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**NEUROBIOLOGICAL EFFECT OF SELECTIVE BRAIN COOLING AFTER
CONCUSSIVE INJURY**

A Thesis in
Kinesiology
by
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Abstract

The search for effective treatment facilitating recovery from concussive injury, as well as reducing risk for recurrent concussion, is an ongoing challenge. This study aimed to determine: a) feasibility of selective brain cooling to facilitate clinical symptoms resolution, and b) biological functions of the brain within athletes in acute phase of sports-related concussion. Selective brain cooling for 30 minutes using WELkins sideline cooling system (at 5°C) was administered to student-athletes suffering concussive injury (n=24) and those without history of concussion (n=24). Magnetic resonance imaging (MRI), such as functional magnetic resonance imaging (fMRI) and arterial spin labeling (ASL) sequences, or electroencephalography (EEG) and virtual reality (VR) testing was done before and immediately after cooling to better understand the mechanism by which cooling affects neurovascular coupling. Concussed subjects self-reported temporary release of physical symptoms immediately after the cooling session. EEG results revealed no significant differences pre- to post-cooling for either group; however there was a significant interaction of cooling on ROI and condition, $F(1.083, 23.821) = 12.982$, $p < 0.005$ in the delta frequency regardless of group. Concussed subjects also showed a differential response to cooling compared to the normal controls. For VR, one-third of concussed subjects could not tolerate testing due to reoccurrence of symptoms, but those who did complete testing had decreased scores post-cooling. There were no differences in the number or strength of functional connections within Default Mode Network (DMN) between groups prior to cooling. However, we observed a reduction in the strength and number of connections of the DMN with other ROIs in both groups after cooling. ASL sequences for the concussed group revealed a significant increase ($p < 0.05$) in relative cerebral blood flow (relCBF) in cortical and subcortical cortex post-cooling while the normal group had significantly decreased ($p < 0.05$) relCBF post-cooling. We suggest that compromised neurovascular coupling in acute phase of injury may be

temporary restored by cooling to match CBF with surges in the metabolic demands of the brain.

Upon further validation, selective brain cooling could be a potential clinical tool in the identification pathological changes after concussion.

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Abbreviations

TBI	Traumatic brain injury
mTBI	Mild traumatic brain injury
MRI	Magnetic resonance imaging
ASL	Arterial spin labeling
fMRI	Functional magnetic resonance imaging
rs-fMRI	Resting-state functional magnetic resonance imaging
MRS	Magnetic resonance spectroscopy
DMN	Default Mode Network
EEG	Electroencephalography
VR	Virtual reality
CBF	Cerebral blood flow
relCBF	Relative cerebral blood flow
ROI	Region of interest
BBB	Blood-brain barrier
APP	Amyloid precursor protein
SCS	Sideline Cooling System
EC	Eyes closed
EO	eyes open
FFT	fast Fourier transform
K ⁺	Potassium
Na ⁺	Sodium
Ca ²⁺	Calcium
ATP	Adenosine triphosphate

Preface

This thesis was written specifically to fulfill thesis requirements. Part of this work has been submitted for publication as Walter, A., Finelli, K., Bai, X., Johnson, B., Neuberger, T., Seidenberg, P., Bream, T., Hallett, M., Slobounov, S. (2017). Neurobiological Effect of Selective Brain Cooling After Concussive Injury. *Brain Imaging and Behavior* [in review].

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Introduction

Overview

Mild traumatic brain injury (mTBI), commonly referred to as concussion, affects 1.6 to 3.8 million people a year, which may even be an underestimation due to the lack of recognition or reporting (Langlois, Rutland-Brown, & Wald, 2006). These injuries can result in long-term effects, including impaired executive, vestibular, and cognitive functioning, which may be present in both the acute and chronic phases of injury. Despite the high prevalence of concussion, there is little to no consensus on the diagnosis, treatment, and duration of symptoms in the subacute, acute, and chronic phases of injury.

Biomechanics of Concussion

Two types of forces are commonly used to describe the forces associated with mTBI: contact and inertial. Contact and inertial forces occur when the head strikes a surface but only inertial (acceleration) occurs from impulsive head motions (Meaney & Smith, 2011). Inertial forces (acceleration), at the moment of impact are the focus of most studies involving concussions, as the common belief is that they are the primary cause of these injuries. Within these forces, two components of acceleration occur: linear and rotational. Linear acceleration is thought to result from a transient intracranial pressure gradient and rotational acceleration is thought to result from strain response (Rowson & Duma, 2013). These two accelerations, although commonly studied separately, combine in force during head impacts (Rowson, Broolinson, Goforth, Dietter, & Duma, 2009). A critical factor in understanding concussive injuries is understanding how the mechanical energy from accelerations is transferred to the brain and vascular tissue. Since the brain is largely water, it is more resistant to changes in its shape, but brain tissue deforms very easily with shearing forces (Meaney & Smith, 2011). The

internal combination of pressure and shearing are the two critical factors in understanding brain changes in response to mechanical forces. However, at this point, there is no commonly used or agreed upon way to predict the strain (rotational acceleration) in the brain due to the necessity of advanced computational and experimental methods.

Physiological Changes During Concussion

During concussion, biomechanical forces cause the brain to undergo various coup-contre coup motions within the skull. This induces various physiological and neurologic dysfunctions. The forces distort cellular membranes causing transient membrane defects and ionic flux in neurons (Giza & Kutcher, 2014). Further neuronal depolarization occurs with an efflux of potassium and influx of calcium and sodium due to mechanoporation of lipid membranes (Giza & Hovda, 2014) leading to various acute and subacute changes (see Giza & Hovda, 2001 for an visual overview). As extracellular $[K^+]$ increases, more neuronal depolarization occurs causing additional release of excitatory amino acids and additional release of K^+ (Giza & Hovda, 2001). In an effort to restore ionic balance, more membrane pumps are activated, (including the sodium-potassium pump, Na^+-K^+ , which requires additional ATP), causing an increase in glucose use and metabolism. Hyperglycolysis occurs leading to increased lactate production and oxidative metabolism is impaired.

Cerebral blood flow (CBF) is normally coupled to neuronal activity and cerebral glucose metabolism. Within normal levels of CBF, the brain is able to extract 50% of the oxygen and 10% of the glucose from arterial blood and high-energy phosphates, like ATP, are the brain's main energy source. These phosphates are produced almost exclusively by the oxidative metabolism of glucose (Zauner & Muizelaar, 1997). Normally, these levels remain relatively constant in the brain, but if a reduction occurs, it results in the release of chemical mediators.

After injury, CBF can be reduced up to 50% of its normal values (Yamakami & McIntosh, 1989; Yuan, Prough, Smith, & DeWitt, 1988). The increased demand for energy occurring with the increase in glucose metabolism, in combination with reduced cerebral blood flow, results in an uncoupling between energy supply and demand – the cellular energy crisis (Giza & Hovda, 2001). Functional hyperemia or neurovascular coupling (Roy & Sherrington, 1890) is a decrease in neuronal activity and, consequently, the cerebral metabolic rate of oxygen, that is tightly related to corresponding decreases in regional CBF. Due to post-injury decreases in CBF, failure to appropriately match CBF with surges in metabolic demands of the brain can lead to temporary or permanent alterations in neurological functioning (Verlade, Fisher, & Hovda, 1992).

More chronically, calcium accumulation in neurons can last for 2 to 4 days post injury (Cortez, McIntosh, & Noble, 1989; McIntosh, 1993). Depolarization, along with the K^+ efflux, triggers the excitatory amino acids that then cause activation of NMDA receptors (Katayama, Becker, Tamura, & Hovda, 1990). These NMDA receptors form a pore shape and allow Ca^{2+} to enter the cell. The excess intracellular Ca^{2+} is appropriated to the mitochondria resulting in impaired oxidative metabolism and energy failures (Giza & Hovda, 2001). While the increased Ca^{2+} does not necessarily lead to cell death, it does negatively affect mitochondrial metabolism. The short term solution of sequestering calcium in the mitochondria results in mitochondrial dysfunction, worsens problems with oxidative metabolism, and worsens the cellular energy crisis (Giza & Hovda, 2014). Additionally, cerebral glucose levels remain low for up to 5 to 10 days post-injury in animal studies. The cascade of events throughout injury can continue, leading to delayed cell death, axonal disconnection, and alterations in neurotransmission (Yoshino, Hovda, Kawamata, Katayama, & Becker, 1991).

Pathology Meets Concussive Symptoms

Giza and Hovda (2014) suggested that physiological perturbations after concussion could have clinical correlates including: ionic flux and migraine, energy crisis and vulnerability to second injury, and axonal injury and impaired cognition and slowed processing. With concussion, symptoms can range from the short-term (acute) to the long-term (chronic) including, but not limited to: loss of consciousness, nausea/vomiting, feeling of fogginess, headache, problems sleeping, behavior changes, problems concentrating and remembering, and oculo-motor deficits (Grubenhoff et al., 2014; McCrory et al., 2013). It is generally accepted that these symptoms usually resolve within 7 to 10 days post-injury (Lovell, Collins, Iverson, Johnston, & Bradley, 2004) and clinical neuroimaging studies are normal in the vast majority of concussed individuals at this time point (M. J. Ellis et al., 2015).

However, there is growing evidence that suggests these effects could be more chronic and long-term deficits from concussions may include impaired executive (Montenigro et al., 2017), vestibular/balance (Helmich, Berger, & Lausberg, 2016), and cognitive functioning (Alexander, Shuttleworth-Edwards, Kidd, & Malcolm, 2015). Recent imaging studies in both high school and collegiate athletes (Johnson et al., 2012; Talavage, Nauman, & Leverenz, 2016; K. Zhang et al., 2010) have shown structural and functional abnormalities present far beyond 10 days. This indicates that symptom resolution in both self-reported and clinical assessment tools may not indicate physiological brain recovery after concussive injury.

Since clinical manifestations of this injury are proposed to be mediated by neuronal dysfunctions and/or cerebrovascular dysregulation (Giza & Hovda, 2001, 2014) and functional hyperemia or neurovascular coupling (Roy & Sherrington, 1890), procedures aimed at reducing or restoring an imbalance in cerebral blood flow and neurovascular coupling may offer great potential in the treatment of concussion. An examination of neurovascular coupling, upon

validation, may be considered as a valuable diagnostic tool for an accurate assessment of concussive injury. Procedures aimed at reducing or restoring an imbalance in CBF and neurovascular coupling may offer great potential in the treatment of concussion.

Temperature as a Modulator of Neuronal Activity

Temperature is a well-known modulator of neuronal activity. Cooling has been demonstrated to reduce synaptic transmission efficacy (Aronov & Fee, 2011; Volgushev, Vidyasagar, Chistiakova, & Eysel, 2000) at the physiological and structure levels (Micheva & Smith, 2005; Taschenberger & von Gersdorff, 2000). The electrical conductivity of tissue is proportional to temperature; with decreased tissue temperature, conductivity also decreases. Hypothermia is theoretically attractive since it can cause a 7% decrease in the cerebral metabolic rate for oxygen per 1°C decrease in temperature without a corresponding decrease in cerebral oxygen supply (Rosomoff & Holaday, 1954). It has been shown to decrease brain volume and protect against anoxic injury, most likely related to the reduction of cerebral oxygen consumption and cerebral blood flow (Williams & Spencer, 1958). Mild hypothermia (a decrease in body temperature by 2 – 4°C) ameliorates brain damage caused by transient ischemia (Minamisawa, Smith, & Siesjö, 1990). Direct brain cooling has also been shown to reduce scalp, ear canal, and oral temperatures in normal populations. Oral temperatures only decreased 0.2 – 0.6 °C after 30 minutes of selective cooling, while intracranial blood flow significantly decreased during the first 10 minutes of cooling (Ku, Montgomery, & Webbon, 1996). Using MRS, with one hour of intranasal brain cooling, brain temperature fell significantly by a mean of 1.7°C throughout the brain (Covaciu et al., 2011).

The Use of Mild Hypothermia in mTBI

Induced hypothermia has been used for years as a way to treat brain injury, whether by whole body cooling or selective head and neck cooling. The influence of hypothermia on TBI (Crossley et al., 2014; Gu et al., 2015; B. Harris et al., 2012; J. H. Lee et al., 2014; W. G. Liu et al., 2006; Titus, Furones, Atkins, & Dietrich, 2015), stroke (Harris et al., 2012; Titus et al., 2015), cardiac arrest (Ponz et al., 2016), and perinatal hypoxic-ischemic injury (Smith, Rosenkrantz, & Fitch, 2016; Wood et al., 2016) have been previously demonstrated to have positive patient outcomes. This offers the promise that hypothermia may be a potential treatment modality for concussion as well. However, selective brain cooling of the head and neck has not been well demonstrated in mTBI or concussion.

Mild therapeutic hypothermia (32°C – 34°C) is a medical treatment that lowers a patient's body temperature in order to help reduce the risk of the ischemic injury following a period of insufficient blood flow. It exerts significant neuroprotection and attenuates secondary cerebral insult after TBI. Variations in temperature can significantly alter pathological response to injury (Chatzipanteli, Alonso, Kraydieh, & Dietrich, 2000; Jia, Mao, Liang, & Jiang, 2009) and can reduce the degree of diffuse axonal damage (Koizumi & Povlishock, 1998; Maxwell et al., 1999).

It is commonly used in the acute (early) phase as a prophylactic neuroprotection and in the sub-acute (late) phase to control brain edema (Urbano & Oddo, 2012). However, the use of mild hypothermia with TBI has been met with some controversy, especially its role as an early neuroprotectant. Animal models have shown benefits (L. Liu & Yenari, 2007), but its translation into human studies is difficult and has not been clearly demonstrated (G. L. Clifton et al., 2001; Guy L. Clifton et al., 2011; Shiozaki et al., 2001).

In the acute phase of injury (minutes to hours), the aim of mild hypothermia is to reduce ischemia, excitotoxicity, energy failure, and cellular death cascades (Urbano & Oddo, 2012). Due to the damage associated with TBI, mild hypothermia can diminish the cerebral metabolic rate of oxygen by approximately 6.5%/°C. These decreases in CBF, and therefore oxygen delivery, prevent an energy crisis from occurring as there is a matched reduction in oxygen demand (Bacher, Illievich, Fitzgerald, Ihra, & Spiss, 1997; Urbano & Oddo, 2012). Mild hypothermia, through its ability to reduce the brains demand for oxygen and glucose, may attenuate post-injury ischemia and energy dysfunction and help preserve ATP energy stores (Oddo et al., 2009; Soukup et al., 2002). The neuroprotection from mild hypothermia arises from its ability to reduce calcium influx into the cells and the accumulation and release of excitatory amino acids (Dietrich & Bramlett, 2010). Increases in intracellular calcium can cause excitotoxicity and oxidative stress, so by targeting it, hypothermia can mitigate some of the damage (Maeda, Katayama, Kawamata, & Yamamoto, 1998; Takata, Nabetani, & Okada, 1997). It also can inhibit early molecular cascades that affect secondary cerebral damage, by altering early gene expression and suppressing stress responses (Truettner, Alonso, Bramlett, & Dietrich, 2011). Hypothermia also can affect levels of various amino acids. It has been shown that moderate hypothermia can attenuate increases in CSF acetylcholine (Lyeth, Jiang, Robinson, Guo, & Jenkins, 1993) and glutamate levels (Marion et al., 1997) and brain interstitial levels of glutamate and aspartate (Globus, Alonso, Dietrich, Busto, & Ginsberg, 1995).

In the subacute phase of injury (24 hours to 7 days), brain edema and swelling and blood-brain barrier (BBB) disruption are the main targets of mild hypothermia. Vasogenic and cytotoxic edema cause brain swelling post-injury (Marmarou, 2007), while inflammatory cytokines, such as interleukin-1 β and macrophages, contribute to inflammatory cascades and secondary cerebral

damage and repairs (Scherbel et al., 1999). By applying mild hypothermia, reductions in BBB disruptions are seen, indicating a preservation of vascular endothelial functioning (Lotocki et al., 2009). It also can reduce cerebral blood flow (minimizing brain swelling (Schreckinger & Marion, 2009)), decrease inflammatory cell infiltration, and decrease production of damaging free radicals (Chatzipanteli et al., 2000). With cerebral inflammation, proinflammatory cytokines (such as Interleukin (IL)-6 and IL-10) and nitric oxide are produced by microglia, but research has suggested hypothermia will suppress the production of these cytokines (Matsui & Kakeda, 2008). Free radicals, generally generated from enzymatically catalyzed mechanisms and deregulated by electron transporters in the mitochondria (Lewén, Matz, & Chan, 2000), attack proteins causing oxidation of the sulfhydryl groups and leading to decreased protein thiol concentrations (Bayir et al., 2009). These markers found in the CBF could make it a useful tool for monitoring treatment effects on oxidative stress. Hypothermia has also been shown to decrease endogenous antioxidant consumption and lipid peroxidation. Therefore, by targeting oxidative stress with hypothermia, beneficial effects on the prevention of mitochondrial failure and reduction in excitotoxicity could occur.

Both phases of TBI, with the use of mild hypothermia, offer promising results. Major medical societies recommend temperature management as the standard of care therapy for many critically ill or surgical patients, including those suffering acute hypoxic-ischemic injuries (Clifton et al., 2015). Most current applications use whole body cooling, which has numerous potential drawbacks, including coagulation abnormalities and impaired immune function (Moore, Nichol, Bernard, & Bellomo, 2011). Existing solutions also may easily miss the treatment window because they are not practical in the pre-hospital setting.

Making the Case for Selective Brain Cooling

Selective brain cooling offers a more specific way of inducing mild hypothermia. It is a simpler, more direct way of providing mild hypothermia. Brain hypothermia is thought to result in different brain temperature gradients based on the mode of cooling used. The cerebrovascular response to selective brain cooling is less agreed upon. It is speculated that the response may depend on the method of cooling the head, due to temperature gradients within the brain (Walter, Bauer, Kuhnen, Fritz, & Zwiener, 2000). Specifically, Walter et al. (2000) suggest that surface cooling could lead to profound drops in peripheral brain temperature so that an increase in cortical blood flow occurs. Additionally, many animal models have shown increases in cerebral blood flow with selective head cooling in rats (Kuluz et al., 1993) and newborn swine (Laptook, Shalak, & Corbett, 2001).

Using simple, non-invasive ways of direct brain cooling have proven effective in lowering brain temperature. MRS studies have shown that head cooling produced significant brain temperature reductions after only 30 minutes (Harris, Andrews, Marshall, Robinson, & Murray, 2008). This could be confounded due to the neck's cooling effect on carotid vasodilation. If neck cooling does induce carotid vasodilation, the increase in flow of warmer blood from the body could reduce the rate of brain cooling (Harris et al., 2008). Other studies have reproduced these effects showing that non-invasive brain cooling lowers brain temperature to 33 – 35 °C in less than 2 hours while maintaining normal rectal temperature (37°C), thus preventing complications to organs (Liu et al., 2006). One study, using the first version of the cooling helmet this study used (see methods for details on the cooling system), showed a mean brain temperature decrease of 1.6°C while the core temperature did not drop below 37°C until 4 – 5 hours after cooling began (Wang et al., 2004). On average, a 1.84°C reduction in brain temperature was seen within 1 hour of using the helmet and it took a mean of 3.4 hours to

achieve a brain temperature lower than 36°C and 6.67 hours before hypothermia (34°C) (Wang et al., 2015). Thus, this system allows rapid and selective brain cooling.

