

ADVANCED MRI APPROACHES TO ASSESSING
MILD TRAUMATIC BRAIN INJURY

ADVANCED MAGNETIC RESONANCE IMAGING (MRI)
APPROACHES TO ASSESSING MILD TRAUMATIC BRAIN
INJURY (MTBI) IN CHILDREN

By

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A Thesis

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Advanced Magnetic Resonance Imaging (MRI) Approaches to Assessing Mild
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Abstract

Despite the high incidence and potential cognitive, physical and emotional consequences of mild traumatic brain injury (mTBI), its pathophysiology and recovery time are not well understood. Furthermore, some mTBI patients' post-concussion symptoms reappear with physical activity. In this project, three advanced MRI techniques (resting state functional magnetic resonance imaging (rs-fMRI), diffusion tensor imaging (DTI), and proton magnetic resonance spectroscopy (^1H -MRS)) were used to investigate brain changes following mTBI in children and acute aerobic exercise in healthy adults.

Children with mTBI were examined longitudinally at 1, 4 and 7 months post-injury. Functional connectivity of resting state networks (RSNs) was investigated using probabilistic independent component analysis. Statistical significance was discovered in three RSNs: decreased functional connectivity in the auditory network; and increased functional connectivity in the default mode network and sensorimotor network. There was some functional recovery observed at the second scan; however, a persistent increase in connectivity in the default mode network was observed. Furthermore, DTI whole-brain analysis was performed using tract-based spatial statistics. A statistically significant decrease in

fractional anisotropy was found in the left inferior longitudinal fasciculus, inferior fronto-occipital fasciculus, superior longitudinal fasciculus and forceps major. Moreover, ¹H-MRS was acquired from the right frontal white matter (FWM) and anterior cingulate cortex. A statistically significant increase in glutamate in the FWM was found relative to controls. Our findings show that mTBI in children causes abnormalities in the brain's RSNs, white matter tracts and neurometabolites, and these disruptions persist for at least 7 months following injury.

The last part of this project involved the assessment of the effects of acute exercise on brain functional connectivity. It is well known that exercise can potentiate post-concussion symptoms. Thus, the goal of the last study was to assess the healthy brain changes, in preparation for examining patients following mTBI. Rs-fMRI was used to investigate the effects of acute aerobic exercise on the healthy brain. Statistical significance was discovered in three RSNs: increased functional connectivity in the executive control network and fronto-parietal network, and decreased functional connectivity in the sensorimotor network. Our findings provide evidence of changes in brain resting state functional connectivity following acute aerobic exercise. This may shed light on mTBI cases where post-concussion symptoms reappear with exercise.

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Abbreviations

$^1\text{H-MRS}$	Proton Magnetic Resonance Spectroscopy
AAN	American Academy of Neurology
ACRM	American Congress of Rehabilitation Medicine
ADC	Apparent Diffusion Coefficient
AN	Auditory Network
BOLD	Blood Oxygen Level Dependent
CHESS	Chemical Shift Selective
CNS	Central Nervous System
Cr	Creatine
CRLB	Cramer-Rao Lower Bound
CSF	Cerebrospinal Fluid
CT	Computed Tomography
dHb	Deoxyhaemoglobin
DMN	Default Mode Network
DTI	Diffusion Tensor Imaging
DWI	Diffusion Weighted Imaging
ECN	Executive Control Network

EPI	Echo-Planer Imaging
FA	Fractional Anisotropy
fMRI	Functional Magnetic Resonance Imaging
FPN	Fronto-Parietal Network
FWM	Frontal White Matter
GABA	Gamma-Aminobutyric Acid
GCS	Glasgow Coma Scale
Gln	Glutamine
Glu	Glutamate
GM	Grey Matter
GPC	Glycerophosphocholine
ICA	Independent Component Analysis
IR	Inversion Recovery
Lac	Lactate
LCModel	Linear Combination Model
LOC	Loss of Consciousness
MD	Mean Diffusivity
MELODIC	Multivariate Exploratory Linear Optimized Decomposition into Independent Components
mI	Myo-inositol
MRS	Magnetic Resonance Spectroscopy
mTBI	Mild Traumatic Brain Injury

NAA	N-Acetyl Aspartate
NAAG	N-Acetyl Aspartyl Glutamate
NMR	Nuclear Magnetic Resonance
oHb	Oxyhaemoglobin
PCh	Phosphocholine
PCr	Phosphocreatine
PCS	Post-Concussion Syndrome
PCSS	Post-Concussion Symptom Scale
PNS	Peripheral Nervous System
ppm	Parts Per Million
PRESS	Point Resolved Spectroscopy
PTA	Post-Traumatic Amnesia
RF	Radio Frequency
ROI	Region of Interest
rs-fMRI	Resting State Functional Magnetic Resonance Imaging
RSN	Resting State Network
SMN	Sensorimotor Network
SNR	Signal to Noise Ratio
STEAM	Stimulated Echo Acquisition Mode
T1	Time to Inversion
TBI	Traumatic Brain Injury
TBSS	Tract-Based Spatial Statistics

TE	Echo Time
WM	White Matter

Thesis Format

This PhD thesis follows the ‘sandwich’ thesis format described in the Guide for the Preparation of Master's and Doctoral Theses by the School of Graduate Studies. The thesis contains a general introduction and objectives (chapters 1 to 4), followed by three articles with self-contained references (chapters 5 to 7), followed by overall conclusions (chapter 8) and references.

CHAPTER 1

The Human Brain

1.1 Brain Function and Anatomy

One of the final frontiers of science is the human brain. Even though we are progressively unravelling new secrets, our journey to fully understanding the brain's functions is not yet complete. The first footsteps of this journey began around 400 BC [1] [2]. It then took us more than 2000 years to gradually put together the complexity and versatility of the human brain, and it seems that we have only scratched the surface.

The human brain is extremely complex. Although little is known about the complex functioning of the brain, quite a lot is known about its anatomy [3]. The brain controls the central nervous system (CNS), the peripheral nervous system (PNS), and regulates all human activity. The CNS consists of the brain and spinal cord, the control centres for the nervous system. The PNS is composed of nerves

leading to and from the CNS. Neural tissue is specialized for the conduction of electrical impulses that convey instructions or information from one area of the body to another. Around 98% of neural tissue is concentrated in the brain and spinal cord.

At the cellular level, the nervous system is defined by the presence of a special type of cell called the neuron. Neurons transmit information in the form of nerve impulses from one part of the body to another. Neurons are composed of three main parts: cell body, axon, and dendrites. The cell body, which includes the nucleus, regulates the functioning and supports the chemical processing of the neuron. Dendrites are attached to the cell body, and receive impulses from other neurons at synapses. Axons are long structures that transmit the impulses away from the cell body and dendrites. Axons are wrapped by a thin layer of connective tissue known as the endoneurium. Groups of axons are bundled together into tracts, or fascicles, by a thin boundary known as the perineurium.

The human brain is made of different elements including white matter (WM), grey matter (GM), and cerebrospinal fluid (CSF). WM contains the axonal nerve fibres or neurons covered by a myelin sheath. The WM axons are highly organized and tightly packed together. The WM connects various GM areas (the locations of nerve cell bodies) of the brain to each other and carries nerve impulses between neurons. It is essentially the cabling that joins the different parts of the cortex and other parts of the brain together. GM contains the neuron cell bodies, dendrites, and final parts of axons, i.e. most of the computational

structure. The cerebral cortex is extensively folded to increase its surface area without increasing its volume. CSF occupies the ventricular system around and inside the brain, which cushions the brain against shear forces within the skull. In the healthy brain, the composition of CSF is constant, being broadly similar to blood serum, while lacking protein.

The brain is further protected by the blood-brain barrier, a physical barrier formed from the junctions between brain capillary endothelial cells. The blood-brain barrier provides control of the chemical environment in the brain by facilitating the transport of certain key molecules (such as sugars and amino acids) into the brain, and blocking unwanted molecules from crossing the barrier.

1.2 Regions of the Brain

The human brain has three primary subdivisions: the cerebral hemispheres, cerebellum, and brainstem. The brainstem, from the spinal cord up, begins with the medulla, which leads up to the pons, which is connected to the cerebellum (see Figure 1.2.1). The medulla is responsible for the basic processes, such as breathing, heart rate, and certain reflexes [4]. The pons acts as the relay system between the cerebellum and the rest of the brain. Moreover, it is responsible for some auditory and visual processing, as well as coordinating eye movements in relation to balance. The cerebellum is involved in supervised learning, controlling skilled movements, coordination, precision, and accurate timing [5].

The midbrain is positioned above the pons, and it contains the thalamus, the hypothalamus, and the inferior and superior colliculi. The thalamus functions as a station for information passing throughout the brain. The hypothalamus is involved in the regulation of hormones, temperature, hunger and thirst. The inferior and superior colliculi are involved in auditory and visual processing, respectively [4].

Above the midbrain are the basal ganglia and limbic system. The basal ganglia are comprised of several different regions, including the globus pallidus, substantia nigra, and striatum, which contains the caudate and putamen. The basal ganglia are responsible for motor control and action selection, and are also involved in reinforcement learning [5]. The limbic system is comprised of the hippocampus, amygdala, septum, and hypothalamus. It is responsible for the experience and expression of emotions.

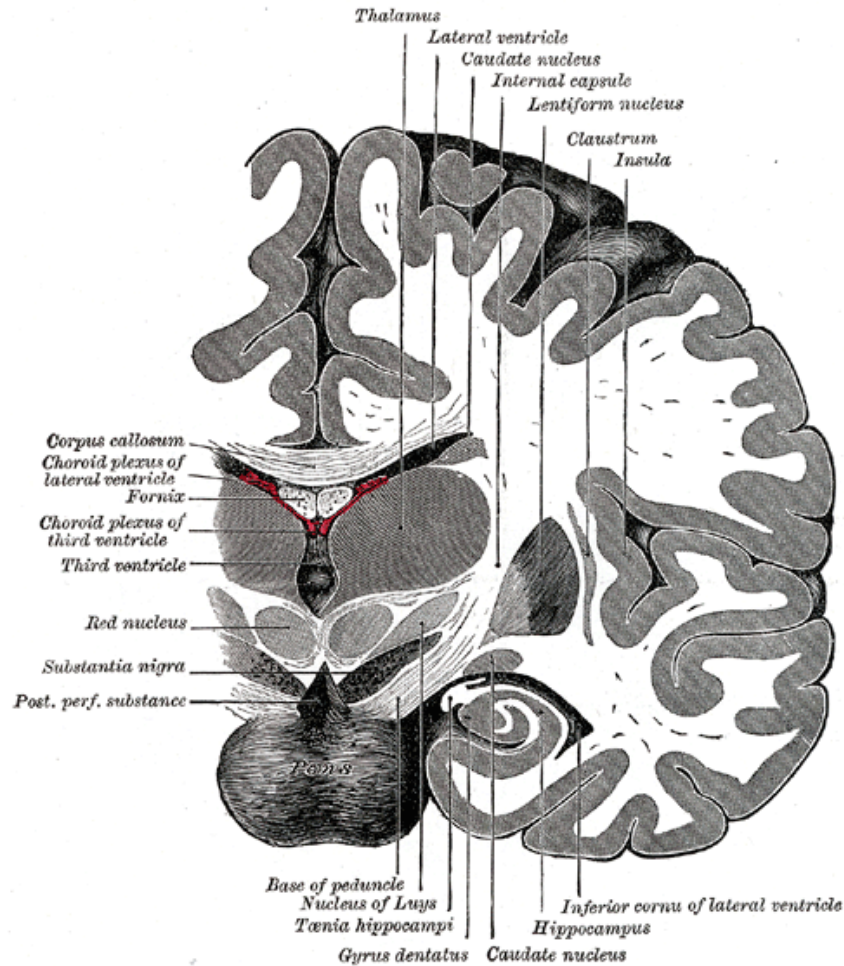


Figure 1.2.1: Coronal section of the brain. [3]

The cerebral cortex is responsible for many of the higher order thoughts that take place in the brain. It is divided into two cerebral hemispheres: the left and right hemispheres. They are connected together by a wide band of nerve fibres called the corpus callosum. Visual inspection of the cerebral cortex allows us to divide it into sections, known as lobes (see Figure 1.2.2). Each hemisphere is

made of four lobes: occipital, temporal, parietal, and frontal. Two additional regions exist as folds within the cortex: the cingulate cortex and the insular cortex.

The occipital lobes are at the back of the brain and are mainly responsible for vision. They include the primary visual areas, which receive inputs from visual pathways that start in the retina of the eye, and pass through the thalamus where some processing occurs, before arriving at the primary visual cortex. It can be divided into at least thirty extensively interconnected areas concerned with vision, which integrate the different dimensions of vision to result in our remarkable perception of the outside world.

The temporal lobes are on the most lateral parts of the cortex. They contain the auditory cortex, which is the main sensory area devoted to hearing. They also contain Wernicke's area, which is involved in language. The temporal lobes contain areas associated with tasks such as recognizing and categorizing faces, objects, animals and so on. They also contain the hippocampus and amygdala. The hippocampus is involved in memory, while the amygdala links memories and visual information with emotions. The temporal lobes contain regions important in emotions, personality, and sexual behaviour.

The parietal lobes, located behind the central sulcus, are largely responsible for sensory perception. The primary somatosensory system contains the sensory areas that respond to receptors in the skin, muscles, and joints. The sensations detected by the somatosensory system are touch, pressure, temperature,

pain, and proprioception. The rest of the parietal lobe combines information from several sensory systems, to provide an integrated perception of the outside world.

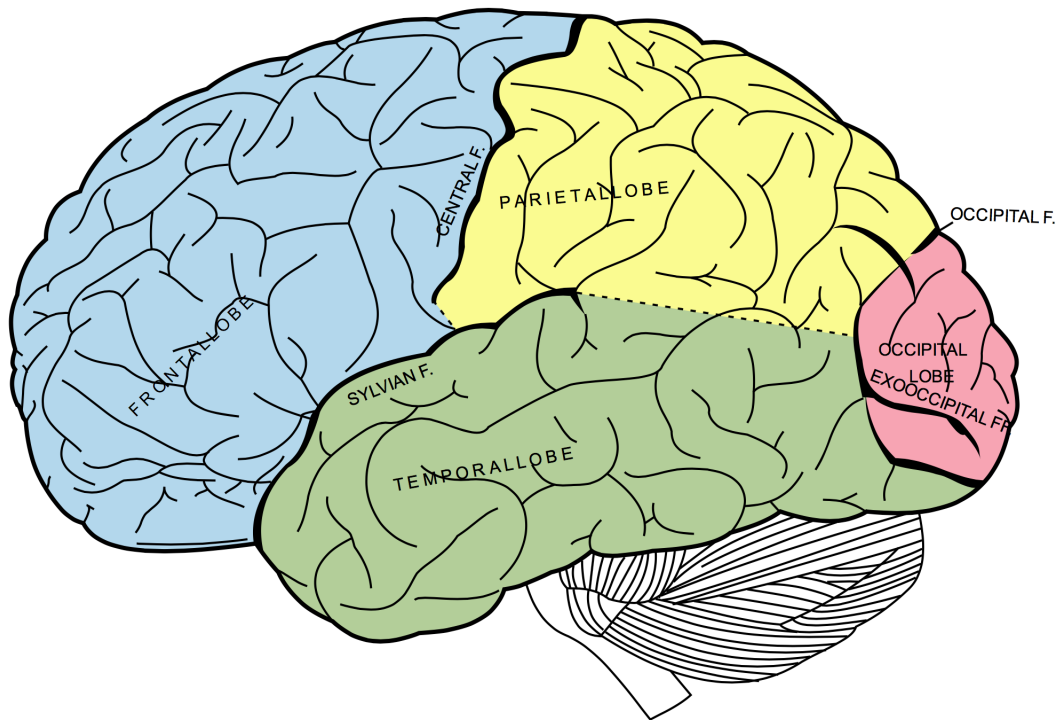


Figure 1.2.2: Principal fissures and lobes of the cerebrum viewed laterally. [3]

The frontal lobes, located in front of the central sulcus, are the largest lobes of the human cerebral cortex. Their size may be fundamental to aspects of brain functions that are regarded as uniquely human. They include areas that play vital roles in high level processes such as reasoning, planning, emotions, speech, navigating social norms and situations, problem solving, and generating ideas. Other parts of the frontal lobes have more complex functions, including aspects of memory, and strategic planning of behaviour, i.e. inhibiting behaviour that would

seem immediately rewarding. At the posterior end of the frontal lobe is the primary motor area, termed the motor cortex, which is responsible for issuing motor commands.

The insular cortex is believed to be involved in a wide range of functions including pain perception, processing basic emotions (such as anger, fear, disgust, joy, and sadness), and conscious desires (such as the active search for food). The cingulate cortex acts as an interface between emotion and cognition, and more specifically in the conversion of feelings into intentions and actions. It is also involved in higher functions including controlling one's emotions, recognizing one's mistakes, and making adaptive responses to changing conditions.

CHAPTER 2

Advanced MRI Applications to Brain Physiology

2.1 Functional MRI (fMRI)

2.1.1 The BOLD Effect

Functional MRI (fMRI) is an imaging technique that relies on the relationship between neuronal function and blood flow to identify patterns of brain activations. These activations are associated with specific cognitive or behavioural events. Most brain fMRI studies use the blood oxygen level dependent (BOLD) signal to infer cerebral function. The BOLD signal is dependent upon changes in cerebral blood flow, cerebral blood volume and oxygen consumption. BOLD signal reflects magnetic field inhomogeneities

caused by changes in the oxygenation state of haemoglobin. Neuronal activation causes an increase in cerebral blood flow and thereby an increase in local blood oxygenation, with a relative decrease in deoxyhaemoglobin (dHb). Oxyhaemoglobin (oHb) is diamagnetic (has no unpaired electrons) so its presence in the blood weakly reduces the local magnetic field [6]. On the other hand, dHb is paramagnetic (has 4 unpaired electrons) so it distorts the local magnetic field homogeneity. The difference in susceptibility between oHb and dHb creates the contrast for BOLD fMRI [7]. When neurons in a region are activated, there is an initial decrease in the oHb:dHb ratio. Then shortly after, there is a relatively large increase in this ratio as vasodilatation brings in fresh oxygenated blood (see Figure 2.1.1).

Generally, the initial decrease in the oHb:dHb ratio is not reliably detectable, but it is more evident at higher field strengths [8]. Although its origin remains under discussion, it is most likely due to an initial utilization of oxygen before oxygenated blood perfuses the tissue [9] [10]. Most fMRI studies use the subsequent increase in the oHb:dHb ratio, i.e. the hemodynamic response, to measure BOLD contrast. The exact mechanism of how neuronal activity results in the BOLD response is still being examined; however, the general steps are agreed upon. Neuronal signalling uses a neurotransmitter (for example glutamate) in the synapse; and the neurotransmitter is taken up by astrocytes. This results in a calcium signal cascade leading to release of several vasodilators including nitric

oxide [8] [11] [12]. Vasodilatation results in oxygenated blood, thus increasing the oHb:dHb ratio, with a concurrent decrease in local magnetic susceptibility.

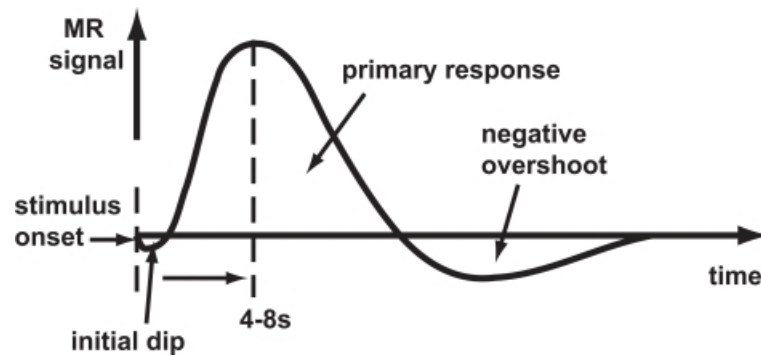


Figure 2.1.1: MR signal over time of BOLD signal. Initial reduction in oHb:dHb ratio, followed by a large increase and then a negative overshoot. [13]

Measuring the BOLD Effect

The hemodynamic response causes susceptibility changes by altering the oHb:dHb ratio, hence altering the ratio of diamagnetic and paramagnetic substance. BOLD fMRI uses parameters sensitive to changes in $T2^*$ (rate of decay of transverse magnetization commonly caused by spin-spin interactions and magnetic field inhomogeneities). This is accomplished using a gradient echo pulse sequence [14]. To measure the hemodynamic response as fast as possible, it is essential to use a fast phase and frequency encoding technique. The most common types of fMRI acquisition techniques are echo-planar imaging (EPI) [15] and spiral acquisitions [16]. Both techniques can acquire a single slice of data in roughly 70 ms, depending on the required resolution, readout bandwidth, and

gradient performance. The main difference between EPI and spiral acquisitions is the approach to k-space (i.e. raw data) navigation and filling. EPI involves filling all lines of k-space by multiple gradient reversals, producing multiple gradient echoes in a single acquisition during one $T2^*$ decay from a single radio frequency (RF) pulse. On the other hand, spiral acquisitions involve filling k-space through a spiral, from the inside out or outside in, by using oscillating gradients. Following phase corrections to account for left-right sampling directions, EPI raw data can be 2D Fourier transformed directly into an image. On the other hand, spiral raw data requires transformation to the Cartesian framework before applying the 2D Fourier transform. The high temporal resolution of these acquisition methods comes at the expense of spatial resolution. Furthermore, since the BOLD signal is of the same order of magnitude as the noise, larger voxels are necessary to increase signal to noise ratio (SNR), as $SNR \propto \text{voxel volume}$ [17].

2.1.2 Task-Based fMRI

Task-based fMRI experiments typically involve collecting MRI images while inducing one or more neurofunctional states from cognitive or sensory stimuli [16]. They are commonly arranged in blocks, i.e. periods of activity and periods of contrasting activity or rest. Typically, block lengths are 10 to 30 seconds. This induces a BOLD response in areas activated by the task. This task type is called ‘block design’. Another more complex task type is called ‘event related’, which allows for randomization of the order of conditions presented and

also variation in the time between stimuli presentation. Once the data is acquired, the BOLD response is statistically compared to the stimulus model, usually using a general linear model [18], to produce a statistical correlation map between each voxel's data and the stimulus model. Then this map may be overlaid onto the anatomical data to identify areas of high correlation.

Although frequently used, there are limitations and design challenges that come with using task-based fMRI methods [19]. For example, they require the formation of appropriate control tasks and the need to ensure equivalent task performance between groups. Furthermore, for a thorough experimental design, control and patient groups are demographically matched, especially age and years of education. The activation tasks need to be standardized to produce more reliable results. Also, some required tasks may be demanding for patients with various health problems.

2.1.3 Resting State fMRI

A promising area of fMRI is aimed at mapping resting state functional connectivity. In contrast to task-based fMRI, resting state fMRI does not require the subject to participate in a specific paradigm. Therefore, it could help overcome the limitations of task-based fMRI. Accordingly, task-based fMRI often has a higher intra-subject variability and lower inter-subject reliability [20].

Even when our brain is at rest, many anatomically separate brain areas are functionally connected and show a considerable amount of spontaneous neuronal

activity [21]. Resting state functional connectivity analysis usually targets low frequency (<0.1 Hz), synchronized activations (also known as low-frequency BOLD fluctuations) in spatially separated areas of the brain [22]. These synchronized neurophysiological events, active at rest, represent structurally and functionally connected networks, termed resting state networks (RSNs). RSNs refer to the temporal coherence between different areas of the brain at rest, i.e. in the absence of task driven activations. Resting state functional connectivity, as measured by BOLD signal fluctuations, has a neurophysiological basis and is sensitive to various neurological and psychiatric abnormalities [23]. Therefore, it is useful in understanding the behaviour of both normal and pathological subjects. In healthy subjects, a RSN is characterized by high metabolism during resting state [11] [24], functional connectivity during rest [25], and deactivation during various attention-demanding cognitive tasks [26] [27]. Several RSNs have been identified and are discussed in section 2.1.4.

