CHARACTERIZING SLEEP-RELATED CHANGES AND THEIR EFFECT ON RECOVERY FOLLOWING CONCUSSION IN COLLEGIATE ATHLETES

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By

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ABSTRACT

Sleep is an essential component following a concussion. A concussion can be defined as a traumatic brain injury induced by biomechanical forces to the head, neck, or spine. Management of a concussion consists of an evidence-based, multimodal approach consisting of clinical measures of neurocognitive function, balance, and self-reported symptoms. Although up to 80% of high school and collegiate athletes will experience a resolution of symptoms within 10 days, up to 20% may experience symptoms for several weeks or months. For this reason there is significant interest in the modifiable factors that contribute to the length of recovery following a concussion to lessen the risk of a prolonged recovery for you and adult athletes.

Collegiate athletes experience a variety of symptoms following a concussion which may include sleep symptoms such as excessive drowsiness, difficulty maintain sleep, or changes in the quality and/or quantity of sleep. Sleep symptoms are endorsed by nearly 70% of collegiate athletes, and research has shown these symptoms to be associated with greater number of days to report symptom free. While clinical studies of concussion have demonstrated sleep disturbances at various time points throughout recovery (i.e.- days to months), a detailed explanation of how sleep influences recovery has yet to be established, particularly in the acute phase (<72 hours) of the injury.

In the following studies, we utilized advanced statistical modeling to examine how the severity of sleep symptoms influences the severity of all other symptoms commonly experienced following a concussion. Research has indicated symptom severity (i.e.- the sum severity of all symptoms reported) to be the primary predictor of prolonged recovery, however, none has examined how disrupted sleep may predict a higher

symptom severity. Following this, in a first of its kind study, we utilized non-invasive, sensor-derived measures of sleep to determine differences in stages of sleep to examine how sleep is affected immediately following a concussion and throughout recovery. Finally, we utilized advanced neuroimaging techniques to measure levels of neuroinflammation in anatomical regions of the brain that are involved in the regulation of sleep to determine an explanation as to why sleep disturbances occur following a concussion.

Our results demonstrated that following a concussion, sleep is an essential component of recovery. Sleep symptoms were shown to be the strongest predictor of overall symptom severity in the acute phase of injury. Athletes with a concussion also had significantly less time in the stage of Deep sleep in the acute phase, compared to athletes without a concussion. Moreover, following the resolution of symptoms, athletes with a concussion had higher levels of neuroinflammation the sleep-wake areas of the brain.

Overall, these studies address a critical gap in research and the initial step in demonstrating how systemic alteration in the sleep-wake cycle can influence recovery following a concussion. The innovative methodology utilized has produced the first-of-its-kind data to demonstrate how changes in sleep, as measured though wearable technology and advanced neuroimaging, may be leveraged to facilitate recovery from concussion.

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APPROVAL OF THE DISSERTATION

This dissertation, "Characterizing Sleep-Related Changes and Their Effect on Recovery Following Concussion in Collegiate Athletes" has been approved by the Graduate Faculty of the School of Education and Human Development in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

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TABLE OF CONTENTS

Page

SECTION III: APPENDICES

LIST OF TABLES

MANUSCRIPT I

MANUSCRIPT II

MANUSCRIPT III

APPENDIX C

LIST OF FIGURES

SECTION II: MANUSCRIPT I

SLEEP SYMPTOM SEVERITY INFLUENCES ACUTE SYMPTOM BURDEN FOLLOWING CONCUSSION

ABSTRACT

Objective: Sleep disturbances are a common complaint in individuals following a concussion. Changes in sleep quality and quantity have been reported both acutely (i.e.<72 hours after injury) and chronically (i.e.- >3 months). It has yet to be determined how sleep disturbances during the acute period following a concussion influence recovery time. Examining factor analytics of common symptom constructs, including sleep, in the acute phase of injury may help explain how sleep influences the severity of symptoms reported following concussion. The purpose of this study was to examine the effects of somatic, cognitive, affect, and sleep symptoms have on acute symptom burden. We hypothesized acute symptom burden would be best explained by sleep disturbances.

Methods: Participants consisted of Division 1 Collegiate athletes who sustained a concussion between the 2016 and 2022 sport seasons. Acute symptom burden was determined by the sum severity of all symptoms reported, as indicated on the revised head injury scale (HIS-r). Structural equation modeling (SEM) approaches were employed to determine the path association for symptom constructs and acute symptom burden.

Results: A total of 196 participants (72 female, 124 Male) with a mean age of 19.1±1.3 years were included in the analysis. The final path model demonstrated acceptable fit indices (CFI= .937, TLI= .920, RMSEA= .079 [90% confidence interval =.064-.093]). Higher acute symptom burden was found to be most significantly associated with increased reports of sleep symptoms.

Conclusion: Results of this study suggest that sleep disturbances experienced in the first 72 hours following concussion play a critical role in the severity of symptoms an individual may experience. Specifically, the higher the severity of sleep symptoms endorsed, the higher overall acute symptom burden the individual may demonstrate.

INTRODUCTION

Following a concussion, individuals may experience a myriad of symptoms that may take days, weeks, or even months to resolve.[1–3] Though this length of recovery may vary, most high school and collegiate athletes will experience symptom resolution within 10 days of their diagnosis, while a subset (<20) of athletes may experience persisting symptoms.[1,2] For this reason, it is important to identify factors that contribute to the length of recovery. Factors that contribute to clinical recovery can be pre-injury (i.e.- history of previous concussion, diagnosis of mental health disorder, diagnosis of ADHD), or post-injury (i.e.- symptom severity or specific symptoms).[4]

Individually, specific symptoms have been shown to be predictors of recovery, including but not limited to headache, dizziness, and sleep disturbances.[4–6] Of the multitude of symptoms that may be experienced following concussion, sleep-related symptoms are endorsed by up to 70% of individuals.[6] More specifically, individuals may experience excessive drowsiness, difficulty maintaining sleep, or general changes in sleep quality and/or quantity. Outside of concussion literature, deficiencies in sleep have been linked to increased risk for depression, decreased quality of life, and impaired cognitive performance.[7,8] These symptoms have also been shown to be further compounded in individuals following a diagnosed concussion.[9,10] Additionally, sleep-related symptoms in the first three weeks of injury have been found to be associated with recovery periods up to three to four times longer than in individuals who did not experience sleep-related symptoms.[11] Though sleep has been implicated at varying time points throughout recovery, much of the research surrounding this has examined sleep past the acute phase of injury (>72 hours).[10–14] The varying time points at which sleep has been assessed

following concussion make it difficult to determine the degree to which acute sleep disturbances impact clinical recovery time.

As previously mentioned, individuals will experience a variety of symptoms following injury.[1,2] Of the many factors that may influence recovery (pre- and post), initial symptom severity, which is the sum of severity of all symptoms reported, has been demonstrated to be the strongest predictor of length of recovery in days.[15–18] However, following a concussion, one individual may report the same symptom severity as another individual by having different symptoms rated with different severities. As such, initial symptom severity does not fully explain how specific symptoms and their subsequent severity are related to recovery from concussion. Varying symptom constructs have been proposed as a way to characterize clusters of symptoms.[19–21] Such symptom constructs include cognitive, somatic, affect, and sleep.[19–21] Provided that the relationship between sleep and recovery from concussion is likely multifaceted, factor analytic findings of the aforementioned four symptom constructs may explain how sleep influences the severity of symptoms endorsed following injury. Therefore, the purpose of this study was to determine if sleep symptoms were most predictive of acute symptom severity compared to cognitive, somatic, and affect symptoms.