Only one study, by (Miyachi, Wei, & Povlishock, 2014), was found regarding mild hypothermia/selective head cooling and concussion or repetitive mTBI. This study, done in rats, examined repetitive brain injury and the minimal level of hypothermia needed to achieve protection post-injury. They found impairment of vascular reactivity in the control rats, but significant preservation of vascular reactivity in the mild hypothermia (35°C) group. Additionally, there was an increase of APP-positive axons in the control group, but no increase in the hypothermia group. This held true as well when they administered hypothermia 1 hour after injury. This indicates that even mild hypothermia can be protective in repetitive mTBI against axonal and vascular damage.

Currently, there is no research indicating the effect of selective brain cooling on cognitive or biological function and symptoms resolution related to the mild spectrum of brain injury or concussion. Accordingly, this research aimed to examine the feasibility and effects of selective brain cooling on clinical symptoms and biological functions of the brain in the acute phase of concussive injury.

Hypotheses

Cooling the brain may reduce self-reported clinical symptoms in the acute phase of concussive injury, as well as produce a reduction of neuronal activity and blood flow assessed via advanced magnetic resonance imaging (MRI), electroencephalography (EEG), and virtual reality (VR). Here, we aim to examine the effect of selective brain cooling on clinical symptoms and biological functions in recently concussed Division 1 athletes. Our global hypothesis was that cooling the brain would modulate clinical symptom resolution that would correlate with

associated changes in neural and metabolic underpinnings observed via EEG and MRI. We also expected to see beneficial effects of cooling in concussed athletes suffering from residual cognitive and balance dysfunctions, observed via virtual reality.

More specifically, we aimed to study if selective brain cooling had an effect on clinical symptoms by examining dynamic balance and neurocognitive functions (primarily spatial memory) assessed via virtual reality and we expected these functions to improve post-cooling in both groups. In addition, EEG measures, which document brain function, would show changes post-cooling with increased activation in alpha frequencies decreased activation in the theta frequencies that were more pronounced in the concussed group.

Additionally, by using MRI sequences, such as functional MRI (fMRI) and arterial spin labeling (ASL), in conjunction with selective cooling, a better understanding into the mechanism by which cooling affects the neurovascular coupling and overall physiological state of the brain would be obtained. Ultimately, direct evidence of the effects of selective cooling on a concussed brain would provide insight into the physiological changes of injury and how cooling could be used as a potential treatment modality in the acute phase of injury.

Methods

Subjects and Inclusion Criteria

All subjects (n=48) were college-aged athletes (both club and varsity sport) at the Pennsylvania State University. Subjects were separated into two groups based on their previous history of concussion: normal controls and concussed. The normal control group (n=12 for MRI; n=12 for EEG/VR) was athletes with no history of previous concussions. The concussed group (n=12 for MRI; n=12 for EEG/VR) was athletes who were diagnosed with a concussion on the sideline by Pennsylvania State University medical personnel and who participated in this study within 5+/-3

days post-injury. Then, subjects were randomly placed in one of two groups: MRI (total n=24) or EEG/VR (total n=24). Subjects were excluded from the study based on previous diagnosis of: (a) spinal cord injury; (b) developmental disorder; (c) psychiatric disorder; (d) neurological disorder (including headache/migraine diagnosis); or (e) pregnancy. Subject details regarding background information, as well as a list of clinical symptoms prior to all sessions, can be found in Table 1 A and B.

Table 1. (A): Subjects demographic information; (B): Concussion checklist administered prior to initial scans and cooling (note: all subjects were still symptomatic at the time of testing).

(A)

	Normal Subjects		Concussed Subjects	
	MRI	EEG	MRI	EEG
	n=12	n=12	n=12	n=12
Gender	7F; 5M	5F; 7M	1F; 11M	4F; 8M
Age	20.8 ± 1.30	19.7 ± 1.18	20.8 ± 2.44	20.3 ± 1.18
Sport	Track and field (n=12)	Track and Field (n=6) Football (n=4) Volleyball (n=1) Soccer (n=1)	Football (n=5) Hockey (n=2) Softball (n=1) Soccer (n=1) Rugby (n=1) Fencing (n=1) Skiing/Snowboarding (n=1)	Skiing/Snowboarding (n=2) Ice Hockey (n=2) Cheerleading (n=1) Track and Field (n=1) Gymnastics (n=1)
Previous Concussion	0 (n=12)	0 (n=12)	0 (n= 0)	0 (n= 0)
			1 (n= 5)	1 (n= 6)
			2 (n= 4)	2 (n= 4)
			3 (n= 2)	3 (n= 2)
			4 (n= 1)	4 (n= 0)

(B)

Concussed Subjects' Symptom Presence at Time of Testing		
Symptom	Number of Subjects who reported it (out of 12): MRI Group	Number of Subjects who reported it (out of 12): EEG Group
Momentary Disorientation	9	10
Difficulty Concentrating	11	11
Memory problems	6	5
Headache	9	12
Nausea/Vomiting	4	5
Blurred Vision	7	5
Skull Fracture	0	0
Brief LOC	3	2
Prolonged LOC	0	0
Sleep Disturbance	3	5
Mood Changes	3	4
Other	2	2

This study followed ethical guidelines set forth by The Pennsylvania State University whose Institutional Review Board approved the protocol before testing began. All subjects signed an informed consent form before participating in this study.

Overview of Study Design

The study design that all 48 subjects followed is outlined in Figure 3.

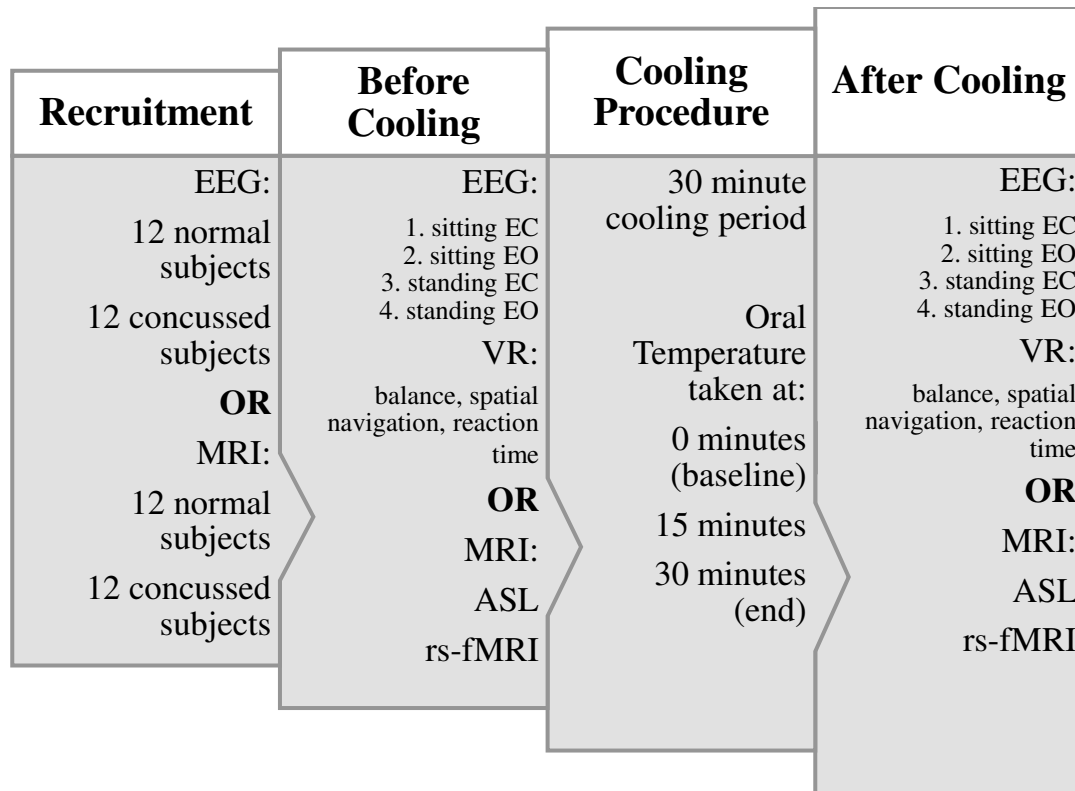


Figure 1. Outline of the study design. EC = eyes closed; EO = eyes open

Measures and Apparatus

Welkins Sideline Cooling System: The cooling system used in this study was the Welkins Sideline Cooling System (Spartan Medical Inc, Silver Spring, MD) which is FDA exempt by K121720. The Sideline Cooling System (SCS) provides a way of rapidly and selectively cooling the subjects' temperature (Figure 4).



Figure 2. The WElkins Sideline Cooling System used in this study. The conditioning unit contains the top housing (control panel and battery tray), center housing (heat exchanger and ice cartridge housing), and bottom housing (hydraulic and pneumatic components) sections.

Using a temperature controlled coolant through the umbilical tubing, the coolant (containing water, propylene glycol, disinfectant, and surfactant) is delivered to the cooling headliner which is directly on the subjects' head and neck. The coolant is chilled and circulated by the conditioning unit and can apply temperatures ranging from -20°C (-4°F) to 54°C (130°F) by using the control knob in the bottom housing component. The system was used for 30 minutes after the subjects' baseline MRI scans or baseline EEG recordings and VR testing were completed. Subjects were fitted with the cooling headliner around the neck and covering the head. During cooling, subjects were allowed to relax freely (sitting or supine) as long as the cooling headliner remained fitted to their head. It should be noted that previously published studies using this cooling system, suggest that a 30-minute cooling period was enough to impact brain temperature with mild hypothermia. Specifically, on average, a 1.84°C reduction of brain

temperature was observed within 1 hour of helmet application (Wang et al., 2004). Additionally, using the cooling cap for a period of 30 minutes reduced brain temperature 1 – 1.5°C (Bagić et al., 2008). There were even cases where a 2°C drop of the brain temperature was observed after 15 minutes of helmet application. Additionally, before the cooling system was turned on, at the halfway point (15 minutes) and at the end (30 minutes), an oral temperature was obtained as a subject safety precaution, not as a measure of core or brain temperature per say.

Electroencephalography (EEG): EEG recordings were acquired with a noninvasive Ag/AgCl 128-electrode high-density Hydrocel Geodesic Sensor Net (Electrical Geodesics Inc., Eugene, OR). Standard electrode net application procedures were followed for net preparation and application and impedance was checked at the beginning of the session. Brain activity was recorded using Net Station 1.3 software (EGI, Eugene, OR). Subjects were instructed to remain completely still while sitting during the set-up process and underwent four segments of testing (2 minutes each; total time 8 minutes): (1) sitting, eyes closed, (2) sitting, eyes open, (3) standing, eyes closed, and (4) standing, eyes open. EEG data were processed offline using Net Station 4.4.2 software and custom-made Matlab code. Data were first run through a 70Hz low-pass filter, 0.3 Hz high-pass filter, and 60 Hz notch filter. It was then run through BrainVision Analyzer 2.0 to remove artifact and eye blinks (ICA and raw data inspection) and custom-made Matlab codes to create plots within Delta, Theta, Alpha, and Beta frequency bands, as well as individual region of interest (ROI; anterior/frontal and posterior/occipital) absolute signal power. Additionally, for each frequency band, the absolute and relative power was calculated (Delta (1-4 Hz), Theta (4-7 Hz), Alpha (8-15 Hz), Beta (16-31 Hz)). Major ROIs were constructed:

- frontal (around Fz: 11, 16, 12, 5, 10, 18)

- central (around Cz: reference, 31, 55, 80, 7, 106)
- parietal (around Pz: 62, 61, 78, 72, 77)
- occipital (around Oz: 75, 70, 71, 76, 83)

These ROIs were chosen to simplify the analyses and increase power to detect regional differences. The electrodes are grouped into clusters of 5 – 6 electrodes per the region of interest. Statistical analysis was done using SPSS v23 and paired t-tests (significance, $p < 0.05$) were run between groups at each frequency and at each ROI and a repeated measures ANOVA (significance, $p < 0.05$) was run to examine if there was an interaction between cooling, group, and frequency band.

Virtual Reality (VR): *HeadRehab's* VR system (www.HeadRehab.com) was used. VR is an interactive, computer-generated 3D environment that can simulate real world environments and provide a sense of immersion. It can be used as an assessment tool to examine neurocognitive, executive, and motor functioning and its sensitivity and specificity have previously been validated (Teel, Gay, Arnett, & Slobounov, 2016; Teel & Slobounov, 2015). In this study, participants were tested using the Rockwell-Collins Head mount display (Figure 5D) in the modules of balance (5A), spatial navigation (5B), and reaction time (5C).

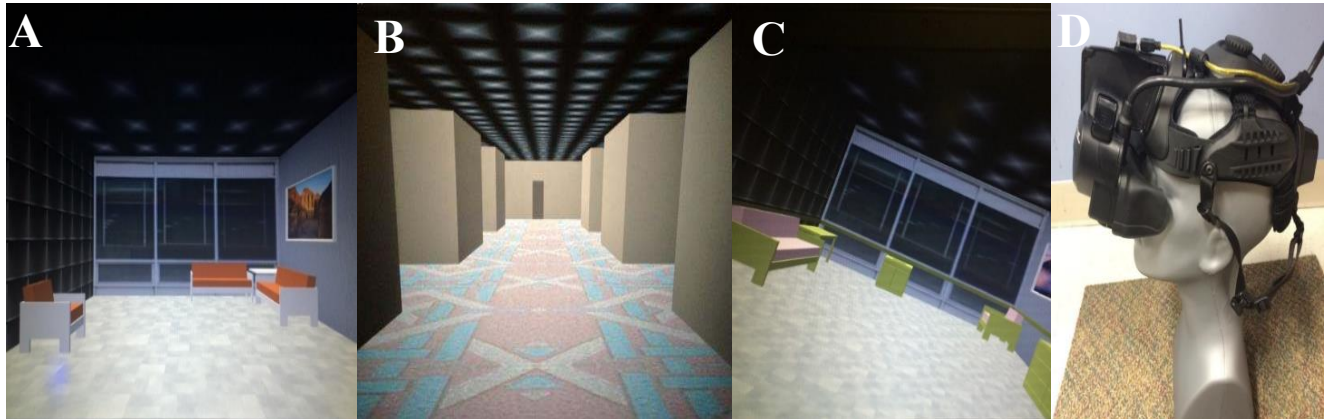


Figure 3. The various virtual reality scenes used throughout testing (A: balance; B: spatial navigation; C: reaction time) and the headset the subjects wore during testing (D). Inside the goggles are two screens projecting 3D images at the participant.

Balance Task (Figure 5A): Subjects are asked to stand in the tandem Romberg position, with either foot in the front, and hands on their hips. A total of seven trials are performed, with the first trial presenting a stationary virtual room to obtain a baseline balance score. The remaining 6 trials involve the room moving in the following directions: (a) forward-backwards oscillatory translation; (b) roll around heading y-axis between 10 and 30° at 0.2 Hz; (c) pitch around interaural x-axis between 10 and 30° at 0.2 Hz; (d) yaw around vertical z-axis between 10 and 30° at 0.2 Hz; (e) translation along x-axis within 18 cm displacement at 0.2 Hz. In between each trial (around 30 seconds), subjects are given a 10 second break and have the option to switch the order of their feet.

Spatial Navigation Task (Figure 5B): This task assesses memory function (specifically spatial memory) using a 3D representation of a visual corridor (see Figure 1). The subjects are shown a route to and from a door (encoding stage) then immediately following are asked to navigate the same route using a joystick. Subjects are able to move in a forward, backward, left and right direction. A total of three trials are allowed to successfully complete the pathway.

Reaction Time Task (Figure 5C): In this task, executive functioning specifically, reaction time was measured. This module was designed to measure the full body response to unpredictable manipulation of optic flow (see Figure 6). The subject was asked to sway forward and backward (anterior-posterior) with the movement of the room. At a randomized point during each trial, an unpredictable change to the left or right (medial-lateral direction) was introduced. The subject was asked to respond to this change of direction with a whole-body motion. The system measures both reaction time (in ms) and errors of anticipation (wrong direction of response).

Scoring: Scores are calculated on a scale of 0 (fail) to 10 (perfect) for each module, as well as a comprehensive score of all three modules completed. These individual module scores were used for analysis. Paired t-test (significance, $p < 0.05$) were run for between subject comparisons and two-sample t-test (significance, $p < 0.05$) were run for between group analyses.

Magnetic Resonance Imaging (MRI): All scans were performed in a 3 Tesla Magnetom Prisma fit scanner (Siemens Medical Systems, Erlangen, Germany) using a 20-channel phased array head coil and high performance gradients (max. gradient strength 80mT/m, 200mT/m/s rise time). Subjects were placed in the scanner in a supine position and were secured using a foam and headphone to minimize movement. Subjects were asked to lie with their eyes open with their arms at their side and were supported by foam padding both at the arms and under the legs. Subjects were able to communicate with the MRI technician while in the scanner at all times and at various time points during the scan were asked how they were doing.

Arterial Spin Labeling (ASL) Acquisition: ASL imaging was performed using a turbo gradient spin echo pulsed sequence (3D GRASE, Siemens advanced 3D ASL package), field of view 192mm, matrix 64x64, slice thickness 3, 40 slices, TE=15.6, bandwidth 2694Hz/pixel and 6 segments (acquisition time=6:02). For each 3D ASL scan, a relative cerebral blood flow map (relCBF) was automatically calculated by using the single TI method in the Siemens advanced 3D ASL package. Parameters of this method are used with a formula as described in JJ-Wang et al. (J. Wang et al., 2003):

$$f = \frac{\lambda \Delta M}{2\alpha M_0 T I_1 \exp(-T I_2 / T_{1\alpha})}$$

Where f is relCBF mL/100g/min regional cerebral blood flow, λ is 0.9 mL/g blood/tissue water partition coefficient, 0.9, α is 98% inversion efficiency for pASL, M_0 is fully relaxed image intensity, ΔM is signal difference (control/label), $T I_1 / T I_2$ are 700/1990 ms inversion times, $T I_2 = T I_1 + w$ (transit time), and $T_{1\alpha}$ is 1650 ms at 3T, longitudinal relaxation time of blood.

ASL data were preprocessed and analyzed by using AFNI (Analysis of Functional NeuroImages, <http://afni.nimh.nih.gov/>) and MATLAB (2013b) scripts written in-house. Preprocessing procedure included co-registration to the T1-weighted anatomical image, co-registration between pre- and post- 3D ASL images and spatial normalization to standard space (TT_N27+trlc) using a 12 parameter affine registration. For each subject, a difference relCBF map was calculated (3Dcalc), $f_{\text{diff}} = f_{\text{pre}} - f_{\text{post}}$. The mean was calculated for pre-cooling and difference relCBF by averaging f_{pre} , and f_{diff} of all voxels within the volume of interest (1,069,707 voxels). For group analysis, mean maps of pre relCBF, post relCBF and f_{diff} were calculated for the normal controls and concussed groups. A one-way ANOVA followed by

Tukey's Honestly Significant Difference (HSD) method (significance $p < 0.005$) was performed to assess the group differences of pre-cooling and difference relCBF.

Resting State-fMRI: Two-dimensional BOLD echo planar rs-fMRI sequence were acquired in the axial plane parallel to the anterior and posterior commissure axis covering the entire brain (2.0 x 2.0 x 2.0mm resolution, TR=2000ms, TE=35ms, acquisition time=8:04). Three-dimensional isotropic T1 weighted magnetization prepared rapid gradient echo (MP-RAGE) anatomical images were acquired in the sagittal plane parallel with the longitudinal fissure covering the entire brain (1mm x 1mm x 1mm resolution, TE= 3.46ms, TR= 2300ms, TI= 900ms, flip angle= 9°, 160 slices, iPAT= none, NSA= 1).