Methods of Analysis

Resting state fMRI can be analyzed by two different approaches: model-dependent seed-based methods or model-free methods. Model-dependent methods examine the functional connectivity of a specific brain region, based on subjective placement of a seed-voxel. On the other hand, model-free methods are used to examine connectivity patterns without the need for defining any a priori seed region. Each method has its own advantages and disadvantages.

Most studies to date have used a seed-based approach to examine the functional connections of a specific brain region. This approach uses the extracted BOLD time course from a specific brain region and finds the temporal correlation between this signal and the time course from all other brain voxels [21]. This creates a whole-brain functional connectivity map of covariance with the seed region. This method is widely used and relatively simple. However, the information on the functional connectivity maps is limited to the functional connections of the chosen seed region, making it difficult to examine functional connectivity patterns on a whole-brain scale. Moreover, there is no standard method of choosing the regions and this may result in selection bias. This also makes comparisons between studies more difficult.

Model-free methods are used to examine brain connectivity patterns without the need for defining an a priori seed region. The most commonly used model-free methods are independent component analysis (ICA) based methods (for example probabilistic ICA) and have been reported to show a high level of consistency [21]. ICA is a mathematical algorithm from the group of algorithms dedicated to blind source separation. It attempts to identify a set of source signals from a set of mixed signals, without knowing any information about the source signals or the mixing process. A classic example of a source separation problem is the ‘cocktail party effect’, where one attempts to follow one of the many conversations taking place in the same room.

Probabilistic ICA looks for underlying sources that can explain the resting state patterns, searching for the existence of maximally independent spatial sources of resting state signals. Probabilistic ICA divides a dataset into different maximally independent components [28]. Spontaneous activity is automatically separated from noise (such as head motion) or physiological confounds (such as cardiac pulsation and respiration). Probabilistic ICA is advantageous because it evaluates and compares the coherence of activity in multiple distributed voxels, and divides RSNs into different independent components [28]. Limitations with the ICA approach include the difficulty of assigning a statistical framework that would enable activation networks to be tested against specific hypotheses, assigning the number of components ICA should pick out, and the difficulty of assessing the regionally specific nature of brain responses [29]. While there is currently no agreement as to which method (ICA or seed-based) is superior for assessing resting state fMRI [30], a comparison study between the two methods found ICA to be the superior method [31].

2.1.4 Resting State Networks

Over time, researchers have identified a number of RSNs that are steadily found in the resting brain, even across studies using different data acquisition and analysis techniques (for example, [32] [33] [34] [35]). The default mode network, sensorimotor network, executive control network, lateralized fronto-parietal

network, medial visual network and auditory network are amongst the most commonly found RSNs (see Figure 2.1.2).

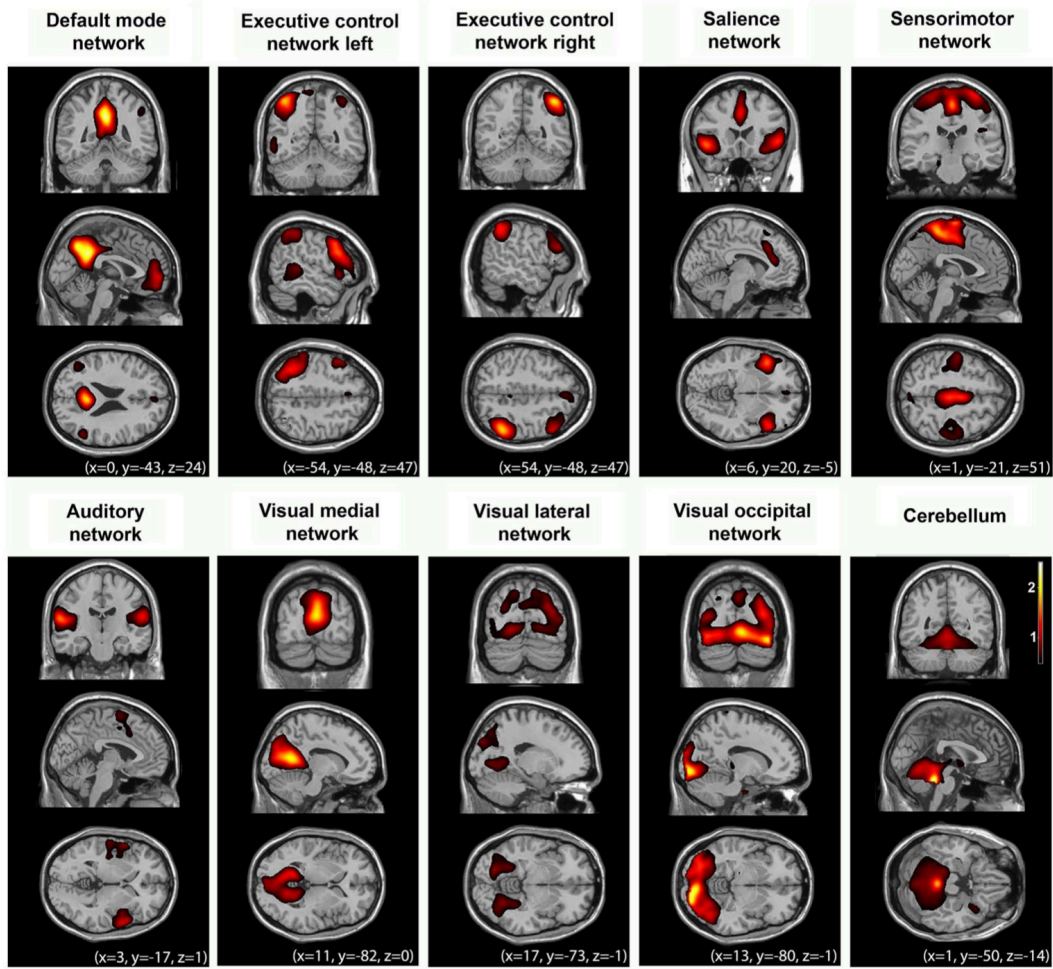


Figure 2.1.2: Ten RSNs generated using ICA of resting state fMRI in 10 healthy subjects. Group-level spatial maps (z values) are rendered on a structural underlay, where x,y and z values indicate MNI coordinates of the represented sections. [35]

Default Mode Network (DMN)

The DMN involves the precuneus/posterior cingulate, lateral parietal cortex, and mesial prefrontal cortex [34]. It has by far received the greatest attention from both the research and clinical community. In general, the DMN is more activated during rest and relatively less activated during demanding tasks that require focused attention. The DMN has been associated to introspective mental processes, stimulus-independent thoughts (also known as mind wandering), retrieving one's past or envisioning one's personal future, and self-reference.

Sensorimotor Network (SMN)

The SMN involves the precentral gyrus, postcentral gyrus, and supplementary motor area [34]. It is characterized by the involvement of brain regions that anatomically correspond to motor as well as sensory areas. It has been found that the activity of the sensorimotor cortex in the resting state exhibits a degree of hemispheric lateralization which correlates with the lateralization of activity in the same areas that emerge during an active finger tapping task [36]. This shows that the SMN is also associated with functionally relevant neural activity, which subserves active motor tasks.

Executive Control Network (ECN)

The ECN involves the medial frontal gyrus, superior frontal gyrus, and anterior cingulate cortex [34]. In some cases it also includes the lateral parietal areas. In general, the ECN is involved in tasks relying on executive functions, such as control processes and working memory. It has been found that intrinsic connectivity throughout the ECN is correlated with performance on the trail-making test, a neuropsychological test of executive functioning [37]. This shows that there is a tie between individual differences in intrinsic connectivity and the variability seen in the fundamental features of cognitive functioning.

Fronto-Parietal Network (FPN)

Two strongly lateralized components are commonly found, one in the right and one in the left hemisphere of the brain. They involve the inferior frontal gyrus, medial frontal gyrus, precuneus, inferior parietal gyrus, and angular gyrus [34]. The FPN has been associated to different functions including memory, language, attention, and visual processes [32] [38] [39] [40]. The precise role of the lateralized FPN remains less clear compared to the other RSNs.

Auditory Network (AN)

The AN involves the superior temporal gyrus, Heschl's gyrus, insula, and postcentral gyrus. The superior temporal gyrus contains the primary auditory cortex, which processes sound and contributes to our ability to hear. It also

contains Wernicke's area, which is involved in processing speech into an understandable language.

Visual Networks

Three distinct visual networks have been reported throughout the literature [34]. The first component is characterized by activity in the mesial visual areas, namely striate cortex and extra-striate regions typically mesial, such as lingual gyrus. The second component is associated with lateral visual areas such as the occipital pole and occipito-temporal regions. The third component is associated with activity in the striate cortex and in polar visual areas.

2.2 Diffusion Tensor Imaging (DTI)

2.2.1 Diffusion Physics

Diffusion is the microscopic phenomenon that describes the random motion of particles when suspended in a fluid. Diffusion may be driven by a concentration gradient, where particles move from a region of high concentration to a region of low concentration. This is described using Fick's first law [41]:

$$J = -D \nabla C$$

where J is the diffusion flux i.e. the rate of flow per unit area, D is the diffusion coefficient, and ∇C is the concentration gradient. Fick's first law states that the

flux of particles in a system is proportional to the gradient of the particle concentration. The negative sign is the basis for the understanding of diffusion as the tendency of particles in a system to move from regions of higher concentration to regions of lower concentration.

Diffusion may also arise from thermal motion in which molecules migrate randomly. Molecular diffusion motion is also known as Brownian motion. It is named after botanist Robert Brown who in 1827 observed the random motion of pollen grains in water under a microscope. Einstein's relation develops a time distance relationship for a particle undergoing Brownian motion in free space [42]:

$$r^2 = 6 D t$$

where r is the mean displacement, D is the diffusion coefficient, and t is the time during which a particle undergoes motion.

If the environment is restrictively bounded, then the particles undergoing Brownian motion are displaced with larger magnitudes in directions parallel to boundaries, and smaller magnitudes in directions that are perpendicular to boundaries [43]. Hence, directionally dependent Brownian motion reflects the underlying structure of a bounded environment. When displacement due to Brownian motion is directionally dependent, the diffusion is said to be anisotropic. In cases of anisotropic diffusion, Einstein's relation is modified to take into account directional dependence [43]:

$$\mathbf{D} = \frac{1}{6t} \hat{r} \hat{r}^t$$

where $\mathbf{D}$ is known as a diffusion tensor, and $\hat{r}$ is a displacement vector indicating both the magnitude and direction of Brownian motion.

Generally, the diffusion tensor, $\mathbf{D}$, depends on the particle mass, temperature and structure of the medium [44]. In DTI, the particle mass of water molecules and the temperature at which measurements are conducted are assumed to be constant. Therefore, the spatial fluctuation of the diffusion tensor is interpreted solely in terms of local anatomical structure.

2.2.2 DTI and White Matter

Groups of axons in the human brain are bundled together in what are known as tracts, or fascicles. The portion of the fascicles that contains white fatty myelinated oligodendrocytes forms the white matter. The white matter axons are highly organized and tightly packed together. Water diffusion across tracts with myelinated boundaries is restricted. This causes the water to diffuse in larger amounts in a direction parallel to fibre tracts [45] [46] [47]. This physical situation is what is measured by diffusion weighted imaging to construct diffusion tensors and resulting tractography estimates.

2.2.3 Diffusion Imaging

Diffusion weighted imaging (DWI) utilizes diffusion gradient pulses to estimate a relative amount of water diffusion in a measurement direction $\hat{g}$.

Diffusion weighted images are the raw data source used to calculate the diffusion tensor. Diffusion weighted images are produced using the Stejskal-Tanner method. This approach may be implemented by adding diffusion gradient pulses to spin echo EPI MRI pulse sequences. A simple example is using a spin-echo sequence with the addition of two diffusion-encoding gradients [47].

The addition of two gradient pulses symmetrically positioned at the 180° refocusing RF pulse allows for the measurement of spin drift in a given direction, $\hat{g}$. Spins not undergoing motion in the $\hat{g}$ direction are refocused by the application of a 180° phase refocusing pulse, and then a repetition of the gradient pulse, g . Spins undergoing Brownian motion at the time between gradient pulses do not refocus completely at the application of the second gradient pulse, resulting in signal loss.

In practice, diffusion weighted images are usually acquired using echo-planar imaging (EPI) sequences to reduce acquisition time [48]. EPI sequences use only one RF excitation to obtain the complete set of planar k-space measurements used to construct a planar DWI image. Although EPI sequences offer better acquisition times and reduce motion artefacts, they are subject to distortions as a result of the eddy currents introduced by the large diffusion gradients used in DWI measurements.

The Stejskal-Tanner equation describes the relationship between loss of phase coherence in the transverse spin RF signal and the gradient pulse, g [47]:

$$S_i = S_0 \exp[-b \hat{g}_i^T D \hat{g}_i]$$

where S_0 is the signal intensity in the absence of any gradients, S is the intensity after the application of the Stejskal-Tanner diffusion gradients, D is the diffusion coefficient, and b is the diffusion weighting factor given by:

$$b = \gamma^2 \delta^2 [\Delta - (\delta/3)] |g|^2$$

where γ is the gyromagnetic ratio, δ is the gradient pulse width, Δ is the time interval between gradient pulses, and $|g|$ is the strength of the diffusion gradient pulses.

When measurements are made in vivo, the measured diffusion coefficient is referred to as apparent diffusion coefficient (ADC). This is because the presence of cell membranes or molecules hinders the free diffusion of water; therefore, what is measured is the apparent decrease in diffusion resulting from restricted water motion [49] [50].

The Diffusion Tensor

Diffusion may be isotropic or anisotropic. If diffusion is isotropic, i.e. not varying in magnitude according to the direction of measurement, then it can be characterized by a sphere (see Figure 2.2.1) [51]. If diffusion is anisotropic, i.e. varying in magnitude according to the direction of measurement, usually due to the presence of barriers that restrict the free motion, then it can be characterized by an ellipsoid (see Figure 2.2.1). The longest axis of the ellipsoid corresponds to the direction of greatest diffusion. Anisotropic diffusion can be represented by a diffusion tensor, $\mathbf{D}$, given as:

$$\mathbf{D} = \begin{bmatrix} D_{xx} & D_{xy} & D_{xz} \\ D_{yx} & D_{yy} & D_{yz} \\ D_{zx} & D_{zy} & D_{zz} \end{bmatrix}$$

where the diagonal elements, D_{xx} , D_{yy} , D_{zz} , represent diffusion along the x, y and z axes, respectively; while the off-diagonal elements carry information about the rotations of diffusion constants. The diffusion tensor is symmetric ($D_{ij} = D_{ji}$), hence six parameters are needed to characterize it. So as to measure six diffusion constants along six independent axes, at least six diffusion weighted images are needed. Then, the diffusion tensor can be calculated using multivariate linear regression [52].

Diagonalization of the diffusion tensor results in a set of three eigenvectors, $\hat{v}_1$, $\hat{v}_2$, $\hat{v}_3$, with associated eigenvalues, λ_1 , λ_2 , λ_3 , and together they characterize diffusion along an ellipsoid. The eigenvalues give the magnitudes of diffusion, while the eigenvectors give diffusion directions. The primary eigenvector is $\hat{v}_1$ as it corresponds to the principle axis of diffusion [42].

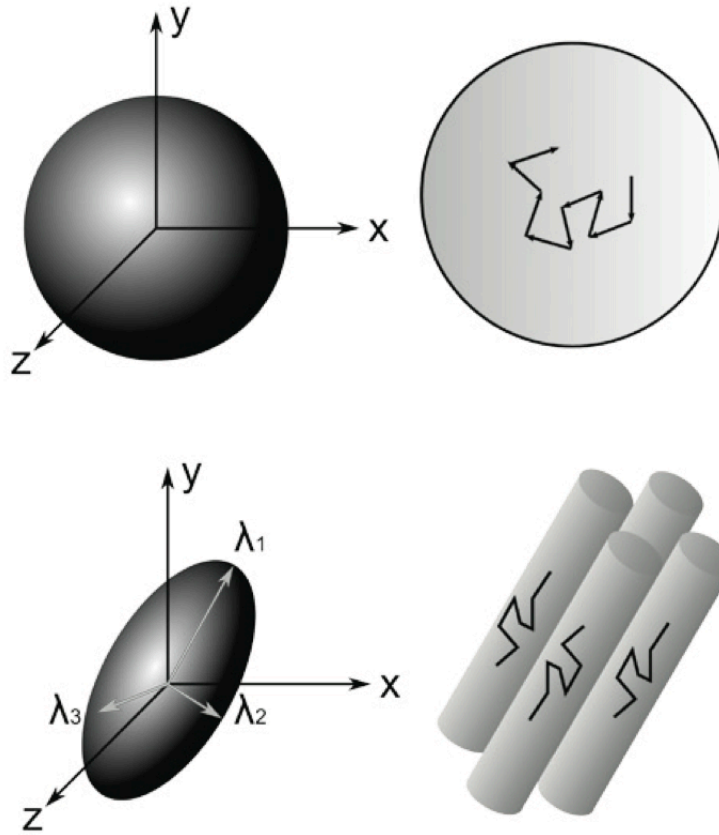


Figure 2.2.1: Diffusion sphere and ellipsoid. In isotropic diffusion (top), diffusion is equal in all directions and can be represented as a sphere; whereas anisotropic diffusion (bottom) can be visualized as an ellipsoid. [52]

The mean diffusivity (MD), also known as ADC, is the arithmetic mean of the three eigenvalues:

$$MD = ADC = \frac{\lambda_1 + \lambda_2 + \lambda_3}{3}$$

The degree of anisotropy in the diffusion tensor is commonly represented by the fractional anisotropy (FA) scalar metric:

$$FA = \sqrt{\frac{3}{2}} \sqrt{\frac{(\lambda_1 - \lambda_m)^2 + (\lambda_2 - \lambda_m)^2 + (\lambda_3 - \lambda_m)^2}{\lambda_1^2 + \lambda_2^2 + \lambda_3^2}}$$

where λ_m is the mean of the eigenvalues i.e. MD. FA scales between zero and one.

In an extreme case, a FA value of zero indicates a completely isotropic diffusion (as in a sphere), where $\lambda_1 = \lambda_2 = \lambda_3$. In the opposite extreme case, a FA value of one indicates a completely anisotropic diffusion (as in a cylinder) in the direction of the principle eigenvector, where $\lambda_1 \gg \lambda_2 = \lambda_3$.

Methods of Analysis

DTI can be used to study brain structure either on a regional or a whole-brain level. Each method has its own advantages and disadvantages. Regional analyses include both region of interest (ROI) analyses and tractography. ROI analyses involve choosing an a priori area of interest for study, whereas tractography involves using an a priori region of interest to define a white matter tract for study. In both ROI analyses and tractography, average diffusion values, such as FA, are extracted from voxels within the ROIs or tracts for subsequent analysis. Regional analyses are widely used and relatively simple. Most studies to date have used ROI analyses to examine the white matter of a specific brain region. However, regional analyses are restricted to the assessment of the a priori defined regions and, as a result, only a small amount of the total white matter is usually investigated. This makes it difficult to examine white matter on a whole-brain scale. Furthermore, the reliability of regional analyses depends on accurate

and reproducible spatial localization of ROIs or tracts across subjects. Moreover, there is no standard method of choosing the regions and this may result in selection bias. This also makes comparisons between studies more difficult. On the other hand, whole-brain analysis methods are used to examine whole-brain diffusion patterns without the need for defining an a priori region of interest. The most commonly used whole-brain analysis methods are voxel-based morphometry (VBM) and tract-based spatial statistics (TBSS).

2.3 Magnetic Resonance Spectroscopy (MRS)

2.3.1 Spectroscopy Background

Magnetic resonance spectroscopy is an analytical method that enables the identification and quantification of metabolites in a sample. Whilst conventional MRI provides anatomical information, MRS provides physiological and chemical information. Both MRS and MRI have their origin in Nuclear Magnetic Resonance (NMR). Nobel Prize winners Edward Purcell, from Harvard University, and Felix Bloch, from Stanford University, first described NMR in 1946. At that point in time, NMR was mainly used by physicists to determine the nuclear magnetic moments of nuclei. In the mid 1970s, NMR began to be used in vivo when Lauterbur, Mansfield, and Grannell introduced gradients into the magnetic field enabling them to determine the location of the emitted signal,

producing an image instead of a spectrum. NMR imaging was later renamed MRI because the term ‘nuclear’ was regularly, and incorrectly, associated with nuclear medicine. For the same reason, in vivo NMR spectroscopy was renamed MRS. In the 1980s, the first MRI medical scanners became available for clinical use, and improvements are continually being made over time.

2.3.2 Physical Basis

Various nuclei may be used to obtain MR spectra, including hydrogen (^1H), carbon (^{13}C), sodium (^{23}Na), phosphorus (^{31}P) and fluorine (^{19}F). ^1H is the most widely used nuclei for clinical MRS, mainly because of its high abundance in human tissues. ^1H -MRS is conveniently acquired using the standard RF coils and a dedicated software package. However, for other nuclei, RF coils tuned to the Larmor frequency of that nuclei, matching preamplifiers, hybrids and broadband power amplifier are required.

^1H -MRS is based on a principle known as chemical shift. When an atom is placed in an external magnetic field, its nucleus will resonate at a frequency, f , that is given by the Larmor equation:

$$f = \gamma B_0$$

where γ is the gyromagnetic ratio, and B_0 is the external magnetic field. Since γ is a constant of each nuclear species, the spin frequency of a certain nuclei depends on B_0 and the local microenvironment. The electric shell interactions of these nuclei with the surrounding molecules produce a change in the local magnetic

field resulting in a change in the spin frequency of the atom - this is known as chemical shift. The value of this difference in resonance frequency provides information about the molecular group carrying ^1H . The chemical shift position of a nucleus is ideally expressed in parts per million (ppm), since it is independent of the field strength (for instance, choline will be positioned at 3.2 ppm at 1.5 T or 3 T). The x-axis of an MR spectrum represents the metabolite frequency in ppm according to the chemical shift, while the y-axis represents the peak amplitude (see Figure 2.3.1). A few metabolites have several peaks (doublets or triplets) instead of single peaks. These peaks are broken down into more complex peaks and are explained by J-coupling, also known as spin-spin coupling. The J-coupling phenomenon occurs when the molecular structure of a metabolite is such that protons are found in different atomic groups, for example CH_3 - and CH_2 -. The local magnetic fields of these groups are slightly different; therefore, each ^1H resonates at a frequency characteristic of its position in the molecule, leading to multiple peaks.

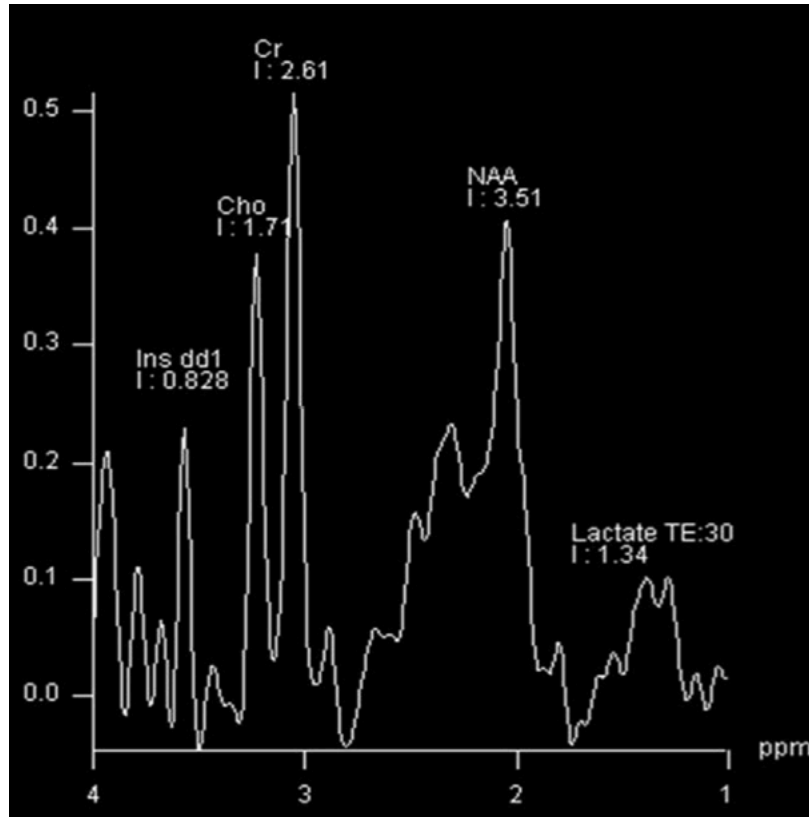


Figure 2.3.1: MR spectrum showing various metabolite peaks. [53]

2.3.3 Brain Metabolites

There are around 15 to 20 brain metabolites that can be detected with relative confidence using in vivo ^1H -MRS and a clinical 3T scanner [54], some of which will be discussed here in detail. Most of the peaks from metabolites that can be recorded are between 0 and 4.0 ppm. This is because suppressing the extreme water signal, which occurs around 4.7 ppm, usually results in higher signals being destroyed. In clinical MRS, the largest chemical peaks are produced from N-acetyl aspartate, creatine and choline.