METHODS

Participants

Data were collected between January 2016 and January 2023 through retrospective chart review of National Collegiate Athletic Association (NCAA) Division 1 collegiate athletes who were diagnosed with a sport concussion (SC) by an athletic trainer (AT) or physician

at a large public university. Participants were evaluated in a manner consistent with the university Athletic Department's NCAA approved concussion management protocol. The definition of SC was adopted from the most recent international consensus statement on concussion in sport at the time of each participant's injury. [1] Participants were excluded if they had incomplete symptom data within the first 72 hours of injury or at the time of reporting symptom-free to their respective AT or physician.

Measures

Revised Head Injury Scale

The revised Head Injury Scale (HIS-r) is a 22-item symptom inventory related to SC. The HIS-r has demonstrated a sensitivity of 77.5% and specificity of 100% in collegiate athletes.[22] In order to complete the HIS-r, participants were instructed to first answer "yes" or "no" to indicate whether they did or did experience one of the 22 SC-related symptoms during the 24 hours prior to their assessment. If the participant responded "yes" to any of the 22 symptoms, then they were instructed to rate the duration of the symptom over the previous 24 hours on a Likert scale which ranged from 1 ("briefly") to 6 ("always"). "Briefly" was defined as 15 minutes or less. Participants also completed a separate Likert scale to rate each endorse symptom's severity that ranged from 0 ("not severe") to 6 ("as severe as possible"). In addition to the total number of symptoms endorsed (ranging from 0 to 22), total symptom duration and total symptom severity scores were calculated separately by summing each respective column with values ranging from 0-132.

Procedures:

Following the diagnosis of a concussion, athletes were administered the HIS-r on a daily basis by their athletic trainer (AT) in alignment with university concussion management protocol. For this study, each participant's first-completed HIS-r within the initial 72 hours after their injury was used. Symptoms associated with their respective latent variable can be found in Table 1. The observed variable of acute symptom burden was determined by the sum of the severity of all items in Table 1. As mentioned the HIS-r consists of 22 symptoms related to a concussion, however for the purpose of this study the items "experienced numbness", "experienced tingling sensations", and "neck pain", were omitted as these items are more strongly linked to musculoskeletal injury rather than a concussive injury. The variable "Acute Symptom Burden" was determine by the sum of severity for all symptoms endorsed.

Statistical Analysis

Preliminary statistical analysis: Since the HIS-r was developed it has been a valuable tool for assessing the symptoms commonly experienced following a concussion.[19,20] However, limited psychometric testing has been done in which the factor structure of the HIS-r has been empirically examined in collegiate athletes following a diagnosed concussion . Factor analysis is a requisite for determining symptom subscales, and for having a theoretical model basis for a structural equation model. We conducted a preliminary confirmatory factor analysis (CFA), in which the theoretically derived model of the HIS-r was assessed for goodness-of-fit. The goodness-of-fit indices include: Comparative Fit Index (CFI;≥ .95), Tucker Lewis Index (TLI; ≥ .95), and Root Mean Square Error of Approximation (RMSEA; ≤ .08).

Primary statistical analysis: To measure the effect of sleep on initial symptom severity, participant's symptom severity scores were categorized into 4 latent variables that represent symptom constructs (Cognitive, Somatic, Affect, Sleep). A structural equation model (SEM) including these four latent variables was used to predict initial symptom severity. Symptoms comprising of each latent variable can be found in Table 1. Goodness of fit indices used were the Comparative Fit Index (CFI; ≥.95), Tucker Lewis Index (TLI; ≥.95), and Root Mean Square of Approximation (RMSEA;≤.08).

RESULTS

From the 282 collegiate athletes diagnosed with a concussion, a total of 196 (72 female, 124 male) with a mean age of 19.1±1.3 years and Acute Symptom Burden of 19.2 ±15.62, were included in analysis. Preliminary CFA of the data yielded a 4 factor model with good fit indices (CFI=.879, TLI = .854, RMSEA= .082). With respect to the subsequent SEM, the initial four factor model included 18 items, however model modifications were made on the basis of content validity of individual items. The item of "feelings of depression" was redundant with the item "feelings of sadness", and thus was removed. The resulting 4-factor SEM represented an acceptable fit to the 17-item HIS-r (CFI= .937, TLI= .920, RMSEA= .079 [90% confidence interval =.064-.093]). All four latent variables (Somatic, Cognitive, Sleep, and Affect) were significant predictors of Acute Symptom Severity, however the latent variable of Sleep was the largest predictor (7.00, p<0.001) (Figure 1.) When standardized, a one unit increase in Sleep symptom severity was associated with a 0.391 standard deviation change in Acute Symptom Severity (p<0.001). A one unit increase Somatic, Cognitive, and Affect symptom severity

was associated with a 0.359, 0.231, and 0.148 standard deviation change in Acute Symptom Severity, respectfully.

DISCUSSION

Of the variety of symptoms that an individual may experience following a concussion, our results suggest that the severity of sleep-related symptoms endorsed during the acute phase of injury (<72 hours) are the best predictor of acute symptom severity in collegiate athletes. To our knowledge, this is the first study to use structural equation modeling to predict initial symptom severity. Initial symptom severity has been considered the strongest predictor of recovery following a concussion and our findings add increase granularity to what may contribute to that symptom severity.[15–18,23,24]

As previously mentioned, the majority of current literature has examined sleep outside the acute phase of concussion (>72 hours). When examined beyond the acute phase, it becomes difficult to determine whether individuals have increased symptom severity due to the concussion itself, or due to the sleep disturbances, as the effects of sleep disturbances (e.g., cognitive deficits or changes in mood) are also recognized symptoms of a concussion. A systematic review concluded that there was strong evidence to suggest that initial symptom severity is a strong predictor of recovery.[4] Additionally, higher symptom severity scores have been reported in individuals experiencing increased or decreased amounts of sleep following injury. [11–13,25,25] In the present study, our results suggest that higher symptom severity of sleep-related symptoms is predictive of overall symptom severity.

In addition to being the strongest predictor of initial symptom severity, the latent variable of sleep symptoms significantly influenced the Cognitive, Somatic, and Affect latent variables as well. This influence that sleep had on these latent variables was not surprising given how sleep has been shown to influence each of them. Though an individual may be physically asleep, the brain itself is not; expending increased amounts of energy to maintain sleep as well as carry out the restorative effects associated with different sleep phases.[28] The alteration between non rapid eye movement (NREM) and rapid eye movement (REM) is referred to as a sleep cycle.[26] A sleep cycle typically lasts 90-120 minutes, occurs approximately four to five times each night and is needed to achieve the restorative benefits of sleep.[26] Specifically following a traumatic brain injury (TBI) it has been shown that individuals spent less time in REM sleep; the stage of sleep responsible for memory consolidation and information processing.[29] This decreased time in REM may contribute to the cognitive impairments commonly experienced following concussion such as deficits in working memory or increased reaction time. Moreover, cognition has been shown to be influenced by mood-based factors such as depression or anxiety, of which our model showed sleep to be predictive of. Sleep disruption has been shown to be associated with increased feelings of depression and confusion, as well as feelings of frustration and irritability- all of which are common symptoms experienced following a concussion.[30,31]

CONCLUSION

To date, research has implicated sleep in recovery following a concussion at numerous time points, however it has remained unclear if it was the symptoms causing the sleep-related changes or the sleep-related changes causing the symptoms. Our results

suggest the latter, in that the more severe the sleep symptoms are in the acute phase of injury, the greater the acute symptom severity will be. Moreover, it suggests the significant influence that sleep has on the severity of all other symptoms.