For data analysis Statistical Parametric Mapping (SPM) version 8 (<http://www.fil.ion.ucl.ac.uk/spm/software/spm8/>), in addition to Functional Connectivity (CONN) toolbox (<http://web.mit.edu/swg/software.htm>), software were used. Images were first preprocessed, which included realignment, co-registration, segmentation, normalization and band filtering. During preprocessing, images were motion-corrected, registered with structural images, and normalized to the standard brain template from the Montreal Neurological Institute (MNI). White matter, cerebrospinal fluid (CSF), and physiological noise source reduction were taken as confounds, following the implemented CompCor strategy (Behzadi, Restom, Liau, & Liu, 2007). Whole brain BOLD signal was excluded as a regressor to eliminate erroneous anti-correlations (Murphy, Birn, Handwerker, Jones, & Bandettini, 2009). The CONN toolbox performs seed-based correlation analysis based on the temporal low-frequency fluctuations of BOLD signals. Region of interest (ROI) evaluations include those structures identified by Raichle et al. (2015) to make up the Default Mode Network (DMN). Specifically, four ROIs were evaluated: right

lateral parietal (RLP), precuneus (PCC), medial pre-frontal cortex (MPFC), and left lateral parietal (LLP). Bi-variate correlations were then calculated between each pair of ROIs as reflections of connections. Fisher transformed Z-scores are introduced to validate multiple comparisons and SPM functions are called by the CONN toolbox for spatial statistical tests. ROI based analysis was then performed for all subjects' data with a general linear model (GLM) test to determine significant resting state DMN connections. An unpaired t-test with a threshold set at P -value <0.05 was used to determine significant DMN connections.

Results

Oral Temperature Data

Oral Temperature decreases ranged, on an individual level, from 0.0 – 1.1 °F after 30 minutes of selective cooling (Table 2), which is consistent with previous studies (Ku et al., 1996). Paired t -test revealed no significant changes ($p>0.05$) in body temperature via oral thermometer from pre- to post-cooling in both normal controls and concussed subjects (Table 3).

Table 2. Mean Temperatures during 3 time points for all subjects.

	Before Cooling		15 minutes of Cooling		30 minutes of Cooling (end of session)	
	Mean	Standard Deviation	Mean	Standard Deviation	Mean	Standard Deviation
All subjects	98.79	0.71	98.92	0.56	98.78	0.64
Normal Group	98.98	0.65	99.08	0.49	98.85	0.55
Concussed Group	98.61	0.71	98.74	0.56	98.70	0.70

Table 3. Individual changes (increase, decrease, no change) in oral temperature for concussed subjects versus normal subjects at varying time points.

	Normal (n=24)			Concussed (n=24)		
	After 15 min	After 30 min	Overall Change	After 15 min	After 30 min	Overall
No change	2	2	1	2	3	1
Increase	15	8	9	12	11	11
Decrease	7	14	14	10	9	11
N/A					1	1

Subjects Self-Reports of Clinical Symptoms

It should be noted that all concussed subjects were still symptomatic, as indicated by the symptoms reported in Table 1B, and were not cleared by Penn State medical staff for sports participation at the time of participation in this study. Common symptoms reported include: headache, sleep problems, fatigue, inability to concentrate, dizziness and balance problems, light sensitivity, feeling of mentally “foggy” and slowed down. The vast majority of concussed subjects (n=8) reported, verbally to investigators, reduced clinical symptom presentation during and immediately after cooling, including headache and dizziness. However, it should be noted that immediately after the post-cooling scanning session, concussed subjects reported re-occurrence of primarily physical symptoms, mainly headache and dizziness.

MRI Data

ASL Group Results

ASL finding suggest that concussed subjects prior to brain cooling had lower relCBF (mean of $f_{pre} = 49.9$ mL/100g/min, SD = 6.9 shown in Figure 6B) compared to normal controls (mean of $f_{pre} = 58.3$ mL/100g/min, SD = 12.8 shown in Figure 6B). ANOVA revealed a main effect of group, $p = 0.006$, in support of this observation.

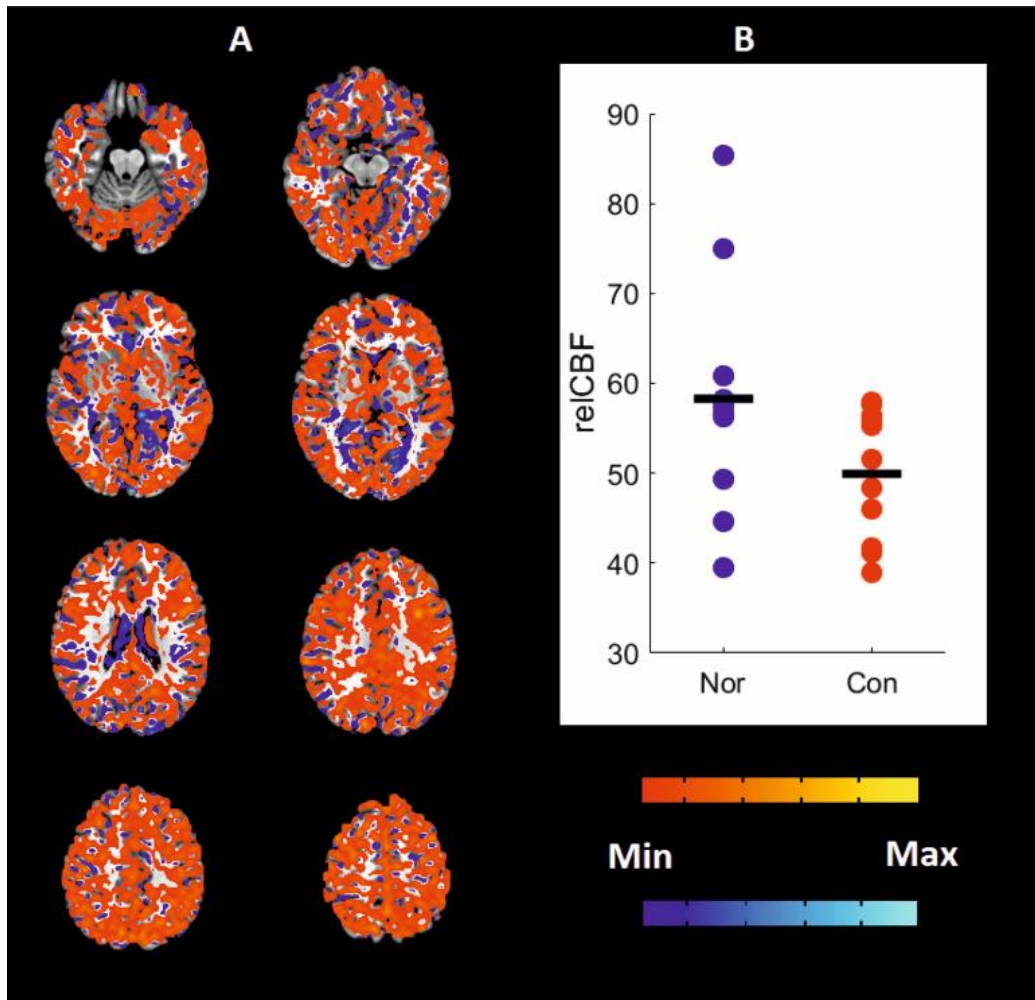


Figure 4. Differences of the pre-cooling relCBF maps of normal and concussed groups. (A) difference of mean of pre-relCBF of normal vs. concussed, warm color: normal (mean of pre-relCBF) > concussed (mean of pre-relCBF), Cold color: normal < concussed, and cluster size = 20. (B) Mean of pre-relCBF of normal (Nor) and concussed (Con) groups, all mean values were calculated by using a volume of interest (1069707 voxels), relCBF: ml/100g/min, black bar: group mean, one-way ANOVA followed by Tukey's Honestly Significant Difference (HSD) method, $p = 0.006$.

Unexpectedly, ASL findings suggest that concussed subjects showed a global increase of the relCBF after brain cooling (mean of $f_{\text{diff}} = -4.67$ mL/100g/min, SD = 6.63 shown in Figure 7D). In contrast to concussed subjects, normal controls group showed a global decrease of the relCBF after brain cooling in similar cortical and sub-cortical regions after brain cooling (mean of $f_{\text{diff}} = 2.99$ mL/100g/min, SD = 3.9 shown in Figure 7D). One-way ANOVA revealed main

effect of time (pre-post-cooling, $p < 0.001$). We observed differential effect of 30 minutes brain cooling on CBF. ANOVA revealed the main effect of groups, $p = 0.003$.

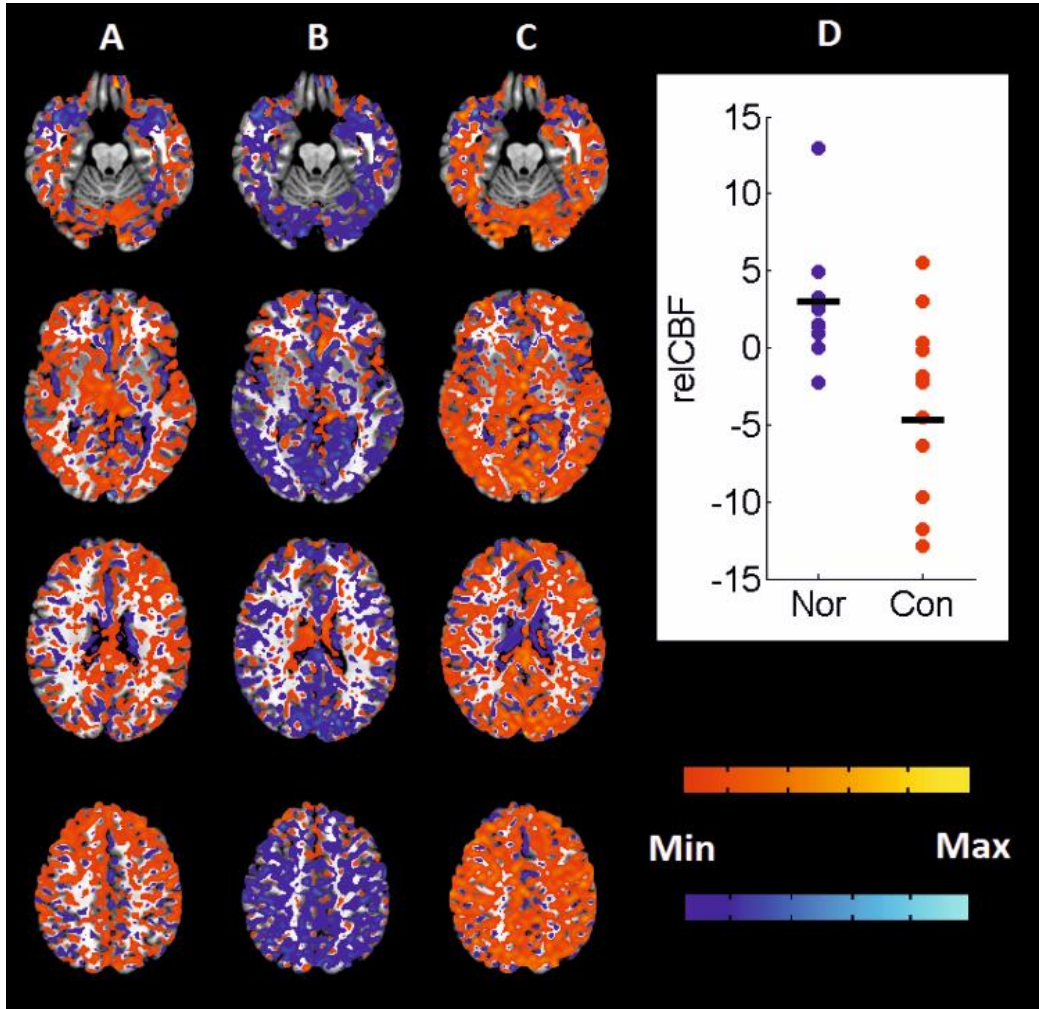


Figure 5. Differences of the pre- and post-relCBF maps of normal and concussed groups. (A) mean of diff-relCBF of normal controls group, (B) the mean of diff-relCBF of concussed group, $\text{diff-relCBF} = \text{pre-relCBF} - \text{post-relCBF}$, warm color: $\text{pre-relCBF} > \text{post-relCBF}$, Cold color: $\text{pre-relCBF} < \text{post-relCBF}$, and cluster size = 20. (C) normal controls vs. concussed group, warm color: normal (mean of diff-relCBF) $>$ concussed (mean of diff-relCBF), cold color: normal $<$ concussed, and cluster size = 20. (D) Mean of diff-relCBF of normal (Nor) and concussed (Con) groups, all mean values were calculated by using a volume of interest (1069707 voxels), relCBF: ml/100g/min, black bar: group mean, one-way ANOVA followed by Tukey's Honestly Significant Difference (HSD) method, $p = 0.003$.

rs_fMRI Group Results

No significant differences in the number and strength of functional connections within the Default Mode Network between groups were observed prior to cooling (Figure 8; $p > 0.05$). For each group, no significant differences were observed between pre and post cooling within the Default Mode Network when corrected for false discovery rate ($p > 0.05$). Both groups showed similar trends in terms of reducing the number and strength of functional connections between DMN and other ROIs after cooling. Also, visually, the reduction of number and strength of functional connections between DMN and other ROIs was more pronounced in concussed group in the post cooling scans compared to pre cooling.

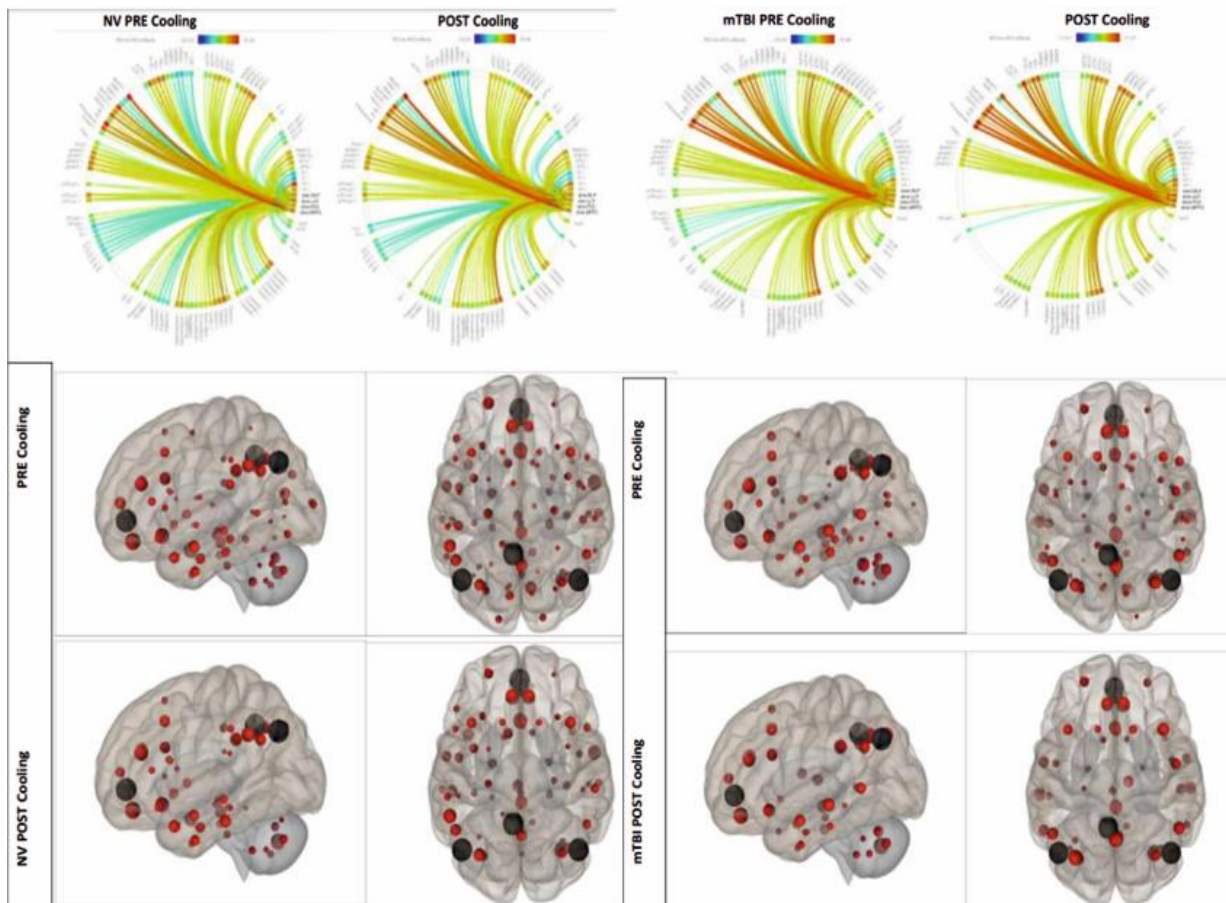


Figure 6. (A) Represents results from Normal volunteers (NV) and (B) from mTBI group. Top row reflects connectome maps based on anatomical ROIs for functional connectivity pre and post cooling. Bottom rows represents anatomical representation of connectome maps, with pre cooling on top and post cooling functional connectivity on the bottom. Black spheres represent seed ROIs of the DMN, and red spheres

represent functional connections. Visualization of rs-fMRI results show decreased number of connections in the mTBI group before cooling compared to NV, and both groups showing less functional connectivity after cooling. Images created and exported from CONN Functional Connectivity Toolbox.

VR Data

Virtual Reality findings showed increases in scores post-cooling for normal controls in all modules except balance (Table 4). The increases in spatial navigation post-cooling were significant ($p < 0.05$). In the concussed group, virtual reality scores for all modules decreased post-cooling (Table 5), but none of the difference in scores for any of the modules or the comprehensive score reached significance ($p > 0.05$). However, it is important to note that only 8 of the 12 subjects in the concussion group were able to successfully finish VR testing due to the presence of concussive symptoms. Therefore, only 8 subjects were included in statistical analyses of VR data for the concussed group.

Table 4. Normal Subjects (n=12) and Concussed Subjects (n=8) Average VR Scores Pre- and Post-Cooling for All Modules and Comprehensive Score.

	Normal Subjects				Concussed Subjects			
	Before Cooling	After Cooling	Before Cooling	After Cooling	Before Cooling	After Cooling	Before Cooling	After Cooling
	Average		Standard Deviation		Average		Standard Deviation	
Comprehensive	7.09	7.98	1.30	1.16	7.70	7.05	1.49	2.05
Spatial Navigation	6.78	8.68	2.90	1.67	7.44	7.24	2.87	3.13
Balance	9.37	9.12	0.66	0.92	8.05	7.92	2.72	1.82
Reaction Time	5.20	5.92	2.44	1.86	7.24	5.92	2.25	2.21

EEG Data

Grand-averaged 2D EEG plots were obtained for all subjects across the same time scale pre- and post-cooling, presented in Figures 9, 10, 11, and 12. Visually, there were changes both in terms of topology and spectral power in each frequency band.

Figure 7. 2D plots of EEG overall Alpha power during the four different recording conditions: (1) sitting eyes closed, (2) sitting eyes open, (3) standing eyes closed, (4) standing eyes open. Both concussed and control group averages were graphed.