N-Acetyl Aspartate (NAA)

NAA is a free amino acid that is present at a high concentration in the healthy brain. NAA is produced in the mitochondria of neurons then transported into neuronal cytoplasm and along axons. NAA is a marker of neuronal integrity and viability. It also acts as an intracellular osmolite (for the removal of large amounts of water from signalling), a storage form of aspartate and acetate, and a precursor of N-acetyl aspartyl glutamate (NAAG) [55]. NAAG is involved in excitatory neurotransmission, and is a source of glutamate. It is believed that a decrease in NAA concentration is a sign of neuronal loss or neuronal dysfunction [56]. The most prominent peak for NAA is from a singlet at 2.01 ppm, from the three protons of an N-acetyl CH₃ group.

Creatine (Cr)

The Cr peak represents a combination of molecules including creatine and phosphocreatine (PCr). They are present in the brain in both neurons and glial cells. Cr plays a role in the energy metabolism of tissues. Higher levels of Cr are found in grey matter than in white matter. The most prominent peak for Cr is from a singlet at 3.03 ppm, from methyl-protons. The concentration of Cr has been found to be relatively constant across age and a variety of diseases. Consequently, it is often used as a concentration reference for calculating metabolite ratios.

However, this practice has been questioned because reduced Cr levels have been found in several conditions, such as tumours and renal disease [53].

Choline (Cho)

Cho, which consists of phosphocholine (PCh) and glycerophosphocholine (GPC), is a constituent of cell membranes. It is a marker of membrane synthesis, inflammation or demyelination. The most prominent peak for Cho is from a singlet at 3.2 ppm, from nine protons of a trimethyl group. Pathological changes in membrane turnover result in an increase in Cho concentration. Increased Cho level in the brain has been associated with head injury, Alzheimer's disease, and cancer [56].

Glutamate (Glu) and Glutamine (Gln)

Glu is the most abundant amino acid found in the healthy brain and acts as the major excitatory neurotransmitter. Gln, located in astrocytes, is an amino acid that acts as a precursor and storage form of Glu. Glu has a complex spectrum resulting in low intensity spectral peaks despite its relative abundance. Furthermore, these signals overlap with resonances from NAA, gamma-aminobutyric acid (GABA) and Gln, from 2.04 to 2.35 ppm. Due to the difficulty in separating these signals at field strengths lower than 3 T, Glu and Gln contributions are sometimes combined and referred to as Glx.

Myo-inositol (mI)

mI is a cyclic sugar alcohol and is a marker of neuroglial cells, being present in the astrocytes of brain tissue. mI is the most important osmolyte in astrocytes. It may also represent a product of myelin degradation. Increased mI concentration occurs with proliferation of glial cells or with increased glial cell size as found in inflammation. mI is typically elevated in Alzheimer's disease and head injury [53]. The most prominent peak for mI is from a doublet-of-doublets at 3.52 ppm and a triplet at 3.61 ppm, from nine protons of a trimethyl group.

Lactate (Lac)

Lac is the end product of anaerobic glycolysis. It is present in the healthy brain at very low concentrations. Lac concentration increases when the brain cells are denied oxygen, for example following a stroke, trauma, or in tumours. It has also been observed to increase briefly with hyperventilation [54]. The peak for Lac is a doublet at 1.31 ppm.

2.3.4 Water Suppression

The most abundant compound in the human brain is water. Generally, the water content is in the range of 73% in white matter, 82% in grey matter, and greater than 95% in CSF. Water content is used as an internal concentration reference since it changes moderately between different pathologies. Water concentration in the brain is approximately 10,000 times greater than the

metabolites of interest. This large water resonance results in baseline distortions and unwanted signals from vibration-induced signal modulation [54].

Water suppression is typically accomplished with CHESS (Chemical Shift Selective) or IR (Inversion Recovery) technique. CHESS technique uses a very narrow bandwidth frequency-selective pulse applied at the exact Larmor frequency of water to selectively excite the water signal [57]. Then, gradient pulses are applied to dephase any resulting transverse magnetization [17]. These gradients may be repeated a few times in different directions to maximize the effectiveness.

In IR, a 180° RF pulse is first applied to invert the hydrogen spins into the longitudinal axis. Then after a period of time, named time to inversion (TI), a 90° pulse is applied to flip the longitudinal magnetization into the x-y axis. During the TI, the longitudinal vectors of various metabolites begin to grow according to their T1 growth curve. At a set time, when $z = 0$ (termed the null point), the growth curve of water molecules crosses the x-y plane. If a 90° pulse is applied at that point, the longitudinal magnetization for water will be zero and stay zero. Meanwhile, other metabolites will acquire a longitudinal magnetization that can be measured. The water signal will have been suppressed [58].

2.3.5 Single Volume Localization

Localizing ^1H -MRS to a specified region of interest (ROI) is essential for eliminating unwanted signals from outside the ROI. Furthermore, it allows for the

minimization of ‘partial volume effects’, where the signal from one compartment is contaminated by a signal from another compartment. Moreover, it decreases magnetic field inhomogeneities, resulting in narrower spectral lines and more precise signal excitation and reception.

In general, two imaging sequences are utilized for single voxel ^1H -MRS: point resolved spectroscopy (PRESS) [59] and stimulated echo acquisition mode (STEAM) [60]. The key difference between them is that PRESS has a better SNR, and STEAM has better localization [61]. PRESS and STEAM sequences both share the following properties: three selective RF pulses; crusher gradients to dephase spins and remove all unwanted echoes; and they both produce stimulated echoes.

Analysis of Spectra

Linear combination model (LCModel) is the most commonly used method to estimate the areas under the peaks for metabolite quantitation [62]. Developed by Stephen Provencher, LCModel is an operator-independent spectral analysis software that obtains approximate maximum likelihood estimates of the metabolite concentrations and their uncertainties.

The basic methodology used in LCModel has been presented by S. Provencher [63]. The method analyzes an in vivo spectrum as a Linear Combination of Model in vitro spectra from individual metabolite solutions. Complete model spectra, rather than individual resonances, are utilized in order to

include maximum prior information into the analysis. LCModel uses a nearly model-free constrained regularization method that automatically accounts for the baseline and line shape in vivo, without inflicting a restrictive parameterized form on them. Approximate maximum-likelihood estimates of the metabolites concentrations and their uncertainties, i.e. Cramer-Rao lower bounds (CRLB), are obtained. The CRLB act as estimated standard deviations, expressed in percent of estimated concentrations. CRLB less than 20% are widely used as a rough criterion for estimates of acceptable reliability [64].

2.3.6 Quantification

In order to obtain ‘absolute’ metabolite levels, a method similar to that described by Gasparovic et al [65] is used. This method uses the unsuppressed ‘internal’ water signal, tissue fractions, and water-tissue and metabolite relaxation times. It assumes that the signal of the metabolite in question, which comes from WM and GM, will be proportional to the number of moles of the molecules in the voxel multiplied by the number of observed hydrogen atoms contributing to the signal. This signal can be related to the water signal from the same voxel by the following equation:

$$\frac{S_M}{S_{H_2O\ GM/WM}} = \frac{[M] \times \#H_M}{[H_2O]_{GM/WM} \times 2}$$

where the subscripts refer to water (H₂O) or metabolite (M) in the parenchyma (GM/WM) of the voxel, #H_M is the number of protons that give rise to the

metabolite peak of interest, 2 is the number of water protons and $[H_2O]$ is the molal concentration of MR-visible water in the metabolite solution which is assumed to be that of pure water (i.e. 55.51 mol/kg). $S_{H_2O GM/WM}$ is derived from the observed water signal, $S_{H_2O obs}$, by relating the signal to the fully relaxed water signal according to the expression:

$$S_{H_2O obs} = f_{GM} \times S_{H_2O} \times R_{H_2O GM} + f_{WM} \times S_{H_2O} \times R_{H_2O WM} + f_{CSF} \times S_{H_2O} \times R_{H_2O CSF}$$

where f_{GM} , f_{WM} and f_{CSF} are the tissue molal water fractions of GM, WM and CSF, respectively. The relaxation attenuation factors are given by:

$$R_{H_2O obs} = \exp[-TE/T2_{H_2Ox}](1 - \exp[-TR/T1_{H_2O}])$$

where $T1_{H_2O}$ and $T2_{H_2Ox}$ are the T1 and T2 relaxation times of water tissue fraction x (GM, WM or CSF). To obtain $S_{H_2O GM/WM}$, we solve for S_{H_2O} and subtract from it the fraction that is CSF:

$$S_{H_2O GM/WM} = \frac{S_{H_2O obs} (1 - f_{CSF})}{f_{GM} \times R_{H_2O GM} + f_{WM} \times R_{H_2O WM} + f_{CSF} \times R_{H_2O CSF}}$$

The tissue molal water fractions can be related to tissue volume fractions by assuming relative densities of MR-visible water in GM, WM and CSF to be 0.78, 0.65 and 0.97, respectively [66]. For example, for GM:

$$f_{GM} = \frac{f_{GMvol} \times 0.78}{f_{GMvol} \times 0.78 + f_{WMvol} \times 0.65 + f_{CSFvol} \times 0.97}$$

where f_{GMvol} , f_{WMvol} and f_{CSFvol} are the tissue volume fractions for GM, WM and CSF, respectively. The relaxation attenuation of the fully relaxed metabolite signal S_M can be estimated from the mean T1 and T2 values of the metabolite protons since the values do not appear to differ considerably in GM and WM [67] [68] [69]. Hence, $S_M \approx S_{Mobs}/R_M$, where:

$$R_M = \exp[-TE/T2_M](1 - \exp[-TR/T1_M])$$

Lastly, by combining the equations we obtain:

$$[M] = \frac{S_{Mobs} \times (f_{GM} \times R_{H2O\ GM} + f_{WM} \times R_{H2O\ WM} + f_{CSF} \times R_{H2O\ CSF})}{S_{H2O\ obs}(1 - f_{CSF}) \times R_M} \times \frac{2}{\#H_M} \times [H2O]$$

Since LCModel calculates metabolite concentrations using the following equation [64]:

$$[M] = \frac{S_{Mobs}}{S_{H2O\ obs}} \times \frac{R_{H2O}}{R_M} \times \frac{2}{\#H_M} \times [H2O]$$

where LCModel assumes that $[H_2O] = 35880$, $R_M = 1.0$ (for basis sets acquired with $TR > 6000$ ms), and $R_{H2O} = 0.7$ (for $TE = 30$ ms). Thus, in order to obtain $[M]$ we must undo LCModel's default values for $[H_2O]$, R_M , and R_{H2O} , and use the corrected values (see Table 2.3.1), along with the tissue volume fractions. Therefore, with the concentration outputs from LCModel, $[M]_{LCModel}$, the 'absolute' concentration can be obtained by:

$$[M] = \frac{[M_{LCModel}]}{0.7 \times 35880} \times \frac{(f_{GM} \times R_{H2O\ GM} + f_{WM} \times R_{H2O\ WM} + f_{CSF} \times R_{H2O\ CSF})}{(1 - f_{CSF}) \times R_M} \times 55510$$

For each metabolite, $R_{H2O\ GM}$, $R_{H2O\ WM}$, $R_{H2O\ CSF}$, and R_M values can be calculated using the T1 and T2 values from Table 2.3.1.

Table 2.3.1: T1 and T2 relaxation times for tissue and metabolites [70] [71]

		T1 (ms)	T2 (ms)
Tissue			
	GM	1470	110
	WM	1060	74
	CSF	3000	200
Metabolite			
	NAA	1515	274
	PCr + Cr	1365	170
	GPC + PC	1230	221
	mI	1040	200
	Glu	1230	200

CHAPTER 3

Traumatic Brain Injury

3.1 Epidemiology of Traumatic Brain Injury

"Women and elephants never forget an injury." – Saki 1910

Traumatic brain injury (TBI) is a major health problem in Canada and around the world. A TBI occurs suddenly and without warning. We participate in everyday activities that put us at risk of sustaining a TBI; for example, a fall on the pavement while walking to the grocery store, a car accident while driving to work, or a collision while playing sports. TBI is defined as “a non-degenerative, non-congenital insult to the brain from an external mechanical force, possibly leading to permanent or temporary impairment of cognitive, physical, and psychosocial functions, with an associated diminished or altered state of consciousness” [72]. As defined by the Centres for Disease Control and

Prevention, a TBI “is caused by a bump, blow or jolt to the head or a penetrating head injury that disrupts the normal function of the brain” [73]. TBI in children and adults is caused by either: impact loading (where the head hits a stationary object or is struck by a moving object) or impulsive loading (when the head moves as a result of the motion to some other part of the body).

According to the Brain Injury Association of Canada, there are approximately 18,000 hospitalizations associated with TBI diagnosis annually in Canada, and an estimated 1.4 million Canadians are living with TBI [74]. According to the Centres for Disease Control and Prevention, an estimated 1.7 million people sustain a TBI annually in the United States [73]. Around 75% of them sustain a mild traumatic brain injury (mTBI). Furthermore, up to 3.8 million sports-related TBI are estimated to occur each year in the United States [75]. These numbers may be underestimates since mTBI is often under-diagnosed, and many sports-related and mild TBI patients do not seek medical care.

TBI has been referred to as the ‘silent epidemic’ due to its high incidence but frequent under-diagnosis. However, more and more people are becoming aware of its potentially devastating and insidious effects [76]. TBI recently attracted considerable attention from academia and mass media, particularly on the dangers of sports-related mTBI and the growing concern for increased vulnerability to repeat injuries.

TBI is caused mainly by falls (35%), motor vehicle and traffic accidents (17%), struck by or against events, which include colliding with a moving or

stationary object (16%), and assaults (10%) [73]. Sports and recreational activities are also a sizeable source of TBI. Furthermore, males are twice as likely to sustain TBI than females [74]. Additionally, children aged 0 to 4 years, adolescents aged 15 to 19 years, and adults aged 65 years and older are most likely to sustain a TBI than any other age group for all hospitalization and emergency department visits [73]. For hospitalizations only, adults aged 75 years and older have the highest rates of TBI related hospitalization.

3.2 Severity of Traumatic Brain Injury

The classification of the severity of a TBI is important to both clinicians and researchers. It is essential in providing the appropriate acute medical care for patients, and in predicting recovery and outcome. The most widely used indices for classifying TBI severity include the Glasgow Coma Scale (GCS), duration of post-traumatic amnesia (PTA), and length of loss of consciousness (LOC) (see Table 3.2.1). Medical personnel utilize these measures soon after a TBI to assess the patient's level of injury and responsiveness. The TBI is then categorized as mild, moderate, or severe. This outcome is also frequently used for patient triage management [77].

The GCS was published in 1974 and is one of the most commonly used indices to assess TBI severity [78]. The GCS assesses a patient's response to simple stimuli, including their best motor response, best verbal response, and degree of eye opening. To assign the GCS score, the results from these three

scores are combined. Head injuries associated with a GCS score of 13 to 15 are generally classified as mild, 9 to 12 as moderate, and 8 or less as severe. When using the GCS to assess the severity of a TBI, it is critical to consider the effects of alcohol, drug use, medications, or any other factors that may lead to inaccurate scores. Furthermore, the time between injury and GCS assessment should be recorded and taken into consideration. Ideally, GCS assessment should be performed right after the injury, or as soon as medical personnel arrive. The time of assessment is particularly important when considering mTBI since these patients could improve considerably within the first few hours after injury.

Another widely used index is PTA, which considers the duration of post-traumatic amnesia. Post-traumatic amnesia is the state of confusion or disorientation following a TBI, where the injured person is conscious but behaving in an uncharacteristic manner, or has impaired concentration, or an inability to consolidate new memories. Head injuries associated with a PTA duration of less than one hour are generally classified as mild, up to 24 hours as moderate, and over 24 hours as severe. When using the duration of PTA to assess the severity of a TBI, it is critical to consider the effects of alcohol, drug use, or medications that may lead to inaccurate estimates.

LOC has historically been used as an indicator of TBI severity. However, it is crucial to note that diagnosis of mTBI does not require any loss of consciousness, and significant TBI does not necessarily lead to loss of consciousness [79]. With these caveats in mind, head injuries associated with a

LOC of less than 30 minutes are generally classified as mild, up to 24 hours as moderate, and over 24 hours as severe.

Table 3.2.1: Severity indices commonly used in classifying TBI severity.

TBI Severity Index	Measurement Approach	Severity Category		
		Mild	Moderate	Severe
Glasgow Coma Scale (GCS)	Mental status, neurologic deficits	13-15	9-12	3-8
Post-traumatic Amnesia (PTA)	Mental status, memory and recall	< 1 hour	1 to 24 hours	> 24 hours
Loss of Consciousness (LOC)	Mental status, alertness	< 30 min	0.5 to 24 hours	> 24 hours

A variety of radiological techniques, such as x-rays of the skull and computed tomography (CT), are also utilized to assess TBI severity. Currently, consensus is lacking regarding the imaging of mTBI. To minimize controversy about the use of CT for patients with mTBI, a highly sensitive decision rule, named ‘The Canadian CT Head Rule’, was developed [80]. The Canadian CT Head Rule states that a CT is only required for patients with minor head injuries (GCS score of 13–15) with any one of the following: GCS score < 15 at 2 hours after injury; suspected open or depressed skull fracture; any sign of basal skull fracture (haemotympanum, ‘racoon’ eyes, cerebrospinal fluid

otorrhoea/rhinorrhoea, Battle's sign); vomiting $\geq$ two episodes; age $\geq$ 65 years; amnesia before impact $>$ 30 minutes or dangerous mechanism (pedestrian struck by motor vehicle, occupant ejected from motor vehicle, fall from height $>$ 3 feet or five stairs).

3.3 Mild Traumatic Brain Injury

Throughout the literature, definitions of mTBI are similar but not identical, and several organizations have developed diagnostic criteria for mTBI. The American Academy of Neurology (AAN) defines mTBI as: “a trauma-induced alteration in mental status that may or may not involve loss of consciousness” [81]. This definition indicates that the injury may not necessarily involve a direct blow to the head, or loss of consciousness.

The Mild Traumatic Brain Injury Committee of the American Congress of Rehabilitation Medicine (ACRM) developed a definition of mTBI, which is: “a patient with mild traumatic brain injury is a person who has had a traumatically induced physiological disruption of brain function, as manifested by at least one of the following:

1. any period of loss of consciousness;
2. any loss of memory for events immediately before or after the accident;
3. any alteration in mental state at the time of the accident (e.g., feeling dazed, disoriented, or confused);
4. focal neurological deficit(s) that may or may not be transient;

but where the severity of the injury does not exceed the following:

- loss of consciousness of approximately 30 minutes or less;
- after 30 minutes, an initial Glasgow Coma Scale (GCS) of 13-15; and
- post-traumatic amnesia (PTA) not greater than 24 hours.” [82]

This mTBI definition includes the head being struck, the head striking an object, and the brain undergoing an acceleration/deceleration movement (i.e. whiplash) without direct external trauma to the head. It excludes stroke anoxia, tumour and encephalitis.

According to the Centres for Disease Control and Prevention, the conceptual definition of mTBI is: “an injury to the head as a result of blunt trauma or acceleration or deceleration forces that result in one or more of the following conditions:

Any period of observed or self-reported:

- Transient confusion, disorientation, or impaired consciousness
- Dysfunction of memory around the time of injury
- Loss of consciousness lasting less than 30 minutes

Observed signs of neurological or neuropsychological dysfunction, such as:

- Seizures acutely following injury to the head
- Among infants and very young children: irritability, lethargy, or vomiting following head injury
- Symptoms among older children and adults such as headache, dizziness, irritability, fatigue or poor concentration, when identified soon after injury,

can be used to support the diagnosis of mild TBI, but cannot be used to make the diagnosis in the absence of loss of consciousness or altered consciousness.” [83]

3.4 Post-Concussion Syndrome

*"That a man with a hurt brain should have a disturbed mind is to be expected." –
Sir Charles Symonds 1942*

Generally, patients who sustain mTBI will recover within days to months [84]. However, up to 15% of patients diagnosed with mTBI experience persistent problems [73]. The consequences for these individuals may include delayed return to work or school, reduced functional ability and heightened emotional distress [84]. When symptoms persist, the term post-concussion syndrome (PCS) is used.

The symptoms following TBI, without detectable structural brain injury, have been historically identified. As early as the 1800s, the term traumatic neurasthenia, or ‘railway brain’, was used to describe symptoms of railway trauma [85]. The symptoms were reported as sleeplessness, irritability, depression, inability to do mental or physical work, memory disturbance, headache, tinnitus, nervousness, vasomotor disturbance, excessive sweating, eye strain, enlarged pupils, spinal pain and twitches, irregular pulse, and lateralized sensory deficit [85]. In 1889, Hermann Oppenheim in Germany developed the term ‘vasomotorischen symptomenskomplex’ to describe similar symptoms that

were not due to an obvious structural impairment [86]. Symptoms included headaches, dizziness, vasomotor instability, and intolerance of alcohol. These symptoms were assumed to be the result of disordered intracranial blood flow.

In 1928, Sir Charles Symonds described a symptom complex of headache, giddiness, inability to concentrate, defective memory, indecision, loss of emotional control, and fatigue that followed head injury. A “temporary vascular embarrassment” was assumed to be the cause of these symptoms [87]. This symptom complex was later named ‘postcontusional syndrome’ [88]. Then in the early 1930s Russell [89], and Strauss and Savitsky [90] [91] developed the term ‘postconcussional syndrome’, which was used to describe individuals with persistent headache, dizziness, loss of memory, nervousness, or sleeplessness approximately six months post-head injury.

Nowadays, the symptoms that follow mTBI are often organized into three broad categories: cognitive problems, physical complaints, and behavioural/emotional changes. Some of the more common symptoms of each category are presented in Table 3.4.1. Cognitive problems associated with PCS include feeling slowed down, feeling ‘in a fog’ or dazed, difficulty concentrating or difficulty remembering. Tasks that are most likely to show deficits are those that require fast processing, attention, executive functions, working memory, and declarative long-term memory [92] [93] [94]. Physical complaints include fatigue, headache, nausea, vomiting, blurred or double vision, seeing stars or lights, balance problems, dizziness, sensitivity to light or noise and tinnitus. Fatigue and

headache are often the most commonly reported symptoms following mTBI [84]. They may affect cognition, social interactions and interfere with return to work or school [95]. Behavioural and emotional symptoms include drowsiness, lethargy, irritability, depression, anxiety, sleeping more than usual and difficulty falling asleep.

