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THE EFFECTS OF CONCUSSION ON MOTOR NEURON REFLEX EXCITABILITY

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THE EFFECTS OF CONCUSSION ON MOTOR NEURON REFLEX
EXCITABILITY

BY

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ABSTRACT

Objective: Individuals who participate in high impact sports are prone to concussive injuries. Assessments used to measure concussion severity mainly examine cognitive domains and only minimally assess the motor system. Testing the motor system is important for athletes whose performance and safety rely heavily on motor control. To our knowledge, there are limited studies assessing motor dysfunction at the spinal level. The goals of this study were to assess the effect that a head injury has on 1) resting spinal motor neuron reflex excitability (MNRE) and 2) MNRE levels over time when preparing for a volitional movement (Motor Preparation).

Participants and Methodology: Sixty-four participants were recruited from high risk club sports teams at The University of Rhode Island and received baseline reflex excitability testing. MNRE was assessed using the standardized soleus H-reflex technique (Riego and Ruegg, 1987). Motor preparation (MP) was evaluated by randomly eliciting a series of H-reflexes before and after a "GO" signal was given to the participant. H-reflexes were elicited at 11 sampling times from 300ms before to 200ms after the “GO” signal at 50ms intervals. Three evoked H-reflex waves were measured and averaged for each sampling time. H-reflexes were elicited at 15-25% of M_max, amplified 1000X and digitized at a sampling frequency of 4000Hz. Follow up experiments were conducted on four participants who reported a head or neck injury that may have resulted in concussion. The returning participants completed three post-injury evaluations at three separate times, 2-3 (P1), 6-12 (P2) and 13+ (P3) days post injury. Sixty-four participants were baseline tested. Data analyses were conducted on these four data sets. The change in H-reflex amplitude was used to assess spinal
MNRE at rest and during MP. Descriptive statistics, paired t-tests and Cohen D effect size (ES) were used to detect changes between pre- and post- concussion values.

**Results:** Resting MNRE showed a moderate to large reduction in post-concussion values when compared with baseline for two of the three post-injury testing sessions (P1, ES = 0.448; P2 ES=0.842). By the third follow up study, resting MNRE was approaching values similar to those obtained at baseline (P3, ES= 0.025) MP showed a moderate inhibition (~25% decline) of the H-reflex across all sampling times for the P2 testing session (ES= 0.45-0.76). This flat depressive response was not characteristic of the normal response observed at baseline testing.

**Conclusion:** These results show that head injury may affect normal MNRE at rest and modulation of MNRE in preparation for volitional movement. The inhibition of reflex activity appears to continue until 6-12 days post injury. If soleus muscle findings in this study are extrapolated to other muscles, our results suggest that mTBI/concussion may contribute to decreased motor function that can last days to weeks after injury. Therefore, we suggest athletes who sustain a concussion have a full motor evaluation before being allowed to return to play or wait approximately 12 days after injury. Further study is needed to determine what the functional outcome our results may impose on sports performance.
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# TABLE OF CONTENTS

ABSTRACT ................................................................................................................................. ii

ACKNOWLEDGEMENTS ........................................................................................................ iv

TABLE OF CONTENTS ............................................................................................................ v

LIST OF FIGURES .................................................................................................................. vi

LIST OF TABLES ................................................................................................................... vii

INTRODUCTION ..................................................................................................................... 1

METHODOLOGY ...................................................................................................................... 8
  
  Participants .............................................................................................................................. 8

  Electromyography (EMG) ..................................................................................................... 9

  H-Reflex ............................................................................................................................... 9

  Force Transduction .......................................................................................................... 10

  Motor Preparation .......................................................................................................... 11

  Procedure ......................................................................................................................... 11

  Statistical Analysis ......................................................................................................... 15

RESULTS ................................................................................................................................ 17

DISCUSSION ........................................................................................................................... 19

Figure 1a-c ............................................................................................................................ 24

Figure 2 .................................................................................................................................. 25

Figure 3 .................................................................................................................................. 26

Figure 4 .................................................................................................................................. 27

Figure 5 .................................................................................................................................. 28

Figure 6 .................................................................................................................................. 29

Figure 7 .................................................................................................................................. 30

Figure 8 .................................................................................................................................. 31

Table 1 .................................................................................................................................... 32

BIBLIOGRAPHY ....................................................................................................................... 33
LIST OF FIGURES

Figure 1a-c: 1a: Example of EMG output wave for Mmax, 1b: Example of EMG output wave for the H-reflex with the corresponding M-wave, 1c: Example of EMG output wave for the H-reflex recording set at stimulus intensity for H_{test} ................................24

Figure 2: Bar graph representation of the average MNRE measured as the H_{test}/Mmax amplitude in mV at each testing date. Change in H-reflex amplitude was quantified by calculating Cohen’s d effect size: P1 ES= 0.50, P2 ES= 0.82, P3 ES= 0.01. * p-value ≤ 0.05 ................................................25