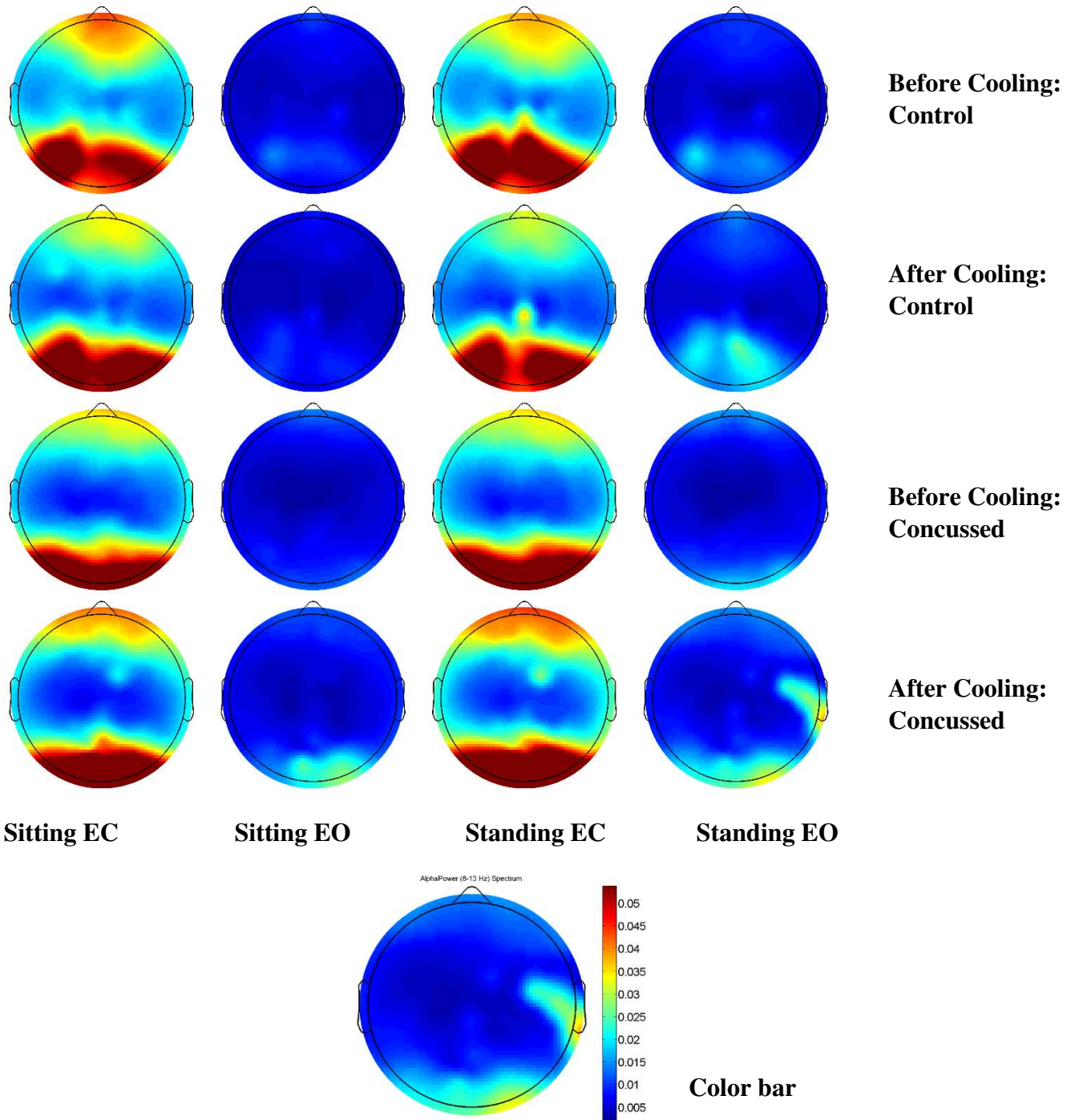


Figure 8. 2D plots of EEG overall *Beta* power during the four different recording conditions: (1) sitting eyes closed, (2) sitting eyes open, (3) standing eyes closed, (4) standing eyes open. Both concussed and control group averages were graphed.

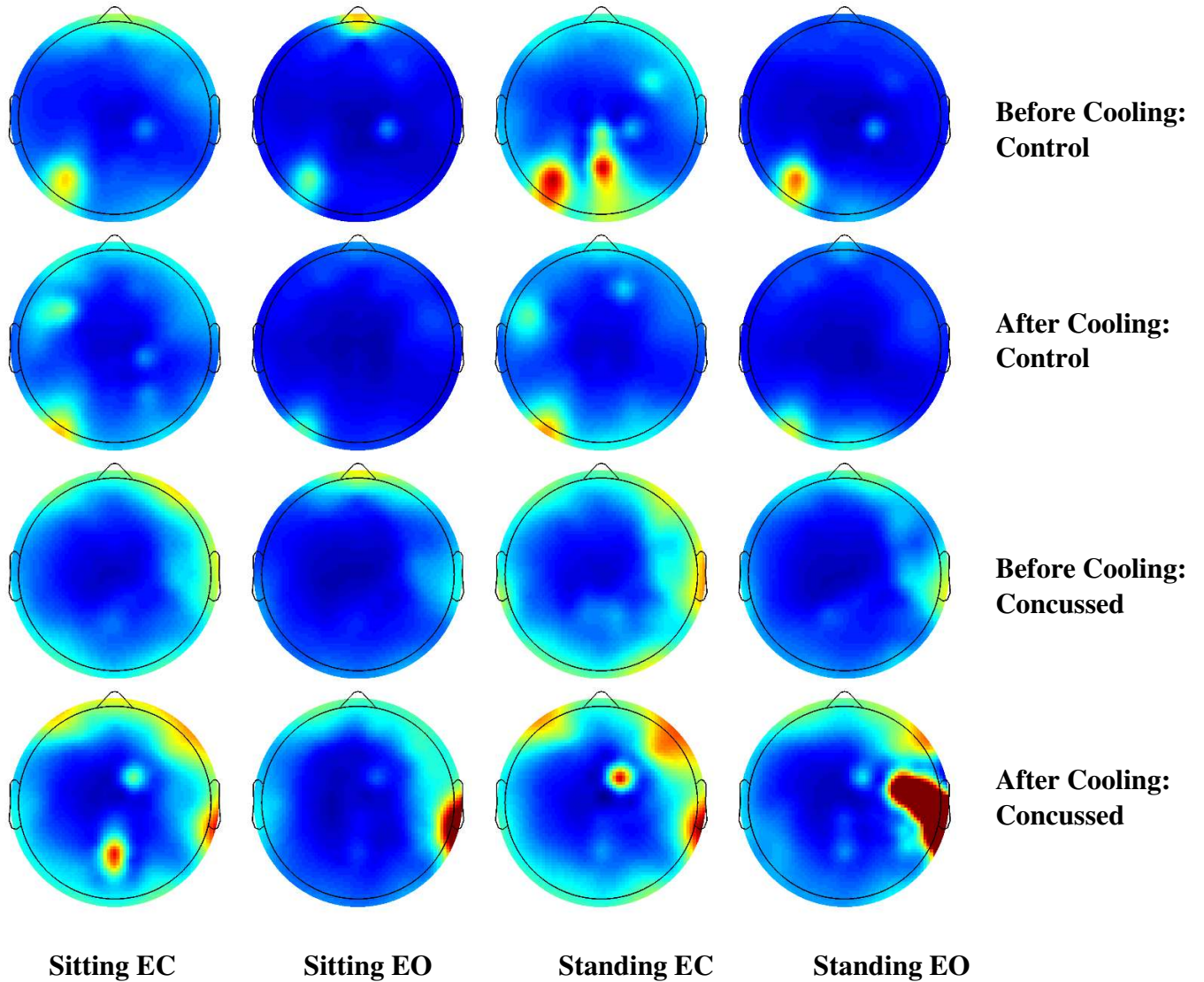
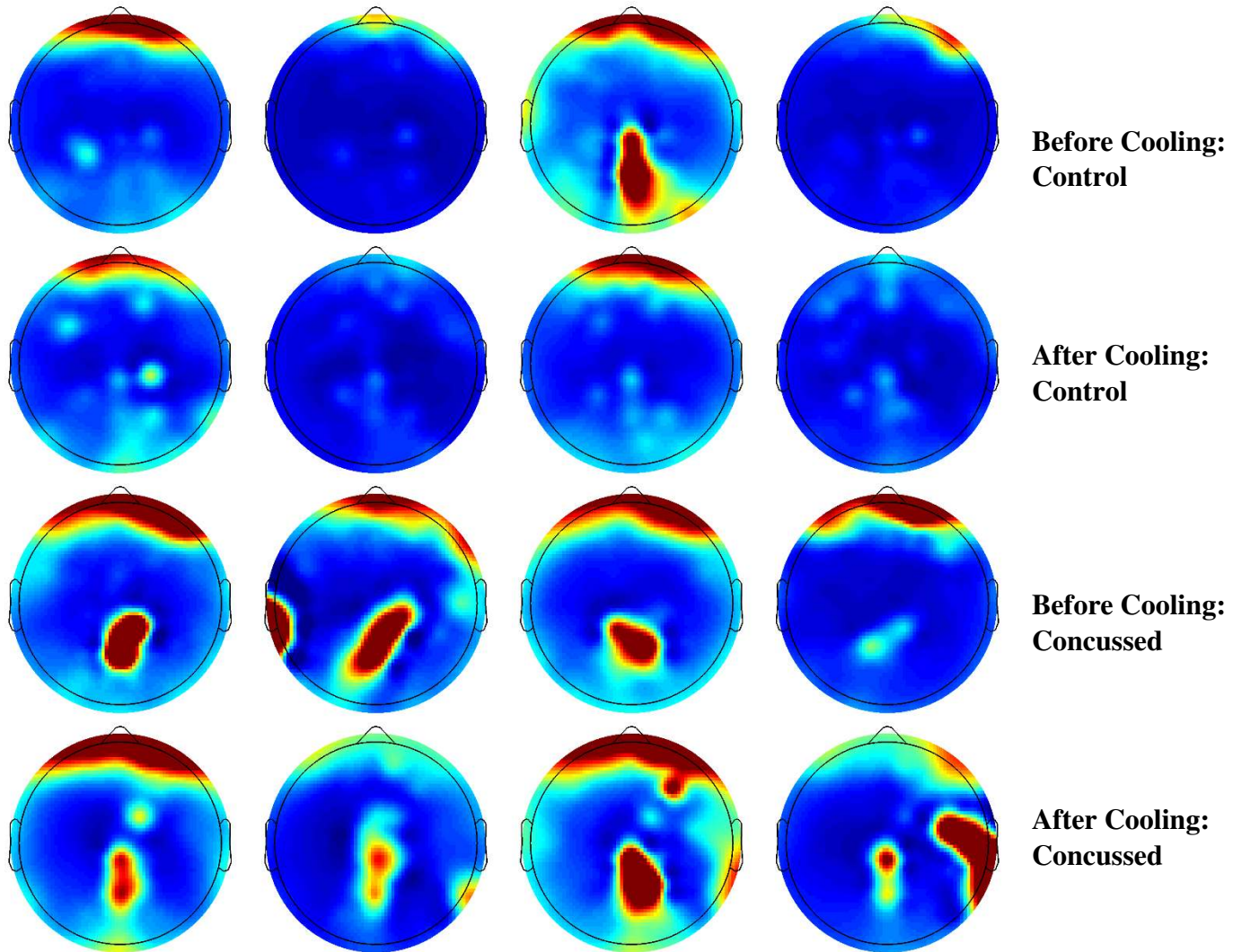


Figure 9. 2D plots of EEG overall Delta power during the four different recording conditions: (1) sitting eyes closed, (2) sitting eyes open, (3) standing eyes closed, (4) standing eyes open. Both concussed and control group averages were graphed.

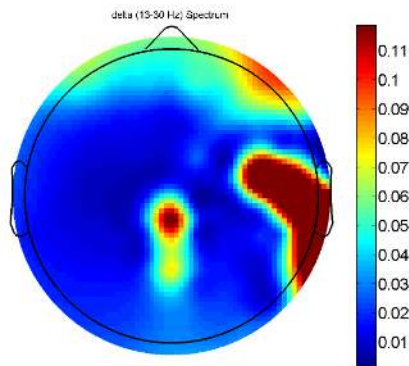


Sitting EC

Sitting EO

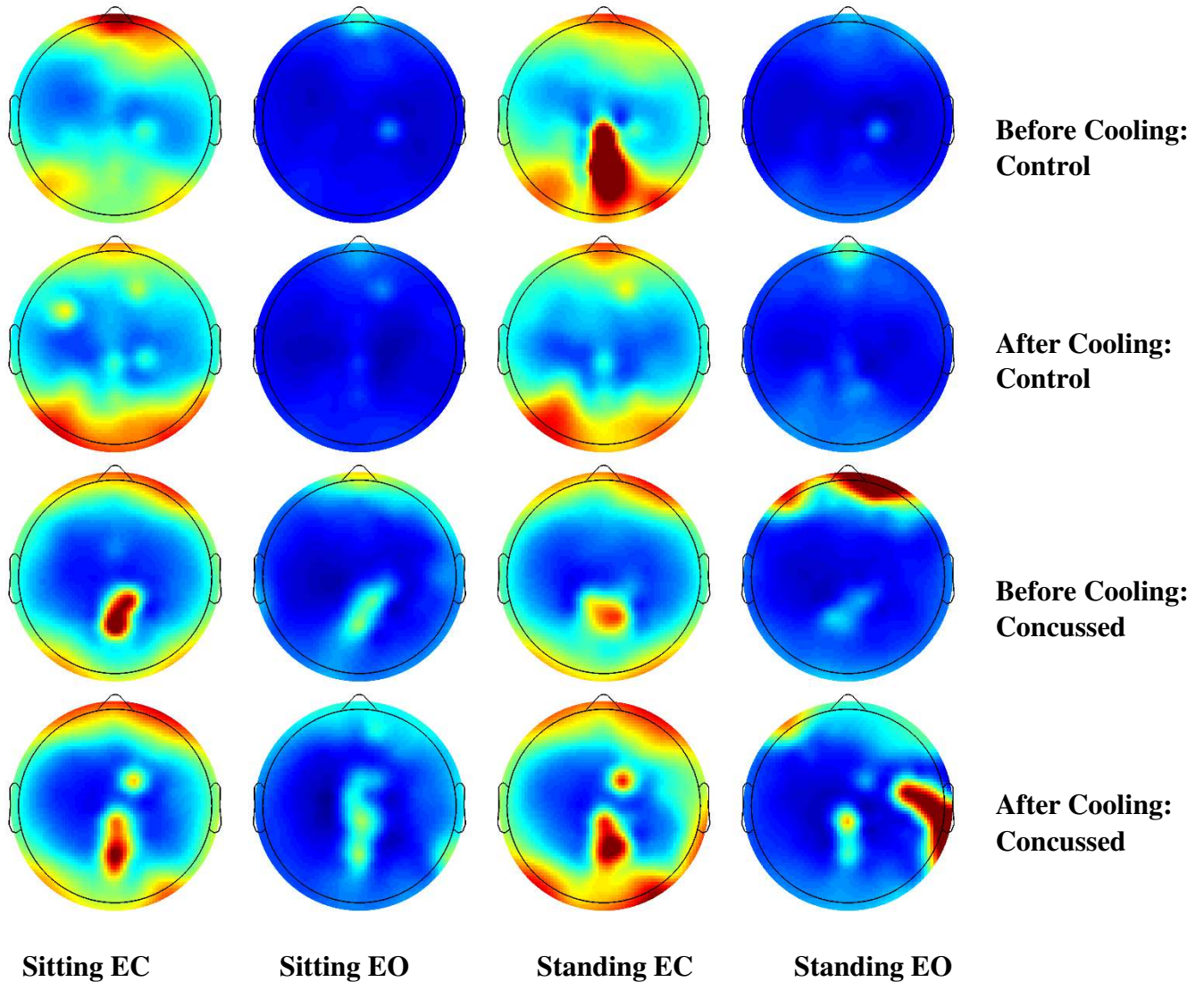
Standing EC

Standing EO



Color bar

Figure 10. 2D plots of EEG overall Theta power during the four different recording conditions: (1) sitting eyes closed, (2) sitting eyes open, (3) standing eyes closed, (4) standing eyes open. Both concussed and control group averages were graphed.



Using raw fast Fourier transform (FFT) values, obtained from the ROIs selected, group averages were calculated across the same time scale and for each frequency band. These FFT values represent an average value across the 6 electrodes in each ROI. Table 6 describes group average FFT values for alpha (A), delta (B), and theta (C) frequencies. Beta frequency was not analyzed due to its minimal visual changes seen in Figure 10.

Table 5. Frequency Band Mean and Standard Deviations for Concussed and Control Groups for the 4 Main ROIs.

(A). Alpha Frequency: FFT values for each individual in either the concussed or control group were averaged together for each segment and their mean and standard deviation values for 4 main ROIs (frontal, central, parietal, occipital) are presented.

B1= before cooling segment 1 (sitting eyes closed); B2= before cooling segment 2 (sitting eyes open); B3= before cooling segment 3 (standing eyes closed); B4= before cooling segment 4 (standing eyes open); A1= after cooling segment 1 (sitting eyes closed); A2= after cooling segment 2 (sitting eyes open); A3= after cooling segment 3 (standing eyes closed); A4= after cooling segment 4 (standing eyes open)

Alpha Frequency FFT Values								
Concussed								
	Frontal		Central		Parietal		Occipital	
	Mean	Std Dev	Mean	Std Dev	Mean	Std Dev	Mean	Std Dev
B1	0.0249	0.0156	0.0093	0.0073	0.0313	0.0209	0.0701	0.0691
B2	0.0065	0.0035	0.0030	0.0018	0.0068	0.0038	0.0098	0.0069
B3	0.0249	0.0108	0.0100	0.0065	0.0329	0.0181	0.0784	0.0609
B4	0.0070	0.0043	0.0030	0.0024	0.0076	0.0054	0.0143	0.0150
A1	0.0265	0.0189	0.0093	0.0060	0.0356	0.0426	0.0736	0.1000
A2	0.0078	0.0075	0.0044	0.0035	0.0087	0.0117	0.0217	0.0511
A3	0.0295	0.0183	0.0112	0.0089	0.0380	0.0382	0.0898	0.1156
A4	0.0087	0.0073	0.0050	0.0059	0.0090	0.0110	0.0227	0.0374
Control								
	Frontal		Central		Parietal		Occipital	
	Mean	Std Dev	Mean	Std Dev	Mean	Std Dev	Mean	Std Dev
B1	0.0345	0.0254	0.0167	0.0135	0.0429	0.0454	0.0479	0.0429
B2	0.0067	0.0061	0.0048	0.0059	0.0093	0.0126	0.0080	0.0094
B3	0.0314	0.0241	0.0178	0.0151	0.0569	0.0585	0.0529	0.0427
B4	0.0088	0.0096	0.0038	0.0044	0.0109	0.0141	0.0120	0.0139
A1	0.0275	0.0237	0.0138	0.0139	0.0377	0.0414	0.0572	0.0583
A2	0.0053	0.0060	0.0045	0.0050	0.0071	0.0089	0.0078	0.0103
A3	0.0247	0.0223	0.0164	0.0188	0.0391	0.0462	0.0531	0.0589
A4	0.0103	0.0146	0.0062	0.0078	0.0199	0.0298	0.0180	0.0214

(B). Delta Frequency: FFT values for each individual in either concussed or control group were averaged together for each segment and their mean and standard deviation values for 4 main ROIs (frontal, central, parietal, occipital) are presented.