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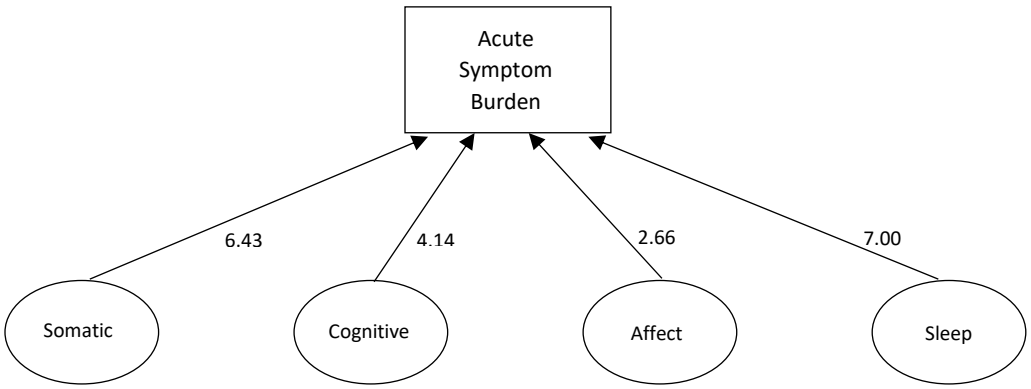
TABLES

Table 1. Latent variables of Somatic, Cognitive, Sleep, and Affect and their observed variables (symptoms).

Somatic	**Cognitive**	**Sleep**	**Affect**
Headache Nausea Dizziness Difficulty balancing Sensitivity to light Sensitivity to noise Blurred vision	Difficulty concentrating Difficulty remembering Feeling "slowed down" Feeling like "in a fog"	Fatigue Drowsiness Sleep disturbances	Sadness Nervousness Irritable

FIGURES

Figure 1. Path diagram of observed variable Acute Symptom Burden, and four latent variables Somatic, Cognitive, Affect, and Sleep symptoms.



SECTION II: MANUSCRIPT II

CHANGES IN SLEEP ARCHITECTURE FOLLOWING CONCUSSION IN COLLEGIATE ATHLETES

ABSTRACT

Objective: Sleep has been suggested to be a modifier of recovery following a concussion. Sleep symptoms are endorsed by 70% of individuals following a concussion and have been found to associate with a greater number of days until symptom resolution and return to play. However, the physiological mechanism for why sleep disturbances occur remains poorly understood. Alterations in time spent in stages of a sleep cycle following a concussion may contribute to recovery.. The purpose of our study was to use a non-invasive, sensor derived measure of sleep stages (Light, Deep, REM sleep, and time awake) to determine differences between collegiate athletes with or without a concussion, immediately following injury (<72 hours).

Methods: Division 1 Collegiate athletes diagnosed with a concussion were compared to healthy-match controls based on demographics and sport. Individuals in both groups were provided with and instructed to wear an OURA ring actigraphy device within 72 hours of their concussion. Differences in sensor derived time spent in Light, Deep, REM sleep, and time awake between groups was determined using independent t-tests. e Analyses were performed with α=0.05.

Results: A total of 21 athletes were included in our analyses (12 concussed, 9 controls) with an average age of 19.3$\pm$ 1.3 years. Individuals with a concussion spent less time in deep sleep (t=1.36, p=0.03, Cohen's d = .600), and more time awake (t= -2.31, p=0.02, Cohen's d= -1.020) compared to individuals without a concussion. No significant differences were found for time spent in Light or REM sleep.

Conclusion: Acutely following concussion, individuals may demonstrate changes in sleep stages. Our results suggest that time spent in different stages of sleep may be a potential mechanism underlying recovery from concussion. Our results provide an important step in using wearable sensors to better understand sleep disturbances following concussion to help mitigate risk of a prolonged recovery.

INTRODUCTION

Concussion related sleep disturbances are a known modifier of recovery following a diagnosed concussion. [1–5] Through the use of self-reported symptoms and actigraphy, research has demonstrated that individuals who experience sleep-related symptoms take a greater number of days to report symptom free and ultimately return-to-play following a diagnosed concussion. [2–6] For example, In adolescents (11-18 years of age), a fourfold increase in recovery time was observed if sleep symptoms (e.g.- difficulty falling asleep or staying asleep) were reported in the first week following injury when compared to those that did not endorse the same symptoms. [5]

Continued sleep disturbances beyond the typical symptom resolution period (i.e- 10-14 days) following a concussion are common and present similarly to trends observed in the acute and sub-acute phases of the injury. More specifically, a decreased sleep efficiency and increased time spent awake has been reported up to one year after injury. [6] Research examining sleep disturbances immediately following concussion (<72 hours) remains limited. In one study of collegiate athletes, it was found that those with a concussion demonstrated longer time to fall asleep, compared to healthy-matched controls. Moreover, those with a concussion demonstrated variability in the number of minutes awake after having initially fallen asleep (i.e.- wake after sleep onset [WASO]) and greater total sleep time, when compared to healthy controls.[7] Though these data support the role of sleep in recovery following concussion, the physiological mechanism of why sleep disturbances occur following injury remain poorly understood.

A sleep cycle can be separated into two stages: nonrapid eye movement (NREM), and rapid eye movement (REM). More specifically, a sleep cycle consists of three NREM stages and one REM stage. Typically individuals will experience four to five sleep cycles each night, with each cycle lasting between 70-120 minutes. However, inadequate sleep cycles (i.e., time spent in each stage) can diminish the restorative benefits of sleep. The current gold standard for assessing sleep is polysomnography (PSG). [8] PSG is used to monitor sleep stages and cycles to determine if or when sleep patterns are disrupted and why. Parameters of sleep measured include: total sleep time (TST); wake after sleep onset (WASO) ; REM sleep; NREM sleep; sleep onset latency (SOL).[8] Although PSG allows researchers to collect physiologic parameters of sleep, the time and cost of the technology prevent it from being widely used, especially after a concussion.

Due to the barriers associated with PSG, , alternative measures of sleep based on actigraphy, such as a FitBit™ or Apple Watch™, have been developed and used to indirectly measure sleep.[9–11] However, these actigraphy devices measure overall time spent asleep opposed to the different stages of sleep.[9,10] Additionally, the accuracy of wearable devices used to measure sleep has been brought into question. Wearable devices used to measure sleep primarily use a single accelerometer thereby only measuring movement (or lack thereof) to determine when an individual is awake or asleep. Provided this limitation, actigraphy devices, such as FitBit or Apple Watch, have demonstrated to have limited reliability and have been observed to underestimate sleep duration by up to 90 minutes, and overestimate wake duration by up to 77 minutes when compared to PSG, demonstrating high (90%) sensitivity and low (36%) specificity.[10,12] The OURA ring (Oulu, Finland) is a smart ring purported to quantify biometric data specific to sleep such

as body temperature, heart rate variability, as well as time spent in the various sleep stages (REM, NREM) through use of three sensors. When compared to PSG, the OURA ring was demonstrated to have high sensitivity (96%) and moderate specificity (48%) for detecting sleep, making it a better option to assess sleep as compared to traditional actigraphy devices. [13]

Thus far, research has demonstrated that subjective and objective measures of sleep disturbances such as night time awakenings, increased daytime drowsiness, and reports of insomnia are associated with prolonged recovery from concussion. [1–5,7,14] Despite the advancements in our understanding of sleep as a modifier of recovery, these studies have not identified how stages of sleep may be associated with recovery, particularly in the acute phase of injury (<72 hours). As sleep is a known modifier of recovery following a diagnosed concussion, examining differences in stages of sleep may provide previously unknown evidence that sleep-wake cycles are affected by concussion. Therefore, the purpose of our study is to use a non-invasive, sensor derived measure of sleep (Oura ring), to observe differences in sleep stages immediately following a diagnosed concussion in collegiate athletes.