Table 3.4.1: Common symptoms following mTBI. [84]

Cognitive	Behavioural/Emotional	Physical
Difficulty concentrating	Drowsiness	Headache
Difficulty remembering	Lethargy	Nausea
Feeling “slowed down”	Irritability	Vomiting
Feeling “in a fog” or “dazed”	Depression	Blurred or double vision
	Anxiety	Seeing stars or lights
	Sleeping more than usual	Balance problems
	Difficulty falling asleep	Dizziness
		Sensitivity to light or noise
		Tinnitus
		Fatigue

The Diagnostic and Statistical Manual of Mental Disorders includes a proposed set of diagnostic criteria for PCS [96]. The proposed criteria include: A) a history of head trauma that has caused significant cerebral concussion; B) evidence from neuropsychological testing or quantified cognitive assessment of

difficulty in attention (concentrating, shifting focus of attention, performing simultaneous cognitive tasks) or memory (learning or recall of information); C) three or more of the following occur shortly after the injury and last at least 3 months: becoming fatigued easily, disordered sleep, headache, vertigo or dizziness, irritability or aggression on little or no provocation, anxiety, depression, or affective instability, changes in personality, apathy or lack of spontaneity. Lastly, the criteria state that the disturbance must result in significant impairment in social or occupational functioning, and result in a significant decline from previous level of functioning. In children, a significant worsening in school performance dating from the trauma may represent impairment.

There is still, however, a lack of consensus on the definition of PCS and how many symptoms are needed to diagnose it. Moreover, PCS is sometimes difficult to diagnose because the symptoms found to persist following mTBI may also occur in other medical conditions, such as depression, post-traumatic stress disorder or chronic pain [84]. These symptoms are also observed, to varying degrees, amongst healthy individuals.

Although males are more likely to sustain a TBI, females may be at increased risk for developing PCS [97]. A meta-analysis of eight studies on mild to severe TBI found that the overall outcome for women was worse than men [98]. This may be related to the differences in the mechanisms of injury; sports-related injuries exhibit less persistent symptoms than motor vehicle accidents [99]. A greater percentage of females than males sustain head injuries in motor

vehicle accidents, whereas a greater percentage of males sustain sports-related head injuries. Furthermore, the elderly are more susceptible to persistent post-concussion symptoms. The risk of persistent symptoms following a TBI is twice as high at age 40 than at age 30 [100]. However, there are differences in the cause of injury in the youth and elderly. Young adults are more likely to be injured in motor vehicle accidents, while older adults are more likely to be injured due to falls [100].

The post-concussion symptom scale (PCSS) is a commonly used measure of perceived symptoms associated with mTBI. The patient is asked to rate the current severity of 22 symptoms, via a 7-point Likert scale from 0 (no symptoms) to 6 (severe). The symptoms rated are: headache, nausea, vomiting, balance problems, dizziness, fatigue, trouble falling asleep, sleeping more than usual, sleeping less than usual, drowsiness, sensitivity to light, sensitivity to noise, irritability, sadness, nervousness, feeling more emotional, numbness or tingling, feeling slowed down, feeling mentally foggy, difficulty concentrating, difficulty remembering and visual problems. The natural distribution of PCSS has been examined and classification ranges were created that reflect proportions of normative subjects.

3.5 Management of Mild TBI

Generally, the basis of mTBI management is physical and cognitive rest until the acute symptoms resolve, then a graded program of exertion prior to

medical clearance and return to activities [101]. Although studies examining the effect of rest after mTBI are sparse, an initial period of rest during the acute symptomatic period following injury may be beneficial [102]. Further research is required to evaluate the long-term outcome of rest, and the optimal amount and type of rest. Additionally, identifying potential prognostic factors (for e.g. age) affecting recovery after mTBI is important for improved management and return to play decisions after mTBI [103]. It is also important to educate children and adults about mTBI and its potential consequences [104] [105].

A ‘return to play’ protocol has been developed by the Consensus Statement on Concussion in Sport and updated during the 4th International Conference on Concussion in Sport held in Zurich in 2012 [102]. This protocol can be applied to children down to the age of 13 years. Its approach involves the gradual stepwise return to work and social activities (prior to contact sports) in a way that does not result in a significant exacerbation of symptoms (see Table 3.5.1). Patients begin with complete rest, followed by light exercise. Then they progress to individual sport-specific activity, followed by team sports practice with no contact. From there, patients participate in team sports with contact, and then full return to activity. Patients should progress from one stage to the next only if they remain asymptomatic at the previous one. If any post-concussion symptoms occur while in the stepwise program, then the patient should drop back to the previous asymptomatic level and try to progress again after one day of rest.

Table 3.5.1: Graduated ‘return to play’ protocol developed by the Consensus

Statement on Concussion in Sport. [102]

Rehabilitation stage	Functional exercise at each stage of rehabilitation	Objective of each stage
1. No activity	Symptom limited physical and cognitive rest	Recovery
2. Light aerobic exercise	Walking, swimming or stationary cycling keeping intensity <70% maximum permitted heart rate. No resistance training.	Increase heart rate
3. Sport-specific exercise	Skating drills in ice hockey, running drills in soccer. No head impact activities.	Add movement
4. Non-contact training drills	Progression to more complex training drills, e.g. passing drills in football and ice hockey. May start progressive resistance training.	Exercise, coordination and cognitive load
5. Full-contact practice	Following medical clearance participate in normal training activities	Restore confidence and assess functional skills by coaching staff
6. Return to play	Normal game play	

CHAPTER 4

Project Objectives and Methods

4.1 Objectives

Using non-invasive advanced imaging technology, MRI can be used to examine not only the brain's anatomy but also its function, white matter architecture, and neurometabolites. In this project we utilized three advanced MRI methods, resting state fMRI, DTI and ^1H -MRS, to assess children with recent mTBI. The work proposed in this project revolved around the hypothesis that these advanced techniques can assess and monitor the progress of recovery in mTBI children.

Mild TBI is often referred to as a 'silent epidemic', partly due to its high incidence and frequent under-diagnosis. Yet in recent years, people are becoming increasingly aware of the potential cognitive, physical, emotional, and social consequences of mTBI. Nevertheless, its pathophysiology and recovery time are

not well understood. In this study we chose to examine children because not many mTBI studies have been performed on this age group, although they are one of the age groups that are most likely to sustain a TBI. Furthermore, we chose a longitudinal design for our study to examine the brain changes over time and to advance our understanding of mTBI recovery, given that many of the previous mTBI studies have a cross-sectional design.

In our first study, we investigated the whole-brain functional connectivity and white matter structural changes at three different time points in children with mTBI, and compared them to healthy age and gender matched controls. Our hypothesis was that we would find alterations in resting state networks (RSNs) and white matter tracts between patients and controls, and that full recovery would not be achieved until the third patient visit. In our second study, we examined the neurometabolite levels in the frontal white matter and anterior cingulate cortex in children with mTBI, at three different time points, and compared them to healthy controls. Our hypothesis was that we would find statistically significant differences in brain metabolites between patients and controls, and that full recovery would not be achieved until the third patient visit.

The motivation for our final study came from the present-day mTBI management guidelines. Most healthcare professionals prescribe physical rest until the acute post-concussion symptoms resolve. However, even then, the post-concussion symptoms sometimes reappear with exercise. There is a lack of studies on the effects of acute exercise on the brain's functional connectivity, and

therefore it is unclear why post-concussion symptoms may resurface with exercise. In our final study we used resting state fMRI to investigate the effect of acute exercise on the healthy brain. We examined whole-brain functional connectivity changes before and after an acute bout of aerobic exercise in healthy adults. Our hypothesis was that exercise would result in RSNs alterations.

4.2 Methods

All scans were performed on a General Electric 3 Tesla HD MRI scanner with a 32-channel phased array head coil (GE Healthcare, Milwaukee, WI). Six subjects with sports-related mTBI, between the ages of 13 and 15 years old, were recruited through the Acquired Brain Injury Clinic at McMaster Children's Hospital. Healthy control subjects matched by age and gender were recruited from the general community through advertisements. Exclusion criteria for patients and controls included psychiatric illnesses and neurological disorders. None of the enrolled subjects were taking prescription medications for treatment of post-concussion symptoms at the time of the study. The Research Ethics Board at St. Joseph's Healthcare approved this study. Each patient was scanned at three different time points: 1, 4 and 7 months post-injury. Before initiating all studies, parents/legal guardians provided written informed consent and participating children provided written assent. Post-concussion symptoms were evaluated at each scan using the post-concussion symptom scale. Patients followed CanChild's

concussion management guidelines, which outlines return to activity and return to school guidelines for children and youth.

For the first study (chapter 5) on functional connectivity and white matter changes following mTBI, resting state BOLD fMRI and DTI data were acquired for the whole brain. Resting state fMRI data processing was performed using the FSL software package MELODIC (Multivariate Exploratory Linear Optimized Decomposition into Independent Components). Probabilistic independent component analysis was performed on all subjects, at a single-group level, to decompose the 4D data sets into separate spatial maps. Subject-specific RSNs were then mapped onto each subject using the FSL software Dual Regression. This software package first used the spatial maps obtained from MELODIC to find temporal dynamics associated with each map. The time courses were then used as a set of temporal regressors in a general linear model to find the subject-specific maps. The different sets of spatial maps were collected across subjects into single 4D files (one per RSN). Group differences were then tested using the FSL software Randomise permutation-testing tool. For DTI data, pre-processing was performed using tools from FMRIB Software Library version 5.0. The data was corrected for the effects of eddy current and head motion using FMRIB's Diffusion Toolbox. Both regional and whole-brain analyses were completed. Regional analysis was performed on the genu and splenium of the corpus callosum of mTBI patients. Statistical analyses were performed using SPSS version 22.0. Whole-brain voxel-wise analysis of the DTI data was carried out

using tract-based spatial statistics (TBSS), part of FSL. Fractional anisotropy images were created by fitting a tensor model to the raw diffusion data, and then all subjects' FA data were aligned using the nonlinear registration tool FNIRT. The mean FA image was then created and thinned (non-maximum suppression, perpendicular to the local tract structure) to create a mean FA skeleton, which represents the centres of all tracts common to the group. Each subject's aligned FA data was then projected onto this skeleton. Voxel-wise statistics of group differences were then performed using the FSL software Randomise permutation-testing tool.

For the study (chapter 6) on metabolic brain changes following mTBI, in vivo ^1H -MRS data was acquired from two separate single-voxel regions: the anterior cingulate cortex and right frontal white matter (two commonly chosen regions in adult mTBI studies). For each spectroscopy voxel, the tissue volume fractions were determined through tissue segmentation of the T1-weighted images into three different tissue types (grey matter, white matter, and cerebrospinal fluid). Tissue volume fractions were used to correct LCModel values and obtain 'absolute' metabolite levels (see chapter 2.3.6 for details). Statistical analyses were performed using SPSS version 22.0.

For the final study (chapter 7), we recruited ten healthy adults with an average age of 25.6 ± 3 years. Subjects with a history of traumatic brain injury, neurological disorders or psychiatric illnesses were excluded from the study. The Research Ethics Board at St. Joseph's Healthcare approved this study. Each

subject was scanned at two different times: before and after exercise. Exercise took place inside the MRI scanner using an MRI-compatible up/down ergometer (Lode, Groningen, Netherlands). The exercise protocol consisted of a 30 seconds warm-up, followed by 3.5 minutes of pedalling at 5 Watts. This power and duration were chosen because they were adequate for all subjects to significantly increase their heart rate. The imaging protocol included an anatomical scan, followed by two resting state BOLD fMRI scans, one before exercise and one immediately after exercise. Whole-brain resting state fMRI data processing was performed using probabilistic independent component analysis using the FSL software package MELODIC.

The last chapter of this thesis (chapter 8) recaps the main experimental conclusions and provides suggested future directions.

CHAPTER 5

Functional Connectivity and White Matter Changes Following Mild Traumatic Brain Injury: A Longitudinal Study of Children

Raghda Hasswa, Carol DeMatteo, John Connolly, Michael D. Noseworthy

5.1 Context of the Paper

Recent evidence suggests that mild traumatic brain injury may result in resting state functional connectivity and white matter tracts abnormalities. We further probed this longitudinally in children through two methods: functional connectivity analysis of all identifiable resting state networks using independent component analysis, and whole-brain white matter structural analysis using tract based spatial statistics.

5.2 Declaration Statement

Raghda Hasswa as principal author wrote the article, performed analysis, and created figures and tables as appropriate. Dr. Michael Noseworthy, as corresponding author, provided guidance, funding and advice, and performed proofreading/editing of the manuscript for publication. Prof. Carol DeMatteo provided patient recruitment, guidance and advice. Dr. John Connolly provided guidance, commentary and advice.

5.3 Paper

Functional Connectivity and White Matter Changes Following Mild Traumatic Brain Injury: A Longitudinal Study of Children

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ABSTRACT

Background. The pathophysiology and recovery time from mild traumatic brain injury (mTBI) are not well understood, especially in children. Thus the goal of this study was to investigate the longitudinal changes in brain functional connectivity and white matter structure in children following mTBI.

Materials and Methods. Resting state functional MRI and diffusion tensor imaging (DTI) were done on children who had sustained a mTBI (mean age = 14.5 ± 0.8 years). Temporal changes were assessed at 1, 4 and 7 months following injury using a 3T MRI and comparisons were made between mTBI patients and age and gender matched controls. Resting state network (RSN) functional connectivity was investigated using probabilistic independent component analysis (PICA), while DTI whole-brain voxel-wise analysis was performed using tract-based spatial statistics.

Results. Statistically significant decreased functional connectivity (mTBI < control) was noted in the auditory network, while increased functional connectivity was found in the default mode network (DMN) and sensorimotor network, at the first patient visit. RSN differences were no longer notable in the auditory or sensorimotor networks by 4 months post-injury. However, increased functional connectivity in the default mode network (compared to healthy controls) was still visible 7 months post-injury. This was accompanied by a statistically significant decrease in fractional anisotropy in the left inferior longitudinal fasciculus, inferior fronto-occipital fasciculus, superior longitudinal

fasciculus and forceps major. No return of fractional anisotropy to that comparable in healthy levels was observed over the duration of the study.

Conclusions. With the exception of the DMN, a modest recovery of functional connectivity was noted 4 months post-injury, compared to controls. However, a persistent decreased fractional anisotropy in the white matter was observed at 7 months post-injury. These results may indicate that functional connectivity recovery may occur before white matter structural recovery when the brain is healing after mTBI.

KEY WORDS: Mild traumatic brain injury (mTBI), paediatric, resting state, functional magnetic resonance imaging (fMRI), diffusion tensor imaging (DTI).

INTRODUCTION

Traumatic brain injury (TBI) is a major health problem in Canada and around the world. TBI is caused mainly by falls (35%), motor vehicle and traffic accidents (17%), collisions with moving or stationary objects (16%), and assaults (10%) [1]. According to the Brain Injury Association of Canada, there are approximately 18,000 hospitalizations associated with TBI diagnosis annually in Canada, and an estimated 1.4 million Canadians are living with TBI [2]. The Centres for Disease Control and Prevention in the United States has estimated that 1.7 million people sustain a TBI annually in the United States, while around 75% of those receive a mild TBI (mTBI) [1].

The American Academy of Neurology (AAN) defines mTBI as “a trauma-induced alteration in mental status that may or may not involve loss of consciousness” [3]. Throughout the literature there is a lack of consensus on the definition of mTBI, and several organizations have developed similar but not identical diagnostic criteria for mTBI (for example World Health Organization [4], Centres for Disease Control and Prevention [5] and American Congress of Rehabilitation Medicine [6]). Furthermore, the pathophysiology of mTBI is not well understood, despite its high incidence and the considerable attention it has attracted from academia and mass media. Moreover, the research studies are largely in adults and have a cross-sectional design. This limited breadth of research is a problem that must be redressed [7]. A paediatric population merits attention because they represent a population that is at greater risk for sustaining an mTBI and of experiencing life disrupting consequences from it. At the same time, the neural plasticity of adolescents that makes them vulnerable also offers greater hope for recovery from injuries such as mTBI [8].

Whenever the details of mTBI are finally comprehended it is agreed that magnetic resonance imaging (MRI), and MR related techniques, will undoubtedly play a key role in the understanding of the pathophysiological processes [9]. Good techniques for examining brain function and structure include functional MRI (fMRI) and diffusion tensor imaging (DTI), respectively.

A promising application of fMRI is aimed at mapping resting state functional connectivity. Even when the brain is at rest, many regions exhibit

temporal synchrony (i.e. the anatomy is functionally connected) and show a considerable amount of spontaneous neuronal activity [10]. Resting state functional connectivity analysis usually targets low frequency (<0.1 Hz), synchronized activations (also known as low-frequency blood oxygen level dependent (BOLD) fluctuations) in spatially separated areas of the brain [11]. These synchronized neurophysiological events represent structurally and functionally connected networks, termed resting state networks (RSNs). RSNs refer to the temporal coherence between different areas of the brain at rest, i.e. in the absence of task driven activations. Resting state functional connectivity, as measured by BOLD signal fluctuations, has a neurophysiological basis and is sensitive to various neurological and psychiatric abnormalities [12]. Therefore, it is useful in understanding the behaviour of both normal and pathological subjects. In normal subjects, a RSN is characterized by high metabolism during resting state [13] [14], functional connectivity during rest [15], and decreased activation during various attention-demanding cognitive tasks [16] [17].

Researchers have identified a number of RSNs that are steadily found in the resting brain, even across studies using different data acquisition and analysis techniques, for example [18] [19] [20]. The default mode network (DMN), sensorimotor network (SMN), executive control network (ECN), auditory network (AN), fronto-parietal network (FPN), and medial visual network are amongst the most commonly found RSNs. The DMN involves the precuneus/posterior cingulate, lateral parietal cortex, and mesial prefrontal cortex

[20]. It has by far received the greatest attention from both the research and clinical community. The DMN has been associated to introspective mental processes, stimulus-independent thoughts (also known as mind wandering), retrieving one's past or envisioning one's personal future, and self-reference. The SMN involves the precentral gyrus, postcentral gyrus, and supplementary motor area [20]. It is characterized by the involvement of brain regions that anatomically correspond to motor as well as sensory areas. The ECN involves the medial frontal gyrus, superior frontal gyrus, and anterior cingulate cortex [20]. In some cases it also includes the lateral parietal areas. In general, the ECN is involved in tasks relying on executive functions, such as control processes and working memory. The AN involves the superior temporal gyrus, Heschl's gyrus, insula, and postcentral gyrus. The superior temporal gyrus contains the primary auditory cortex, which processes sound and contributes to our ability to hear. It also contains Wernicke's area, which is involved in processing speech into an understandable language. The FPN includes the inferior frontal gyrus, medial frontal gyrus, precuneus, inferior parietal gyrus, and angular gyrus [20]. The FPN has been associated to different functions including memory, language, attention, and visual processes [18].

Resting state functional connectivity can be analyzed by two different approaches: model-dependent seed-based methods and model-free methods. Model-dependent methods examine the functional connectivity of a specific brain region, based on subjective placement of a seed-voxel. Model-free methods,

however, are used to examine whole-brain connectivity patterns without the need for defining any *a priori* seed region.

DTI delineates brain structural organization, based on the degree of water proton mobility or restricted mobility. Brain white matter (WM) axons are highly organized and tightly packed together. Due to the hydrophobic nature of myelin, water diffusivity transverse to axons is restricted. The result is water diffusivity being greater parallel to fibre tracts [21] [22] [23]. By encoding diffusion weighting as a rank-2 tensor (in the most simplest sense) the biophysical nature of water and hence the tissue microstructural integrity can be estimated [24]. The rotational invariant nature of the tensor allows indirect measurement of tissue anisotropy and structural orientation. Anisotropy, commonly represented by the scalar metric fractional anisotropy (FA), is typically linked to WM integrity, where reduced FA is linked to abnormal WM structure. Mathematically, FA scales between zero and one where one represents an infinitely long cylinder (i.e. complete anisotropic diffusion) and zero represents a sphere indicating isotropic diffusion [25].

Both resting state fMRI and DTI have emerged as potential clinically useful tools in the assessment of diseases, such as multiple sclerosis, Alzheimer's disease and schizophrenia [26] [27]. The purpose of this study was to apply these techniques to investigate changes in the paediatric brain following mTBI. The patients were examined at 1, 4 and 7 months after mTBI in order to study the temporal changes and progress of recovery.

MATERIALS AND METHODS

Participants

The study was performed at the Imaging Research Centre at St. Joseph's Healthcare in Hamilton, Ontario, Canada. The Research Ethics Board at St. Joseph's Healthcare approved this study. Parents/legal guardians provided written informed consent while participants provided written assent, followed by careful screening for MRI compatibility.

This longitudinal study consisted of children between the ages of 13 and 15 who had sustained mTBI (N = 6, 4 females and 2 males, mean age = 14.5 ± 0.8 years). The cause of mTBI was impact loading (i.e. where the head hits a stationary object or is struck by a moving object). All patients had a sports-related mTBI (such as ice hockey, volleyball, basketball and football). Recruitment occurred through the Acquired Brain Injury Clinic at the McMaster Children's Hospital. The diagnosis of mTBI was made by a licensed healthcare professional. Inclusion criteria for the mTBI group included time since injury of less than or equal to 1 month. Exclusion criteria included psychiatric illnesses and neurological disorders. None of the enrolled subjects were taking prescription medications for treatment of post-concussion symptoms at the time of the study. Each patient was scanned at three different time points: 1 month post-injury (mean = 29.2 ± 3.7 days), 4 months post-injury (mean = 125 ± 5.9 days), and 7 months post-injury (mean = 210.8 ± 11.8 days). A healthy control population

matched by age and gender was recruited after enrolment of the mTBI patient ($N = 6$, 4 females and 2 males, mean age = 14.3 ± 1.0 years).

Clinical Measures

A concussion information form was administered to obtain detailed information about the cause of head injury, as well as number of previous mTBI, if any. Post-concussion symptoms were evaluated at each scan using the post-concussion symptom scale (PCSS), which is a commonly used measure of perceived symptoms associated with mTBI, where the patient is asked to rate the current severity of 22 symptoms, via a 7-point Likert scale from 0 (no symptoms) to 6 (severe). The symptoms rated are: headache, nausea, vomiting, balance problems, dizziness, fatigue, trouble falling asleep, sleeping more than usual, sleeping less than usual, drowsiness, sensitivity to light, sensitivity to noise, irritability, sadness, nervousness, feeling more emotional, numbness or tingling, feeling slowed down, feeling mentally foggy, difficulty concentrating, difficulty remembering and visual problems. Patient PCSS scores are listed in **Table 1**. Although some patients showed improvement over time in self-rated post-concussion symptoms, others continued to experience symptoms 7 months following injury.

Table 1: PCSS score and classification [28] of 6 patients, taken before each MRI scan.

Patient	Scan 1		Scan 2		Scan 3	
	PCSS	Classification	PCSS	Classification	PCSS	Classification
1	78	very high	32	high	1	normal
2	57	very high	50	very high	60	very high
3	48	very high	15	high	39	very high
4	43	very high	8	normal	2	normal
5	0	low-normal	0	low-normal	1	normal
6	51	very high	3	normal	11	unusual

As part of standard practice, all children/families enrolled in the study received education and written material on the CanChild concussion management guidelines outlining return to activity and return to school guidelines for children and youth [29]. This approach involves the gradual stepwise return to activity and school in a way that does not result in a significant exacerbation of symptoms. The mTBI patients began with complete rest, followed by light exercise. Patients progressed from one stage to the next only if they remained asymptomatic at the previous one. If any post-concussion symptoms occurred while in the stepwise program, then the patient was instructed to drop back to the previous asymptomatic level and try to progress again after one day of rest.