B1= before cooling segment 1 (sitting eyes closed); B2= before cooling segment 2 (sitting eyes open); B3= before cooling segment 3 (standing eyes closed); B4= before cooling segment 4 (standing eyes open); A1= after cooling segment 1 (sitting eyes closed); A2= after cooling segment 2 (sitting eyes open); A3= after cooling segment 3 (standing eyes closed); A4= after cooling segment 4 (standing eyes open)

Delta Frequency FFT Values								
Concussed								
	Frontal		Central		Parietal		Occipital	
	Mean	Std Dev	Mean	Std Dev	Mean	Std Dev	Mean	Std Dev
B1	0.00182	0.00103	0.00076	0.00029	0.00176	0.00066	0.00273	0.00091
B2	0.00156	0.00101	0.00058	0.00028	0.00121	0.00074	0.00187	0.00087
B3	0.00195	0.00108	0.00089	0.00040	0.00226	0.00164	0.00326	0.00096
B4	0.00145	0.00065	0.00063	0.00026	0.00149	0.00095	0.00200	0.00082
A1	0.00221	0.00148	0.00102	0.00059	0.00383	0.00747	0.00300	0.00208
A2	0.00171	0.00135	0.00082	0.00058	0.00115	0.00058	0.00172	0.00144
A3	0.00214	0.00142	0.00098	0.00056	0.00189	0.00071	0.00312	0.00175
A4	0.00210	0.00215	0.00089	0.00042	0.00150	0.00107	0.00215	0.00105
Control								
	Frontal		Central		Parietal		Occipital	
	Mean	Std Dev	Mean	Std Dev	Mean	Std Dev	Mean	Std Dev
B1	0.00198	0.00093	0.00093	0.00046	0.00163	0.00116	0.00231	0.00175
B2	0.00134	0.00067	0.00059	0.00033	0.00088	0.00059	0.00119	0.00071
B3	0.00204	0.00080	0.00178	0.00265	0.00482	0.00946	0.00431	0.00381
B4	0.00130	0.00081	0.00058	0.00028	0.00115	0.00077	0.00223	0.00245
A1	0.00160	0.00075	0.00085	0.00053	0.00142	0.00104	0.00246	0.00191
A2	0.00107	0.00056	0.00055	0.00038	0.00086	0.00066	0.00142	0.00125
A3	0.00154	0.00067	0.00083	0.00053	0.00150	0.00122	0.00279	0.00243
A4	0.00120	0.00069	0.00058	0.00037	0.00115	0.00098	0.00251	0.00388

(C). Theta Frequency: FFT values for each individual in either concussed or control group were averaged together for each segment and their mean and standard deviation values for 4 main ROIs (frontal, central, parietal, occipital) are presented.

B1= before cooling segment 1 (sitting eyes closed); B2= before cooling segment 2 (sitting eyes open); B3= before cooling segment 3 (standing eyes closed); B4= before cooling segment 4 (standing eyes open); A1= after cooling segment 1 (sitting eyes closed); A2= after cooling segment 2 (sitting eyes open); A3= after cooling segment 3 (standing eyes closed); A4= after cooling segment 4 (standing eyes open)

Theta Frequency FFT Values								
Concussed								
	Frontal		Central		Parietal		Occipital	
	Mean	Std Dev	Mean	Std Dev	Mean	Std Dev	Mean	Std Dev
B1	0.01026	0.00444	0.00706	0.00449	0.01824	0.02014	0.01418	0.01096
B2	0.00677	0.00564	0.00465	0.00520	0.00888	0.01427	0.00739	0.00475
B3	0.01113	0.00440	0.00653	0.00353	0.01745	0.01798	0.01535	0.00911
B4	0.00932	0.01182	0.00365	0.00236	0.00699	0.00536	0.00735	0.00495
A1	0.01120	0.00544	0.01010	0.00883	0.01931	0.01892	0.01589	0.01564
A2	0.00723	0.00599	0.01073	0.01583	0.00903	0.01062	0.00731	0.00672
A3	0.01272	0.00629	0.01082	0.01065	0.02183	0.02489	0.01985	0.02165
A4	0.00713	0.00531	0.00737	0.01183	0.00738	0.00611	0.00812	0.00758
Control								
	Frontal		Central		Parietal		Occipital	
	Mean	Std Dev	Mean	Std Dev	Mean	Std Dev	Mean	Std Dev
B1	0.01566	0.01722	0.00923	0.01252	0.01414	0.02288	0.01533	0.02086
B2	0.00538	0.00264	0.00334	0.00308	0.00363	0.00311	0.00408	0.00242
B3	0.01509	0.01589	0.01514	0.02630	0.03973	0.09028	0.02321	0.02794
B4	0.00604	0.00306	0.00301	0.00177	0.00479	0.00305	0.00642	0.00447
A1	0.01225	0.01251	0.00901	0.01251	0.01358	0.02015	0.02119	0.03983
A2	0.00538	0.00217	0.00365	0.00203	0.00434	0.00285	0.00507	0.00354
A3	0.01408	0.01808	0.00857	0.01249	0.01204	0.01798	0.01962	0.04085
A4	0.00676	0.00375	0.00443	0.00356	0.00690	0.00554	0.00759	0.00576

Paired t-tests were run for each group (concussed or control) and ROI (frontal, central, parietal, occipital) to examine if any relation existed pre- and post-cooling for each segment (1 – 4).

In the alpha frequency (see Figures 13 – 16), for the concussed group, there were no significant changes from pre- to post-cooling for all segments in the frontal ($p>0.05$), central ($p>0.05$), parietal ($p>0.05$), and occipital ($p>0.05$) ROIs. In the alpha frequency, for the control group, there were no significant changes from pre- to post-cooling for any segment in the central ($p>0.05$), parietal ($p>0.05$), and occipital ($p>0.05$) ROIs. There was a significant change in average FFT value post-cooling in the frontal B2-A2 (sitting eyes open) paired t-test ($p<0.05$).

In the delta frequency (see Figures 13 – 16), for the concussed group, there were no significant changes from pre- to post-cooling for any segment in frontal ($p>0.05$), parietal ($p>0.05$), and occipital ($p>0.05$) ROIs. In the central ROI, the B4-A4 (standing eyes open) condition approached significance ($p=0.054$). In the delta frequency, for the control group, there were no significant changes from pre- to post-cooling for any segment in central ($p>0.05$), parietal ($p>0.05$), and occipital ($p>0.05$) ROIs. In the frontal ROI, the B3-A3 (standing eyes closed) condition approached significance ($p=0.057$).

In the theta frequency (see Figures 13 – 16), for the concussed group, there were no significant changes from pre- to post-cooling for any segment in frontal ($p>0.05$), central ($p>0.05$), parietal ($p>0.05$), and occipital ($p>0.05$) ROIs. In the theta frequency, for the control group, there were no significant changes from pre- to post-cooling for any segment in frontal ($p>0.05$), central ($p>0.05$), parietal ($p>0.05$), and occipital ($p>0.05$) ROIs.

Figure 11. The Frontal ROI average FFT values for each frequency (alpha, theta, and delta) and each group (control and concussed). There are obvious increases post-cooling in the alpha frequency in the concussed group and decreases in alpha frequency in the control group (except B4-A4, which increases). In theta concussed group, there are increases in the eyes closed conditions (B1-A1 and B3-A3) and decreases in the eyes open conditions (B2-A2 and B4-A4). In the control group, theta frequency, there are decreases across all conditions. For delta, concussed group, there are increases in the eyes open conditions (B1-A1 and B3-A3) and decreases in the eyes open conditions (B2-A2 and B4-A4). For the control group, there are decreases in the eyes closed conditions (B1-A1 and B3-A3) and increases in the eyes open conditions (B2-A2 and B4-A4).

B1= before cooling segment 1 (sitting eyes closed); B2= before cooling segment 2 (sitting eyes open); B3= before cooling segment 3 (standing eyes closed); B4= before cooling segment 4 (standing eyes open); A1= after cooling segment 1 (sitting eyes closed); A2= after cooling segment 2 (sitting eyes open); A3= after cooling segment 3 (standing eyes closed); A4= after cooling segment 4 (standing eyes open).

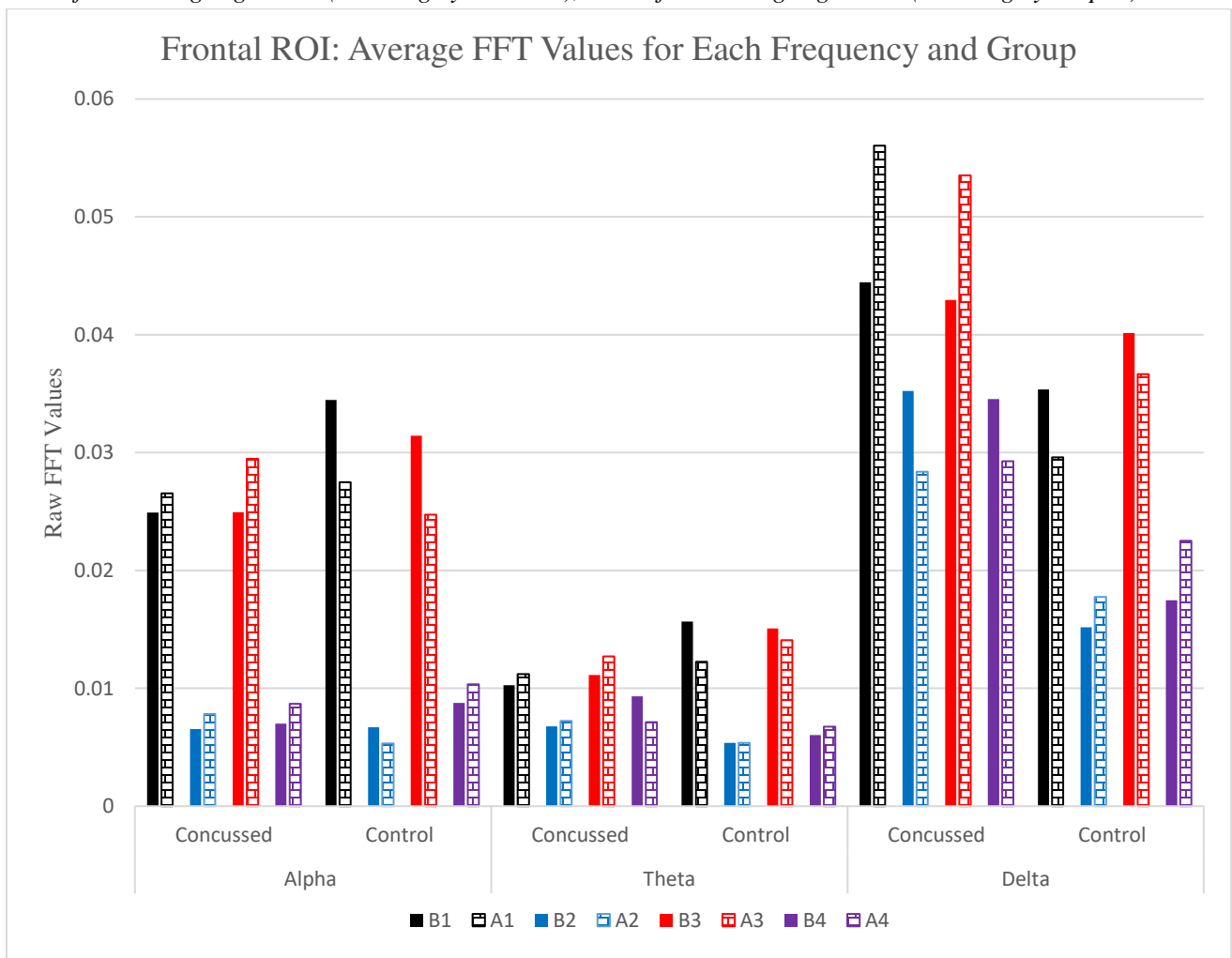


Figure 12. The Central ROI average FFT values for each frequency (alpha, theta, and delta) and each group (control and concussed). There are increases post-cooling in the alpha frequency in the concussed group and decreases in alpha frequency in the control group (except B4-A4, which increases). In theta, concussed group, there are increases in all conditions. In the control group, theta frequency, there is no consistency: there are increases, decreases, or it remains the same. For delta, concussed group, there are large increases in all conditions. For the control group, there are increases in the sitting conditions (B1-A1 and B2-A2) and decreases in the standing conditions (B3-A3 and B4-A4).

B1= before cooling segment 1 (sitting eyes closed); B2= before cooling segment 2 (sitting eyes open); B3= before cooling segment 3 (standing eyes closed); B4= before cooling segment 4 (standing eyes open); A1= after cooling segment 1 (sitting eyes closed); A2= after cooling segment 2 (sitting eyes open); A3= after cooling segment 3 (standing eyes closed); A4= after cooling segment 4 (standing eyes open).

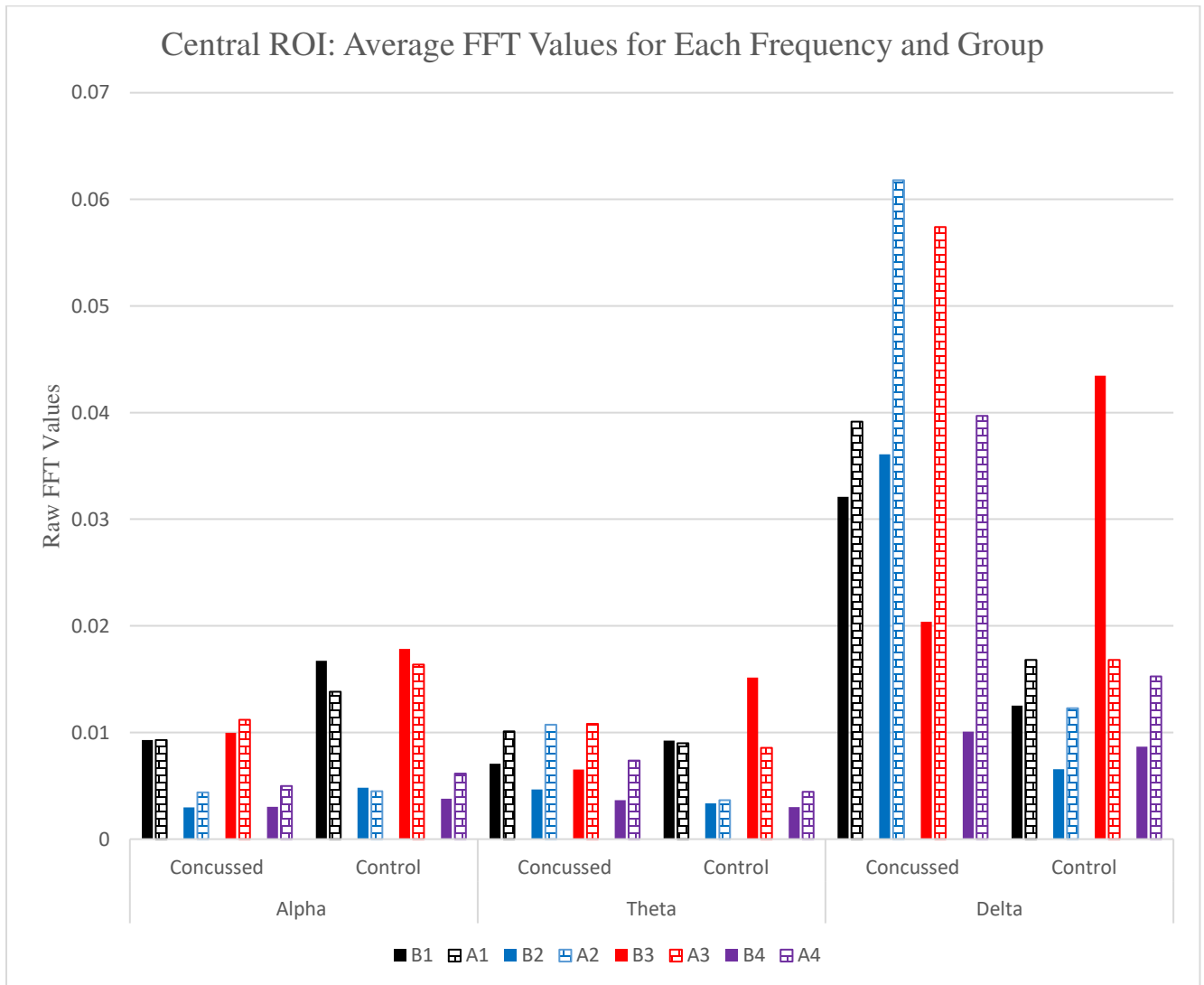


Figure 13. The Parietal ROI average FFT values for each frequency (alpha, theta, and delta) and each group (control and concussed). There are increases post-cooling in the alpha frequency in the concussed group and decreases in alpha frequency in the control group (except B4-A4, which increases). In theta, concussed group, there are relatively no changes in all conditions, except B3-A3 which increases post-cooling. In the control group, theta frequency, there is no consistency: there are increases, decreases, or it remains the same. For delta, concussed group, there are large decreases in the sitting conditions (B1-A1 and B2-A2) and increases in the standing conditions (B3-A3 and B4-A4). For the control group, there are increases in the sitting conditions (B1-A1 and B2-A2) and decreases in the standing conditions (B3-A3 and B4-A4).

B1= before cooling segment 1 (sitting eyes closed); B2= before cooling segment 2 (sitting eyes open); B3= before cooling segment 3 (standing eyes closed); B4= before cooling segment 4 (standing eyes open); A1= after cooling segment 1 (sitting eyes closed); A2= after cooling segment 2 (sitting eyes open); A3= after cooling segment 3 (standing eyes closed); A4= after cooling segment 4 (standing eyes open).