METHODS

Participants

Data were collected on Division 1, National Collegiate Athletic Association (NCAA) athletes who were active in their respective sport, between the 2021-22' and 2022-23' academic years. For the concussed group, athletes were diagnosed with a concussion by an athletic trainer (AT) or team physician. Concussion diagnosis was

adopted from the most recent international consensus statement on concussion in sport at the time of diagnosis.[15] Following the diagnosis of a concussion, athletes were referred to study personnel for recruitment within 72 hours post-injury. Upon successful recruitment, a control participant was identified and matched to their injured counterpart by sport, position played, height, weight, age, and the absence of acute medical complications (i.e., illness or musculoskeletal injury) or an active injury. Participants in both groups were excluded if they had any prior traumatic brain injury (TBI) requiring hospitalization, presence of brain tumor, a concussion within six months of study participation (excluding present concussion), neurologic or neurodevelopmental disorder (epilepsy, dementia, autism, migraine, but not attention-deficit disorder). Additionally, potential participants with a concussion were excluded if they had a Glascow Coma Scale (GCS) score <13 on initial evaluation, traumatic injury requiring intensive care unit (ICU) monitoring or operative repair, a structural abnormality on brain CT (if obtained), or any additional comorbidity (i.e.- ACL tear). All participants signed an informed consent form prior to participation in this study. This study was approved by the university's institutional review board.

Measures

OURA Ring

Following enrollment, participants were issued an OURA Ring (City, State) and instructed to wear it on a nightly basis, for the duration of the study. The OURA ring is consumer based health tracking device that measures body signals (i.e.- heart rate, heart rate variability, temperature), as well as sleep-specific metrics (i.e.- time in light, deep,

REM sleep, total sleep time, sleep onset latency, and sleep after wake onset). [13] Aforementioned body signals are measured via infared photoplethysmography (PPG), negative temperature coefficient (NTC), and a triaxial accelerometer. Accelerometer data is sampled at a frequency of 50Hz.[13] Sleep is measured in five minute epochs, with sleep onset being defined as the first five consecutive minutes of persistent sleep. Previous studies have demonstrated a sensitivity (ability to detect sleep) of 96%, and a specificity (ability to detect wake) of 48% in the OURA ring when compared to PSG. [13]

Revised Head Injury Scale

The Revised Head Injury Scale (HIS-r) consists of 22 symptoms related to concussion, and measures symptom quantity, duration, and severity over a 24-hour period. To complete the HIS-r, participants first indicated which of the 22 symptoms (yes/no) they had experienced during the previous 24 hours that was atypical for them. For each symptom endorsed, the participant then rated the symptom duration and severity over the past 24 hours. Duration is rated on a Likert scale from 1 “briefly” to 6 “always”. Severity is rated on a Likert scale that ranges from 0 “not severe” to 6 “as severe as possible”. In addition to the total number of symptoms endorsed (ranging from 0 to 22), total symptom duration and total symptom severity scores were calculated by summing the respective individual duration and severity scores for each endorsed symptom, resulting in total duration and severity scores each ranging from 0 to 132. The HIS-r has been demonstrated to have high sensitivity (77.5%) and specificity (100%) in recognizing collegiate athletes diagnosed with a concussion acutely after injury.[16]

Procedure

Upon diagnosis of a concussion by the athlete's athletic trainer or team physician, participants were recruited within 72 hours of injury. Participants provided consent to participate in the study, and were sized for an OURA ring to be worn on their second, third, or fourth digit (on either hand). [13] Additionally, at this time participants were instructed to download the OURA ring application on their personal smartphone. The application allowed for continuous collection of data from the ring while being encrypted. OURA system preferences were set so that participants could not see any of their sleep data in the application. OURA ring sleep parameters examined in this study included time (in minutes) spent in Light sleep, Deep sleep, REM sleep, and awake.

Statistical Analysis

Independent T-tests were performed to determine if duration of time in Light, Deep, or REM sleep, time awake, total sleep time, and sleep onset latency, in the first 72 hours of injury was statistically different between groups. Data were analyzed using SPSS version 26.0 (Armonk, NY) with $\alpha=0.05$.

RESULTS

A total of 21 collegiate athletes (10 female, 11 male) were included in the study (12 concussed, 9 healthy match control). Significant differences in time spent awake were found between groups, with individuals with a concussion spending an average of 77.8±35.23 minutes awake, compared to match controls who spent an average of 49.3± 12.22 minutes awake (t=-2.31,p=0.021, Cohen's d= -1.020) Additionally, differences were observed in deep sleep duration, with individuals in the concussed group spending significantly less time (108.0± 34.81 minutes) than matched controls (134.4± 54.2

minutes[*t*=1.36, p=0.027, Cohen's d=.600]). No significant differences between groups were observed in duration of Light sleep (p=0.709) or REM sleep (p=0.096). (Table 1)

DISCUSSION

This study is the first of its kind to examine duration of sleep stages in the acute phase of a concussion using the OURA ring. Overall, our results suggest that collegiate athletes with a concussion spend less time in deep sleep and increased time awake in the acute phase of injury, compared to athletes without a concussion. Importantly, our results suggest that time spent in different sleep stages may be a potential mechanism underlying recovery from concussion that had not previously been examined in the collegiate population.

In our study, there were no significant differences between groups for total sleep time, with both groups demonstrating an average of 6.5-7 hours of sleep. This finding is in line with previous research in which total sleep time differences were not observed between individuals with and without a concussion.[6,17] This suggests that total sleep time may not play a large role in recovery following a concussion, but rather the length of time spent in different stages may be a more defining factor. An individual with a concussion may demonstrate the same hours of sleep as an individual without a concussion, and as such total sleep time does not fully explain the frequency or duration of sleep stages that comprised the total sleep time. Additionally, we observed that individuals with a concussion spent more time awake during the night than those without a concussion. Following a concussion, subjective reports of frequent nighttime wakenings and increased time spent awake via actigraphy are commonly seen throughout research.[3,5,6,14]

However, many of these studies included a wide range of time from injury to enrollment, ranging from weeks to months, rather than acutely after injury. [1,3–6,14]

The decreased duration of deep sleep in the concussed group immediately following injury may be explained by factors not analyzed in this study. The regulation of sleep is driven by the interaction of two separate biological mechanisms: the homeostatic need for sleep (Process S), and the circadian timing of when sleep happens (Process C).[18–22] The regulation of sleep and wakefulness by Process S and Process C is referred to as the 2 Process Model, and is reflective of the inhibition or activation of various neurotransmitters.[18–20] The excitement of sleep-promoting neurons or inhibition of wake-promoting neurons may prompt sleep. Conversely, waking can be promoted through the excitement of wake-promoting neurons and or the inhibition of sleep-promoting neurons.[18,20–22] Specifically, during sleep, various neurotransmitters are released throughout each stage that promote and maintain sleep.[20] These include, histamine, acetylcholine, serotonin, norepinephrine, dopamine, hypocrite, and gamma-Aminobutyric acid (GABA).[20] Changes in the inhibition or activation of these neurotransmitters can lead to a either a decreased or an increased amount of time spent in a specific sleep stage, as well as frequency of that stage.[18,20] Disrupted sleep stages, particularly stage three of non-rapid eye movement (NREM) otherwise known as deep sleep, are reflective of decreased homeostatic drive for sleep (Process S).[23] This stage of deep sleep is of great importance as it is the primary stage of sleep in which the glymphatic system is active.[23] The glymphatic system is a series of perivascular channels throughout the brain that serve to clear away and expel waste in the central nervous system (CNS). [24,25]Disruptions in sleep stages and their duration, particularly deep sleep, may therefore result in decreased

glymphatic clearance of metabolic waste (i.e.- cellular debris) following injury. However, further research is needed to confirm this in a concussed population.

This study was not without limitations. Our sample size was small and unequal, however given the limited number of prospective studies assessing sleep in the acute phase of a concussion, this study does provide valuable information regarding duration of sleep stages in individuals with or without a concussion. Additionally, we acknowledge that being a consumer wearable device, the OURA ring is limited in its accuracy of measurement.

CONCLUSION

This study extends the current literature by using a novel, wearable device to assess sleep acutely following concussion, during which there is limited data. Individuals with a concussion demonstrated decreased duration of deep sleep and increased time spent awake. These findings provide new insight into the underlying mechanism by which sleep influences recovery from concussion.