Figure 3: Mean H_{test}/Mmax amplitude (mV) on each test date plotted as a function of sampling time (msec) ........................................................................................................26

Figure 4: Motor preparation response curve obtained from participant a2. H_{test}/Mmax amplitude (mV) plotted as a function of sampling time (msec) ........................................27

Figure 5: Motor preparation response curve obtained from participant B1. H_{test}/Mmax amplitude (mV) plotted as a function of sampling time (msec) ........................................28

Figure 6: Motor preparation response curve obtained from participant l2. H_{test}/Mmax amplitude (mV) plotted as a function of sampling time (msec) ........................................29

Figure 7: Motor preparation response curve obtained from participant w2. H_{test}/Mmax amplitude (mV) plotted as a function of sampling time (msec) ........................................30

Figure 8: Histogram representation of H-reflex amplitude (mV) for sampling times: -100 msec, -50 msec, 100 msec, and 150 msec. Negative values indicate sampling times before the presentation of the GO_{sig}. The differences in amplitude were quantified using Cohen’s d effect size. -100 msec ES= 0.567, -50 msec ES= 0.764, 100 msec ES= 0.657, and 150 msec ES= 0.486 ........................................31
LIST OF TABLES

Table 1: Demographic table on four returning participants……………………32
INTRODUCTION

The incidence of concussion in athletes has become an increasing concern as information regarding negative consequences from head injuries has become more public (McKee, 2014). Athletes who experience multiple concussions are at a higher risk for future and more severe traumatic brain injuries (TBI). The Centers for Disease Control and Prevention have found that at least 300,000 athletes a year suffer from a concussion in the United States alone and 1.6 to 3.8 million concussions occur during sports related or recreational activities (McKee, 2014). In 2010 the estimated cost of TBI was about $76.5 billion dollars in direct and indirect medical expenses. The difficulties associated with diagnosis and assessment of concussion make known incidence rates an under-estimation of their true occurrence (Moser et al., 2007; Johnson, Kegel, and Collins 2011; Putukian, 2011).

Concussion is a form of diffuse mild traumatic brain injury (mTBI) caused by a mechanical force to the body resulting in rapid acceleration and deceleration of the brain. The neuronal tissue becomes distorted and axonal shearing may occur throughout brain (Moser et al., 2007; Putukian, 2011; Ling, Hardy, and Zetterberg, 2015). This injury is followed by clinical symptoms that in many cases are not related to structural damage (Johnson, Kegel, and Collins 2011; Putukian, 2011; McCrory et al., 2012). Common symptoms include: headaches, dizziness, visual disturbance, fatigue, change in mental status, change in sleep patterns, and altered motor control (Giza and Hovda, 2001; Thomas et al., 2015). The accurate diagnosis of a concussion is limited by its dependency on self-report, its covert symptomatology, and the various ways concussion symptoms may present themselves across individuals (Johnson,
Kegel, and Collins 2011; Putukian, 2011; Brooks et al., 2016). Previously published literature on sports related concussion has focused on cognitive and emotional dysfunction following injury. Less information is known about the connection between concussion and motor dysfunction even though an athlete’s career and performance are dependent on enhanced motor control.

Recent concerns about the acute and long-term cognitive implications of concussions have resulted in an increase of computerized neuropsychological tests. These tests measure the cognitive domains most commonly affected by concussion: attention, working memory, visual motor speed, and reaction time (Moser et al., 2007; Johnson, Kegel, and Collins 2011; Livingston et al., 2012). Neuropsychological tests have become more common because they are meant to be an objective and more reliable method for assessing concussion severity compared to assessments using symptom inventories. An individual’s performance on these neuropsychological tests is also used to develop a plan of action for recovery, particularly in the athletic community. The neuropsychological tests used to measure concussion severity only minimally assess motor system function via static balance testing (Moser et al., 2007; Putukian, 2011). No standardized neurophysiological method for assessing concussion severity and monitoring recovery currently exists (Livingston et al., 2012). Clinically, overlooking silent deficits in motor function are potentially putting athletes in danger when returning to play (Thomas et al., 2015; Brooks et al., 2016). The influence concussion has on the motor system should be investigated further in this population to maximize the safety of athletes during return to play decisions.
A persistent decline in athletic performance has been documented in athletes who have sustained a concussion. Wasserman et al. (2015) found that athletes who sustained a concussion had a lower batting average when compared to healthy controls and when compared to their batting averages prior to injury. Recent studies demonstrated that athletes who experienced a concussion adopted a more conservative gait and experienced reduced visuospatial acuity. They are also more likely to sustain successive injuries following a previously reported concussion (Giza and Hovda, 2012; Moser et al., 2007). Brooks et al. (2016) conducted a retrospective cohort study on injury data obtained from a university sports injury monitoring database. They found that athletes were at increased odds of experiencing a lower extremity musculoskeletal injury within 90 days of returning to play. Increased rates of non-impact related injuries in this study were associated with altered motor control, conservative gait, and a decreased capacity for motor planning in otherwise asymptomatic individuals. Reduced implicit motor learning in concussed athletes suggested by Beaumont et al. (2012) may be a contributing factor in poor athletic performance and errors in motor execution.