Imaging

Scanning was performed on a GE 3 Tesla MR750 scanner with 32-channel phased array RF head coil (General Electric Healthcare, Milwaukee, WI). Following head positioning, immobilization and a localizer scan, anatomical images were collected using a three-dimensional IR-prepped fast SPGR T1-weighted scan (TR/TE = 11.4/4.3 ms, T1 = 450 ms, flip angle = 12°, 512 x 256 matrix, 80 slices, 24 cm FOV). Subsequent T2 and T2-FLAIR imaging was included as part of routine clinical TBI scanning. Resting state BOLD fMRI (gradient echo EPI, TR/TE = 2000/35 ms, flip angle = 90°, 64x64 matrix, 31 slices, 180 time points, 24 cm FOV, slice thickness = 4.5 mm) and DTI (dual echo spin echo EPI sequence, TR/TE = 10000/70 ms, b = 900 s/mm², 96x96 matrix, 42 slices, 2.9 mm slice thickness, 24 cm FOV, ASSET = 2) scans were then acquired. During resting state fMRI scans, subjects were asked to remain awake, keep their eyes open, and not to think of anything in particular.

fMRI Data Processing and Analysis

Resting state fMRI data pre-processing was performed using tools from the FMRIB Software Library (version 5.0) [30]. Data from each subject was corrected for interleaved slice acquisition. The skull and other non-brain tissue were removed using Brain Extraction Tool (BET) [31]. Motion correction was performed using MCFLIRT [32]. Using the FSL software package MELODIC (Multivariate Exploratory Linear Optimized Decomposition into Independent

Components) [33], functional images were filtered with a high-pass temporal cutoff of 0.01 Hz (for very low frequency noise removal), and were spatially smoothed with a Gaussian kernel with full width at half maximum of 5 mm (to improve signal-to-noise ratio). All functional data were registered to the subject's high resolution T1-weighted 3D anatomical scans, then subsequently registered to MNI152 standard space at 2 mm resampling resolution.

Using MELODIC, probabilistic independent component analysis (PICA) was performed on all subjects, at a single-group level, to decompose the 4D data sets into separate spatial maps. This was accomplished using a multi-session temporal concatenation approach, which performs a single 2D ICA run on the concatenated data matrix (obtained by stacking all slices of every data set on top of each other). This approach was chosen in order to look for common spatial patterns without assuming that the temporal response is consistent between subjects. The following options were implemented in MELODIC: time-courses were variance-normalized in order to stress voxel-wise temporal dynamics over mean signal; the number of components were automatically estimated; a level of 0.5 was used as the threshold level for independent components (i.e. a voxel 'survives' thresholding as soon as the probability of being in the 'active' class exceeds the probability of being in the 'background' noise class. A threshold level of 0.5 assumes an equal loss on false-positives and false-negatives is placed [34]).

The set of spatial maps obtained from the group-average analysis (MELODIC) was used to generate subject-specific versions of the spatial maps,

and associated time series, using the FSL software Dual Regression [35]. First, for each subject, the group-average set of spatial maps was regressed into the subject's 4D space-time dataset. This results in a set of subject-specific time series, one per group-level spatial map. Next, those time series were regressed into the same 4D dataset, resulting in a set of subject-specific spatial maps, one per group-level spatial map. Group differences were then tested using the FSL software Randomise permutation-testing tool.

DTI Data Processing and Analysis

DTI data pre-processing was performed using tools from FMRIB Software Library version 5.0 [30]. Initially, region of interest (ROI) analysis of the DTI measures in the genu and splenium of the corpus callosum of mTBI patients and matched controls was performed. Statistical analyses were performed using SPSS version 22.0. Comparisons between mTBI patients and matched controls were examined using a two-sided Student's t-test. Differences were considered to be statistically significant if $p < 0.05$. A repeated measures analysis of variance (ANOVA) was used to evaluate significant differences between sets. Sphericity was evaluated using Mauchly's test of sphericity. Adjustment for multiple comparisons was done using Bonferroni correction. The relationship between post-concussion symptoms and fractional anisotropy was examined using Pearson's correlation.

Whole-brain voxel-wise analysis of the DTI measures was carried out using tract-based spatial statistics (TBSS) [36], part of FSL. First, data was corrected for eddy current and head motion using FMRIB's Diffusion Toolbox (FDT). Next, the skull and other non-brain tissue were removed using Brain Extraction Tool (BET) [31]. FA images were created following fitting a tensor model to the raw diffusion data using FDT. All subjects' FA data were then aligned using the nonlinear registration tool FNIRT [37] [38], which uses a b-spline representation of the registration warp field [39]. Next, the mean FA image was created and thinned (non-maximum suppression, perpendicular to the local tract structure) to create a mean FA skeleton, which represents the centres of all tracts common to the group. Each subject's aligned FA data was then projected onto this skeleton. Voxel-wise statistics of group differences were then performed using FSL software Randomise permutation-testing tool. Localization of the significant WM regions was performed by reference to the John Hopkins University and International Consortium of Brain Mapping atlases of human white matter anatomy [40] [41] [42].

Methodological Approaches

In the present study, model-free, PICA approach was used to analyze the resting state fMRI data. Model-free methods (as opposed to model-dependent, seed-based methods) are used to examine whole-brain connectivity patterns without the need for defining an a priori seed region. The most commonly used

model-free methods are ICA-based (e.g. PICA) and have been reported to show a high level of consistency [10]. PICA looks for underlying sources that can explain the resting state patterns, searching for the existence of maximally independent spatial sources of resting state signals and subsequently divides a dataset into different maximally independent components. As such it is capable of isolating cortical connectivity maps from signals such as head motion or physiological confounds (e.g. cardiac pulsation and respiratory oscillation) [34]. PICA is advantageous because it evaluates and compares the coherence of activity in multiple distributed voxels, and divides different RSNs into different independent components [34]. However, limitations with the ICA approach include the difficulty of assigning a statistical framework that would enable activation networks to be tested against specific hypotheses, and the difficulty of assessing the regionally specific nature of brain responses [43]. While there is currently no agreement as to which method (ICA or seed-voxel) is superior for assessing resting state functional connectivity MRI [44], a comparison study between the two methods found ICA to be the superior method [45].

To analyze the DTI data in this study we initially used a ROI approach, and then used a whole-brain approach (TBSS). Although commonly used, the major problem of ROI analysis is how to choose the ROI. Since acquired brain injury, such as mTBI and TBI, are widely varying in etiology, there is currently no way to predict where abnormalities may be most profound. Furthermore, there is no standard method of choosing the ROIs, and this may result in selection bias

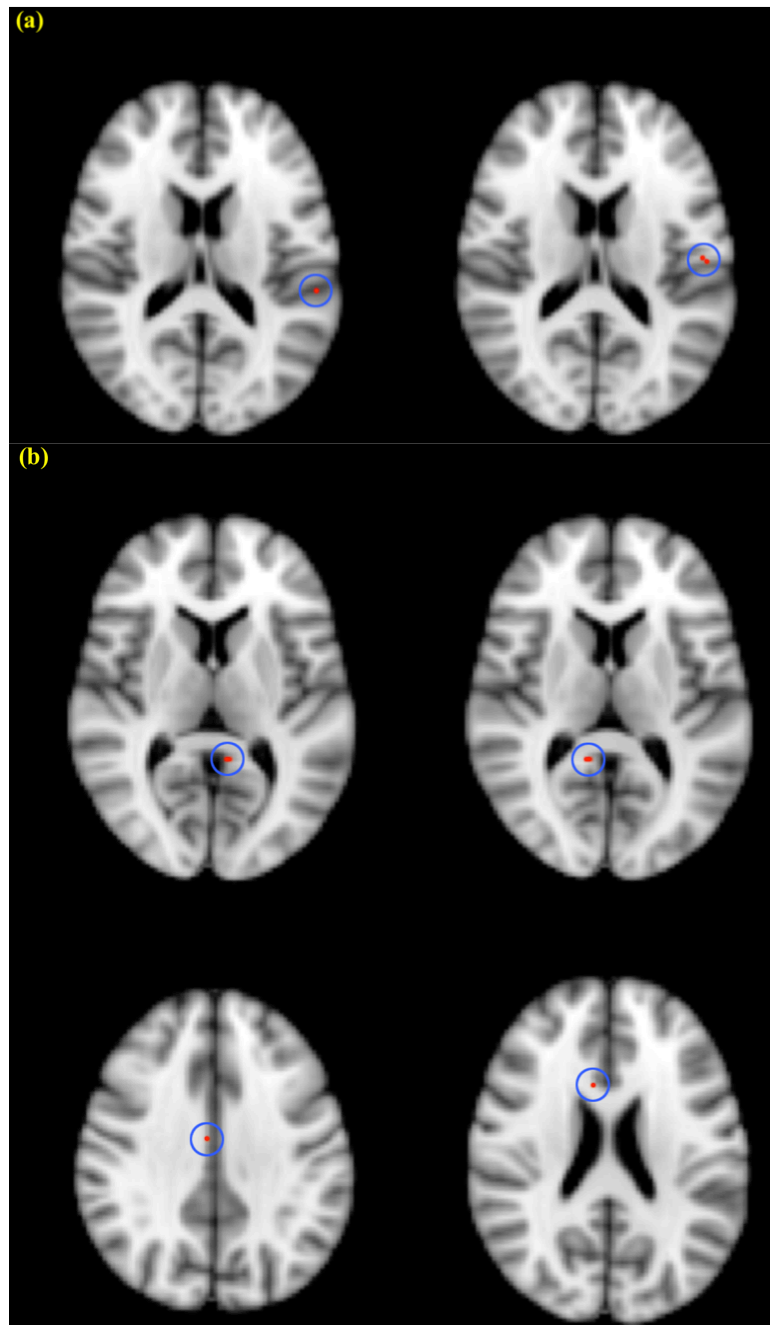
[46]. The examination of a limited number of regions may result in failure to identify significant white matter damage in other brain regions. This also makes comparisons between studies more difficult. Moreover, the reliability of regional analyses depends on accurate and reproducible spatial localization of ROIs. On the other hand, a whole-brain approach (TBSS) examines diffusion patterns of the entire white matter, without the need for specifying or localizing an a priori ROI [36]. This is particularly advantageous since TBI results in a complex pattern of white matter alterations at variable locations, making it difficult to choose an a priori region of white matter disruption.

RESULTS

Resting State Networks

Statistical significance was found in three RSNs (uncorrected $p < 0.005$) following the first MRI scan, relative to controls (**Figure 1**). Decreased functional connectivity (mTBI < Control) in the auditory network (AN) was found in the left superior temporal gyrus in Brodmann area (BA) 42 and left postcentral gyrus in BA 43. Furthermore, increased functional connectivity in the default mode network (DMN) was found in the posterior cingulate cortex in the right BA 23, and left and right BA 29, as well as in the right anterior cingulate in BA 33. Also, increased functional connectivity in the sensorimotor network (SMN) was found in the left paracentral gyrus in BA 5. The complete list of MNI standard brain coordinates of the centre of the significant brain regions is shown in **Table**

2. Elevated DMN functional connectivity, relative to controls, was observed at subsequent visits at 4 and 7 months post-mTBI. However, differences in functional connectivity of the AN and SMN in patients relative to controls were no longer observed at 4 and 7 months post-injury.



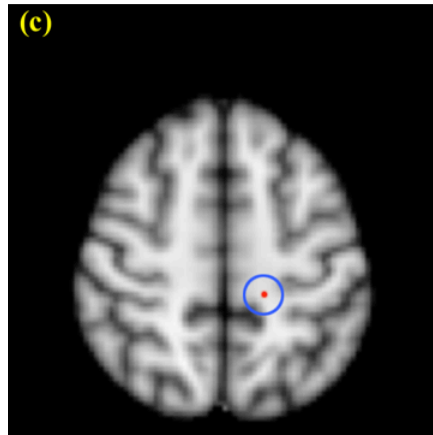


Figure 1: Significant between-group RSN differences, circled in blue, between mTBI patients and controls. Red overlay corresponds to differences found with $p < 0.005$. Images are presented in standard radiological orientation (i.e. the image left side is the right hemisphere). (a) auditory network, mTBI < control; (b) default mode network, mTBI > control; (c) sensorimotor network, mTBI > control.

Table 2: MNI coordinates of the centre of the statistically significant brain regions in mTBI patients, one month post-injury.

Brain region	BA	MNI coordinates		
		x	y	z
Left superior temporal gyrus	42	-56	-32	16
Left postcentral gyrus	43	-52	-15	17
Right posterior cingulate cortex	23	4	-10	34
Right posterior cingulate cortex	29	12	-46	10
Left posterior cingulate cortex	29	-6	-46	8
Right anterior cingulate	33	8	16	22
Left paracentral gyrus	5	-16	-32	52

Diffusion Tensor Imaging

ROI analysis showed no significant longitudinal changes in FA over time in mTBI patients ($p = 0.66$ and 0.70 , in the genu and splenium of the corpus callosum, respectively, using repeated measures ANOVA). There were also no differences found when comparing the FA in the genu and splenium of the corpus callosum of mTBI patients to healthy controls at all three time-points (**Table 3**). Additionally, no correlations were found (using Pearson's correlation) between PCSS scores and FA values in the genu of the corpus callosum at scan 1 ($r = 0.530$, $p = 0.36$), scan 2 ($r = 0.147$, $p = 0.81$), or scan 3 ($r = 0.489$, $p = 0.40$); as well as in the splenium of the corpus callosum at scan 1 ($r = -0.124$, $p = 0.84$), scan 2 ($r = 0.853$, $p = 0.07$), or scan 3 ($r = 0.098$, $p = 0.88$).

Analyses of the DTI data using TBSS showed no significant longitudinal changes in FA over time in the mTBI patients. However, differences were found when comparing mTBI patients to healthy controls at all three time-points. Relative to controls, statistically significant decreases in FA (mTBI < control) were found in the left inferior longitudinal fasciculus, inferior fronto-occipital fasciculus, superior longitudinal fasciculus and forceps major (**Figure 2**), at the first, second and third patient visit. This indicates that WM abnormalities persisted at 7 months post-injury.

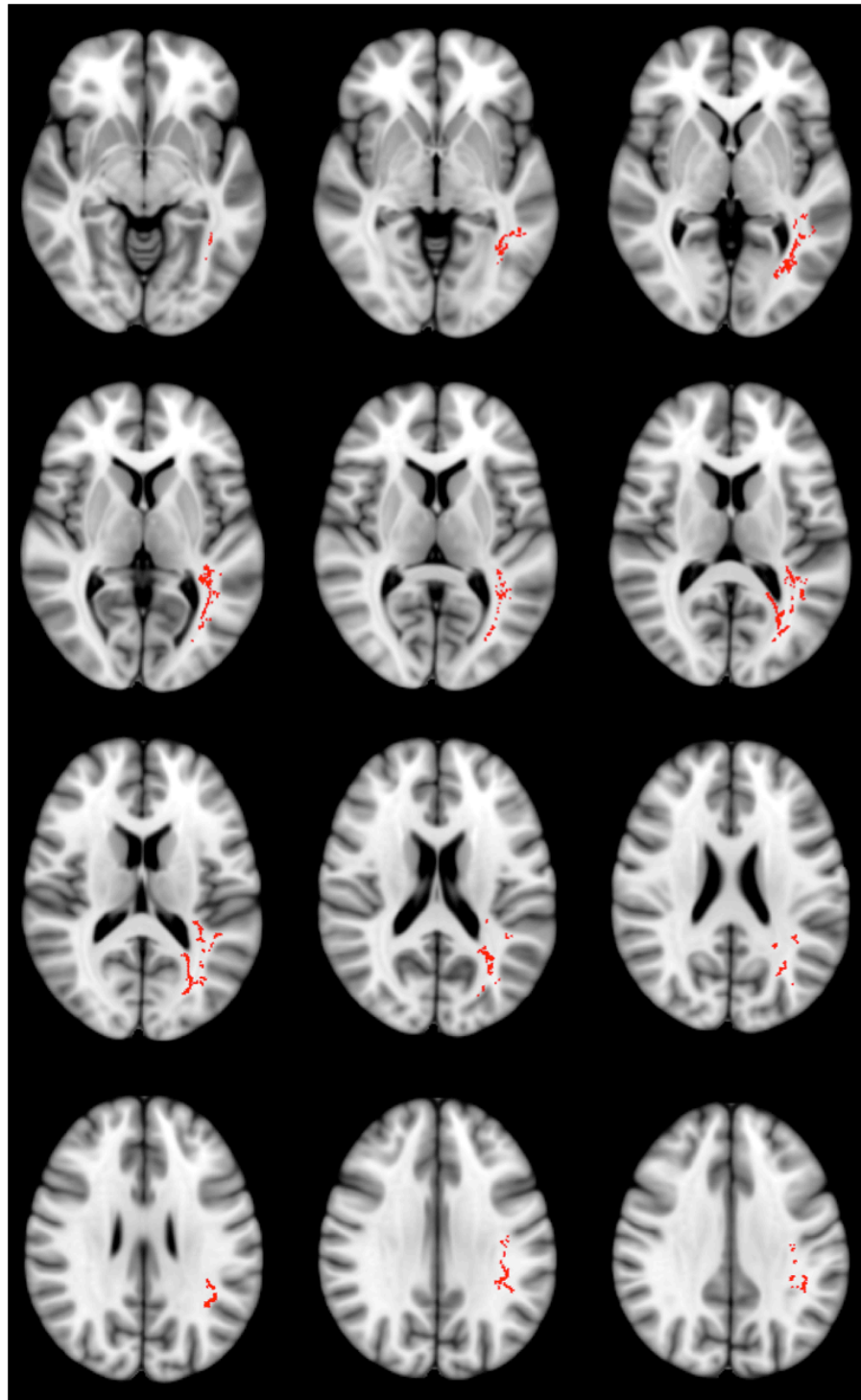


Figure 2: Significant between group white matter differences, between mTBI patients and controls. Red overlay corresponds to fractional anisotropy differences

found (control>mTBI) with corrected $p < 0.05$. The left side of the image refers to the right hemisphere of the brain.

Table 3: FA findings in the corpus callosum of controls and mTBI patients over time, mean (SD).

	Genu	Splenium
Controls	0.66 (0.04)	0.79 (0.03)
Patients Scan 1	0.63 (0.03)	0.78 (0.03)
Patients Scan 2	0.65 (0.03)	0.76 (0.04)
Patients Scan 3	0.63 (0.05)	0.77 (0.05)

DISCUSSION

Resting state fMRI and DTI studies on mTBI mostly focus on adults, have a cross-sectional design, and use seed/regional analysis methods [47] [48]. The present study examined children with mTBI, longitudinally at three time points (1, 4 and 7 months post-injury), using an ICA approach for separation of spatial maps in resting state fMRI, and TBSS whole-brain analysis for the examination of white matter changes in DTI. Statistically significant differences were discovered in several RSNs and white matter tracts, and some abnormalities persisted at 7 months post-injury. Additionally, no statistically significant correlations were found between FA and PCSS scores, at all time points. Hence, even though some

patients recovered from a symptom standpoint, there remains a persistent functional and structural abnormality.

Functional Connectivity

At the first MRI scan, decreased connectivity (mTBI<Control) in the AN in the left BA 42 and left BA 43; increased connectivity in the DMN in the right BA 23, left and right BA 29, and right BA 33; and increased connectivity in the SMN in the left BA 5 were found, relative to controls. At the second patient visit, increased connectivity in the DMN of mTBI patients relative to controls remained present. However, changes were no longer observed in the AN and SMN. At the third patient visit, increased connectivity in the DMN of mTBI patients relative to controls remained present, demonstrating that RSN abnormalities persist at 7 months following mTBI.

Following mTBI, a significant decrease in functional connectivity in the AN was found. The AN is involved in audition, such as tone/pitch discrimination, music, and speech [49]. Specifically, decreased functional connectivity was found in the superior temporal gyrus in the left BA 42, and the postcentral gyrus in the left BA 43. BA 42 plays a role as the secondary auditory cortex, and in the dominant hemisphere it is an element of Wernicke's area. It has been shown to be involved in language syntax cognition, emotions, and somatosensory perception of pain [50]. BA 43 plays a role as the primary gustatory cortex and is also

involved in language perception, speech cognition, execution of actions, and somatosensory perception of pain [50].

On the other hand, a significant increase in functional connectivity in the DMN was found following mTBI. The DMN has been associated with introspective mental processes, stimulus-independent thoughts (also known as mind wandering), retrieving one's past or envisioning one's personal future, and self-reference [20]. Specifically, increased functional connectivity was found in the posterior cingulate cortex in the right BA 23 and in the right and left BA 29, as well as in the anterior cingulate in the right BA 33. BA 23 is involved in emotional processes, visual perception, memory and reasoning [50]. BA 29 has been shown to be involved in emotion, explicit memory and language orthography. BA 33 plays a role in interoception, however little is known about its function [50].

Similarly, a significant increase in functional connectivity in the SMN was found following mTBI. The SMN is characterized by the engagement of regions that anatomically correspond to motor as well as sensory areas [20]. Specifically, increased functional connectivity was found in the paracentral gyrus in the left BA 5. BA 5 plays a role as the secondary somatosensory cortex and is involved in visuo-spatial processing, perception of audition and language orthography [50].

The RSNs findings in the present study may have implications for previous and future resting state fMRI studies on mTBI. Our DMN findings are in line with Sharp *et al.* [51] who investigated whether mild, moderate or severe

TBI in adults results in abnormalities of functional connectivity within key cognitive networks. Patients underwent resting state fMRI at least 6 months post-injury. Using ICA, an increase in functional connectivity of the DMN was found in the TBI group. Thus, the enhanced functional connectivity in the DMN observed following mTBI may represent neural compensation following injury. Other studies have reported abnormalities of the DMN following mTBI [52] [53] [54] [55]. Mayer *et al.* [52] used seed-based analysis to examine the DMN and its relationship with activity in the frontoparietal task-related network in adults with mTBI. Patients were scanned twice, less than 3 weeks post-injury and then after 3 to 5 months of recovery period. Results showed a decrease in functional connectivity within the DMN in the mTBI group, as well as hyper connectivity between the DMN and frontoparietal task-related network. No changes toward normal levels in functional connectivity were seen over the recovery period. Furthermore, functional connectivity measures were predictive of group (mTBI or control) and cognitive complaints within 3 weeks of injury, but did not show changes (toward normal levels) at the later assessment. Another study by Stevens *et al.* [53] characterized the effects of mTBI on multiple integrated neural networks observed during resting state fMRI between 13 and 136 days post-injury. Twelve separate brain networks were identified using ICA. Abnormal functional connectivity in mTBI patients was found in the 12 networks, including visual processing, motor, limbic, DMN, and numerous circuits believed to underlie executive cognition. The abnormalities did not only include decreases in

functional connectivity but also increases, possibly reflecting compensatory neural processes. Additionally, the authors reported that post-concussive symptom severity was related to abnormal regional connectivity within most brain networks identified, particularly the anterior cingulate. Johnson *et al.* [55] examined the DMN functional connectivity in young adults (average age of 20.6 years) with a single episode of mTBI versus multiple episodes. All patients were asymptomatic prior to the resting state fMRI session, which took place 10 days post-injury. Relative to controls, the DMN in mTBI showed an increase in number and strength of connections in the medial prefrontal cortex, and a decrease in number and strength of connections in the posterior cingulate and lateral parietal cortices. Additionally, as the number of mTBI episodes increased, connections between the left dorsolateral prefrontal cortex and left lateral parietal cortex showed a significant decrease in magnitude. Regression analysis also indicated an overall loss of connectivity as the number of mTBI episodes increased. Therefore, these studies that examined the functional connectivity of the DMN in adults with mTBI support our findings of persistent changes in the DMN in children following mTBI.