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TABLES

Table 1. Time spent (minutes) in Light, Deep, and REM sleep, and time awake between groups.
** $p<0.05$

	Concussed	Control	*p*	Cohen's d	95% Confidence Interval Lower	Upper
Time in minutes						
Light Sleep	248.67	208.28	0.709	-.533	-1.406	.355
Deep Sleep	108.04	134.44	0.027*	.600	-.293	1.477
REM	56.29	43.44	0.096	-.377	-1.245	.500
Awake	77.70	49.28	0.021*	-1.020	-1.930	-.086

SECTION II: MANUSCRIPT III

MICROGLIA ACTIVATION IN SLEEP-WAKE CENTERS FOLLOWING CLINICAL RECOVERY FROM CONCUSSION

ABSTRACT

Objective: Following concussion, sleep disturbances are often reported. These changes in sleep include daytime drowsiness, frequent night time wakenings, and or a delayed onset of sleep. Sleep disturbances may last months to years after the clinical resolution of the injury. One plausible mechanism for persisting sleep disturbances may be microglia activation in the hypothalamus and pituitary gland. Recent evidence has demonstrated microglial activation of the central nervous system in collegiate athletes following symptom resolution after a concussion. The purpose of this study was to quantify the extent of microglial activation in sleep-regulating regions of the brain (i.e., the hypothalamus and pituitary gland), in collegiate athletes following a diagnosed concussion.

Methods: Participants consisted of Division 1 collegiate athletes (*n*=8 [19.6±1.2 years of age])) who had sustained a concussion and healthy college students (*n*=10 [21.6±2.7 years of age]). Following symptom resolution and medical clearance to begin a graduated exercise protocol all athletes with concussion and healthy controls completed a full brain MRI and brain PET/CT scan. Independent t-tests were used to compare maximum standard uptake volume (SUV_{max}) of [^{18}F]DPA-714 between groups to depict microglial activation. All analyses were performed with α=0.05.

Results: Participants in the concussed group demonstrated 26% to 41% higher SUV_{max} of [^{18}F]DPA-714 tracer in the pituitary gland and 22% to 44% in the hypothalamus, compared to healthy collegiate students.

Conclusion: Elevated microglial activation was observed in the hypothalamus and pituitary gland that regulate sleep. Though these data are preliminary, they may represent

the physiological process by which circadian rhythm is being restored following concussion in active collegiate athletes.

INTRODUCTION

A concussive force results in neuronal strain of the axonal membrane leading to a pathological response known as the neurometabolic cascade.[1,2] The neurometabolic cascade results in a dysregulation of intracellular and extracellular ions and an overall disruption of homeostasis that typically lasts between 24 hours up to four days, following a concussion.[1,2] Disruption of cellular homeostasis creates an inflammatory response in which microglia are activated and inflammatory cytokines are released to aid in tissue repair and removal of cellular debris. While the initial response of microglia is beneficial, if chronically activated, they can perpetuate the neuroinflammatory response. [3]

Sleep is considered a mediator to the immune response as it acts to maintain homeostasis and restore dysregulated physiological processes (i.e., the neurometabolic cascade), such that pathological inflammation may disrupt the chemical signaling required to maintain sleep. Cytokines can act as sleep regulatory substances and can induce or inhibit sleep depending on the cytokine type and quantity released which provides strong evidence that they are heavily involved in the regulation of sleep.[4] However, unregulated elevated levels of pro-inflammatory cytokines (e.g., interleukin-1 [IL-1 and tumor necrosis factor [TNF]) can lead to sleep disturbances. In this way, a feedback loop is created between microglia activation, cytokine release, and sleep disturbances with further exacerbation of the injury (i.e., heightened symptoms [Figure 1])

Following a concussion (i.e., mild traumatic brain injury [mTBI]) and throughout recovery, sleep disturbances are often reported.[5–7,7–10] These disturbances include, but are

not limited to, delayed onset of sleep, frequent nighttime wakenings, daytime sleepiness, and insomnia.[5,6,8,10–12] Current literature surrounding sleep provides important rationale for examining why sleep disturbances are present immediately following a concussion and may persist years after the injury. Sleep disturbances such as excessive drowsiness or difficulty initiating or maintaining sleep, have been reported in up to 70% of individuals (<25 years of age) following concussion.[5,10,11] Sleep disturbances in adolescents between 13 and18 years of age within the first three weeks of a diagnosed concussion have also been shown to associate with a three to four times longer recovery period when compared to young athletes that did not experience the same symptoms.[8] Moreover, there is substantial evidence demonstrating that sleep disturbances continue long after traumatic brain injury (TBI).[13] In individuals with a moderate traumatic brain injury, approximately 65% of patients experienced sleep disturbances in the first two weeks of injury, and 41% still experienced such disturbances one year later.[13] While the identification of sleep symptoms following concussion have been reported, our understanding of the etiology of these changes in sleep remains limited.

Sleep is a physiological process regulated by circadian and homeostatic systems that modulate the sleep/wake cycle.[14–16] Anatomically, sleep and wakefulness are regulated by sleep- and wake-promoting nuclei located in various regions of the brain including the hypothalamus and pituitary gland .[14,16,17] These nuclei activate or inhibit the release of neurotransmitters (e.g.,noradrenaline, orexin, acetylcholine) that not only help transition the body from a state of sleep to a state of waking (and vice versa), but also control the depth and length of sleep. [14–18] At the onset of sleep, activity in the hypothalamic pituitary axis (HPA) is suppressed, however this activity fluctuates throughout the sleep

cycle with increased activity being found during the sleep state of rapid eye movement (REM) and decreased at the cessation of REM.[19] As the brain transitions from a sleep state to a waking state, the HPA axis is activated, which results in the release of the neurotransmitter noradrenaline (NA) and arousal.[19] Relatively low levels of NA can exert neuroprotective properties, however in higher levels, such as those seen in an inflammatory response, NA can be destructive.[20] In this way, both acute and chronic inflammation may be modulated by NA, which in turn is modulated by sleep.

Outside of concussion literature, maladaptive sleep patterns have been shown to occur as a result of increased activation of the central nervous system, which can persist for days to weeks to months.[4] Sleep disturbances are a common complaint following concussion that also may last months to years following injury. One plausible explanation for persisting sleep symptoms commonly seen following a concussion is neuroinflammation of the hypothalamus and pituitary gland that control the sleep-wake cycle. Recently, increased neuroinflammation was observed in collegiate athletes following the diagnosis of concussion using [^{18}F]DPA-714, a Positron Emission Tomography (PET) imaging agent with high affinity for the translocator protein (TSPO) overexpressed in activated microglia.[21] These findings however did not specifically examine areas of the brain associated with regulation of sleep-wake cycle. We hypothesized that [^{18}F]DPA-714 PET signal would be elevated in collegiate athletes diagnosed with a concussion in the hypothalamus and pituitary gland, when compared to healthy controls. Therefore, the purpose of this study was to determine whether increased microglial activation was evident in regions rich with sleep- and wake-promoting nuclei,

such as in the hypothalamus and pituitary gland, following concussion in collegiate athletes.

METHODS

Participants

Data were collected on Division 1 National Collegiate Athletic Association (NCAA) athletes and healthy, active college students from a large public university. For injured collegiate athletes, the diagnosis of concussion was made by an athletic trainer (AT) or team physician. The diagnosis of concussion was consisted with the most recent international consensus statement on concussion in sport. [22] Control participants consisted of healthy, physically active collegiate students. Control participants were matched by phenotype (i.e., mixed or high affinity). Participants in both groups were excluded if they had any prior TBI requiring hospitalization, presence of a brain tumor, a concussion within the previous three months (excluding present concussion for those in Group 1), neurologic or neurodevelopmental disorder (epilepsy, dementia, autism, migraine, but not attention-deficit disorder), a Glascow Coma Scale (GCS) <13, a TBI requiring intensive care unit monitoring or operative care, a structural abnormality on brain computerized tomography, an injury resulting from physical abuse (e.g.- intimate partner violence), pregnancy, prior adverse reaction to a radiotracer, or if they were a low affinity binder for TSPO ligand. This study was approved by the institutional review board and all participants provided consent prior to data collection.