The nervous system’s pathophysiological response to brain injury is thought to be the basis of observed functional disturbances. The acute period immediately following injury is characterized by a rapid flux of charged ions and neurotransmitters; referred to as a neurometabolic cascade (Giza and Hovda, 2001; Moser et al., 2007; Ling, Hardy, and Zetterberg, 2015). The rapid alteration of metabolic homeostasis influences the efficiency of communication between areas of the nervous system (Giza
Electrophysiological techniques are sensitive enough to detect changes in cortical activity after a concussion.

Studies have attempted to understand the relationship between motor function and head injury using transcortical magnetic stimulation (TMS), motor evoked potentials (MEPs) and other measures of cortical excitability (Boulay et al., 2014; Livingston et al., 2012; Lopez, et al. 2015). Individuals with mild to moderate TBI exhibit altered motor cortex excitability and impaired corticospinal activity (Miller et al., 2014). Miller et al. (2014) used TMS of the motor cortex to monitor acute and longitudinal changes in motor function after a concussion. They found that concussed individuals demonstrated a reduction in MEP amplitude compared to controls. They also found that intra-cortical inhibition (measured by duration of cortical silent period) was greater in the concussed group. This effect did not change over the 2 month period of their study suggesting persistent motor dysfunction lasting beyond the average recovery time required for an athlete to return to play. Evidence of persistent motor cortex abnormalities has been found in other TMS studies measuring similar variables (Beaumont et al., 2011 and 2007; Livingston et al., 2012; Pearce et al., 2014; Chistyakov et al., 2001). Researchers are now proposing electrophysiologic techniques for monitoring and assessing various degrees of TBI.

Cortical excitability changes after injury are implicated in altered balance, gait and postural control (Chistyakov et al., 2001; Beaumont et al., 2011; Guskiewicz et al., 2005). Some studies have suggested altered spinal excitability and desynchronization of descending input volleys to the motoneurons as contributing factors for changes in motor cortex excitability (Livingston et al., 2012; Chistyakov et al., 2001).
To our knowledge, no studies have attempted to measure changes in spinal excitability after head injury. Monitoring changes in spinal motoneuron reflex excitability (MNRE) that coincide with cortical abnormalities previously identified in concussed athletes may help identify a possible mechanism by which the motor system is impacted after injury (Miller et al., 2014; Pearce et al., 2014; Chistyakov et al., 2001). This study uses Hoffmann reflexes to measure the level of motoneuron reflex excitability at any given time. The Hoffmann reflex (H-reflex) is a monosynaptic reflex that is the electrical equivalent to the stretch reflex. The H-reflex is commonly used as a clinical measurement of central nervous system integrity because it is easily accessible and provides an estimate of overall alpha motoneuron excitability with consideration of pre-synaptic inputs and intrinsic properties of the motoneuron pool (Bonnet et al., 1980; Palmieri, Ingersoll, and Hoffman, 2004; Chen et al. 2015). Studies have provided evidence of reciprocal communication between spinal centers and the motor cortex via parallel descending pathways (Cohen et al., 2010; Zinger et al., 2013). The H-reflex has been established as an appropriate measurement for changes in excitatory and inhibitory mechanisms in these pathways. Zinger et al. (2013) found spinal neurons are recruited by direct and/or indirect pathways when recording spinal response to stimulation of finger-related cortical areas in primates. Changes in H-reflex activity while preparing for a voluntary contraction further supports the dynamic interaction between spinal motoneurons and cortical motor areas (Duclos et al., 2008; Cohen et al., 2010; McNeil et al., 2013).

The amplitude of the H-reflex provides a measure of MNRE at any given time and can be interpreted as the level of “readiness” to perform an action when assessed
during a goal directed task (Bonnet et al., 1980; Cohen et al., 2010). Modulation of the H-reflex has been recorded in individuals prior to the initiation of voluntary motor contraction (Frank, 2009; Eichenberger and Ruegg 1983). This set of adjustments is thought to produce an optimal state of muscular tone and attention needed for the execution of an expected motor skill (Bonnet et al., 1981). Studies have demonstrated that spinal motoneurons are modulated in a time dependent pattern when preparing to initiate a voluntary movement (Chen et al., 2015; Frank, 1985). This pattern of motor neuron excitability builds and peaks at approximately 100-160ms before the actual movement begins (Eichenberger and Ruegg, 1983; Frank, 1985). The changes in motorneuron excitability that occur before movement initiation are thought to demonstrate varying amounts of facilitation to spinal motor neuron centers by higher regions of the nervous system. It has been shown to occur in a predictable, phasic manner within the general population (Frank, 1985; Bock and Arnold, 1992; Bonnet et al., 1980). These anticipatory adjustments are thought to function as a priming mechanism that prepares motoneurons involved in the upcoming movement. Athletes must constantly adjust movement and responses to changing environmental factors during play. Accurate anticipatory behavior is important when executing rapid adaptations during play not only to enhance performance but also as a protective factor when bracing for, or avoiding, a possible insult to the body.