In addition to the DMN changes following mTBI, a few studies reported abnormalities in other RSNs in adults with mTBI [56] [57] [58]. Shumskaya *et al.* [56] examined adults with mTBI between 2 and 28 days post-injury and found a decrease in functional connectivity within the motor-striatal network using ICA. Patients also showed deficits in psychomotor speed and in speed of information

processing. Thus, even though disorders in motor function after mTBI are rarely reported, mTBI does indeed have an effect on motor functioning. Furthermore, an increase in functional connectivity in the right frontoparietal network of mTBI patients was found. It was suggested that this may explain the excessive cognitive fatigue reported by patients, and may also underlie the physical post-concussive symptoms such as headache and increased sensitivity to noise and light. Another study by Messe *et al.* [57] examined changes in functional brain networks following mTBI in adults with post-concussion syndrome (PCS). Patients with persistent PCS at 6 months post-injury were compared with patients with no PCS and with healthy controls. All patients were scanned two times, 1 to 3 weeks after injury and then after 6 months. Using graph theory analysis measures, functional networks alterations were found in all mTBI patients relative to controls. mTBI patients showed increased connectivity in the limbic system after the injury, whereas mTBI patients with PCS showed specific early thalamic and temporal, and late frontal changes after the injury. A different study [58] investigated resting state functional connectivity changes and cognitive deficits in adults with mTBI, with high and low post-concussion symptoms. Patients were examined two times, less than 10 days post-injury and then after one month. At the first visit, low symptom mTBI patients had reduced inter-hemispheric functional connectivity (IH-FC) within the lateral parietal lobe. However, at the second visit, high symptom mTBI patients showed reduced IH-FC compared to the control group within the dorsolateral prefrontal cortex, and this was associated

with reduced cognitive performance. Additionally, a couple of other studies reported abnormalities in thalamic resting state networks following mTBI, using seed-based analysis [59] [60]. Therefore, these studies of functional connectivity changes in various RSNs in adults with mTBI support our findings of persistent changes in networks, other than the DMN, in children with mTBI. This confirms that mTBI causes functional connectivity changes in several RSNs in both adults and children following mTBI.

White Matter Integrity

There were no significant longitudinal changes in FA over time in the mTBI patients. However, differences were found when comparing mTBI patients to healthy controls at all three time-points. Relative to controls, statistically significant decreases in FA (mTBI<control) were found in the left inferior longitudinal fasciculus, inferior fronto-occipital fasciculus, superior longitudinal fasciculus and forceps major, at the first, second and third patient visit. This shows that white matter abnormalities persist at 7 months following mTBI.

Our white matter findings in the present study may have implications for previous and future DTI studies on mTBI. Although most mild TBI DTI studies have focused on adults, and most paediatric DTI studies have examined moderate to severe TBI (see [48] [61]), there are a few paediatric DTI studies that have examined mTBI [62] [63] [64] [65] [66] [67]. Wilde et al [62] studied children (age range 14 to 19 years) with mTBI within 6 days of injury and found an

increase in FA in the corpus callosum, relative to controls. The same group examined the integrity of the cingulum bundles 3 days post-injury in 12 mTBI children (age range 14 to 17 years) [63]. They reported a decrease in mean diffusivity (MD) and no changes in FA, relative to controls. Mayer et al [64] examined 15 children (mean age of 13.5 years) on average 16 days post-injury, and then 11 patients returned for a second scan after 127 days from the initial scan. An increase in FA after the first scan was found, and little evidence of recovery in white matter abnormalities was observed at the second scan. An increase in FA in the early days following mTBI was also observed by Chu et al. [65], who examined children (mean age of 15.7 years) between 1 and 6 days post-injury, and by Yallampalli et al [66] who studied the integrity of the fornix within 6 days of injury in mTBI children (mean age of 15 years). The increase in FA observed in those studies, as opposed to the expected decrease, may be attributed to the early timing of imaging when localized inflammatory responses and axonal cytotoxic edema prevail, and white matter degradation has not yet had time to develop. In contrast, Maugans et al [67], who examined 12 children (age range 11 to 17 years) at 72 hours, 14 days, and 30 days or greater following mTBI, did not observe any group differences in the diffusion metrics for any ROI (genu, splenium and body of the corpus callosum, bilateral anterior limb of the internal capsule and posterior limb of the internal capsule). Also, no significant differences over time for any of the ROIs were observed. Therefore, these studies of white matter changes in various ROIs in children with mTBI support our

findings of white matter abnormalities in children. They also show the importance of examining the whole-brain white matter, not only the commonly chosen white matter ROIs such as the corpus callosum.

In addition to those studies of white matter integrity following mTBI in children, several studies have examined adults using whole-brain DTI analysis methods [68] [69] [70] [71] [72] [73] [74] [75]. Smits *et al.* [68], in examining adults with mTBI at an average of 31 days post-injury, found a decrease in FA in the right temporal subcortical white matter, and no differences in MD, relative to controls. Furthermore, FA was reduced in association with the severity of post-concussive symptoms in the uncinate fasciculus, inferior fronto-occipital fasciculus, internal capsule and corpus callosum, as well as in the parietal and frontal subcortical white matter, whereas MD was increased in association with the severity of post-concussive symptoms in the inferior fronto-occipital fasciculus, inferior longitudinal fasciculus and superior longitudinal fasciculus. A study by Lipton *et al.* [69] determined whether frontal white matter diffusion abnormalities can help predict acute executive function impairment within 2 weeks of mTBI. Several areas of lower frontal white matter FA, including the dorsolateral prefrontal cortex, were found in mTBI patients with several areas also demonstrating higher MD. Patients also performed worse on executive function tests relative to controls. Therefore, these studies that used whole-brain analysis methods in adults with mTBI support our method of using whole-brain analyses

in order to achieve a complete picture of the white matter integrity in children with mTBI.

Our findings of persistent decreased FA over time are supported by studies that examined white matter integrity in adults during the chronic stage of mTBI, using whole-brain DTI analyses [70] [71] [72]. Wada *et al.* [70] scanned patients at an average of 35 months post-injury. Relative to controls, a significant decrease in FA was found in the superior longitudinal fasciculus, superior frontal gyrus, insula, and fornix. Lipton *et al.* [71] examined white matter abnormalities in adults with persistent cognitive impairment following an mTBI within the past 8 months to 3 years. Decreased FA and increased MD were found in corpus callosum, internal capsules, subcortical white matter, centrum semiovale, and deep cerebellar white matter. Another study by Kinnunen *et al.* [72] investigated damage to white matter tracts in adults with TBI, at an average of 25 months post-injury. Using TBSS, mTBI patients showed areas of reduced FA, namely the fornices, cingulum bundle bilaterally, corpus callosum, anterior limb of the right internal capsule, left external capsule, inferior fronto-occipital fasciculi, left superior longitudinal fasciculus, forceps major and minor bilaterally, anterior thalamic radiations bilaterally and corticospinal tracts. These findings suggest that abnormal white matter integrity in mTBI patients persists in the chronic stage.

Conclusions

To summarize the main strengths of this work are the temporal examination of children with mTBI, using whole-brain analyses methods. Most mTBI work has focused on adults at a single time point post-mTBI, using ROI analysis. Although the sample size we used is similar to other resting state fMRI and whole-brain DTI studies of mTBI [54] [55] [65] [73] [74] [75], examining the reproducibility of our results in a larger sample will be important.

In conclusion, our study examined children with mTBI longitudinally at three time points, using independent component analysis approach of RSNs, and tract-based spatial statistics of white matter diffusion anisotropy. Our findings suggest that mTBI in children causes abnormalities in the brain's RSNs and white matter tracts, and these disruptions persist at 7 months post-injury. Lastly, based on the DTI findings that white matter structural changes persist out to 7 months post-mTBI, while RSNs return to that of controls (2 of 3 abnormal RSNs had returned to control levels at 7 months post-mTBI), we suggest that functional connectivity recovery may occur before white matter structural recovery when the brain is healing after mTBI.

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CHAPTER 6

Metabolic Brain Changes Following Mild Traumatic Brain Injury: A Longitudinal Magnetic Resonance Spectroscopy (MRS) Study of Children

Raghda Hasswa, Carol DeMatteo, John Connolly, Michael D. Noseworthy

6.1 Context of the Paper

Recent evidence suggests that mild traumatic brain injury may result in neurometabolic abnormalities. We further probed this longitudinally in children through the analysis of neurometabolites in the frontal white matter and anterior cingulate cortex. Specifically, we looked at absolute concentrations of glutamate, N-acetyl aspartate, choline, creatine and myo-inositol through in vivo proton magnetic resonance spectroscopy (MRS).

6.2 Declaration Statement

Raghda Hasswa as principal author wrote the article, performed analysis, and created figures and tables as appropriate. Dr. Michael Noseworthy, as corresponding author, provided guidance, funding and advice, and performed proofreading/editing of the manuscript for publication. Prof. Carol DeMatteo provided patient recruitment, guidance and advice. Dr. John Connolly provided guidance, commentary and advice.

6.3 Paper

Metabolic Brain Changes Following Mild Traumatic Brain Injury: A Longitudinal Magnetic Resonance Spectroscopy (MRS) Study of Children

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ABSTRACT

Background. Despite the high incidence of mild traumatic brain injury (mTBI), the pathophysiology and recovery time are not well understood. The consequences of mTBI may be attributable to brain metabolite abnormalities. The present study used proton magnetic resonance spectroscopy (^1H -MRS) to investigate the longitudinal effects of mTBI on paediatric brain metabolism.

Materials and Methods. Metabolite levels in children who had sustained a mTBI (mean age = 14.5 ± 0.8 years) were examined three times, at 1, 4 and 7 months following injury. A 3 Tesla MRI was used to acquire single voxel PRESS spectra from the right frontal white matter (FWM) and anterior cingulate cortex. Levels of glutamate (Glu), N-acetylaspartate (NAA), choline, myo-inositol and creatine were measured and compared to age and gender matched healthy controls.

Results. Patients with mTBI demonstrated metabolic change in the FWM, where a statistically significant ($p < 0.05$) increase in Glu was found relative to controls at 1 month post-injury. Glu levels remained elevated at the follow-up visits, implying that Glu does not fully recover to control levels by 7 months post-injury. In addition, Glu levels in mTBI patients did not appear related to their post-concussion symptoms.

Conclusions. Our findings show that mTBI in children causes abnormalities in brain metabolites, and these disruptions persist for at least 7 months following injury.

KEY WORDS: ^1H -MRS, mild traumatic brain injury (mTBI), paediatric, brain.

INTRODUCTION

Mild traumatic brain injury (mTBI) is a major health problem in Canada and around the world. TBI is mainly caused by falls (35%), motor vehicle and traffic accidents (17%), collisions with moving or stationary objects (16%), and assaults (10%) [1]. According to the Brain Injury Association of Canada, there are approximately 18,000 hospitalizations associated with TBI diagnosis (including mTBI) annually in Canada, and an estimated 1.4 million Canadians are living with TBI [2]. According to the Centres for Disease Control and Prevention, an estimated 1.7 million people sustain a TBI annually in the United States [1]. Around 75% of them sustain a mild traumatic brain injury (mTBI).

The American Academy of Neurology (AAN) defines mTBI as “a trauma-induced alteration in mental status that may or may not involve loss of consciousness” [3]. Throughout the literature there is a lack of consensus on the definition of mTBI, and several organizations have developed similar but not identical diagnostic criteria for mTBI (for example World Health Organization [4], Centres for Disease Control and Prevention [5] and American Congress of Rehabilitation Medicine [6]). Furthermore, the pathophysiology of mTBI is not well understood, despite the high incidence and considerable attention it has attracted from academia and mass media. To date, most mTBI research studies have been done on adults, use a cross-sectional design, and focus on patterns of

structural injury. Given the large concern of brain injury caused through sport in paediatric populations, it is surprising how little there has been done on this demographic [7]. A paediatric population merits attention because they represent a population at greater risk for sustaining a mTBI and also for subsequently experiencing life altering consequences from it. Neural plasticity of adolescents makes this population more vulnerable to head injury but also offers greater hope for recovery from injuries such as mTBI [8]. The emphasis on structural brain changes from mTBI does not provide the complete aetiology of this problem, leaving a need for exploration of further techniques that ascertain physiological changes as a result of a mTBI.

An excellent non-invasive neuroimaging technique for assessment of brain metabolites is proton magnetic resonance spectroscopy (^1H -MRS) [9] [10]. ^1H -MRS provides an assessment of a number of important metabolites that are likely altered by brain injury. For example, N-acetylaspartate (NAA), an amino-acid derivative produced by mitochondria, is a marker of neuronal integrity that decreases with neuronal loss or dysfunction. Thus, because of its role in neuron health, decreased NAA is typically associated with mTBI [11] [12]. Choline (Cho), which consists of phosphocholine (PCh) and glycerophosphocholine (GPC), is a constituent of cell membranes and is a marker of membrane synthesis or repair, inflammation or demyelination. Pathological changes in membrane turnover result in an increase in Cho concentration. Following mTBI, Cho may be elevated due to breakdown products appearing after the shearing of myelin and

cellular membranes [13]. Myo-inositol (mI) is a marker of neuroglial cells, being present in the astrocytes of brain tissue, and also functions as an osmolyte. Similar to Cho, mI increases after mTBI due to membrane damage [14]. Another commonly measured metabolite is Glutamate (Glu), which is the dominant excitatory neurotransmitter in the brain and is predictive of outcome after a severe TBI [15]. Creatine (Cr) is a key component of brain energy metabolism and functions to supply energy to cells through the creatine-kinase reaction.

Several ^1H -MRS studies have previously shown altered brain metabolites following mTBI, albeit in adults [16] [17] [18] [19]. One group, Maugans *et al.* [20], examined children with mTBI and showed no changes in NAA or lactate relative to controls. However, they did not study or report on any of the other important brain metabolites that are easily measured in brain ^1H -MRS. Therefore, the goal of this longitudinal study was to examine whether temporal changes in NAA, Cho, Glu, mI or Cr occur in paediatric brains following mTBI and during recovery.

MATERIALS AND METHODS

Participants

This longitudinal study consisted of adolescents between the ages of 13 and 15 who had sustained mTBI ($N = 6$, 4 females and 2 males, mean age = 14.5 ± 0.8 years). The cause of mTBI was impact loading (i.e. where the head hits a stationary object or is struck by a moving object). All patients had a sports-related

mTBI (such as ice hockey, volleyball, basketball and football). Recruitment occurred through the Acquired Brain Injury Clinic at McMaster Children's Hospital. The diagnosis of mTBI was made by a licensed healthcare professional. Inclusion criteria for the mTBI group included time since injury of less than or equal to 1 month. Exclusion criteria included psychiatric illnesses and neurological disorders. None of the enrolled subjects were taking prescription medications for treatment of post-concussion symptoms at the time of the study. Each patient was scanned at three different time points: 1 month post-injury (mean = 29.2 ± 3.7 days), 4 months post-injury (mean = 125 ± 5.9 days), and 7 months post-injury (mean = 210.8 ± 11.8 days). A healthy control population matched by age and gender was recruited after enrolment of the mTBI patient ($N = 6$, 4 females and 2 males, mean age = 14.3 ± 1.0 years). The study was approved by our institutional research ethics board. Prior to informed consent, potential subjects were thoroughly screened for MRI compatibility. Parents/legal guardians then provided written informed consent while participants provided written assent.

Clinical Measures

A concussion information form was administered to obtain detailed information about the cause of head injury, as well as the number of previous mTBI, if any. Post-concussion symptoms were evaluated at each scan using the post-concussion symptom scale (PCSS), which is a commonly used measure of

perceived symptoms associated with mTBI. The patient is asked to rate the current severity of 22 symptoms, via a 7-point Likert scale from 0 (no symptoms) to 6 (severe). The symptoms rated are: headache, nausea, vomiting, balance problems, dizziness, fatigue, trouble falling asleep, sleeping more than usual, sleeping less than usual, drowsiness, sensitivity to light, sensitivity to noise, irritability, sadness, nervousness, feeling more emotional, numbness or tingling, feeling slowed down, feeling mentally “foggy”, difficulty concentrating, difficulty remembering and visual problems.

As part of standard practice, all children/families enrolled in the study received education and written material on the CanChild (<http://www.canchild.ca>) concussion management guidelines outlining return to activity and return to school guidelines for children and youth [21]. This approach involves the gradual stepwise return to activity and school in a way that does not result in a significant exacerbation of symptoms. The mTBI patients began with complete rest, followed by light exercise. Patients progressed from one stage to the next only if they remained asymptomatic at the previous one. If any post-concussion symptoms occurred while in the stepwise program, then the patient was instructed to drop back to the previous asymptomatic level and try to progress again after one day of rest.

Imaging

Scanning was performed with a GE 3 Tesla MR750 MRI scanner with a 32-channel phased array RF head coil (General Electric Healthcare, Milwaukee, WI). Following head positioning, immobilization and a localizer scan, anatomical images were collected using a three-dimensional IR-prepped fast SPGR T1-weighted scan (TR/TE=11.4/4.3 ms, T1 = 450 ms, flip angle = 12°, 512 x 256 matrix, 80 slices, 24 cm FOV) parallel to the anterior commissure-posterior commissure (AC-PC) line. Subsequent T2 and T2-FLAIR imaging was done as part of the routine clinical TBI scanning. ¹H-MRS was acquired from two regions of interest: anterior cingulate cortex (ACC) and right frontal white matter (FWM), as shown in Figure 1. A rigorous anatomical localization protocol was used to position the MRS acquisition voxel, where all voxels were placed on an AC-PC-oriented oblique axial slice corresponding to the voxel on the sagittal view. In follow-up scans, a rigorous acquisition voxel placement strategy was applied for consistency. Single-voxel ¹H-MRS spectroscopic measurements were acquired using a PRESS (Point RESolved Spectroscopy) sequence (TE/TR=30/2000ms, 2x2x2 cm voxel, 256 acquisitions, duration 9.3 minutes).

Data Processing and Analysis

LCModel was used for MRS spectral fitting [22]. This is an operator-independent spectral analysis software package that obtains approximate maximum likelihood estimates of the metabolite concentrations and their

uncertainties. A sample of LCModel output from our study is shown in Figure 2. Cr, mI, NAA, Cho and Glu values were only considered significant if the estimated uncertainties, calculated as Cramer-Rao lower bounds (%SD), were less than 20% because levels above that are considered unreliable [23].

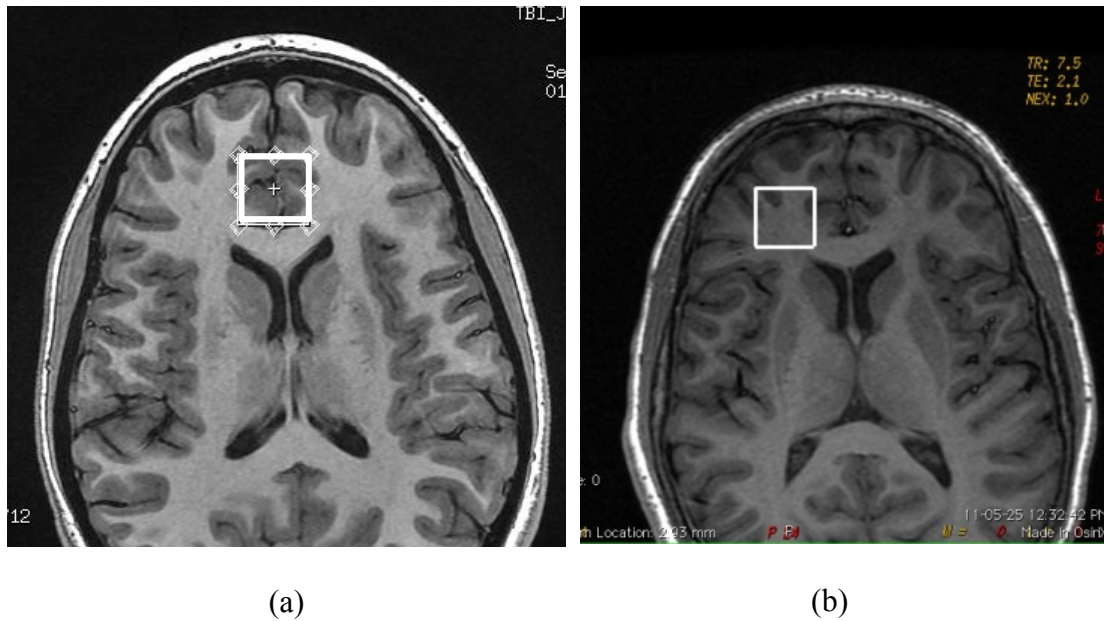


Figure 1. PRESS voxel acquisition locations shown on axial T1-weighted images for (a) ACC; (b) right FWM.

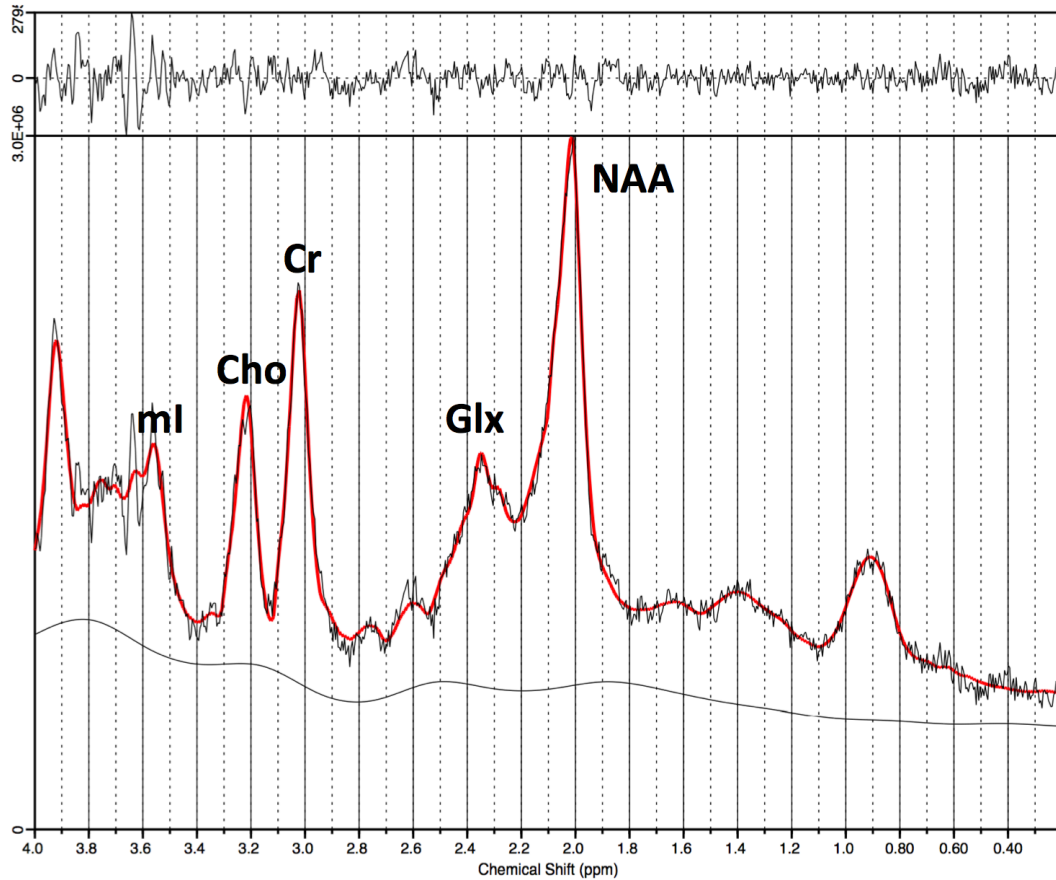


Figure 2. Sample of LCMoDeL fitted spectrum from the ACC of a mTBI subject, showing the peaks corresponding to the metabolites of interest. ml: myo-inositol; Cho: glycerophosphocholine plus phosphocholine; Cr: phosphocreatine plus creatine; Glx: glutamate plus glutamine; NAA: N-acetylaspartate.

For each spectroscopy voxel, the tissue volume fractions were determined through tissue segmentation of the T1-weighted images into three different tissue types (grey matter, white matter, and cerebrospinal fluid). This was performed using an in-house developed UNIX BASH script and the AFNI software [24]. ^1H -MRS voxel coordinates were used to find the specific tissue volume fraction for

the ^1H -MRS voxel of interest in the 3D acquisition volume. In order to obtain ‘absolute’ metabolite levels, the method of Gasparovic *et al.* [25] was followed which uses the unsuppressed internal water signal, tissue fractions, and water-tissue and metabolite relaxation times. The ^1H signal from each metabolite is proportional to its molar content per acquisition volume and the number of MR visible hydrogen atoms contributing to the specific metabolite ^1H signal. This signal is then related to the water ^1H signal from the same voxel. Then, the water ^1H signal from grey matter and white matter is found using tissue molal water fractions and NMR relaxation factors, excluding the contribution from cerebrospinal fluid [26]. The use of these values, instead of LCModel’s default values, has been shown to increase precision and inter-laboratory reproducibility [27]. This detailed correction was performed on both ACC and FWM acquisitions, at each time point (i.e. 1, 4 and 7 months post-mTBI).