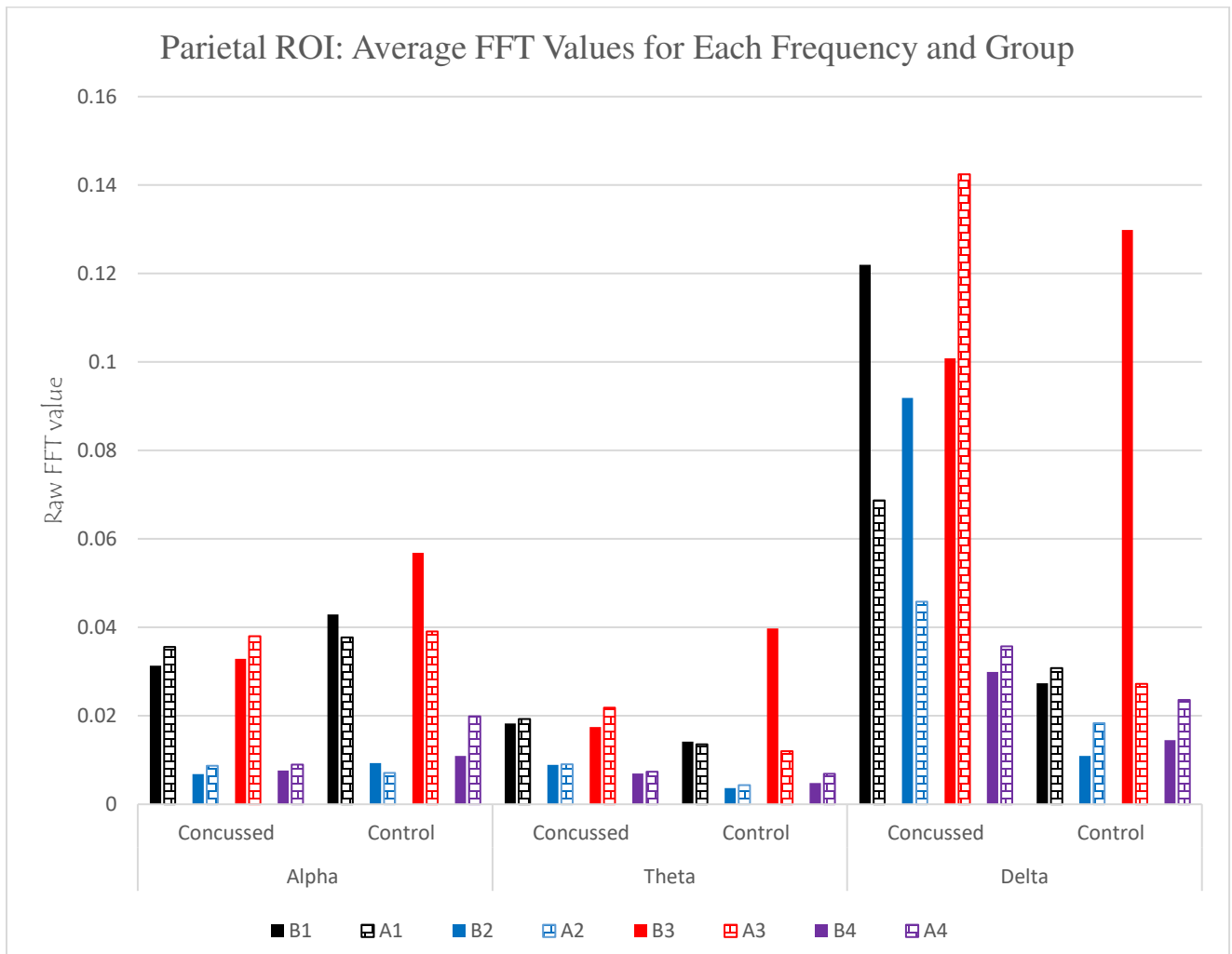
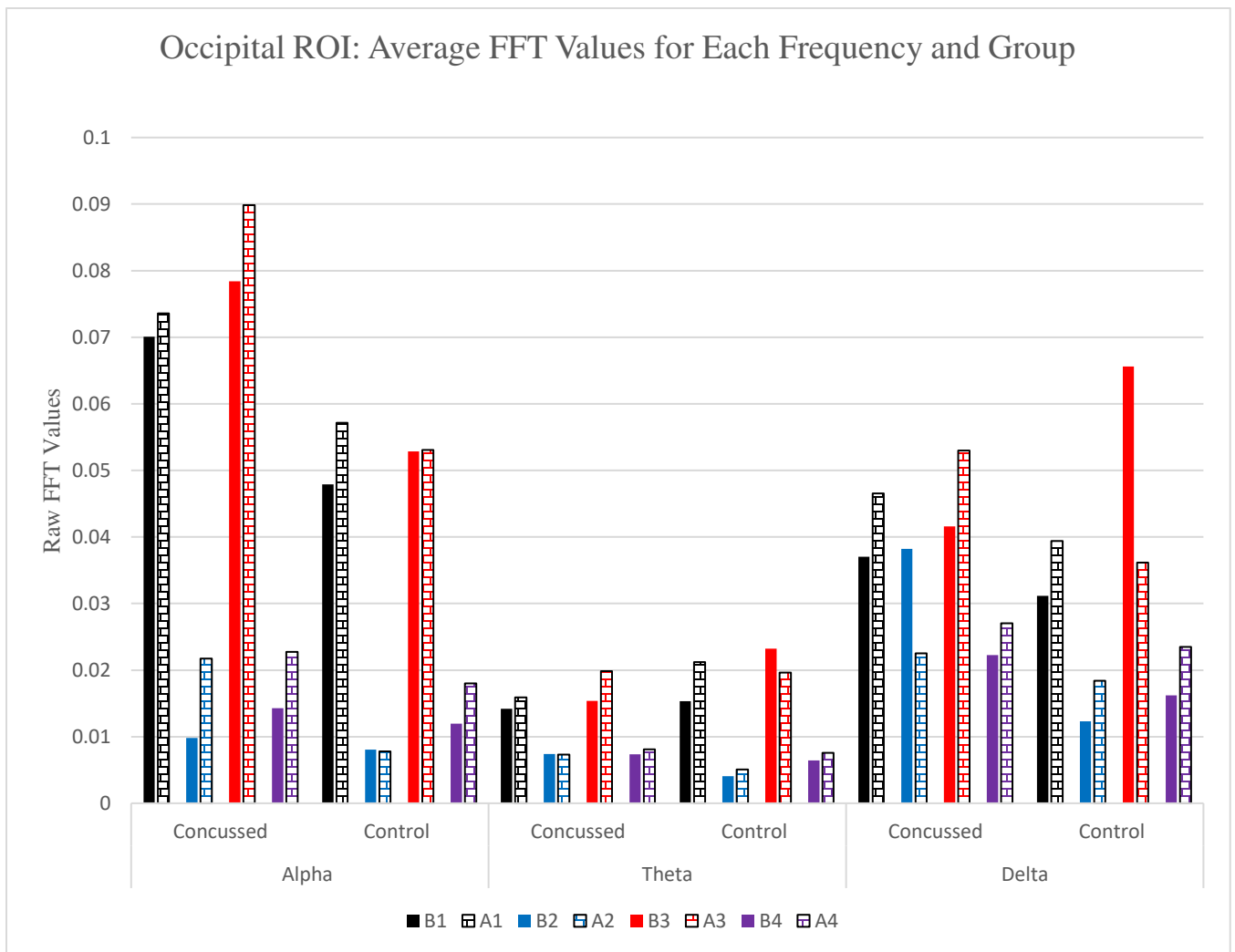


Figure 14. The Occipital ROI average FFT values for each frequency (alpha, theta, and delta) and each group (control and concussed). There are increases post-cooling in all alpha frequency conditions in the concussed group and control group. In theta, concussed group, there are slight or no increases in all conditions. In the control group, theta frequency, there are increases in the sitting conditions (B1-A1 and B2-A2) and decreases in the standing conditions (B3-A3 and B4-A4). For delta, concussed group, there are large increases in all conditions, except B2-A2 which decreases. For the control group, there are increases in all conditions, except B3-A3.

B1= before cooling segment 1 (sitting eyes closed); B2= before cooling segment 2 (sitting eyes open); B3= before cooling segment 3 (standing eyes closed); B4= before cooling segment 4 (standing eyes open); A1= after cooling segment 1 (sitting eyes closed); A2= after cooling segment 2 (sitting eyes open); A3= after cooling segment 3 (standing eyes closed); A4= after cooling segment 4 (standing eyes open).



Since the majority of the changes were non-significant, the overall patterns of change were examined (Table 6). There is a distinctly differential pattern of response between the groups at each frequency in response to cooling.

Table 6. The change from pre to post cooling within each group was examined for each frequency and ROI. Arrows indicated the change in raw FFT power (pre – post) and are indicated as an increase (up arrow) or decrease (down arrow).

		Concussed				Control			
		Sit EC	Sit EO	Stand EC	Stand EO	Sit EC	Sit EO	Stand EC	Stand EO
Alpha	Frontal	↑	↑	↑	↑	↓	↓	↓	↑
	Central	↑	↑	↑	↑	↓	↓	↓	↑
	Parietal	↑	↑	↑	↑	↓	↓	↓	↑
	Occipital	↑	↑	↑	↑	↑	↓	↑	↑
Theta	Frontal	↑	↑	↑	↓	↓	↓	↓	↑
	Central	↑	↑	↑	↑	↓	↑	↓	↑
	Parietal	↑	↑	↑	↑	↓	↑	↓	↑
	Occipital	↑	↓	↑	↑	↑	↑	↓	↑
Delta	Frontal	↑	↓	↑	↓	↓	↑	↓	↑
	Central	↑	↑	↑	↑	↑	↑	↓	↑
	Parietal	↓	↓	↑	↑	↑	↑	↓	↑
	Occipital	↑	↓	↑	↑	↑	↑	↓	↑

A repeated measures ANOVA was run for each frequency band with the between-subject factor of injury (concussion or control) and the within-subject factors of cooling (before and after), ROI (frontal, central, parietal, occipital), and condition (1, 2, 3, 4). Mauchly's Test of Sphericity indicated that the assumption of sphericity had been violated, $\chi^2(44) = 915.485$, $p < 0.005$, and therefore, a Greenhouse-Geisser correction was used. The delta frequency was the

only frequency that revealed significant findings, regardless of injury group. Cooling alone was significant, $F(1, 22) = 11.303, p < 0.005$, as was region ($F(1.009, 22.192) = 14.8, p < 0.005$) and segment ($F(1.093, 24.035) = 15.675, p < 0.005$). The interaction of ROI and condition was significant, $F(1.088, 23.928) = 13.471, p < 0.005$. Additionally, there was a significant effect of cooling on ROI, $F(1.007, 22.154) = 12.890, p < 0.005$ and of cooling on condition $F(1.078, 23.717) = 12.119, p < 0.005$. There also was a significant effect of cooling on ROI and condition, $F(1.083, 23.821) = 12.982, p < 0.005$. No other interactions were statistically significant.

Discussion

Presently, there is no accepted treatment for concussion, with current best practices recommending rest and sleep to manage injuries. The search for effective treatment facilitating recovery from concussive injury, as well as reducing the risk for recurrent concussions is ongoing. Accordingly, this study aimed to determine if selective brain cooling has an effect on clinical symptoms resolution and biological functions of the brain in athletes in the acute phase of sports-related concussion. Using advanced neuroimaging tools and physiological measures (EEG and VR), the potential feasibility of selective brain cooling on brain functions was investigated in cohort of student-athletes suffering from concussion in acute phase of injury.

There are several findings of interest. First, all concussed subjects were clinically symptomatic at the time of this study and were not cleared for sport participation. Immediately following administration of 30 minutes of selective neck and head cooling, the vast majority of concussed subjects (MRI: 67%; EEG: 83%) under study reported a partial resolution of symptoms, predominantly physical symptoms including headache and dizziness. However, the effects from the selective cooling seem to be short lived as symptoms returned shortly after intervention was stopped. We cannot rule out that this could be due to the subjects being placed

back in the scanner for 30 minutes or completing VR testing again, re-triggering their previous clinical symptoms or it may be a placebo effect.

Virtual Reality Discussion

We did not see any significant differences in virtual reality scores from pre- to post-cooling. On average, the control group's scores increased post-cooling, indicating improvements in scores. The concussed individuals however had decreases in scores post-cooling. The effect of hypothermia on cognition is greatly debated. Some studies have shown cognitive impairment after hypothermia, specifically affecting attention (Sun et al., 2012), memory (Racinais, Gaoua, & Grantham, 2008), information processing (Sun, Li, Li, & Jiang, 2011), and attention processing (Gaoua, Racinais, Grantham, & El Massioui, 2011). However, other hypothermia and cold exposure studies contradict these findings and suggest that moderate cold exposure can improve task performance (Doll et al., 2009; Mäkinen, 2007) or has no effect on performance (Lilja et al., 2015). One theory is that general arousal levels are increased by mild to moderate cold exposure, which initially leads to improved performance (Mäkinen, 2007). However, with longer or more severe cooling, arousal can increase to a point where performance becomes degraded (H. D. Ellis, 1982; Van Orden, Ahlers, Thomas, House, & Schrot, 1990). These improvements could suggest that shorter evoked potential latencies could lead to faster CNS processing (Van Orden et al., 1990). This theory supports the findings in our control group, as the average scores increased for reaction time and spatial memory. The findings in the concussed group are more difficult to decipher as only 8 of the 12 subjects were able to complete the testing. This could suggest a possible release of symptoms in some subjects post-cooling that allowed them to successfully complete the VR modules or that some subjects were not as symptomatic as others were; however, this needs to be explored further. Even though they were able to complete testing, their scores still decreased. This finding is more consistent with the

current mTBI and hypothermia research that suggests impaired cognitive performance post-cooling at hypothermic levels (Titus et al., 2015).

EEG Discussion

The EEG data did not reveal many statistically significant findings in regards to the effect of cooling in both groups. Visually, the 2D plots of overall power showed changes from pre- to post-cooling, however many of these changes did not reach significance. We believe this is most likely due to the factor of time. Since the subjects were cooled for 30 minutes and then had to be set up again in the EEG protocol, we believe the time in between finishing cooling and beginning EEG procedures (around 10 – 15 minutes) was long enough to mitigate some of the effects of cooling. Nevertheless, in the following text, I will discuss major findings of interest.

In concussed individuals, EEG results have shown lower power in the alpha frequency (Tebano et al., 1988) and reduced theta power (Montgomery, Fenton, McClelland, MacFlynn, & Rutherford, 1991) after injury. A comprehensive EEG study revealed increased coherence in the frontal-temporal regions, decreased power differences between anterior and posterior cortical regions, and reduced alpha power in the posterior regions (Thatcher, Walker, Gerson, & Geisler, 1989). As I hypothesized, power with the alpha frequency band increased, however non-significantly, post-cooling in the concussed group. This could suggest that selective brain cooling may potentially have a positive effect by mediating some of the damaging effects of a concussive injury, as seen through the alpha frequency power changes.

In many EEG studies, increases in delta and theta rhythms have been commonly associated with drowsiness and fatigue build up, especially in the frontal and central areas (De Gennaro et al., 2007; Makeig & Jung, 1995; Tinguely, Finelli, Landolt, Borbély, & Achermann, 2006). During task related activities, theta tends to increase, as there is a heightened focus on arousal and performance, and it has also been involved with spontaneous resting state. However,

during resting state, increases in theta are associated with generalized slowing and are indicative of injury or damage (Slobounov, Teel, & Newell, 2013). Power within theta frequency band increased, however non-significantly, post-cooling for the concussed group and decreased post-cooling for the controls. The control group showed positive tendencies as they had decreases in theta post-cooling, indicating no slowing and the activation of excitatory mechanisms. However, the concussed groups' increases in theta post-cooling are somewhat contradictory. One hypothesis is that the concussed subjects could be compensating for their injury by requiring more brain resource allocation to complete the same task (Slobounov et al., 2010). This could make a simple standing or sitting task more difficult and could require the requirement of more resources. Therefore, these increases in theta could indicate that the concussed subjects had to recruit more resources to successfully complete this task. Theta power has also been related to postural control (Slobounov, Cao, Jaiswal, & Newell, 2009) and increases in theta could be related to unpredictable conditions and the need to recruit additional brain resources to meet the demands of a postural task (Slobounov, Teel, & Newell, 2013). Therefore, these increases in power post-cooling might indicate a slightly positive effect, as resource allocation and attentional processes may be increasing.

Slowing of delta frequency (increased power values) in concussion is often indicative of injury. Prominent delta power in a concussed individual has even been identified as a biomarker of the injury as MEG studies have shown that injured brain tissues in mTBI show abnormal delta activity: a way to measure, localize, and diagnose concussions (Lee & Huang, 2014). This reduction can persist for months post-injury (Haneef, Levin, Frost, & Mizrahi, 2013) and could be explained by a mechanism that subjects are compensating for cognitive deficits (Balkan, Virji-Babul, Miyakoshi, Makeig, & Garudadri, 2015). MEG has also shown differences in

executive functioning and processing speed as well as a significant correlation between delta activity and these patterns of cognitive function (Robb Swan et al., 2015).

Cooling, if it has a beneficial effect, should theoretically reduce delta values. EEG results revealed that certain frequency bands had decreases in power post-cooling; however, it was not consistent. This reveals a partial support of my hypothesis that brain cooling would reduce power in the delta region post-cooling. There were some decreases in delta, but only in specific regions. The effect of cooling was dependent on the specific ROI chosen, as well as the injury status. The concussed and control subjects responded differently to cooling within delta frequency and ROI.

MRI Discussion

Prior to brain cooling, we observed reduced CBF in our concussed subjects compared to normal controls. This finding is consistent with Maugans et al. (2012), that documented impaired mean resting CBF in the acute phase of pediatric sports-related concussion that persisted at 1 month, despite normalization of neurocognitive testing scores. Also, Bartnik-Olson et al. (2014) observed reductions in CBF and cerebral blood volume in the thalamus of pediatric concussed patients compared to normal controls. Moreover, Meier et al. (2015) detected regional abnormal decreases in resting CBF among collegiate concussed patients during the acute injury phase that were normalized 1 month later, as well as, regional CBF within two regions of interest that correlated with scores on a clinical symptom inventory. Finally, Wang et al. (2016) detected regional reductions in CBF within 24 hours of injury in collegiate athletes that persisted at day 8 post-injury despite normalization of clinical and neurocognitive testing scores. Collectively, these empirical findings support of the notion that failure to appropriately match CBF (due to its reduction) with surges in the metabolic demands of the brain can lead to at least temporary alterations in neurological functioning following concussion (Ellis et al., 2015). If this is true, it may explain why return-to-play too early can be problematic and put an injured athlete at a

higher risk for recurrent concussion. Although, contradictory to our initial hypothesis, we saw a significant increase in CBF assessed by ASL after selective cooling in the concussed subjects compared to the normal controls.

As we expected, we did not observe any differences in number and strength of functional connections within DMN between groups prior to cooling. This is consistent with our previous neuroimaging studies demonstrating the resilience of DMN in concussed athletes in the acute phase of injury (Zhang et al., 2012). Similarly, resilience of DMN in concussed athletes in the acute phase of injury but reduced functional connectivity between different networks was previously reported in the literature (Zhu et al., 2015). However, we observed a reduction in the strength and number of connections of the DMN with other ROIs in both groups, but more obvious, visually, in concussed group after selective cooling.

This reduction in rs-fMRI connectivity, along with reduced CBF in the normal controls, shows a predictable response to hypothermia treatment, and implies a strong relationship between neuronal activity and cerebrovascular response or neurovascular coupling. However, this tight neurovascular coupling was not seen in the concussed group. In contrast to normal controls, concussed subjects showed reduced rs-fMRI functional connectivity along with increased CBF. This relationship between functional connectivity and cerebral blood flow has been previously observed, suggesting that high functional connectivity reflects tighter neurovascular connections (Tak, Polimeni, Wang, Yan, & Chen, 2015). This coupling is reflected in our results, although unlike normal controls, the concussed subjects had a disrupted link. The disruption of connectivity and cerebral blood flow could guide future studies.

Overall Discussion

Observed temporary partial resolution of clinical symptoms despite presence of altered neurophysiology raises major concerns with current clinical management of concussion.

Resolution of clinical symptoms does not adequately represent the presence of a healed injury or brain (Johnson et al., 2012; Johnson, Hallett, & Slobounov, 2015; S. M. Slobounov et al., 2010; Talavage et al., 2014; Zhu et al., 2015). This also points out that other tests, like neuroimaging and brain cooling, may better be suited in return-to-play decisions. If concussion is indeed a complex pathophysiological process affecting the brain, proper diagnostic (physiological, brain imaging in isolation and/or in conjunction with other advanced methods) tools have to be clinically validated and implemented in clinical practice (Slobounov et al., 2014). Without the inclusion of diagnostic tools designed to directly evaluate the underlying complex pathophysiology affecting the brain, our true understanding of recovery from concussion will continue to remain incomplete at best. Selective cooling or other stressors/tests may be better at challenging the neurophysiology/metabolic response and therefore, more sensitive to detecting and predicting the presence of injury long-term.

Considering the fact that concussed subjects reported temporary release of physical post-concussion symptoms, we suggest that compromised neurovascular coupling may be temporary restored to match CBF with surges in the metabolic demands of the brain. Our ongoing research attempts to identify specific brain regions that are most affected by cooling both in terms of alteration of CBF and functional connections (ASL data) and reduced connectivity (fMRI data).