Recovery of motor functions occur at a different rate than cognitive and physical symptoms of concussion (Brooks et al., 2016; Beaumont et al., 2011; Livingston et al., 2012). This demonstrates the complex nature of concussion and why more research using serial testing is needed to understand the underlying mechanisms
influencing motor system dysfunction following injury. In this study we monitored changes in spinal excitability by measuring H-reflex amplitude changes after a head injury. It is hypothesized that spinal motoneuron reflex excitability would increase due to the hyper-excitible state of the nervous system after a concussion (Shaw, 2002; Laskowski et al., 2015). The integrity of signal transduction to spinal motoneurons was determined by measuring the level of H-reflex facilitation during motor preparation (MP). It was hypothesized that motor preparation recordings would be abnormal compared to those obtained at baseline. A negative shift in the characteristic response curve of H-reflex modulation prior to movement was expected to occur in athletes after injury. Impaired H-reflex facilitation during motor preparation would suggest a disruption in the balance of excitatory and inhibitory inputs needed to execute a voluntary motor contraction.
METHODOLOGY

Participants

Sixty-four participants were recruited from high risk club sports teams at URI and received baseline evaluations. All participants signed an informed consent form approved by The University of Rhode Island Institutional Review Board before taking part in the experiment (IRB# 728853-3; HU 1415-150). Athletes who reported a history of lower limb neuromuscular disorders were excluded from the study. A total of 64 participants between the ages of 18 and 32 were tested in this study. The mean age was 21 (SD ± 2.37), there were 33 males and 31 female participants. Participants reported participating in a wide range of sports prone to concussive injuries including: football, volleyball, rugby, hockey, lacrosse, sailing, skating, boxing, snowboarding, softball, and soccer. Thirty-five participants reported involvement in more than one sport.

All participants completed baseline testing at the beginning of the athletic season and were asked to return for follow-up testing if they received any form of head or neck injury. Participants completed a neuropsychological test battery, ImPACT, at baseline and after injury to confirm injury related functional deficits. Four participants returned for three follow-up tests with a suspected concussion. Two of the returning participants were male and 2 were female. Their ages were 21 or 22 years old. They participated in either rugby, lacrosse or football and reported participation in other recreational sports. One participant had no history of prior concussion, the remaining three reported their last diagnosed concussion occurring between 1 or 2
years ago. All laboratory visits included the same testing procedures as those conducted at baseline recording.

Electromyography (EMG)

EMG was used throughout the experimental protocol to monitor and record muscle activity. The skin over the soleus, tibialis anterior and the quadriceps muscles were cleaned and shaven before applying electrodes. Three sets of 9mm Ag/Ag/Cl electrodes were placed longitudinally 3 cm apart over the respective muscles. A 5 x 5 cm metal plate placed over the skin of the lateral malleolus served as the grounding electrode. The H-reflex was measured from the soleus muscle using raw EMG data recorded with a Therapeutics Unlimited EMG-67 amplifier/processer Module (impedance: >15 Megaohms at 100 Hz, common mode rejection: 87db at 60 Hz, noise: 1.5 µV RMS). Myoelectric activity was amplified 1000x at a bandwidth of 3 – 10,000Hz. Raw data was then digitized at 4000 Hz using an ADI data acquisition and analysis system. Muscle activity in the quadriceps and tibialis anterior were monitored using a Biometric DataLog MWX8 acquisition system. EMG raw data was amplified 1000x and digitized at a sampling frequency of 1000 Hz at a bandwidth of 3 Hz- 10,000 Hz.

H-Reflex

The H-reflex peak-to-peak amplitude in microvolts was collected and analyzed as a measure of motoneuron reflex excitability. H-reflex recordings were completed according to the methods established by Hugon (1973) and reviewed by Tucker,
Tuncer, and Turker (2005). H-reflexes were elicited using a 2.5 cm monopolar stimulating cathode ball electrode placed and secured on the skin over the tibial nerve within the popliteal fossa. A 10 x 10 cm sponge reference electrode soaked in 0.09% saline was fixed to the distal anterior thigh. The H-reflex was evoked using a rectangular 1ms pulse at a frequency ≥ 0.2 Hz. Three criteria were used to determine proper electrode placement: 1) the H-reflex was evoked at a lower intensity than the soleus M-wave, 2) the least amount of intensity was required to elicit a maximum H-reflex and 3) the soleus M-wave and H-reflex displayed a consistent configuration and appeared at the appropriate latency from the time of stimulus presentation (Palmieri, Ingersoll, and Hoffman, 2004) (Figures 1a & b). The shape and amplitude of the motor response (M-wave) were monitored throughout the experiment to confirm consistency.