Statistical analyses were performed using SPSS version 22.0 (IBM, Chicago, IL). Comparisons between mTBI patients and matched controls were examined using a two-sided Student's t-test. The equality of variances was evaluated using Levene’s test. Differences were considered to be statistically significant if $p < 0.05$. A repeated measures analysis of variance (ANOVA) was used to evaluate significant concentration differences between successive time points. Sphericity was evaluated using Mauchly's test. Differences were considered to be statistically significant if $p < 0.05$. Possible relationships between metabolite levels and the PCSS scores were examined using Pearson’s correlation.

RESULTS

A neuroradiologist qualitatively reviewed the anatomic MRI images and reported that no patients or controls presented with any detectable abnormality.

The PCSS scores of the mTBI patients are summarized in Table 1. Although some patients showed improvement over time in self-rated post-concussion symptoms, others continued to experience symptoms 7 months following injury.

Table 1. PCSS score and classification [28] of 6 patients, taken before each MRI scan.

Patient	Scan 1		Scan 2		Scan 3	
	PCSS	Classification	PCSS	Classification	PCSS	Classification
1	78	very high	32	high	1	Normal
2	57	very high	50	very high	60	very high
3	48	very high	15	high	39	very high
4	43	very high	8	normal	2	Normal
5	0	low-normal	0	low-normal	1	Normal
6	51	very high	3	normal	11	Unusual

¹H-MRS Metabolite Levels

The metabolite levels found in the FWM and ACC are presented in Table 2 and Table 3, respectively. Mean metabolite levels are reported as institutional levels. Analyses of the ¹H-MRS data showed no significant longitudinal changes in the metabolites levels over time in the mTBI patients, for the two regions

examined. However, differences were found when comparing mTBI patients to healthy controls at all three time-points. A statistically significant ($p < 0.05$) increase in Glu was found in the FWM of mTBI patients relative to controls at 1 month post-injury. The Glu levels in the patients remained elevated relative to controls at 4 and 7 months post-injury. No statistically significant changes were observed in NAA, Cho, mI or Cr levels in the FWM and ACC.

Table 2. Mean and standard deviation values of metabolite levels in the FWM of controls, and mTBI patients at 1, 4 and 7 months post-injury (* $p < 0.05$).

Metabolite	Controls	Patients Scan 1	p Value	Patients Scan 2	p Value	Patients Scan 3	p Value
NAA	14.90 (1.00)	14.51 (1.42)	0.66	15.23 (1.53)	0.73	14.71 (1.47)	0.84
Glu	11.59 (0.79)	12.68 (0.44)	0.03 *	12.92 (0.46)	0.03 *	12.80 (0.56)	0.04 *
Cho	3.53 (0.40)	3.37 (0.46)	0.61	3.58 (0.45)	0.87	3.45 (0.49)	0.80
mI	8.16 (1.42)	7.95 (1.12)	0.81	7.84 (1.04)	0.73	7.78 (1.26)	0.71
Cr	9.72 (1.18)	9.90 (1.31)	0.84	10.52 (1.10)	0.36	10.03 (1.54)	0.76

Table 3. Mean and standard deviation values of metabolite levels in the ACC of controls, and mTBI patients at 1, 4 and 7 months post-injury.

Metabolite	Controls	Patients Scan 1	p Value	Patients Scan 2	p Value	Patients Scan 3	p Value
NAA	18.36 (1.27)	17.39 (1.53)	0.33	17.53 (1.39)	0.39	17.44 (1.55)	0.37
Glu	17.55 (1.00)	17.20 (1.97)	0.76	17.69 (1.68)	0.88	17.59 (1.75)	0.79
Cho	3.66 (0.55)	3.54 (0.78)	0.80	3.52 (0.82)	0.78	3.36 (0.75)	0.53
mI	9.75 (1.16)	9.60 (1.66)	0.88	9.72 (1.29)	0.97	9.68 (1.90)	0.95
Cr	13.88 (1.55)	13.80 (1.66)	0.94	13.73 (1.55)	0.89	13.49 (1.47)	0.71

Correlations between significant metabolite changes and post-concussion symptoms were carried out. No statistically significant correlations were found between Glu and PCSS scores in the FWM and ACC, at all three patient visits. Glu levels in the right FWM and ACC against PCSS scores are shown in Figures 3 and 4, respectively.

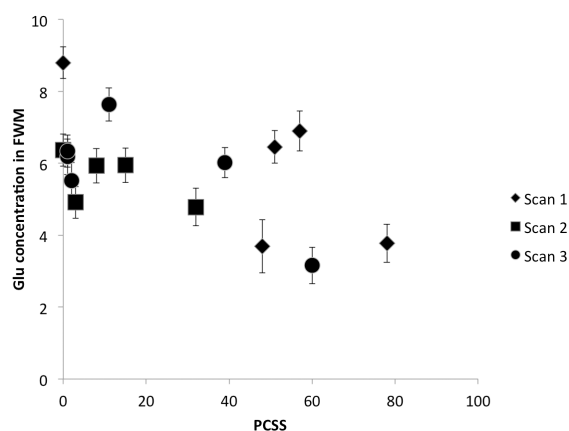


Figure 3. Glutamate levels in the right FWM against PCSS scores of 6 mTBI patients. Longitudinal values are shown from the examination of each patient at 3 different time points. Sample standard deviations are represented by vertical bars.

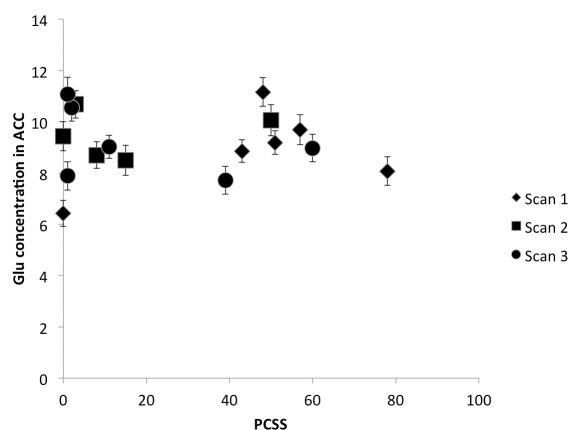


Figure 4. Glutamate levels in the ACC against PCSS scores of 6 mTBI patients. Longitudinal values are shown from the examination of each patient at 3 different time points. Sample standard deviations are represented by vertical bars.

DISCUSSION

As far as we are aware, this is the first study showing changes in brain metabolite concentrations in children following mTBI. Importantly, we found a significantly elevated Glu level in the FWM of mTBI patients, relative to healthy controls, at 1 month post-injury. The FWM Glu level did not return to healthy control levels at subsequent patient visits (i.e. 4 and 7 months post-injury) showing that Glu abnormalities persist even up to 7 months following mTBI.

Glutamate is the major excitatory neurotransmitter in the brain. Not only is it an excitatory neurotransmitter, Glu signalling also regulates myelination and trophic support, which could, in theory, need upregulating as a result of white matter abnormalities following mTBI. Thus, our observed increase in Glu levels in the FWM may be a metabolic link to white matter abnormalities in children following mTBI [29] [30] [31] [32] [33].

Our findings may have implications for previous and future ^1H -MRS studies on mTBI. Although longitudinal ^1H -MRS studies focus largely on adults, a recent study investigated the changes in NAA and lactate in 12 children following a sports-related mTBI [20]. NAA is a marker of neuronal integrity that decreases with neuronal loss or dysfunction [11] [12] and elevated lactate is often seen during hypoxic insult as a result of reduced blood flow or oxygenation. Single voxel ^1H -MRS was acquired from three regions: anterior cingulate gyrus, left frontal white matter and left thalamus. Patients were scanned at 72 hours, 14 days, and 30 days or more, following injury. Relative to controls, no decrease in

NAA and no increase in lactate were found, leading the authors to conclude that there is no evidence of measurable metabolic changes in paediatric sports-related mTBI. However, it could be suggested that their conclusion was premature, as they did not investigate other metabolite levels including Glu, which is known to increase in other forms of brain insult. Our present study demonstrates that paediatric sports-related mTBI has a measurable and significant metabolic injury component.

In agreement with the previous paediatric mTBI study [19], we did not observe measureable changes in NAA. However, decreased brain NAA has been observed in longitudinal adult mTBI studies [16] [17] [18] [19]. One study investigated the effects of sports-related mTBI on ratios of brain metabolites to creatine in 10 athletes where scanning was done twice, 1 to 6 days post-injury and then again after 6 months [17]. Relative to controls, a significantly lower NAA/Cr was found in the dorsolateral prefrontal cortex and motor cortex, in early post-mTBI and even after 6 months. Furthermore, increased Glu/Cr was found in the motor cortex during the early scan, which returned to normal 6 months post-injury. The authors also reported increased ml/Cr in the motor cortex that was only found during the second scan time, suggesting that metabolic changes may have also emerged over time, rather than immediately following injury. However, one assumption in their study was that the brain creatine level is constant, even post-mTBI (hence reporting values relative to Cr). This may not be a valid

standardization procedure seeing as Cr is involved in brain energy metabolism.

There has yet to be definitive evidence showing that Cr is stable post-mTBI.

In another study NAA levels were assessed with ^1H -MRS in 13 non-professional adult athletes following sports-related mTBI [18]. Patients were scanned at 3, 15, and 30 days post-injury where single voxel ^1H -MRS was acquired bilaterally from the frontal lobe white matter. At 3 days post-injury, a decrease in NAA/Cr was found in mTBI patients. Reduced levels were also found 15 days post-injury, although a modest 3% recovery was observed. At 30 days post-injury, NAA/Cr showed full recovery. Hence, based on this study the MRS approach was classified as useful for monitoring recovery from mTBI, where recovery was not necessarily linearly related to time. Furthermore, in the same study, a second head injury was shown to prolong the time of NAA/Cr normalization by 15 days. These results have been supported by a follow up, multi-centre study of 40 adult athletes with mTBI in which scans were performed at 3, 15, 22 and 30 days post-injury [19]. The most significant abnormalities in NAA/Cr and NAA/Cho were found at 3 days post-injury. On average, NAA/Cr and NAA/Cho levels gradually recovered, initially in a slow manner and then faster after 15 days. At 30 days post-injury, full recovery of the metabolite ratios was observed. A different study examined the metabolite concentrations 13 days post-injury and then 4 months following mTBI [16]. An increase in white matter concentrations of Cr and Glx, and a decrease in grey matter concentrations of Glx,

was found at the first scan. Partial normalization of Cr and Glx was found during the semi-acute period of recovery.

Our findings of no changes in NAA following mTBI are in line with a study of mTBI in children [20] but differ from adults [17] [18] [19], therefore this may suggest differences in pathophysiology and neural responses to mTBI between children and adults. The differences between children and adults have been explored by a study that examined the pathophysiological response to diffuse TBI in rats as a function of developmental age [34]. Their MRS findings showed no significant changes in metabolites of juvenile rats with moderate diffuse TBI, in contrast to significant decreases identified in adults.

In our study, some mTBI patients showed improvement over time in self-rated post-concussion symptoms, while others continued to experience symptoms 7 months post-injury. Moreover, no statistically significant correlations or trends were found between Glu and PCSS scores, at all time points, in both the FWM and ACC. This is not surprising given the varied types of injuries and also the highly variable qualitative nature of the PCSS. However, even though some patients recovered from a symptom standpoint, there remains a persistent neurometabolite abnormality at 7 months following mTBI.

The main strengths of the present study are the measurements of children with mTBI, longitudinally at three time points, and the absolute quantification approach that takes into account CSF, grey and white matter tissue fractions. Although the sample size we used is similar to other ^1H -MRS studies in mTBI

[20] [17] [18], examining the prevalence of our results in a larger sample will be important. The main limitations of our study are the wide variation in mTBI severity and type, and the fact that each patient and control had MRS measurements taken from identical locations. Although the data acquisition approach is methodologically robust the acquisition regions may or may not reflect regions of brain damage. Given the fact that mTBI does not present with obvious anatomical abnormalities which could be used to guide PRESS acquisition volumes, any study using MRS would have this difficulty.

In conclusion, our study examined children with mTBI longitudinally at three time points, using ^1H -MRS. Our findings imply that mTBI in children causes abnormalities in brain metabolites, and these disruptions persist up to 7 months post-injury. Our study also provides evidence that metabolic abnormalities may be different in adolescents/children compared to adults. Furthermore, our findings indicate that the resolution of post-concussion symptoms in children does not necessarily indicate brain metabolic recovery. Therefore, we conclude that ^1H -MRS is a useful and important technique to non-invasively assess and monitor recovery from mTBI in children.

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CHAPTER 7

The Effect of Acute Exercise on Brain Resting State Functional Connectivity

Raghda Hasswa, Saman Sarraf, Carol DeMatteo, John Connolly, Mohammed A. Warsi, Michael D. Noseworthy

7.1 Context of the Paper

The long-term benefits of aerobic exercise on brain health are well known. However, no studies have assessed whether exercise acutely can affect the brain. Resting state fMRI analysis offers an in vivo method of studying the changes that take place in the brain following acute aerobic exercise. We used functional connectivity analysis of all identifiable resting state networks using independent component analysis.

7.2 Declaration Statement

Raghda Hasswa as principal author wrote the article, performed analysis, and created figures and tables as appropriate. Dr. Michael Noseworthy, as corresponding author, provided guidance, funding and advice, and performed proofreading/editing of the manuscript for publication. Saman Sarraf provided methodological instructions and guidance. Prof. Carol DeMatteo provided guidance and advice. Dr. John Connolly provided commentary and advice. Dr. Mohammed Warsi provided guidance and commentary.

7.3 Paper

The Effect of Acute Exercise on Brain Resting State Functional Connectivity

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ABSTRACT

Purpose. Aerobic exercise has the potential to be used in everyday life to improve well-being and cognition. However, more research is needed to further understand the effects of acute exercise on healthy individuals and on patients with mild traumatic brain injury (mTBI). Blood oxygen level dependent (BOLD) functional magnetic resonance imaging (fMRI) can rapidly assess brain function immediately during and following physiological perturbation. Using resting state BOLD fMRI, we investigated the brain resting state functional connectivity changes immediately following an acute bout of aerobic exercise.

Materials and Methods. Resting state fMRI was performed on healthy young adults (n=10) before and after an acute bout of exercise, performed using a MRI-compatible up/down ergometer. On average, subjects exercised at 65% of their age-predicted maximum heart rate. Functional connectivity of resting state networks was investigated using probabilistic independent component analysis (PICA).

Results. Statistical significance was found in three resting state networks ($p < 0.001$). Exercise related increases in functional connectivity were found in the executive control network (Brodmann area 24) and fronto-parietal network (Brodmann area 40), while decreased connectivity was observed in the sensorimotor network (Brodmann area 6).

Conclusions. This study provided evidence of changes in brain resting state functional connectivity following an acute bout of exercise. Our findings may

provide insight on how exercise affects the brain function, and lay the foundation for future studies on the effects of acute exercise on mTBI patients.

KEY WORDS: Brain, resting state, fMRI, acute exercise, ergometer

INTRODUCTION

There is a wealth of research demonstrating that regular physical activity provides significant long-term benefits for brain health, function and cognition [1]. A review conducted by the National Institute of Mental Health (NIMH) concluded that exercise is positively related to several indices of mental health [2]. Exercise improves mental health by reducing stress levels, depression and anxiety [3]. Exercise also reduces stroke risk and transient ischemic attacks [4]. Furthermore, the health benefits of exercise have been demonstrated by increases in brain volume in the hippocampus [5], anterior cingulate, supplementary motor area, right inferior frontal gyrus and left superior temporal gyrus [6].

In addition to the long-term benefits of physical activity, studies have shown that a single acute bout of exercise may be beneficial to our health. A single bout of low-moderate intensity aerobic exercise is sufficient to improve the mood and well-being of healthy subjects [7], and patients with depression [8] [9]. Furthermore, acute exercise can facilitate selective aspects of executive functions in healthy subjects [10] [11] [12], and in patients with depression [13] or attention deficit hyperactivity disorder (ADHD) [14]. Exercise may also help manage

children who suffer from psychiatric disorders associated with increased levels of arousal [15]. Moreover, a single bout of aerobic exercise can facilitate goal maintenance processes [16]. Exercise has been linked to improvements in response speed and response accuracy [17]. Following exercise, people are better prepared to engage in action, concentrate and solve complex problems [18]. Additionally, acute exercise may suppress appetite, leading to a lower consumption of high-calorie foods [19]. Thus, all these findings indicate that acute exercise has the potential to be systematically used in everyday life to improve well-being and cognition. However, more research is needed to further understand the mechanisms underlying the beneficial effects of acute exercise on the brain.

Furthering our understanding of the effects of acute exercise on the brain is advantageous not only for healthy individuals but also for patients suffering from mild traumatic brain injury (mTBI). In the present-day mTBI management guidelines, most healthcare professionals prescribe physical rest until the acute post-concussion symptoms resolve. However, even then, the post-concussion symptoms sometimes reappear with exercise. There is a lack of studies on the effects of acute exercise on the brain's functional connectivity, and therefore it is unclear why post-concussion symptoms may resurface with exercise.

A promising area of functional magnetic resonance imaging (fMRI) is aimed at mapping resting state functional connectivity. Resting state functional connectivity research [20] has demonstrated the continued interaction amongst

anatomically separate brain areas even during periods of rest or disengagement from explicit tasks or demands [21]. Resting state functional connectivity analysis usually targets low frequency (<0.1 Hz), synchronized activations (also known as low-frequency blood oxygen level dependent (BOLD) fluctuations) in spatially separated areas of the brain [22]. These synchronized neurophysiological events, active at rest, represent structurally and functionally connected networks, termed resting state networks (RSNs). RSNs refer to the temporal coherence between different areas of the brain at rest, i.e. in the absence of task driven activations. Resting state functional connectivity, as measured by BOLD signal fluctuations, has a neurophysiological basis and is sensitive to various neurological and psychiatric abnormalities [23]. Therefore, it is useful in understanding the behaviour observed in both normal and pathological conditions. Thus, resting state brain functional connectivity is potentially an important measure in evaluating the impact of exercise on brain function. In normal states, a RSN is characterized by high metabolism during resting state [20] [24], functional connectivity during rest [25], and deactivation during various attention-demanding cognitive tasks [26] [27].

Researchers have identified a number of RSNs that are steadily found in the resting brain, even across studies using different data acquisition and analysis techniques, for example [28] [29] [30]. The default mode network (DMN), sensorimotor network (SMN), executive control network (ECN), fronto-parietal network (FPN), medial visual network and auditory network are amongst the most

commonly found RSNs. The DMN involves the precuneus/posterior cingulate, lateral parietal cortex, and mesial prefrontal cortex [30]. It has by far received the greatest attention from both the research and clinical community. The DMN has been associated to introspective mental processes, stimulus-independent thoughts (also known as mind wandering), retrieving one's past or envisioning one's personal future, and self-reference. The SMN involves the precentral gyrus, postcentral gyrus, and supplementary motor area [30]. It is characterized by the involvement of brain regions that anatomically correspond to motor as well as sensory areas. The ECN involves the medial frontal gyrus, superior frontal gyrus, and anterior cingulate cortex [30]. In some cases it also includes the lateral parietal areas. In general, the ECN is involved in tasks relying on executive functions, such as control processes and working memory. The FPN includes the inferior frontal gyrus, medial frontal gyrus, precuneus, inferior parietal gyrus, and angular gyrus [30]. The FPN has been associated to different functions including memory, language, attention, and visual processes [28]. The precise role of the lateralized FPN remains less clear compared to the other RSNs. The auditory network involves the superior temporal gyrus, Heschl's gyrus, insula, and postcentral gyrus. The superior temporal gyrus contains the primary auditory cortex, which processes sound and contributes to our ability to hear. It also contains Wernicke's area, which is involved in processing speech into an understandable language.

Resting state functional connectivity can be analyzed by two different approaches: model-dependent seed-based methods and model-free approaches. Model-dependent methods examine the functional connectivity of a specific brain region, based on the placement of a seed-voxel in a predefined region of interest. Model-free methods, however, are used to examine whole-brain connectivity patterns without the need for defining any *a priori* seed region.

Resting state functional connectivity was originally used as a research tool to investigate the functional architecture of the brain in healthy subjects. However, more recently a number of potential clinical applications have emerged, where resting state fMRI has shown promise of improving our understanding of the functional abnormalities underlying different disease states, and potentially advancing the diagnosis and assessment of patients with various neurological and psychiatric disorders and neurodegenerative diseases (such as Alzheimer's disease, multiple sclerosis and schizophrenia) [31].

In this study we examined the functional connectivity changes in the brain before and after an acute bout of aerobic exercise. We used resting state fMRI to investigate the RSNs alterations following acute exercise in healthy adults. This may shed light on how exercise affects the brain function and cognitive ability. Furthermore, this will be of importance in studying mTBI cases where post-concussion symptoms are evident, but not well understood, following exercise. This is the first study using this approach to study a single acute bout of exercise on the brain.

MATERIALS AND METHODS

Subjects

Ten typically developed healthy participants with no psychiatric disorders or traumatic brain injuries were recruited for this study (see Table 1). The subjects were required to be free from injury or disease that would prevent them from exercising safely.

Table 1. Information of subjects.

Characteristic	Mean (SD)
Age, years	25.6 (3)
Sex, % male	60
Height, cm	172.6 (7)
Weight, kg	65 (14)
Handedness, % right	90
Resting heart rate, bpm	73.0 (4)
Exercise heart rate, bpm	126.4 (15)

Exercise

Exercise took place inside the MRI scanner using an MRI-compatible up/down ergometer (Lode, Groningen, Netherlands). The exercise protocol consisted of a 30 second warm-up, in order to familiarize the subject with the ergometer and prepare them for the exercise intensity. The ergometer power was gradually increased from 0 to 5 Watts during the warm-up. The subject then

pedaled for 3.5 minutes at 5W, which was adequate for all subjects to increase their heart rate significantly. The same procedure was repeated but without exercise as a control condition. Instead of exercising, the subject remained lying quietly in the scanner for the same duration of exercise. The two scan conditions were conducted on different days to ensure that the subjects do not spend too much time in the MRI scanner, which may have compromised the state of the person during the second condition.

Imaging

Imaging was performed using a General Electric 3 Tesla MR750 Discovery MRI scanner and 8-channel phased array RF coil (GE Healthcare, Milwaukee, WI). Anatomical and functional images were acquired. Anatomical images were collected using a three-dimensional IR-prepped fast SPGR T1-weighted sequence (TR/TE=7.5/2.1ms, T1=450ms, flip angle=12°, 512x512 matrix, 140 slices, 24 cm FOV). Two separate resting state BOLD imaging scans (gradient echo EPI, TR/TE=2000/35ms, flip angle=90°, 64x64 matrix, 31 slices, 180 time points, 24 cm FOV) were acquired, one before and one immediately after exercise. During resting state BOLD imaging acquisitions, subjects were asked to remain awake, keep their eyes open, and not to think of anything in particular

Data Processing and Analysis

Preprocessing was performed using tools from FMRIB Software Library version 5.0.2.2 [32]. Data from each subject was corrected for interleaved slice acquisition. The skull and other non-brain tissue were removed using Brain Extraction Tool [33]. Motion correction was performed using MCFLIRT [34]. Using the FSL software package MELODIC (Multivariate Exploratory Linear Optimized Decomposition into Independent Components) [35], functional images were filtered with a high-pass temporal cutoff of 0.01 Hz (for low level noise removal), and were spatially smoothed with a Gaussian kernel with full width at half maximum of 5mm (to improve signal-to-noise ratio). All functional data were registered to the subject's high resolution T1-weighted anatomical scans, then subsequently registered to MNI152 standard space at 2mm resampling resolution.