This study has limitations. First, we must acknowledge the limited sample size (and its bias towards certain sports) and the need for the replication of these results. Second, longevity of clinical symptoms resolution after cooling may be partially influenced by the MRI environment, mainly acoustic noise, although all subjects were given proper noise protection. Additionally, consistent symptom reporting should be implemented in future studies. Finally, we are aware of potential problems regarding overcoming the protective effect of warm blood perfusion. When

the warm blood reaches the brain via the uncooled carotid arterial supply it may block the external cooling wave from advancing to the core of the brain (Dennis, Eberhart, Dulikravich, & Radons, 2003). However, more recent studies have documented the feasibility of WELKINS Cooling System, used in this study to successfully reduce brain temperature after 30 minutes of helmet application (Bagić et al., 2008; Wang et al., 2016). Additionally, CBF could be affected by various postural positions. Studies in healthy subjects have found greater CBF during standing than sitting or supine (Ouchi, Okada, Yoshikawa, Futatsubashi, & Nobezawa, 2001); however, these changes in postures do not appear to have a large functional consequence of CBF (Garrett, Pearson, & Subudhi, 2017).

Our ongoing research aimed at: a) directly assessing brain temperature decreases while cooling using MRS thermometry; and b) further exploring the phenomenon of neurovascular coupling and decoupling in concussed subjects via cooling while scanning. If proven, selective brain cooling could be an important clinical tool in the minimization of symptoms and pathological changes.

As observed in this study, the majority of concussed subjects reported temporary release of physical post-concussion symptoms, such as headache and dizziness. One explanation is that the paradoxical increase in CBF was adequate to meet the metabolic demands of the brain and therefore alleviate clinical symptoms. Dosing and duration of selective cooling needs to be studied further. The combined results of MRI, EEG, and VR suggest that the concussed brain responds differently to cooling than the normal controls. This suggests that the selective cooling system could be a diagnosis tool to help clinicians recognize the presence of injury and the presence of neurovascular decoupling.

Conclusion

Considering the existing evidence of “metabolic crisis” after concussive injury (e.g., reduced blood flow and increased demands for glucose etc., metabolism) (Giza & Hovda, 2001) and the observed increase of blood flow (ASL data) in concussed individuals after 30 minutes of brain cooling administration, we can conclude that brain cooling may potentially be implemented as a diagnostic tool in the acute phase of injury. After analysis, the combination of the three modalities suggest that selective brain cooling reveals differential responses between the concussed and control subjects. The overall decreases in VR scores, the increase of CBF, and the increases in delta frequency post-cooling in the concussed subjects could be indicators of concussive injury, re-emphasizing or exaggerating deficits that exist. Specifics, duration, frequency, time since injury (acute, sub-acute, chronic phases) as well as underlying mechanisms, surely should be explored in future studies.

References

- Alexander, D. G., Shuttleworth-Edwards, A. B., Kidd, M., & Malcolm, C. M. (2015). Mild traumatic brain injuries in early adolescent rugby players: Long-term neurocognitive and academic outcomes. *Brain Injury, 29*(9), 1113–1125.
<https://doi.org/10.3109/02699052.2015.1031699>
- Aronov, D., & Fee, M. S. (2011). Analyzing the dynamics of brain circuits with temperature: design and implementation of a miniature thermoelectric device. *Journal of Neuroscience Methods, 197*(1), 32–47. <https://doi.org/10.1016/j.jneumeth.2011.01.024>
- Bacher, A., Illievich, U. M., Fitzgerald, R., Ihra, G., & Spiss, C. K. (1997). Changes in oxygenation variables during progressive hypothermia in anesthetized patients. *Journal of Neurosurgical Anesthesiology, 9*(3), 205–210.
- Bagić, A., Theodore, W. H., Boudreau, E. A., Bonwetsch, R., Greenfield, J., Elkins, W., & Sato, S. (2008). Towards a non-invasive interictal application of hypothermia for treating seizures: a feasibility and pilot study. *Acta Neurologica Scandinavica, 118*(4), 240–244.
<https://doi.org/10.1111/j.1600-0404.2008.01008.x>
- Balkan, O., Virji-Babul, N., Miyakoshi, M., Makeig, S., & Garudadri, H. (2015). Source-domain spectral EEG analysis of sports-related concussion via Measure Projection Analysis. *Conference Proceedings: ... Annual International Conference of the IEEE Engineering in Medicine and Biology Society. IEEE Engineering in Medicine and Biology Society. Annual Conference, 2015*, 4053–4056. <https://doi.org/10.1109/EMBC.2015.7319284>
- Bartnik-Olson, B. L., Holshouser, B., Wang, H., Grube, M., Tong, K., Wong, V., & Ashwal, S. (2014). Impaired neurovascular unit function contributes to persistent symptoms after concussion: a pilot study. *Journal of Neurotrauma, 31*(17), 1497–1506.
<https://doi.org/10.1089/neu.2013.3213>

- Bayir, H., Adelson, P. D., Wisniewski, S. R., Shore, P., Lai, Y., Brown, D., ... Kochanek, P. M. (2009). Therapeutic hypothermia preserves antioxidant defenses after severe traumatic brain injury in infants and children. *Critical Care Medicine*, *37*(2), 689–695.
<https://doi.org/10.1097/CCM.0b013e318194abf2>
- Behzadi, Y., Restom, K., Liau, J., & Liu, T. T. (2007). A component based noise correction method (CompCor) for BOLD and perfusion based fMRI. *NeuroImage*, *37*(1), 90–101.
<https://doi.org/10.1016/j.neuroimage.2007.04.042>
- Chatzipanteli, K., Alonso, O. F., Kraydieh, S., & Dietrich, W. D. (2000). Importance of posttraumatic hypothermia and hyperthermia on the inflammatory response after fluid percussion brain injury: biochemical and immunocytochemical studies. *Journal of Cerebral Blood Flow and Metabolism: Official Journal of the International Society of Cerebral Blood Flow and Metabolism*, *20*(3), 531–542.
<https://doi.org/10.1097/00004647-200003000-00012>
- Clifton, G. L., Miller, E. R., Choi, S. C., Levin, H. S., McCauley, S., Smith, K. R., ... Schwartz, M. (2001). Lack of effect of induction of hypothermia after acute brain injury. *The New England Journal of Medicine*, *344*(8), 556–563.
<https://doi.org/10.1056/NEJM200102223440803>
- Clifton, Guy L., Valadka, A., Zygun, D., Coffey, C. S., Drever, P., Fourwinds, S., ... Okonkwo, D. O. (2011). Very early hypothermia induction in patients with severe brain injury (the National Acute Brain Injury Study: Hypothermia II): a randomised trial. *The Lancet. Neurology*, *10*(2), 131–139. [https://doi.org/10.1016/S1474-4422\(10\)70300-8](https://doi.org/10.1016/S1474-4422(10)70300-8)
- Clifton, W., Donnino, M., Fink, E., Romergryko, G., Golan, E., Kern, K., ... Zimmerman, J. (2015). Part 8: Post-Cardiac Arrest Care. American Heart Association. Retrieved from

<https://eccguidelines.heart.org/index.php/circulation/cpr-ecc-guidelines-2/part-8-post-cardiac-arrest-care/>

- Cortez, S. C., McIntosh, T. K., & Noble, L. J. (1989). Experimental fluid percussion brain injury: vascular disruption and neuronal and glial alterations. *Brain Research*, *482*(2), 271–282.
- Covaciu, L., Weis, J., Bengtsson, C., Allers, M., Lunderquist, A., Ahlström, H., & Rubertsson, S. (2011). Brain temperature in volunteers subjected to intranasal cooling. *Intensive Care Medicine*, *37*(8), 1277–1284. <https://doi.org/10.1007/s00134-011-2264-7>
- Crossley, S., Reid, J., McLatchie, R., Hayton, J., Clark, C., MacDougall, M., & Andrews, P. J. D. (2014). A systematic review of therapeutic hypothermia for adult patients following traumatic brain injury. *Critical Care (London, England)*, *18*(2), R75. <https://doi.org/10.1186/cc13835>
- De Gennaro, L., Marzano, C., Veniero, D., Moroni, F., Fratello, F., Curcio, G., ... Rossini, P. M. (2007). Neurophysiological correlates of sleepiness: a combined TMS and EEG study. *NeuroImage*, *36*(4), 1277–1287. <https://doi.org/10.1016/j.neuroimage.2007.04.013>
- Dennis, B. H., Eberhart, R. C., Dulikravich, G. S., & Radons, S. W. (2003). Finite-element simulation of cooling of realistic 3-D human head and neck. *Journal of Biomechanical Engineering*, *125*(6), 832–840.
- Dietrich, W. D., & Bramlett, H. M. (2010). THE EVIDENCE FOR HYPOTHERMIA AS A NEUROPROTECTANT IN TRAUMATIC BRAIN INJURY. *Neurotherapeutics : The Journal of the American Society for Experimental NeuroTherapeutics*, *7*(1), 43. <https://doi.org/10.1016/j.nurt.2009.10.015>
- Doll, H., Truebel, H., Kipfmueller, F., Schaefer, U., Neugebauer, E. A. M., Wirth, S., & Maegle, M. (2009). Pharyngeal selective brain cooling improves neurofunctional and

- neurocognitive outcome after fluid percussion brain injury in rats. *Journal of Neurotrauma*, 26(2), 235–242. <https://doi.org/10.1089/neu.2008.0741>
- Ellis, H. D. (1982). The effects of cold on the performance of serial choice reaction time and various discrete tasks. *Human Factors*, 24(5), 589–598.
<https://doi.org/10.1177/001872088202400509>
- Ellis, M. J., Leiter, J., Hall, T., McDonald, P. J., Sawyer, S., Silver, N., ... Essig, M. (2015). Neuroimaging findings in pediatric sports-related concussion. *Journal of Neurosurgery. Pediatrics*, 16(3), 241–247. <https://doi.org/10.3171/2015.1.PEDS14510>
- Gaoua, N., Racinais, S., Grantham, J., & El Massioui, F. (2011). Alterations in cognitive performance during passive hyperthermia are task dependent. *International Journal of Hyperthermia: The Official Journal of European Society for Hyperthermic Oncology, North American Hyperthermia Group*, 27(1), 1–9.
<https://doi.org/10.3109/02656736.2010.516305>
- Garrett, Z. K., Pearson, J., & Subudhi, A. W. (2017). Postural effects on cerebral blood flow and autoregulation. *Physiological Reports*, 5(4). <https://doi.org/10.14814/phy2.13150>
- Giza, C. C., & Hovda, D. A. (2001). The Neurometabolic Cascade of Concussion. *Journal of Athletic Training*, 36(3), 228–235.
- Giza, C. C., & Hovda, D. A. (2014). The New Neurometabolic Cascade of Concussion. *Neurosurgery*, 75(0 4), S24–S33. <https://doi.org/10.1227/NEU.0000000000000505>
- Giza, C. C., & Kutcher, J. S. (2014). An Introduction to Sports Concussions. *Continuum : Lifelong Learning in Neurology*, 20(6 Sports Neurology), 1545–1551.
<https://doi.org/10.1212/01.CON.0000458975.78766.11>

- Globus, M. Y., Alonso, O., Dietrich, W. D., Busto, R., & Ginsberg, M. D. (1995). Glutamate release and free radical production following brain injury: effects of posttraumatic hypothermia. *Journal of Neurochemistry*, *65*(4), 1704–1711.
- Grubenhoff, J. A., Deakne, S. J., Brou, L., Bajaj, L., Comstock, R. D., & Kirkwood, M. W. (2014). Acute concussion symptom severity and delayed symptom resolution. *Pediatrics*, *134*(1), 54–62. <https://doi.org/10.1542/peds.2013-2988>
- Gu, X., Wei, Z. Z., Espinera, A., Lee, J. H., Ji, X., Wei, L., ... Yu, S. P. (2015). Pharmacologically Induced Hypothermia Attenuates Traumatic Brain Injury in Neonatal Rats. *Experimental Neurology*, *267*, 135–142. <https://doi.org/10.1016/j.expneurol.2015.02.029>
- Haneef, Z., Levin, H. S., Frost, J. D., & Mizrahi, E. M. (2013). Electroencephalography and quantitative electroencephalography in mild traumatic brain injury. *Journal of Neurotrauma*, *30*(8), 653–656. <https://doi.org/10.1089/neu.2012.2585>
- Harris, B. A., Andrews, P. J. D., Marshall, I., Robinson, T. M., & Murray, G. D. (2008). Forced convective head cooling device reduces human cross-sectional brain temperature measured by magnetic resonance: a non-randomized healthy volunteer pilot study. *British Journal of Anaesthesia*, *100*(3), 365–372. <https://doi.org/10.1093/bja/aem405>
- Harris, B., Andrews, P. J. D., Murray, G. D., Forbes, J., Moseley, O., Harris, B., ... Moseley, O. (2012). *Systematic Review of Head Cooling in Adults After Traumatic Brain Injury and Stroke*. NIHR Journals Library.
- Helmich, I., Berger, A., & Lausberg, H. (2016). Neural Control of Posture in Individuals with Persisting Postconcussion Symptoms. *Medicine and Science in Sports and Exercise*, *48*(12), 2362–2369. <https://doi.org/10.1249/MSS.0000000000001028>

- Jia, F., Mao, Q., Liang, Y.-M., & Jiang, J.-Y. (2009). Effect of post-traumatic mild hypothermia on hippocampal cell death after traumatic brain injury in rats. *Journal of Neurotrauma*, 26(2), 243–252. <https://doi.org/10.1089/neu.2008.0670>
- Johnson, B., Hallett, M., & Slobounov, S. (2015). Follow-up evaluation of oculomotor performance with fMRI in the subacute phase of concussion. *Neurology*, 85(13), 1163–1166. <https://doi.org/10.1212/WNL.0000000000001968>
- Johnson, B., Zhang, K., Gay, M., Horovitz, S., Hallett, M., Sebastianelli, W., & Slobounov, S. (2012). Alteration of brain default network in subacute phase of injury in concussed individuals: resting-state fMRI study. *NeuroImage*, 59(1), 511–518. <https://doi.org/10.1016/j.neuroimage.2011.07.081>
- Katayama, Y., Becker, D. P., Tamura, T., & Hovda, D. A. (1990). Massive increases in extracellular potassium and the indiscriminate release of glutamate following concussive brain injury. *Journal of Neurosurgery*, 73(6), 889–900. <https://doi.org/10.3171/jns.1990.73.6.0889>
- Koizumi, H., & Povlishock, J. T. (1998). Posttraumatic hypothermia in the treatment of axonal damage in an animal model of traumatic axonal injury. *Journal of Neurosurgery*, 89(2), 303–309. <https://doi.org/10.3171/jns.1998.89.2.0303>
- Ku, Y. T., Montgomery, L. D., & Webbon, B. W. (1996). Hemodynamic and thermal responses to head and neck cooling in men and women. *American Journal of Physical Medicine & Rehabilitation*, 75(6), 443–450.
- Kuluz, J. W., Prado, R., Chang, J., Ginsberg, M. D., Schleien, C. L., & Busto, R. (1993). Selective brain cooling increases cortical cerebral blood flow in rats. *The American Journal of Physiology*, 265(3 Pt 2), H824-827.

- Langlois, J. A., Rutland-Brown, W., & Wald, M. M. (2006). The epidemiology and impact of traumatic brain injury: a brief overview. *The Journal of Head Trauma Rehabilitation*, *21*(5), 375–378.
- Laptook, A. R., Shalak, L., & Corbett, R. J. (2001). Differences in brain temperature and cerebral blood flow during selective head versus whole-body cooling. *Pediatrics*, *108*(5), 1103–1110.
- Lee, J. H., Wei, L., Gu, X., Wei, Z., Dix, T. A., & Yu, S. P. (2014). Therapeutic effects of pharmacologically induced hypothermia against traumatic brain injury in mice. *Journal of Neurotrauma*, *31*(16), 1417–1430. <https://doi.org/10.1089/neu.2013.3251>
- Lee, R. R., & Huang, M. (2014). Magnetoencephalography in the diagnosis of concussion. *Progress in Neurological Surgery*, *28*, 94–111. <https://doi.org/10.1159/000358768>
- Lewén, A., Matz, P., & Chan, P. H. (2000). Free radical pathways in CNS injury. *Journal of Neurotrauma*, *17*(10), 871–890.
- Lilja, G., Nielsen, N., Friberg, H., Horn, J., Kjaergaard, J., Nilsson, F., ... Cronberg, T. (2015). Cognitive function in survivors of out-of-hospital cardiac arrest after target temperature management at 33°C versus 36°C. *Circulation*, *131*(15), 1340–1349. <https://doi.org/10.1161/CIRCULATIONAHA.114.014414>
- Liu, L., & Yenari, M. A. (2007). Therapeutic hypothermia: neuroprotective mechanisms. *Frontiers in Bioscience: A Journal and Virtual Library*, *12*, 816–825.
- Liu, W. G., Qiu, W. S., Zhang, Y., Wang, W. M., Lu, F., & Yang, X. F. (2006). Effects of selective brain cooling in patients with severe traumatic brain injury: a preliminary study. *The Journal of International Medical Research*, *34*(1), 58–64.