**Force Transduction**

An Omegadyne Inc. LC101 series force transducer (Output: 3mV/V ±0.0075 mV/V, Resistance: 350 ± 10Ω) was used to measure load when participants plantar flexed against a foot plate in response to a visual stimulus. The Force transducer output was digitized at a sampling frequency of 1000 Hz using the same ADI data acquisition and analysis system employed in H-reflex recording. In this study, force data were used to detect movement initiation and to monitor the participant’s movement profile during each of the experiment trials. If no force was recorded during a trial then that specific response was deleted from the data record.
Motor Preparation

The concept of preparation and readiness for movement, addressed in this study as motor preparation, refers to a set of ritualized sensorimotor and psychological adjustments occurring when an individual is expected to perform a voluntary motor response (Bonnet et al., 1981; Frank, 1985). Motor preparation is measured from the ongoing spinal motoneuron recruitment pattern that occurs when preparing to initiate a voluntary movement during a simple reaction time task (Frank, 1985). In this study participants were asked to press down on the foot plate when a bright flash of light appeared on the oscilloscope screen in front of them (Go sig). H-reflexes were elicited to the soleus muscle at 50 ms intervals over a 300 ms period before Go sig to measure motor preparation. H-reflexes were also recorded at 50 ms intervals over a period of 200 ms after Go sig to observe changes in facilitation right before and during movement initiation. The H-reflex stimulus was delivered at random and triggered to occur at 11 inter-stimulus sampling times: -300, -250, -200, -150, -100, -50, 0, 50, 100, 150, 200. The positive integers represent H-reflex stimuli occurring after the GO sig while the negative numbers indicate times where the H-reflex was elicited before. At time zero the H-reflex stimulus was elicited simultaneously with the Go sig. Three H-reflexes were recorded at each inter-stimulus interval. The peak-to peak amplitude was measured for the three recordings and averaged as a measure of excitability over time.

Procedure

After completion of the ImPACT test, participants were seated with their dominant leg positioned and secured in a specially designed chair. Participants
remained in a semi-reclined position with their knee bent at 120°. The foot was stabilized and secured while resting on a footplate connected to a force transducer that measured isometric force. Once seated properly, electrical stimulation began to determine proper electrode placement (Tucker, Tuncer, and Turker, 2005). The stimulating electrode was secured with a Velcro strap once H-reflex parameters, mentioned previously, were met. Participants were asked to wear noise cancelling headphones to prevent anticipation of the H-reflex stimulus. Participants listened to white noise while the experimenter elicited the H-reflex. At this time, the experimenter began gradually increasing H-reflex stimulus intensity until a supramaximum M-response was elicited, M\text{max} (Figure 1a). Five M\text{max} recordings were made and recorded. H-reflex stimulation was then decreased until H-reflex amplitude reached 15-25% of M\text{max} (H\text{test}). At this intensity a small M-wave was usually present (Figure 1c). If the configuration and/or size of this small M-wave changed by more than 2 SDs the experiment was concluded and the data deleted. Ten reflexes were recorded and then averaged. This reflex measure served as the baseline level that all data in future experiments were compared with.

The experiment consisted of two phases: a “learning” and an experimental phase. The learning phase consisted of two parts: 1) learning the proper ankle movement technique and 2) learning how to accurately initiate the movement on the Go\text{sig}.

*Learning phase:* It was common for individuals to activate their quadriceps muscle when performing a ballistic isometric plantar-flexion movement. To limit quadriceps involvement, participants went through a procedure where they practiced
limiting their quadriceps muscle activity while maximally plantar flexing their ankle. Participants were asked to watch a monitor displaying their muscle activity (EMG). They were instructed to keep the raw EMG signal for the quadriceps as low as possible during practice trials. Practice continued until the participant was consistently able to minimize quadriceps involvement during the plantar-flexion movement at the presentation of Go_{sig}. Participants also experienced difficulty ignoring the H-reflex stimulus and contracting only when presented with the GOS_{sig}. To minimize this from happening, participants went through a learning procedure that emulated the actual experiment where they could practice initiating the plantar-flexion contraction appropriately on the Go_{Sig} and not the H-reflex test stimulus. The procedure continued until the participant initiated the voluntary contraction appropriately.

