contributes to the events reported in this study. While it is unlikely that implanting an electrode into the brain was the sole cause of the seizures we observed (as evidenced by the fact that some animals did not have seizures or the corresponding post-ictal hyperoxia), it could be possible that it contributed to generation of seizures, and that without the damage from the implant, animals wouldn't have seizures at all. That is, the damage from the implant may be necessary but not sufficient for the generation of seizures. If this true, then the observations reported in this study may not be representative of concussions or mTBIs under "natural" conditions. To at least somewhat circumvent this, it could be possible to simply not implant a thick, deep electrode and only implant an optrode which is implanted less deep into the brain and is significant thinner than the electrode. We have shown that post-traumatic seizures are followed by post-traumatic, post-ictal hyperoxia and this hyperoxia seems to be responsible for behavioural deficits. Consequently, if we implant only the optrode and still observe hyperoxia and changes in behavioural function, it would imply that the damage caused by the electrode isn't causative of those events.

This study made use of only male rats to characterize the presented phenomena. It may be possible that conducting the same experiments on female rats may result in different outcomes. Estrogen is both a genomic and non-genomic neuroprotectant as well as an anticonvulsant. It may be possible that the increased amount of estrogen in females will protect them from seizures by either stopping them from occurring entirely, or by reducing the probability of them occurring (resulting in a lower incidence).

Conclusion

This study reported the presence of an immediate, electrographic post-traumatic seizure and subsequent hyperoxic episode, that's reversal, rescued some of the behavioural deficits commonly seen after RmTBI. We propose that the post-traumatic seizure is an important part of what differentiates a symptomatic mTBI, or concussion, from one without persistent symptomology, and that hemodynamic perturbations resulting in changes to oxygenation of the brain are key etiological factors for many of the deficits seen after concussion. Future studies should evaluate blood flow and oxygenation changes at the level of the whole brain to ascertain the extent of homeostatic dysregulation after concussion and attempt to correlate changes in oxygenation of certain brain structures to corresponding deficits. Moreover, determining how to reverse these oxygenation changes may be an important tool in relieving some of the symptoms of concussion.

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