- Lotocki, G., de Rivero Vaccari, J. P., Perez, E. R., Sanchez-Molano, J., Furones-Alonso, O., Bramlett, H. M., & Dietrich, W. D. (2009). Alterations in blood-brain barrier permeability to large and small molecules and leukocyte accumulation after traumatic brain injury: effects of post-traumatic hypothermia. *Journal of Neurotrauma*, *26*(7), 1123–1134. <https://doi.org/10.1089/neu.2008.0802>
- Lovell, M. R., Collins, M. W., Iverson, G. L., Johnston, K. M., & Bradley, J. P. (2004). Grade 1 or “ding” concussions in high school athletes. *The American Journal of Sports Medicine*, *32*(1), 47–54. <https://doi.org/10.1177/0363546503260723>
- Lyeth, B. G., Jiang, J. Y., Robinson, S. E., Guo, H., & Jenkins, L. W. (1993). Hypothermia blunts acetylcholine increase in CSF of traumatically brain injured rats. *Molecular and Chemical Neuropathology*, *18*(3), 247–256.
- Maeda, T., Katayama, Y., Kawamata, T., & Yamamoto, T. (1998). Mechanisms of excitatory amino acid release in contused brain tissue: effects of hypothermia and in situ administration of Co²⁺ on extracellular levels of glutamate. *Journal of Neurotrauma*, *15*(9), 655–664. <https://doi.org/10.1089/neu.1998.15.655>
- Makeig, S., & Jung, T. P. (1995). Changes in alertness are a principal component of variance in the EEG spectrum. *Neuroreport*, *7*(1), 213–216.
- Mäkinen, T. M. (2007). Human cold exposure, adaptation, and performance in high latitude environments. *American Journal of Human Biology: The Official Journal of the Human Biology Council*, *19*(2), 155–164. <https://doi.org/10.1002/ajhb.20627>
- Marion, D. W., Penrod, L. E., Kelsey, S. F., Obrist, W. D., Kochanek, P. M., Palmer, A. M., ... DeKosky, S. T. (1997). Treatment of Traumatic Brain Injury with Moderate

- Hypothermia. *New England Journal of Medicine*, 336(8), 540–546.
<https://doi.org/10.1056/NEJM199702203360803>
- Marmarou, A. (2007). A review of progress in understanding the pathophysiology and treatment of brain edema. *Neurosurgical Focus*, 22(5), E1.
- Matsui, T., & Kakeda, T. (2008). IL-10 production is reduced by hypothermia but augmented by hyperthermia in rat microglia. *Journal of Neurotrauma*, 25(6), 709–715.
<https://doi.org/10.1089/neu.2007.0482>
- Maugans, T. A., Farley, C., Altaye, M., Leach, J., & Cecil, K. M. (2012). Pediatric sports-related concussion produces cerebral blood flow alterations. *Pediatrics*, 129(1), 28–37.
<https://doi.org/10.1542/peds.2011-2083>
- Maxwell, W. L., Donnelly, S., Sun, X., Fenton, T., Puri, N., & Graham, D. I. (1999). Axonal cytoskeletal responses to nondisruptive axonal injury and the short-term effects of posttraumatic hypothermia. *Journal of Neurotrauma*, 16(12), 1225–1234.
<https://doi.org/10.1089/neu.1999.16.1225>
- McCrory, P., Meeuwisse, W. H., Aubry, M., Cantu, R. C., Dvořák, J., Echemendia, R. J., ... Turner, M. (2013). Consensus statement on concussion in sport--the 4th International Conference on Concussion in Sport held in Zurich, November 2012. *PM & R: The Journal of Injury, Function, and Rehabilitation*, 5(4), 255–279.
<https://doi.org/10.1016/j.pmrj.2013.02.012>
- McIntosh, T. K. (1993). Novel pharmacologic therapies in the treatment of experimental traumatic brain injury: a review. *Journal of Neurotrauma*, 10(3), 215–261.
<https://doi.org/10.1089/neu.1993.10.215>

- Meaney, D. F., & Smith, D. H. (2011). Biomechanics of Concussion. *Clinics in Sports Medicine*, 30(1), 19–vii. <https://doi.org/10.1016/j.csm.2010.08.009>
- Meier, T. B., Bellgowan, P. S. F., Singh, R., Kuplicki, R., Polanski, D. W., & Mayer, A. R. (2015). Recovery of cerebral blood flow following sports-related concussion. *JAMA Neurology*, 72(5), 530–538. <https://doi.org/10.1001/jamaneurol.2014.4778>
- Micheva, K. D., & Smith, S. J. (2005). Strong effects of subphysiological temperature on the function and plasticity of mammalian presynaptic terminals. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience*, 25(33), 7481–7488. <https://doi.org/10.1523/JNEUROSCI.1801-05.2005>
- Minamisawa, H., Smith, M. L., & Siesjö, B. K. (1990). The effect of mild hyperthermia and hypothermia on brain damage following 5, 10, and 15 minutes of forebrain ischemia. *Annals of Neurology*, 28(1), 26–33. <https://doi.org/10.1002/ana.410280107>
- Miyachi, T., Wei, E. P., & Povlishock, J. T. (2014). Evidence for the Therapeutic Efficacy of Either Mild Hypothermia or Oxygen Radical Scavengers after Repetitive Mild Traumatic Brain Injury. *Journal of Neurotrauma*, 31(8), 773–781. <https://doi.org/10.1089/neu.2013.3181>
- Montenigro, P. H., Alosco, M. L., Martin, B. M., Daneshvar, D. H., Mez, J., Chaisson, C. E., ... Tripodis, Y. (2017). Cumulative Head Impact Exposure Predicts Later-Life Depression, Apathy, Executive Dysfunction, and Cognitive Impairment in Former High School and College Football Players. *Journal of Neurotrauma*, 34(2), 328–340. <https://doi.org/10.1089/neu.2016.4413>

- Montgomery, E. A., Fenton, G. W., McClelland, R. J., MacFlynn, G., & Rutherford, W. H. (1991). The psychobiology of minor head injury. *Psychological Medicine*, *21*(2), 375–384.
- Moore, E. M., Nichol, A. D., Bernard, S. A., & Bellomo, R. (2011). Therapeutic hypothermia: benefits, mechanisms and potential clinical applications in neurological, cardiac and kidney injury. *Injury*, *42*(9), 843–854. <https://doi.org/10.1016/j.injury.2011.03.027>
- Murphy, K., Birn, R. M., Handwerker, D. A., Jones, T. B., & Bandettini, P. A. (2009). The impact of global signal regression on resting state correlations: are anti-correlated networks introduced? *NeuroImage*, *44*(3), 893–905. <https://doi.org/10.1016/j.neuroimage.2008.09.036>
- Oddo, M., Frangos, S., Milby, A., Chen, I., Maloney-Wilensky, E., Murtrie, E. M., ... Levine, J. M. (2009). Induced Normothermia Attenuates Cerebral Metabolic Distress in Patients With Aneurysmal Subarachnoid Hemorrhage and Refractory Fever. *Stroke*, *40*(5), 1913–1916. <https://doi.org/10.1161/STROKEAHA.108.534115>
- Ouchi, Y., Okada, H., Yoshikawa, E., Futatsubashi, M., & Nobezawa, S. (2001). Absolute changes in regional cerebral blood flow in association with upright posture in humans: an orthostatic PET study. *Journal of Nuclear Medicine: Official Publication, Society of Nuclear Medicine*, *42*(5), 707–712.
- Ponz, I., Lopez-de-Sa, E., Armada, E., Caro, J., Blazquez, Z., Rosillo, S., ... Lopez-Sendon, J. L. (2016). Influence of the temperature on the moment of awakening in patients treated with therapeutic hypothermia after cardiac arrest. *Resuscitation*, *103*, 32–36. <https://doi.org/10.1016/j.resuscitation.2016.03.017>

- Racinais, S., Gaoua, N., & Grantham, J. (2008). Hyperthermia impairs short-term memory and peripheral motor drive transmission. *The Journal of Physiology*, *586*(19), 4751–4762.
<https://doi.org/10.1113/jphysiol.2008.157420>
- Raichle, M. E. (2015). The brain's default mode network. *Annual Review of Neuroscience*, *38*, 433–447. <https://doi.org/10.1146/annurev-neuro-071013-014030>
- Robb Swan, A., Nichols, S., Drake, A., Angeles, A., Diwakar, M., Song, T., ... Huang, M.-X. (2015). Magnetoencephalography Slow-Wave Detection in Patients with Mild Traumatic Brain Injury and Ongoing Symptoms Correlated with Long-Term Neuropsychological Outcome. *Journal of Neurotrauma*, *32*(19), 1510–1521.
<https://doi.org/10.1089/neu.2014.3654>
- Rosomoff, H. L., & Holaday, D. A. (1954). Cerebral blood flow and cerebral oxygen consumption during hypothermia. *The American Journal of Physiology*, *179*(1), 85–88.
- Rowson, S., Brolinson, G., Goforth, M., Dietter, D., & Duma, S. (2009). Linear and angular head acceleration measurements in collegiate football. *Journal of Biomechanical Engineering*, *131*(6), 061016. <https://doi.org/10.1115/1.3130454>
- Rowson, S., & Duma, S. M. (2013). Brain Injury Prediction: Assessing the Combined Probability of Concussion Using Linear and Rotational Head Acceleration. *Annals of Biomedical Engineering*, *41*(5), 873–882. <https://doi.org/10.1007/s10439-012-0731-0>
- Roy, C. S., & Sherrington, C. S. (1890). On the Regulation of the Blood-supply of the Brain. *The Journal of Physiology*, *11*(1–2), 85–158.17.
- Scherbel, U., Raghupathi, R., Nakamura, M., Saatman, K. E., Trojanowski, J. Q., Neugebauer, E., ... McIntosh, T. K. (1999). Differential acute and chronic responses of tumor necrosis

- factor-deficient mice to experimental brain injury. *Proceedings of the National Academy of Sciences of the United States of America*, 96(15), 8721–8726.
- Schreckinger, M., & Marion, D. W. (2009). Contemporary management of traumatic intracranial hypertension: is there a role for therapeutic hypothermia? *Neurocritical Care*, 11(3), 427–436. <https://doi.org/10.1007/s12028-009-9256-2>
- Shiozaki, T., Hayakata, T., Taneda, M., Nakajima, Y., Hashiguchi, N., Fujimi, S., ... Sugimoto, H. (2001). A multicenter prospective randomized controlled trial of the efficacy of mild hypothermia for severely head injured patients with low intracranial pressure. Mild Hypothermia Study Group in Japan. *Journal of Neurosurgery*, 94(1), 50–54. <https://doi.org/10.3171/jns.2001.94.1.0050>
- Slobounov, S., Bazarian, J., Bigler, E., Cantu, R., Hallett, M., Harbaugh, R., ... Vagnozzi, R. (2014). Sports-related concussion: ongoing debate. *British Journal of Sports Medicine*, 48(2), 75–76. <https://doi.org/10.1136/bjsports-2013-092362>
- Slobounov, S., Cao, C., Jaiswal, N., & Newell, K. M. (2009). Neural basis of postural instability identified by VTC and EEG. *Experimental Brain Research*, 199(1), 1–16. <https://doi.org/10.1007/s00221-009-1956-5>
- Slobounov, S. M., Teel, E., & Newell, K. M. (2013). Modulation of cortical activity in response to visually induced postural perturbation: combined VR and EEG study. *Neuroscience Letters*, 547, 6–9. <https://doi.org/10.1016/j.neulet.2013.05.001>
- Slobounov, S. M., Zhang, K., Pennell, D., Ray, W., Johnson, B., & Sebastianelli, W. (2010). Functional abnormalities in normally appearing athletes following mild traumatic brain injury: a functional MRI study. *Experimental Brain Research*, 202(2), 341–354. <https://doi.org/10.1007/s00221-009-2141-6>

- Smith, A. L., Rosenkrantz, T. S., & Fitch, R. H. (2016). Effects of Sex and Mild Intrainsult Hypothermia on Neuropathology and Neural Reorganization following Neonatal Hypoxic Ischemic Brain Injury in Rats. *Neural Plasticity*, 2016. <https://doi.org/10.1155/2016/2585230>
- Soukup, J., Zauner, A., Doppenberg, E. M. R., Menzel, M., Gilman, C., Bullock, R., & Young, H. F. (2002). Relationship between brain temperature, brain chemistry and oxygen delivery after severe human head injury: the effect of mild hypothermia. *Neurological Research*, 24(2), 161–168. <https://doi.org/10.1179/016164102101199710>
- Sun, G., Li, L., Li, M., & Jiang, Q. (2011). Hyperthermia impaired pre-attentive processing: an auditory MMN study. *Neuroscience Letters*, 502(2), 94–98. <https://doi.org/10.1016/j.neulet.2011.07.029>
- Sun, G., Yang, X., Jiang, Q., Liu, K., Li, B., Li, L., ... Li, M. (2012). Hyperthermia impairs the executive function using the Attention Network Test. *International Journal of Hyperthermia: The Official Journal of European Society for Hyperthermic Oncology, North American Hyperthermia Group*, 28(7), 621–626. <https://doi.org/10.3109/02656736.2012.705217>
- Tak, S., Polimeni, J. R., Wang, D. J. J., Yan, L., & Chen, J. J. (2015). Associations of resting-state fMRI functional connectivity with flow-BOLD coupling and regional vasculature. *Brain Connectivity*, 5(3), 137–146. <https://doi.org/10.1089/brain.2014.0299>
- Takata, T., Nabetani, M., & Okada, Y. (1997). Effects of hypothermia on the neuronal activity, [Ca²⁺]_i accumulation and ATP levels during oxygen and/or glucose deprivation in hippocampal slices of guinea pigs. *Neuroscience Letters*, 227(1), 41–44.

- Talavage, T. M., Nauman, E. A., Breedlove, E. L., Yoruk, U., Dye, A. E., Morigaki, K. E., ...
Leverenz, L. J. (2014). Functionally-detected cognitive impairment in high school
football players without clinically-diagnosed concussion. *Journal of Neurotrauma*, *31*(4),
327–338. <https://doi.org/10.1089/neu.2010.1512>
- Talavage, T. M., Nauman, E. A., & Leverenz, L. J. (2016). The Role of Medical Imaging in the
Recharacterization of Mild Traumatic Brain Injury Using Youth Sports as a Laboratory.
Frontiers in Neurology, *6*. <https://doi.org/10.3389/fneur.2015.00273>
- Taschenberger, H., & von Gersdorff, H. (2000). Fine-tuning an auditory synapse for speed and
fidelity: developmental changes in presynaptic waveform, EPSC kinetics, and synaptic
plasticity. *The Journal of Neuroscience: The Official Journal of the Society for
Neuroscience*, *20*(24), 9162–9173.
- Tebano, M. T., Cameroni, M., Gallozzi, G., Loizzo, A., Palazzino, G., Pezzini, G., & Ricci, G. F.
(1988). EEG spectral analysis after minor head injury in man. *Electroencephalography
and Clinical Neurophysiology*, *70*(2), 185–189.
- Teel, E. F., Gay, M. R., Arnett, P. A., & Slobounov, S. M. (2016). Differential Sensitivity
Between a Virtual Reality Balance Module and Clinically Used Concussion Balance
Modalities. *Clinical Journal of Sport Medicine: Official Journal of the Canadian
Academy of Sport Medicine*, *26*(2), 162–166.
<https://doi.org/10.1097/JSM.0000000000000210>
- Teel, E. F., & Slobounov, S. M. (2015). Validation of a virtual reality balance module for use in
clinical concussion assessment and management. *Clinical Journal of Sport Medicine:
Official Journal of the Canadian Academy of Sport Medicine*, *25*(2), 144–148.
<https://doi.org/10.1097/JSM.0000000000000109>

- Thatcher, R. W., Walker, R. A., Gerson, I., & Geisler, F. H. (1989). EEG discriminant analyses of mild head trauma. *Electroencephalography and Clinical Neurophysiology*, 73(2), 94–106.
- Tinguely, G., Finelli, L. A., Landolt, H.-P., Borbély, A. A., & Achermann, P. (2006). Functional EEG topography in sleep and waking: state-dependent and state-independent features. *NeuroImage*, 32(1), 283–292. <https://doi.org/10.1016/j.neuroimage.2006.03.017>
- Titus, D. J., Furones, C., Atkins, C. M., & Dietrich, W. D. (2015). Emergence of cognitive deficits after mild traumatic brain injury due to hyperthermia. *Experimental Neurology*, 263, 254–262. <https://doi.org/10.1016/j.expneurol.2014.10.020>
- Truettner, J. S., Alonso, O. F., Bramlett, H. M., & Dietrich, W. D. (2011). Therapeutic hypothermia alters microRNA responses to traumatic brain injury in rats. *Journal of Cerebral Blood Flow and Metabolism: Official Journal of the International Society of Cerebral Blood Flow and Metabolism*, 31(9), 1897–1907. <https://doi.org/10.1038/jcbfm.2011.33>
- Urbano, L. A., & Oddo, M. (2012). Therapeutic hypothermia for traumatic brain injury. *Current Neurology and Neuroscience Reports*, 12(5), 580–591. <https://doi.org/10.1007/s11910-012-0304-5>
- Van Orden, K. F., Ahlers, S. T., Thomas, J. R., House, J. F., & Schrot, J. (1990). Moderate cold exposure shortens evoked potential latencies in humans. *Aviation, Space, and Environmental Medicine*, 61(7), 636–639.
- Verlade, F., Fisher, D., & Hovda, D. A. (1992). Fluid percussion injury induces prolonged changes in cerebral blood flow, 9(402).

- Volgushev, M., Vidyasagar, T. R., Chistiakova, M., & Eysel, U. T. (2000). Synaptic transmission in the neocortex during reversible cooling. *Neuroscience*, *98*(1), 9–22.
- Walter, B., Bauer, R., Kuhnen, G., Fritz, H., & Zwiener, U. (2000). Coupling of Cerebral Blood Flow and Oxygen Metabolism in Infant Pigs during Selective Brain Hypothermia. *Journal of Cerebral Blood Flow & Metabolism*, *20*(8), 1215–1224.
<https://doi.org/10.1097/00004647-200008000-00007>
- Wang, H., Olivero, W., Lanzino, G., Elkins, W., Rose, J., Honings, D., ... Wang, D. (2004). Rapid and selective cerebral hypothermia achieved using a cooling helmet. *Journal of Neurosurgery*, *100*(2), 272–277. <https://doi.org/10.3171/jns.2004.100.2.0272>
- Wang, H., Wang, B., Jackson, K., Miller, C. M., Hasadsri, L., Llano, D., ... Sutton, B. (2015). A novel head-neck cooling device for concussion injury in contact sports. *Translational Neuroscience*, *6*(1), 20–31. <https://doi.org/10.1515/tnsci-2015-0004>
- Wang, J., Licht, D. J., Jahng, G.-H., Liu, C.-S., Rubin, J. T., Haselgrove, J., ... Detre, J. A. (2003). Pediatric perfusion imaging using pulsed arterial spin labeling. *Journal of Magnetic Resonance Imaging: JMRI*, *18*(4), 404–413. <https://doi.org/10.1002/jmri.10372>
- Wang, Y., Nelson, L. D., LaRoche, A. A., Pfaller, A. Y., Nencka, A. S., Koch, K. M., & McCrea, M. A. (2016). Cerebral Blood Flow Alterations in Acute Sport-Related Concussion. *Journal of Neurotrauma*, *33*(13), 1227–1236.
<https://doi.org/10.1089/neu.2015.4072>
- Williams, G. R., & Spencer, F. C. (1958). The clinical use of hypothermia following cardiac arrest. *Annals of Surgery*, *148*(3), 462–468.

- Wood, T., Osredkar, D., Puchades, M., Maes, E., Falck, M., Flatebø, T., ... Thoresen, M. (2016). Treatment temperature and insult severity influence the neuroprotective effects of therapeutic hypothermia. *Scientific Reports*, *6*, 23430. <https://doi.org/10.1038/srep23430>
- Yamakami, I., & McIntosh, T. (1989). Effects of Traumatic Brain Injury on Regional Cerebral Blood Flow in Rats as Measured with Radiolabeled Microspheres, *9*(1), 117–124.
- Yoshino, A., Hovda, D. A., Kawamata, T., Katayama, Y., & Becker, D. P. (1991). Dynamic changes in local cerebral glucose utilization following cerebral concussion in rats: evidence of a hyper- and subsequent hypometabolic state. *Brain Research*, *561*(1), 106–119.
- Yuan, X., Prough, D., Smith, T., & DeWitt, D. (1988). The effects of traumatic brain injury on regional cerebral blood flow in rats, *5*, 289–301.
- Zauner, A., & Muizelaar, J. P. (1997). Brain metabolism and cerebral blood flow. In *Head Injury* (pp. 89–99). London: Chapman & Hall. Retrieved from <https://www.nationalneurotraumasociety.org/images/media/society-information/head-injury-reilly-and-bullock/Chap05.pdf>
- Zhang, K., Johnson, B., Pennell, D., Ray, W., Sebastianelli, W., & Slobounov, S. (2010). Are functional deficits in concussed individuals consistent with white matter structural alterations: combined FMRI & DTI study. *Experimental Brain Research*, *204*(1), 57–70. <https://doi.org/10.1007/s00221-010-2294-3>
- Zhang, Kai, Johnson, B., Gay, M., Horovitz, S. G., Hallett, M., Sebastianelli, W., & Slobounov, S. (2012). Default mode network in concussed individuals in response to the YMCA physical stress test. *Journal of Neurotrauma*, *29*(5), 756–765. <https://doi.org/10.1089/neu.2011.2125>

Zhu, D. C., Covassin, T., Nogle, S., Doyle, S., Russell, D., Pearson, R. L., ... Kaufman, D. I.

(2015). A potential biomarker in sports-related concussion: brain functional connectivity alteration of the default-mode network measured with longitudinal resting-state fMRI over thirty days. *Journal of Neurotrauma*, 32(5), 327–341.

<https://doi.org/10.1089/neu.2014.3413>