Procedure

Upon diagnosis of a concussion by an athlete's AT or team physician, injured participants were recruited within 48 hours of their injury. Following consent, participants in both groups underwent a 4 mL blood draw for DNA extraction (PureGene® Blood Kit C, Qiagen, Valencia, CA) and TSPO (rs6971) genotyping using the Taqman assay (Applied Biosystems®, Life Technologies, Grand Island, NY). Allelic discrimination plots were generated to create standard curves for three distinct TSPO genotypes: Ala147/Ala147 (C/C), Thr147/Thr147 (T/T), and Ala147/Thr147 (C/T). Homozygotes, C/C and T/T, correspond to the high affinity (HAB) and low affinity binding (LAB) phenotype, respectively. The heterozygotes (C/T) correspond to the mixed affinity binding (MAB) phenotype. If participants were either C/T or C/C, they continued with the study protocol and completed a symptom inventory daily until symptom resolution.

Upon symptom resolution, neuroimaging was scheduled and performed in athletes with concussion. It is important to note that all participants scored within normal limited on clinical measures of balance, neurocognitive function, and remained symptom free prior which is consistent with the university concussion management protocol to begin a return-to-play progression.

All participants underwent a whole brain MRI for anatomical delineation for regions of interest on PET images after PET-MRI co-registration. MRI T1 weighted images (256 pixels x 256 pixels x 192 slices) were obtained on a Siemens Prisma 3T scanner with a 64 channel head coil using the 3D MP-RAGE sequence (cite). Following MRI, all participants underwent 90 minutes of imaging using the time-to-flight Siemens Biograph Mct (PET-CT) scanner. The dynamic PET data (400 pixels x 400 pixels x 110 slices x 38 time frames) corrected for photon attenuation were generated using an iterative

reconstruction algorithm. The 90 minute listmode PET data were binned as follows: (frames, time (s)): (4 x 7-15s; 4 x 20-30s; 3 x 45-60s; 2 x 90-120s; 5 x 180-240s; 12 x 270-300s) To measure microglial activity, the radiotracer [^{18}F]DPA-714 was injected intravenously at the onset of a 90 minute dynamic listmode PET acquisition. Throughout the PET scan, (four 4mL) venous blood samples were collected at 45, 60, 75, and 90 minutes.

Image Analysis

Dynamic imaging was performed with concomitant blood sampling for computing the image derived input function (IDIF) using whole blood radioactivity and correcting for metabolites. After correcting for attenuation and motion, PET images were co-registered with a T1 MR image, which was gathered immediately prior to the PET scan. PET data were averaged across the first 14 frames to create a reference to seed to perform a rigid body transform across the PET frames. The average of all motion corrected PET frames were resliced and co-registered with T1-weighted MRI using non-rigid transform to generate a transformation matrix to generate a co-registered dynamic PET volume. Areas of interest (hypothalamus and pituitary gland) were then determined through image analysis software PMOD (Version 3.9). Maximum standard uptake value (SUV_{max}) was measured in the aforementioned areas of the brain, and then normalized to the SUV_{max} of a separate region of the brain (thalamus).

Statistical analysis

As data was normally distributed, independent T-tests were performed to determine if standard uptake value (SUV) of [^{18}F]DPA-714 was significantly different in the

hypothalamus and pituitary gland between groups. Data were analyzed using SPSS version 26.0 (Armonk, NY) with α=0.05

RESULTS:

A total of 27 participants were recruited for this study, with 18 being included in the final analysis. Participants in the injured group (5 males, 3 females) were on average 19.6$\pm$1.27 years of age. Participants in the health control group (5 males ,5 females) were on average 21.6$\pm$2.71 years of age. Exclusion from the final analysis was due to the following: low-affinity binder (n=2); did not complete imagining protocol (n=2); sustained a head injury two days prior to scheduled imagining protocol (n=1); withdrew from the study (n=4); use of creatine (n=1).

In vivo [^{18}F]DPA-714 PET imaging

No significant differences between healthy student controls and NCAA Division 1 concussed athletes were noted with respect to injected dose of [^{18}F]DPA-714, molar activity, and mass of [^{19}F]DPA-714 (Table 1).

Computed SUV_{max} for each region demonstrated elevated differences in the geometric means in concussed group as compared to the healthy controls. When normalized to genotype, C/T (mixed affinity binder) subjects in the injured group were observed to have a 26% increase in SUV_{max} in the pituitary gland and 44% increase in SUV_{max} of the hypothalamus, as compared to C/T subjects in the health control group (Figure 2). Though elevated, these differences seen were not found to be statistically significant when comparing the population means in the pituitary (t=-1.21, p=.198; d=-.701 [-1.856,.487]) or hypothalamus (t= -1.78, p=.178;d=-1.029[-2.223,.209]). Similarly, C/C (high affinity binder) subjects in the injured group were observed to have a 41%

increase in SUV_{max} in the pituitary gland and 22% increase in the hypothalamus as compared to healthy controls (Figure 3). Despite this difference, it was not deemed to be statistically significant. (*t*= -.956, *p*=.416; d= -.828[-2.569,1.002; *t*=-.795, *p*=.310; d=-.688[-2.409,1.109] respectively)

DISCUSSION

Following a concussion, changes in sleep quantity and quality are often reported and found to persist months to years after the injury.[13,23] The etiology of these chronic symptoms, however, has remained unclear. The relationship between sleep and concussion is likely complex and multifaceted. Sleep is considered a mediator to immune responses, and is tightly regulated by the synchronization of various neurotransmitters and can serve as a mediator for immune responses.[24] Our results represent an important first step in understanding microglial activation associated with a concussion, specifically after clinical recovery (symptom resolution) has occurred in relation to structures implicated in sleep. Our findings of elevated levels of microglia in the regions of the brain that regulate sleep- the pituitary gland and hypothalamus- may be best appreciated by factors not directly measured in our study.

In this study, we hypothesized microglial activation persisted in regions associated with sleep regulator, as evidenced by [^{18}F]DPA-714 signal in the brain. Microglia are immune cells within the central nervous system (i.e.-brain and spinal cord) responsible for immune surveillance and macrophage activities,[3,25] including cytokines and/or chemokine production.[3] Microglia have two adrenergic receptors, β2 which is active in a resting state, and α2A which is active in an inflammatory state.[26] Immediately following a concussion, HPA axis activation occurs and the sympathetic nervous system and subsequent release of

ACTH, which results in an increased release of NA.[27] Interestingly, NA mediates both of the aforementioned adrenergic receptors found on microglia. In a resting state, NA binds to β2 receptors and downregulates the expression of pro-inflammatory cytokines, whereas in an inflammatory state when the α2A receptor is active, NA binding results in increased cytokine production.[28]

The glymphatic system is a series of perivascular channels throughout the brain that serve to clear away and expel waste in the central nervous system. [29]The glymphatic system is most active during sleep, promoting an influx of cerebral spinal fluid into the brain to clear metabolic waste (e.g.- amyloid beta) that has naturally accumulated while awake. The glymphatic system has been implicated in mTBI in which deficient sleep was associated with perivascular space, suggesting impaired waste clearance.[30] The enhanced efficiency of the glymphatic system due to sleep may be intertwined with the actions of NA.[29] During wakefulness, the release of NA, which is a neurotransmitter associated with arousal, acts to suppress glymphatic activity by decreasing perivascular space. Conversely, interstitial space is increased when NA release is inhibited.[29] This poses the theory that increased levels of NA, as a result of the inflammatory response from concussion, may hinder the glymphatic system's ability to expel the cellular debris created from the injury.