**Recording Phase:** The recorded portion of this experiment consisted of two parts: a motor neuron reflex excitability part and motor preparation part. The motoneuron reflex excitability part of the experiment used the ten H_{test} reflexes that were recorded during the experimental sessions. The peak-to-peak amplitude values for these H-reflexes were averaged for each experimental session and compared (pre-concussion and three post-concussion sessions). Any change in amplitude levels were considered as a change in spinal cord motoneuron reflex excitability.

The motor preparation part of the experiment consisted of participants isometrically plantar-flexing their foot as fast and forceful as possible after receiving the Go_{Sig}, while randomly receiving H-reflex stimulations at the previously described inter-stimulus intervals. The protocol elicited random H_{test} reflexes between 0.065 - 0.2Hz, at each of the inter-stimulus intervals until all 11 times had three reflex
recordings. Random stimulation was controlled by a custom designed program written on a National Instruments Labview software. The H_{test} stimulation was randomized in relation to the presentation of the GO_{sig}. Participants were presented with an acoustic warning signal through the noise cancelling headphones. The warning signal occurred randomly before the presentation of the GO_{sig}. The three reflexes were recorded and averaged for data analysis. The experiment was concluded with the collection of ten additional H_{test} and five M_{max} recordings at rest to check experimental stability.

The entire experiment lasted approximately two hours. Each participant was instructed to return to the laboratory after sustaining any head or neck injury for follow-up studies. Every follow-up visit consisted of the same experimental protocol. The first follow up visit occurred within 2-5 days (P1), the second at 6-12 days (P2) and a third at 13-20 days (P3) post injury.
Statistical Analysis

Statistical analysis for resting motor neuron reflex excitability was conducted on the grouped data collected from the four participants who returned for follow-up evaluations. All parametric statistical procedures were conducted on Sigma Stat 2.0 software program and plotted on an Excel spreadsheet. Effect size analysis was manually computed. For each experimental session, ten $H_{\text{test}}$ reflexes were collected at rest and their peak-to-peak amplitudes were measured. These amplitudes were normalized by taking the ratio of $H_{\text{test}}/M_{\text{max}}$. The average $H_{\text{test}}/M_{\text{max}}$ amplitude measure for each experimental session was graphed for visual comparison (Figure 2). A planned comparison was completed for ratio values from the post head injury sessions with those obtained at the baseline session using three paired t-tests. Level of significance was set at $p \leq 0.05$. In addition, Cohen’s $d$ effect size was calculated. The purpose of this analysis was to quantify the size of the difference between the data sets from each test date with a small population sample ($n = 4$).

A non-parametric design was used to analyze the data obtained for the motor preparation task. Motor preparation (MP) was measured by taking the average peak-to-peak $H_{\text{test}}$ amplitudes collected at each sampling time. $H_{\text{test}}$ amplitude was normalized by taking the ratio of $H_{\text{test}}/M_{\text{max}}$. Data was descriptively analyzed by graphing the ratio values from all four experimental sessions as a function of $H_{\text{test}}$ sampling time (Figure 3). The graphical representation was used to observe any appreciable changes that occurred between baseline and post injury tests. MP data from experimental session P2 had the greatest change from baseline values and therefore was chosen for further statistical analysis. In addition, from the eleven
sampling intervals used to monitor H-reflex recordings during the motor preparation testing, four were chosen for statistical analysis. These were 100 and 50 msec prior to the presentation of GO_{sig} as well as 100 and 150 msec after the presentation of GO_{sig}. These intervals were selected based on a study conducted by Frank (2009) that investigated H-reflex facilitation during the “fore period” of a voluntary contraction in response to a reaction time task. Frank (2009) found reflex facilitation to occur 74 msec prior to response initiation. Response initiation in this study occurred approximately 150 msec after presentation of the GO_{sig}. Two values prior to the GO_{sig} were selected to observe any possible changes in facilitation before being prompted to move. Average H-reflex amplitudes were graphed in a histogram plot for ease of comparison (Refer to results section). Cohen’s d effect size was calculated to measure the difference in H-reflex amplitudes between test dates.
RESULTS

Four out of the sixty-four athletes baseline tested returned to complete follow up testing after experiencing a potential concussion (Table 1). Only one participant reported having the injury diagnosed as a concussion (l2). The clinical report provided from the ImPACT test showed functional deficits and an increase in concussion symptoms in all four participants.

*Motor Neuron Reflex Excitability:* A 25%, 40% and 8% change in MNRE was shown between post injury test dates P1, P2, and P3 compared to baseline values respectively (Figure 2). After conducting a paired t-test a significant difference between resting MNRE at rest and after injury was found for test dates P1 and P2. The paired t-test comparing baseline to P3 values showed no significant difference in excitability. Cohen’s d effect size between baseline and P1 was 0.50. An effect size of 0.50 indicates a difference in sample means greater than 0.2 standard deviations. This difference is considered moderate, with a 64% probability that values at P1 would be different than those obtained at baseline. When comparing the level of MNRE between baseline and P2. Cohen’s d effect size was 0.82. This is considered a large difference in the sample means, with a 71 % chance that values obtained at P2 would be different than those found at baseline. No significant difference was found between values obtained at P3 and those obtained at baseline. This is further demonstrated by the small Cohen’s d effect size (0.01).