Using MELODIC, probabilistic independent component analysis (PICA) was performed on all subjects, at a single-group level, to decompose the 4D data sets into separate spatial maps. This was accomplished using a multi-session temporal concatenation approach, which performs a single 2D ICA run on the concatenated data matrix (obtained by stacking all slices of every data set on top of each other). This approach was chosen in order to look for common spatial patterns without assuming that the temporal response is consistent between subjects. The following options were implemented in MELODIC [36]: time-courses were variance-normalized in order to stress voxel-wise temporal

dynamics over mean signal; the number of components were automatically estimated; a level of 0.5 was used as the threshold level for independent components (i.e. a voxel 'survives' thresholding as soon as the probability of being in the 'active' class exceeds the probability of being in the 'background' noise class. A threshold level of 0.5 assumes an equal loss on false-positives and false-negatives is placed [37]).

The set of spatial maps obtained from the group-average analysis (MELODIC) was used to generate subject-specific versions of the spatial maps, and associated time series, using the FSL software Dual Regression [38]. First, for each subject, the group-average set of spatial maps was regressed into the subject's 4D space-time dataset. This results in a set of subject-specific time series, one per group-level spatial map. Next, those time series were regressed into the same 4D dataset, resulting in a set of subject-specific spatial maps, one per group-level spatial map. Group differences (before vs. after exercise) were then tested using the FSL software Randomise permutation-testing tool. Since this is the first ICA study of acute exercise effects on the brain, and no specific RSN was being tested, it was treated as exploratory in nature; thus, correction for multiple-comparisons was not performed.

Methodological Approach

Model-free independent component analysis (ICA) approach was used to analyze the resting state fMRI data. Model-free methods (as opposed to model-

dependent, seed-voxel methods) are used to examine whole-brain connectivity patterns without the need for defining an a priori seed region. The most commonly used model-free methods are ICA-based methods (for e.g. PICA) and have been reported to show a high level of consistency [21]. PICA looks for underlying sources that can explain the resting state patterns, searching for the existence of maximally independent spatial sources of resting state signals. PICA divides a dataset into different maximally independent components [37]. Spontaneous activity is automatically separated from noise (such as head motion) or physiological confounds (such as cardiac pulsation and respiratory motion). PICA is advantageous because it evaluates and compares the coherence of activity in multiple distributed voxels, and divides RSNs into different independent components [37] [39]. On the other hand, limitations with the ICA approach include the difficulty of assigning a statistical framework that would enable activation networks to be tested against specific hypotheses, and the difficulty of assessing the regionally specific nature of brain responses [40]. While there is currently no agreement as to which method (ICA or seed-based) is superior for assessing resting state functional connectivity MRI [41], a comparison study between the two methods found ICA to be the superior method [42].

RESULTS

Exercise

The participants (6 males, 4 females) had a mean age of 25.6 ± 3 years. The mean resting and exercise heart rates were 73.0 ± 4 and 126.4 ± 15 beats per minute (bpm), respectively. This corresponded to an average age-predicted exercise maximum heart rate of 194.4 bpm. Thus, on average, subjects exercised at 65% of their maximum heart rate, which is within the target heart rate. The exercise intensity reached by each subject was within the target heart rate.

Resting State Networks

The following RSNs were identified in all subjects using PICA: executive control network, fronto-parietal network, sensorimotor network, default mode network, salience network, medial visual network, cingulate network and auditory network. Following acute exercise, statistically significant (uncorrected $p < 0.001$) differences were discovered in three RSNs: the executive control network (ECN), fronto-parietal network (FPN) and sensorimotor network (SMN) (shown in Figure 1). Increased functional connectivity (post > pre) in the ECN was found in the cingulate gyrus in the left Brodmann area (BA) 24 ($x = -4\text{mm}$, $y = -10\text{mm}$, $z = 36\text{mm}$ in MNI standard space), as shown in Figure 2a. Increased functional connectivity was also found in the FPN in the inferior parietal lobule in the right BA 40 ($x = 62\text{mm}$, $y = -32\text{mm}$, $z = 30\text{mm}$), as shown in Figure 2b. Decreased functional connectivity in the SMN was noted in the precentral gyrus in the left BA 6 ($x = -$

50mm, $y=-4\text{mm}$, $z=38\text{mm}$), as shown in Figure 2c. Moreover, as expected no significant differences in the RSNs were observed in the control scans.

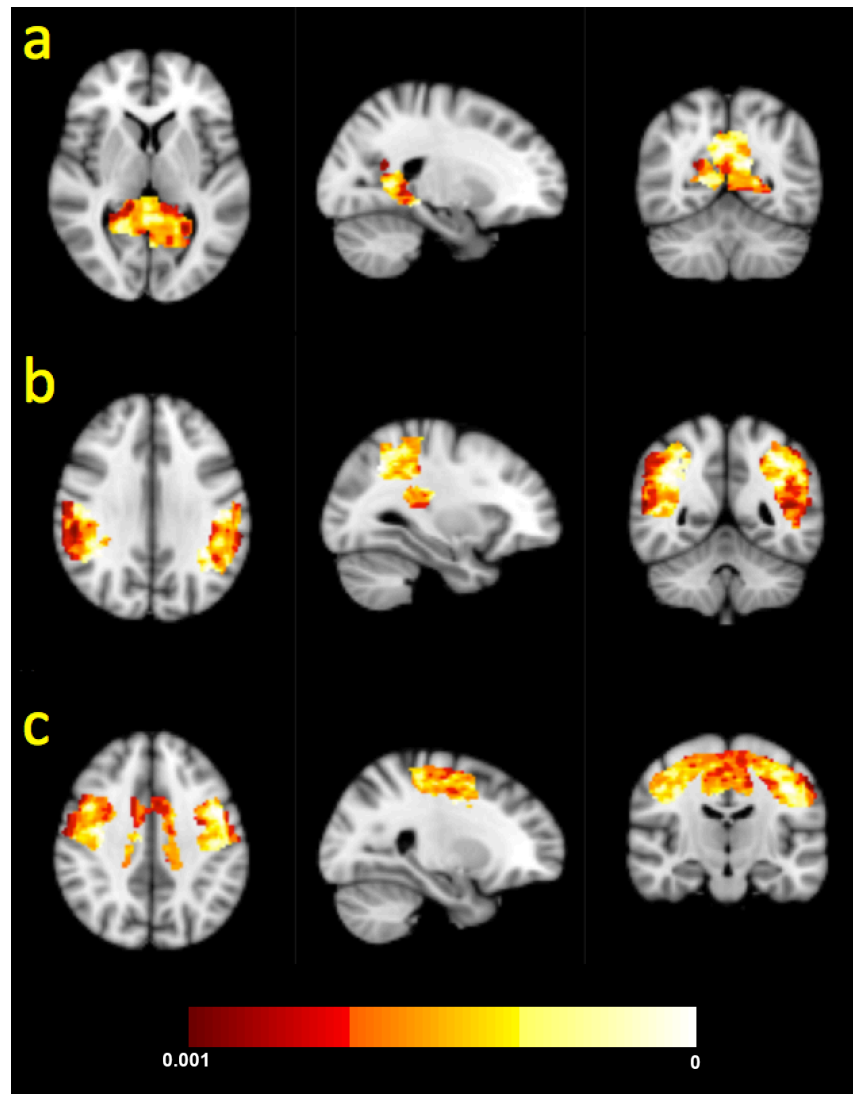


Figure 1. Probabilistic ICA spatial maps of the 3 resting state networks, from the analysis of 10 healthy subjects, before exercise. Colour-bar is p-values, ranging from 0 to 0.001. (a) executive control network; (b) fronto-parietal network; (c)

sensorimotor network. Axial, sagittal and coronal views are shown. The left side of the image refers to the right hemisphere of the brain.

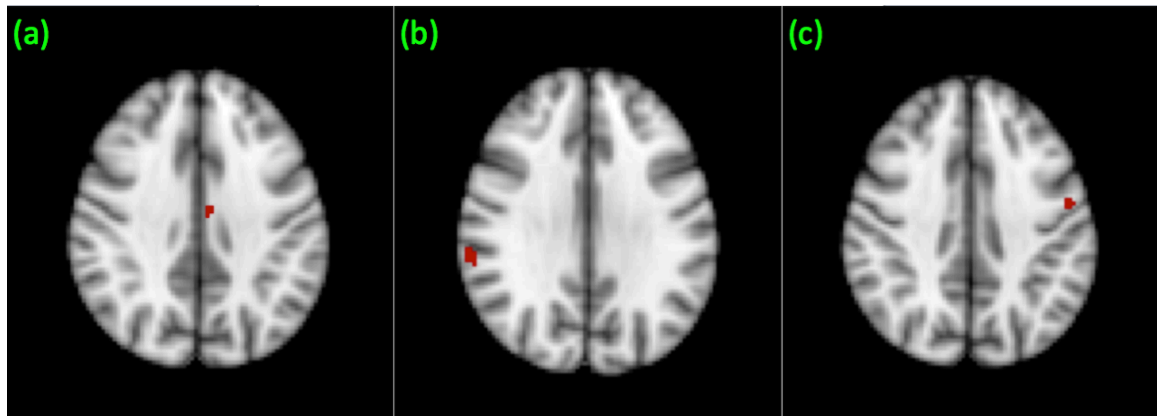


Figure 2. Significant between group RSN differences following an acute bout of exercise. Red overlay corresponds to differences found with $p < 0.001$. The left side of the image refers to the right hemisphere of the brain. (a) executive control network, BA 24, post > pre exercise; (b) fronto-parietal network, BA 40, post > pre exercise; (c) sensorimotor network, BA 6, pre > post exercise.

DISCUSSION

This study is the first to use resting state fMRI to investigate the effects of acute exercise on the brain. Statistical significance was discovered in three RSNs: increased connectivity (post>pre) in the ECN in the cingulate gyrus in the left BA 24, increased connectivity in the FPN in the inferior parietal lobule in the right BA 40, and decreased connectivity in the SMN in the precentral gyrus in the left BA 6, post exercise.

Following acute exercise, a significant increase in functional connectivity in the ECN was found. The ECN is involved in tasks relying on high-level cognitive functions such as control processes, maintenance and manipulation of information in working memory, control of attention judgment and decision-making in the context of goal directed behaviour [30] [43]. Specifically, increased functional connectivity was found in the left BA 24, which has been shown to be involved in the execution, imagination and inhibition of actions, as well as emotional assessment of somatosensory perception and music processing [44].

Similarly, a significant increase in functional connectivity in the FPN was found following exercise. The FPN has been associated to different functions such as memory, language, attention, and visual processes [28]. Specifically, increased functional connectivity was found in the right BA 40, which has been shown to be involved in the execution, imagination, inhibition and observation of actions, as well as spatial discrimination, cognition, attention, working memory and somatosensory perception [44].

On the other hand, a significant decrease in functional connectivity in the SMN was found. The SMN is characterized by the engagement of regions that anatomically correspond to motor as well as sensory areas [30]. Specifically, decreased functional connectivity was found in left BA 6, which has been shown to be mainly involved in motor learning and planning as well as motor activation of the hand (Exner's area) [44].

Our findings have implications for previous and future studies investigating the effects of acute aerobic exercise on the brain. Using various techniques, such as electroencephalography, previous studies showed that acute aerobic exercise has the potential to be systematically used in everyday life to promote well-being and enhance selective aspects of cognition [10] – [17]. The effects of acute exercise on the cognitive function in preadolescent children and young adults were investigated by Hillman et al. [10] [11]. Using event-related brain potentials (ERPs), they found that acute exercise affects electrophysiological manifestations of neural processes underlying executive control through the increased allocation of resources and through changes in cognitive processing and stimulus classification speed [10]. Thus, their findings indicate that a single bout of moderate acute exercise may improve the cognitive control of attention [11]. Additionally, Drollette et al. [12] used ERPs to examine the effects of moderate-intensity aerobic exercise on aspects of cognitive control in children categorized by higher and lower-task performance. They reported that children with lower inhibitory control capacity may benefit the most from acute exercise. A single bout of aerobic exercise may also facilitate goal maintenance processes, as reported by Scudder et al. [16], by allowing individuals to better inhibit extraneous neural activity to allocate more attentional resources toward the updating and revision of goal representations. Hogan et al. [17] studied the effects of aerobic exercise on cognitive functioning using electroencephalography in

adolescents. Their findings suggest that physical fitness and acute exercise may improve cognition by increasing the efficacy of the attentional system.

In addition to the benefits of acute exercise in healthy individuals, studies have shown that acute exercise may be beneficial to patients with mental disorders [13] and psychiatric disorders associated with increased levels of arousal [14] [15]. The effects of acute exercise on executive functions in adults with major depressive disorder were studied by Kubesch et al. [13]. They reported positive effects on executive control processes that appear to be controlled by the anterior cingulate. Furthermore, the neurocognitive and behavioural effects of a single session of moderate-intensity aerobic exercise on preadolescent children with ADHD was investigated by Pontifex et al. [14]. Following exercise, the children exhibited selective enhancements in regulatory processes, greater response accuracy and stimulus-related processing, as well as enhanced performance in the areas of reading and arithmetic. Their findings indicate that exercise may have positive implications for aspects of neurocognitive function and inhibitory control in children with ADHD. Furthermore, Mierau et al. [15] examined the relationship between cortical oscillations, arousal and cognitive performance following physical exercise in preschoolers using electroencephalography. Their findings indicate cortical inhibition and attenuation of arousal in response to visual stimulation following exercise. Future studies using resting state fMRI may help confirm the benefits of acute exercise on the executive functions of children. Similarly, resting state fMRI

may potentially further our understanding of the effects of acute exercise on illnesses and injuries such as mTBI.

In conclusion, our preliminary findings provide evidence of changes in brain resting state functional connectivity following an acute bout of exercise. This preliminary study may shed light on how exercise affects brain function, such as cognitive ability, following acute exercise. Furthermore, our findings lay the foundation for future studies on the effects of acute exercise on mTBI patients, especially the cases where post-concussion symptoms are evident, but not well understood, following exercise.

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CHAPTER 8

Conclusions and Future Directions

8.1 Main Findings and Conclusions

Mild traumatic brain injury (mTBI) is a trauma-induced alteration in mental status that may or may not involve loss of consciousness. It is often referred to as a ‘silent epidemic’, partly due to its high incidence and frequent under-diagnosis. Yet in recent years, mTBI has attracted considerable attention from academia and mass media, and people are becoming increasingly aware of its potential cognitive, physical, emotional, and social consequences. Nevertheless, the pathophysiology and recovery time of mTBI are not well understood. Furthermore, present-day mTBI management guidelines typically include physical rest until the acute post-concussion symptoms resolve. However, even then, the post-concussion symptoms sometimes reappear with physical activity. There is a lack of studies on the effects of acute exercise on the brain’s

functional connectivity, and therefore it is unclear why post-concussion symptoms may resurface with exercise.

In this project we used three advanced MRI techniques: resting state functional magnetic resonance imaging (rs-fMRI), diffusion tensor imaging (DTI), and proton magnetic resonance spectroscopy (^1H -MRS). These promising neuroimaging techniques were used to examine the brain's functional connectivity, white matter architecture and metabolites, which may underlie the observed abnormalities following mTBI. Children who had sustained an mTBI were examined three times (at 1, 4 and 7 months post-injury) i.e. a longitudinal study design, to determine the progress of recovery over time. The cause of mTBI was impact loading; specifically, all patients had a sports-related mTBI. Age and gender matched healthy controls were also evaluated.

Functional connectivity of resting state networks (RSNs) was investigated using probabilistic independent component analysis. Statistical significance was discovered in three RSNs. Firstly, decreased connectivity (mTBI<Control) in the auditory network (AN) was found. The AN is involved in audition, such as tone/pitch discrimination, music, and speech. Secondly, increased connectivity in the default mode network (DMN) was found. The DMN is associated to introspective mental processes, stimulus-independent thoughts (also known as mind wandering), retrieving one's past or envisioning one's personal future, and self-reference. Thirdly, increased connectivity in the sensorimotor network (SMN) was found. The SMN is characterized by the engagement of regions that

anatomically correspond to motor as well as sensory areas. Enhanced functional connectivity following mTBI may represent neural compensation following injury. Furthermore, there was some functional recovery observed at the second scan, where changes were no longer found in the AN and SMN. However, a persistent increase in connectivity in the DMN was observed at 7 months post-injury.

DTI whole-brain voxel-wise analysis was performed using tract-based spatial statistics. Statistical significance was discovered in the fractional anisotropy (FA) of several white matter tracts. A significant decrease (mTBI<control) in fractional anisotropy was found in the left inferior longitudinal fasciculus, inferior fronto-occipital fasciculus, superior longitudinal fasciculus and forceps major. No apparent recovery in the fractional anisotropy of mTBI patients was observed, showing that mTBI in children causes abnormalities in the brain's white matter tracts, and these disruptions persist for at least seven months post-injury.

¹H-MRS was used to examine the levels of glutamate (Glu), N-acetyl aspartate, choline, myo-inositol and creatine, in the right frontal white matter (FWM) and anterior cingulate cortex. mTBI patients demonstrated significantly higher levels of Glu, relative to healthy controls, which never fully recovered by 7 months post-injury. Although no correlations were found between patients' post-concussion symptoms and Glu levels, the persistently higher Glu could have contributed to, or resulted from neurological impairments seen in mTBI patients.

Finally, we used rs-fMRI to investigate the effects of acute aerobic exercise on the healthy adult brain. Exercise was performed using a MRI-compatible up/down ergometer. We examined the whole-brain functional connectivity changes before and after a bout of exercise in healthy adults. On average, subjects exercised at 65% of their age-predicted maximum heart rate. Functional connectivity of RSNs was investigated using probabilistic independent component analysis. Statistical significance was discovered in three RSNs. Firstly, increased connectivity (post>pre-exercise) in the executive control network (ECN) was found. The ECN is involved in tasks relying on high-level cognitive functions such as control processes, maintenance and manipulation of information in working memory, control of attention judgment and decision-making in the context of goal directed behaviour. Secondly, increased connectivity in the fronto-parietal network (FPN) was observed. The FPN has been associated to different functions such as memory, attention and visual processes. Thirdly, decreased connectivity in the SMN was found. The SMN is characterized by the engagement of regions that anatomically correspond to motor as well as sensory areas. Our findings provide evidence of changes in brain resting state functional connectivity following an acute bout of aerobic exercise. This may shed light on how exercise affects brain function, such as cognitive ability, following acute exercise. Furthermore, this will be of importance in studying mTBI cases where post-concussion symptoms are evident, but not well understood, following exercise.

8.2 Contributions of this Work

To our knowledge, this study is the first longitudinal study on children with mTBI that uses a model-free ICA approach for analysis of rs-fMRI. There have been previous reports of model-free rs-fMRI analyses performed on mTBI patients, but those focused on adults, and mostly had a cross-sectional study design. The other studies that performed rs-fMRI on mTBI patients have predominantly used a model-dependent, seed-based analysis approach; thus, these only focused on one or two RSNs due to the limitation of the seed-based approach. Furthermore, to our knowledge, our study is the first longitudinal study on children with mTBI that uses tract-based spatial statistics for a whole-brain analysis of the DTI data. Previously, whole-brain DTI analyses have been performed on mTBI patients, but all were focused on adults, and mostly had a cross-sectional study design. The other studies that performed DTI on mTBI patients have predominantly used a regional analysis approach. Hence, these only examined the white matter structure in a limited number of brain regions. This is particularly disadvantageous since mTBI results in a complex pattern of white matter alterations at variable locations, making it difficult to choose an a priori region of white matter disruption.

Our longitudinal ^1H -MRS study on children with mTBI is the first MRS study that looks at the levels of NAA, Cho, Glu, mI, and Cr in the anterior cingulate cortex and frontal white matter in children with mTBI at 1, 4 and 7

months post-injury. Furthermore, our study was strengthened by the fact that we used an absolute MRS quantification method that took into account CSF, grey matter and white matter tissue fractions. The other study that used MRS on children with mTBI looked at NAA and lactate only, and examined patients in the first month post-injury.

Finally, our exercise study is the first to investigate the effects of acute aerobic exercise on the brain's functional connectivity in healthy adults using a model-free ICA approach for analysis of rs-fMRI. Other studies have examined the effects of acute exercise on various health aspects and predominantly used different techniques, such as electroencephalography.

8.3 Future Work

Studying the functional connectivity changes in the brain following acute exercise may help us understand why resolved post-concussion symptoms sometimes resurface with physical activity. Further studies are needed to fully understand the acute effects of physical activity on the brain function. Our present work, titled 'the effect of acute exercise on brain resting state functional connectivity', lays the foundation for future studies on the effects of acute physical activity on mTBI patients.

Our findings show that mTBI abnormalities observed in the brain's functional connectivity, white matter tracts and metabolite levels persist at 7 months post-injury. In future studies, mTBI children can be followed-up and

examined after this time point to determine if and when full recovery will be reached. Furthermore, future ^1H -MRS studies in mTBI children can examine other potential WM and GM abnormalities in more areas of the brain. Moreover, ^1H -MRS can be acquired from the abnormal WM and GM regions discovered in our DTI and RSNs results, thus providing detailed information about the health of that brain region – anatomically, functionally and metabolically. Additionally, since post-concussion symptoms (PCSS results) were not correlated with any neuroimaging metrics, different neuropsychological assessments can be used to determine if there is a correlation between cognitive/executive functioning test results, and the neuroimaging findings. Future studies can also follow-up and examine the mTBI children 5 – 10 years after injury. This will determine the long-term effects of paediatric mTBI and the long-term consequences, or lack thereof, of sustaining a mTBI during the developmental years.

Lastly, future studies can perform individual mTBI patient assessment. TBI is a heterogeneous injury and is dependent on the mechanism of injury (for example motor vehicle accident versus sports injury). It is inevitable that each injury has different biomechanical features. The current standard practice amongst mTBI researchers is to categorize patients by injury type and perform group comparisons to recruited healthy controls, as was performed in this study. Group comparisons can be insensitive to inter-individual differences. Therefore, the heterogeneity of injury mechanisms may also be captured in future studies by examining individual patients. This will help in the characterization of outliers

who vary from the group in terms of spatial distribution and degree of injury. Furthermore, when the use of advanced MRI techniques in mTBI patients progresses from the research stage to the clinical stage, assessment of individual mTBI patients will be required. This may improve clinical practice by predicting recovery and providing better patient management. One approach to individual patient assessment is to use a normative database along with statistics, such as z-score and receiver operating characteristic (ROC) curves, in order to detect abnormal imaging features at various brain locations. This establishes an injury profile for each individual patient. Moreover, the injury profiles could then be used to perform group analyses based on the degree and extent of injury, without relying on the assumption that the injury pattern is common amongst all patients.

Several projects, for example The Human Connectome Project (www.humanconnectomeproject.org), are in the process of building normative databases of the healthy human brain. A normative database has distinguishing characteristics including age range, sample size per age group, mixture of gender and geographical distribution; thus providing a well-rounded representation of the population. Normative databases share common scientific standards such as tests of reliability, cross-validation tests, and disclosure of the inclusion and exclusion criteria. The data consists of clinically healthy individuals for the purpose of comparison. Normative databases can be used to compare one individual to a population of “normal” individuals in order to detect the measures that are deviant from normal and the magnitude of deviation. Therefore, future studies of

advanced MRI on mTBI patients can perform individual patient assessment using normative databases.

8.4 Concluding Statement

The human brain is extremely complex and our journey to fully understanding its functions is not yet complete. Recent advances in MRI have developed our understanding of the healthy and diseased brain. Each development in this field brings us a little closer to unravelling the mysteries of the brain. We hope that our research on mild traumatic brain injury and exercise is a valuable and welcome addition to this exciting and growing body of literature.

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