Our first-of-their-kind findings enhance our understanding related to microglial activation associated with a concussion, specifically after clinical recovery (symptom resolution) has occurred in structures associated with sleep. While statistical significance was not achieved, this may be due to the temporal dependence of TSPO expression relative to microglial activation. In rodent models, peak TSPO expression occurs approximately 7-14 days post-injury, however in human models this temporal dependency is unknown.

Thus, in our sample, it may be possible that the time of peak TSPO expression had passed, given the range of days since injury (5-16) at which athletes were imaged. Previous studies have measured inflammatory markers in the periphery immediately following concussion and upon medical clearance.[31,32] However, these studies were limited by study design, including study population and period of data sampling. As such, measuring inflammatory markers in the periphery may be limited in their utility for determining physiological recovery from concussion.

Previous imaging studies with retired professional football athletes have also shown increased microglia activation, compared to healthy age-matched controls.[33] Though important, the observed of microglial activation in active and retired professional football athletes likely represents a cohort of individuals with different microglial status as a result of engaging in a career of collision sports and other lifestyle-related factors. Importantly, participants in this study were all medically cleared to being a return-to-play progression and were still observed to have elevated microglial activation as indicated by [^{18}F]DPA-714 PET. It is important to note that the observed microglia activation should not be definitively interpreted as informative of chronic inflammation, as the increase in [^{18}F]DPA-714 PET signal could be the result of a natural, pro-inflammatory response to injury. These observations warrant further study to understand if the increased PET signal in the hypothalamus and pituitary represent a physiological process by which circadian rhythm is being restored following concussion in active collegiate athletes.

Our study was not without limitations. First, our control group consisted of healthy, college students rather than varsity athletes. Though groups were matched demographically, the lives and stressors of collegiate athletes may vary from those of a

collegiate students, and may have contributed to a neuroinflammatory response. Further research is needed to compare non-concussed controls from same sports as those with a concussion. An additional limitation to our study was the use of SUV_{max}. While SUV is a widely used metric for PET imaging studies, SUV presents limited information. Namely, SUV cannot delineate blood pool contamination from tissue specific PET signal, which may be important in perfused brain regions like the pituitary. This decision was founded on anecdotal observations within our study that participants, especially athletes, were averse to blood sampling techniques needed for advanced parametric analyses. Nonetheless, kinetic modeling approaches are needed to validate our findings.

CONCLUSION

In summary, following clinical recovery from concussion, individuals with C/C or C/T genotypes demonstrated elevated levels of microglial activation in the hypothalamus and pituitary, compared to healthy controls. Following concussion, sleep disturbances are often reported and have been reported to persist months to years after the injury. The etiology of these persisting changes of sleep and concussion is likely multifaceted, of which, microglial activation in the hypothalamus and pituitary gland may have an important contribution. Elevated microglia activation may be representative of the physiological restoration of disrupted circadian rhythm consequential to a concussion which has not been previously examined.

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TABLES

Table 1. Participant clinical, demographic, and radiopharmaceutical injection characteristics. Characteristics of Enrolled Subjects. Abbreviations: C/C, high affinity binder; C/T, mixed affinity binder. *P values for t test or Fisher exact test were calculated as appropriate. Threshold for significance, $P < .05$.

Demographic Variable	**Group 1 (*n* = 8)**	**Group 2 (*n*=10)**	***P***
Age (mean[SD])	19.6 (1.2)	21.6 (2.7)	0.06
Mass (kg) (mean[SD)	82.0 (28.6)	80.0 (14.6)	0.68
Rs6971 TSPO genotype (*n)*			
C/C (High affinity binders)	4	2	
C/T (Mixed affinity binders)	6	6	0.88
Injected dose (MBq), (mean[SD])	289(89)	277(30)	0.37
	31.4(56.0)	28.3 (29.0)	0.51

FIGURES

Figure 1. Pathological inflammation may disrupt chemical signaling required for maintenance of sleep. Unregulated, elevated levels of pro-inflammatory cytokines may create feedback loop between microglial activation, increased activity of the sympathetic nervous system and HPA axis, and sleep disturbances.

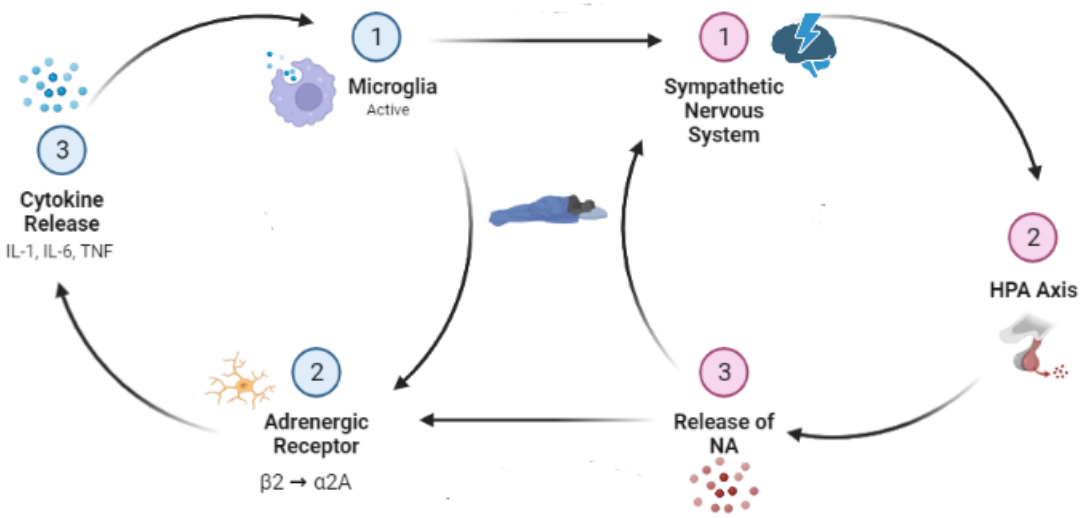
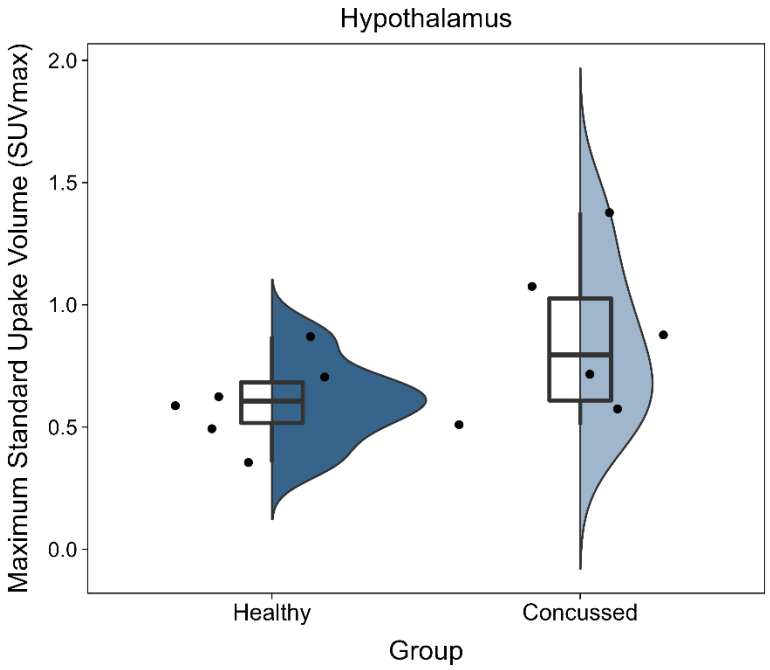


Figure 2. SUV_{max} of [^{18}F]DPA-714 in the hypothalamus and pituitary between subjects of C/T (mixed affinity binder) genotype, in Groups 1 and 2. Individuals with a concussion demonstrated a 42% and 23% increase in SUV_{max} of the hypothalamus and pituitary, respectively.