*Motor Preparation:* At baseline two periods of facilitation were found to occur: 100msec before the presentation of GO_{sig} and 150 msec after the presentation of GO_{sig}. The motor preparation response curve obtained at P2 was substantially
depressed compared to all other test dates (Figure 3). Cohen’s d effect size calculations resulted in a moderate effect size between P2 and baseline data, demonstrating a substantial change in the pattern of facilitation occurring 6-12 days after injury. Effect sizes calculated for the -100, -50, 100, and 150 msec sampling intervals 0.567, 0.764, 0.657, and 0.486 respectively. Figures 4-7 demonstrate the individual responses from all four participants to provide a better understanding of the individual decline in excitability that occurred across all MP values.
DISCUSSION

The purpose of this study was to investigate the effects that concussion has on spinal motoneuron reflex excitability (MNRE) at rest. MNRE was also assessed while preparing and executing a voluntary contraction in a simple reaction time test paradigm (MP). MNRE was measured using the H-reflex experimental technique (Tucker, Tuncer, and Turker, 2005). MNRE was reduced 3-5 days (P1) after head injury and continued diminishing by more than 50% by 6-12 days (P2) post injury when compared with baseline pre-concussion values (Fig. 2). H-reflex amplitudes started to return to baseline 13-20 days (P3) after injury. The motor preparation response curve was lowest when participants completed the P2 follow up testing (Figure 3). Three of the four participants tested showed a complete or partial decline in H-reflex facilitation after concussion. This is indicative of an increase in inhibitory input to the motoneuron pool. Differences in the pattern of H-reflex facilitation are most likely due to the differences in time when the participants returned for follow up tests. History of concussion and injury severity could have also influenced the magnitude of change between baseline and post injury sessions.

It has been suggested that concussion results in a period of cerebral excitation immediately after injury (Shaw, 2002; Guerriero et al., 2015). This excess in excitation is thought to be in response to neurometabolic abnormalities (Giza and Hovda, 2001). Increased glucose metabolism, abrupt release of charged ions, and the excess release of the excitatory neurotransmitter, glutamate, have all been found to occur after a sustained concussion (Guerrio et al., 2015; Giza and Hovda, 2001). Due to this excess in activity, we originally hypothesized that descending input to spinal
motoneurons would result in an increase in MNRE. However, contrary to our hypothesis, the results of this study showed that the occurrence of a head injury had a generalized inhibitory effect on H-reflex amplitude at rest and throughout motor preparation.

Spinal processing of cortical descending input is thought to be crucial for translating a motor command into muscle activation (Zinger et al., 2013). Modulation of spinal excitability prior to movement is caused by either: removal of pre-synaptic inhibition or activation of spinal interneurons (Eichenberger and Ruegg, 1984). Our results suggest that increased cerebral excitation following a concussion results in an increase in spinal inhibition via descending pathways onto spinal interneurons (Livingston et al., 2010; Beaumont et al., 2007). This non-specific inhibition is most likely due to an excess amount of the neurotransmitter, GABA, which would prolong pre-synaptic inhibitory activity. It has been shown, that GABA receptors are in both the dorsal and ventral horns of interneurons and mediate powerful inhibitory mechanisms that suppress MNRE (Malcangio and Bowery, 1996). Increased release of the neurotransmitter GABA and a reduction in reuptake at the synapse has been implicated in many of the presenting symptoms of mTBI (Beaumont et al., 2012; Guerriero et al., 2015).

In the rat model, Nardelli et al. (2016) showed that lowered MNRE is an important contributor to muscle weakness and fatigue. This raises the possibility that reduced MNRE contributes to persistent disability after the resolution of injury (Nardelli et al., 2016). Lowered MNRE and responsiveness at spinal motor centers have also been associated with decreased endurance, conservative/slowed gait, poor
postural control and inaccurate motor execution (Cavanaugh et al., 2005; Ekblom 2010; Fitts 2003). Symptoms relating to motor function often persist long after cognitive symptoms are resolved (Brooks et al., 2016). An athlete needs to make quick movements in response to unexpected events during play. An athlete who has not recovered full motor function could have a reduced ability to execute rapid motor adjustments required to protect oneself as well as others, thereby increasing the risk for musculoskeletal injuries (Beaumont et al., 2011; Brooks et al., 2016; Wasserman et al., 2015).

Concussion and mTBI are also associated with poor judgement due to decreased concentration, confusion and impulsiveness (Bissell, 2018). Judgement errors may cause the athlete to make ill-advised unsafe decisions while playing. This coupled with motor disturbance also increases the risk of receiving another injury including a second mTBI (Giza and Hovda, 2001; Moser et al, 2007; Ling et al., 2015). It has been shown undergoing a second impact before completely recovering from the initial one, can cause devastating cerebral injury, leading to collapse and on occasion death (McLendon, 2016). Thus, accurate assessment of the athlete is paramount before allowing return to play (McLeod, 2017; Matuszak et al, 2016).

Results of this study suggest that soleus spinal motor neuron activity is influenced by a concussive injury and may contribute to decreased motor function that lasts days to weeks post injury. If other muscles react similarly to the findings from the soleus muscle this would account for many of the motor deficits seen in athletes after injury. In summary, concussion resulted in a progressive spinal MNRE decrease, plateauing approximately 6-12 days post injury. Research has shown that after
receiving a concussion, the probability of receiving a second one (or another injury) increases substantially (Hovda, 2014). This may, in part, be due to a reduction in motor responsiveness and motor planning. Health care personnel assessing athletes to return to play should always be cautious in return-to-play decisions. Concussion is a complex central nervous system disorder with symptoms that can manifest in various ways for different people making it difficult to diagnose (Baker C. and Cinelli M, 2014; Carson J. et al., 2014). Motor system deficits are sometimes present in asymptomatic athletes. Further research is needed to understand the physiological mechanisms contributing to concussion symptoms, particularly those affecting the motor system.

**Study limitations:** This study is limited by the small number of participants that returned for follow-up testing. It would be beneficial to collect follow-up data on a matched control group of participants that did not experience a potential concussion. The H-reflex is a standardized test and by normalizing to $M_{\text{max}}$ we are able to compare across participants and test dates. The possible differences in temperature or the state of the individual may have interfered with the standardization of the results. The amplitude of the H-reflex is susceptible to change with the physical state of the person being tested. Participant’s may have experienced fatigue or discomfort due to the time required to complete the experimental protocol. Although participants were asked not to engage in strenuous activity prior to testing there was no way to control for their daily activities prior to testing.

Future studies should collect and monitor the H-reflex recruitment curve in addition to H-reflex variables. Recording the H-reflex recruitment curve and stimulus
intensity would provide additional variables to analyze possible changes in spinal reflex excitability (i.e. motor threshold, reflex threshold, $H_{\text{max}}$). Observed changes would be further supported by the collection and analysis of neuropsychological test scores.
Figure 1a-c: 1a: Example of EMG output wave for Mmax, 1b: Example of EMG output wave for the H-reflex with the corresponding M-wave, 1c: Example of EMG output wave for the H-reflex recording set at stimulus intensity for $H_{test}$
Figure 2: Bar graph representation of the average MNRE measured as the $H_{\text{test}}/M_{\text{max}}$ amplitude in mV at each testing date. Change in H-reflex amplitude was quantified by calculating Cohen’s d effect size: $P1\; \text{ES}= 0.50$, $P2\; \text{ES}= 0.82$, $P3\; \text{ES}= 0.01$. * p-value $\leq 0.05$
Figure 3: Mean H\textsubscript{test}/Mmax amplitude (mV) on each test date plotted as a function of sampling time (msec).
Figure 4: Motor preparation response curve obtained from participant a2. \( H_{ref}/M_{max} \) amplitude (mV) plotted as a function of sampling time (msec).
Figure 5: Motor preparation response curve obtained from participant B1. $H_{test}/M_{max}$ amplitude (mV) plotted as a function of sampling time (msec).
Figure 6: Motor preparation response curve obtained from participant 12. $H_{test}/M_{max}$ amplitude (mV) plotted as a function of sampling time (msec).
Figure 7: Motor preparation response curve obtained from participant w2. $H_{ref}/M_{max}$ amplitude (mV) plotted as a function of sampling time (msec).
Figure 8: Histogram representation of H-reflex amplitude (mV) for sampling times: -100 msec, -50 msec, 100 msec, and 150 msec. Negative values indicate sampling times before the presentation of the GO

The differences in amplitude were quantified using Cohen’s d effect size. -100 msec ES= 0.567, -50 msec ES= 0.764, 100 msec ES= 0.657, and 150 msec ES= 0.486.
Table 1

<table>
<thead>
<tr>
<th>Age</th>
<th>Gender</th>
<th>Sport</th>
<th>Previous Concussions</th>
<th>Post 1 days after injury</th>
<th>Post 2 days after injury</th>
<th>Post 3 days after injury</th>
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<tr>
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<td>Rugby</td>
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<td>6 days</td>
<td>11 days</td>
<td>19 days</td>
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<tr>
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<td>Lacrosse</td>
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<td>2 days</td>
<td>5 days</td>
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</tr>
<tr>
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<td>Rugby</td>
<td>4</td>
<td>5 days</td>
<td>13 days</td>
<td>17 days</td>
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</table>