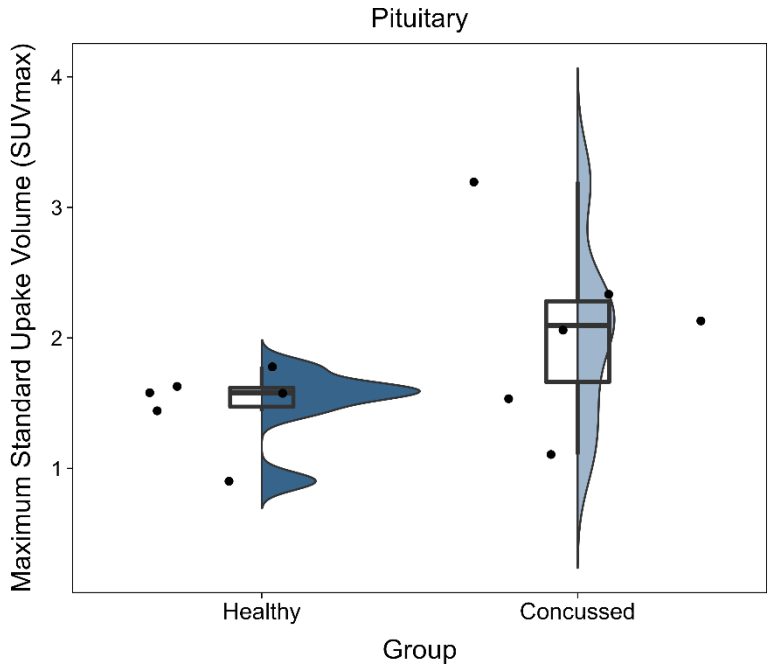
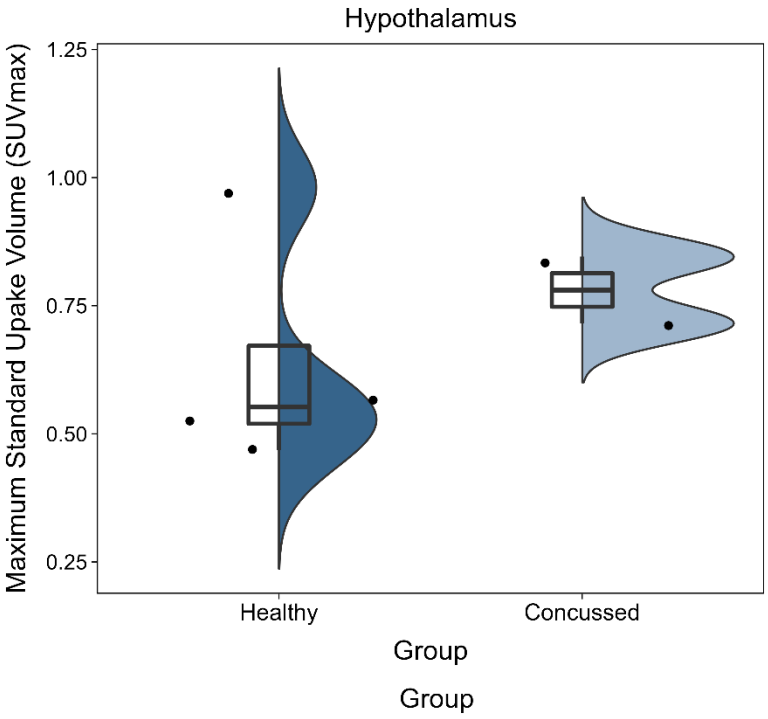


Figure 3. SUV_{max} of [^{18}F]DPA-714 in the hypothalamus and pituitary between subjects of C/C (high affinity binder) genotype, in Groups 1 and 2. Individuals with a concussion demonstrated a 27% and 53% increase in SUV_{max} of the hypothalamus and pituitary, respectively.



SECTION III: APPENDICES

APPENDIX A: THE PROBLEM

STATEMENT OF THE PROBLEM

Millions of concussion are diagnosed each year, impacting athletes participating at all levels of sport, including high school collegiate, and professional. Current management of a concussion consists of an evidence-based, multimodal approach consisting of clinical measures of neurocognitive function, balance, and self-reported symptoms. Length of recovery following a concussion varies, and while most athletes will experience resolution of symptoms within 10-14 days of their diagnosis, a subset of athletes may experience persisting symptoms (i.e. lasting longer than 14 days). For this reason it is important to identify factors that influence time to symptom resolution. Among the predictors of recovery time after concussion are multiple post-injury factors such as greater symptom severity or the presence of specific symptoms such as headache, dizziness, or sleep disturbances.

Following a concussion, sleep-related symptoms, such as excessive drowsiness, difficulty maintaining sleep, or changes in the quality and/or quantity of sleep are often endorsed, with nearly 70% of individuals under the age of 25 reporting sleep disturbances throughout recovery. Research has shown that individuals who experience sleep

symptoms take longer to experience symptom resolution and ultimately an unrestricted return to play. Though this is suggestive that sleep is a modifiable factor of recovery, the majority of this research has been primarily conducted in individuals aged less than 18 years old, and has primarily been assessed after the acute phase (i.e.->72 hours) of injury. Additionally, despite research demonstrating that sleep-related symptoms are associated with longer recovery time, the physiological mechanism for why sleep disturbances occur how they influence recovery remain poorly understood. The pattern and time frame in which sleep-related symptoms present may vary between individuals, and consequently, no evidence-based recommendations currently exist for the management of these symptoms. For this reason, it is important that health care providers understand the physiological link between sleep and concussion, how to evaluate sleep disturbances, and ultimately how to manage athletes with a concussion who are experiencing sleep symptoms to facilitate recovery. Therefore, our study will provide evidence related to the role sleep plays in recovery that has not yet been examined in previous research, particularly in collegiate athletes with and without a diagnosed concussion.

RESEARCH QUESTIONS

1. Does severity of sleep symptoms predict overall symptom severity in collegiate athletes within 72 hours of a diagnosed concussion, compared to the severity of Somatic, Cognitive, and Affect symptoms?
2. Do collegiate athletes with a concussion demonstrate differences in time spent in stages of sleep within 72 hours of injury, as compared to non-concussed matched comparisons?

3. Following resolution of concussion symptoms, do collegiate athletes demonstrate higher levels of neuroinflammation, as indicated by microglia activation, in anatomical regions of brain that regulate the sleep-wake cycle, compared to non-concussed matched controls?

EXPERIMENTAL HYPOTHESIS

1. Overall symptom burden in the acute phase of injury (<72 hours) will be best explained by the severity of Sleep symptoms, with higher Sleep symptom severity being predictive of higher overall symptoms burden.
2. Collegiate athletes with a concussion will demonstrate significant differences in time spent in the stages of Light, Deep, and REM sleep in the acute phase of injury (<72 hours), compared to non-concussed matched controls.
3. Compared to non-concussed matched controls, collegiate athletes with a concussion will demonstrate higher volumes of inflamed brain tissue of the Hypothalamus and Pituitary gland, as measured through advanced neuroimaging.

ASSUMPTIONS

1. Clinical diagnosis of concussion will be appropriate to the most recent international guidelines established by the Concussion in Sport Group.
2. Participants will follow the recommendations of their team's respective athletic trainer and team physicians related to athletic and academic participation following their diagnosed concussion.

3. Participants will wear the OURA ring for the duration of the study and only remove OURA ring when necessary (i.e.- when they are not sleeping).
4. Sensor derived time for each sleep stage as measured by the OURA ring is a reflection of cumulative amount of time spent in stage over the duration of one night of sleep.
5. Participants will accurately an honestly complete the patient-reported questionnaire related to symptomology.
6. The matched non-concussed participants will accurately reflect typical function and demographic of each of the concussed participants.

DELIMITATIONS

1. Concussed participants will be assessed at three pre-specified time points following injury, one patient-specific time point dependent on their reported symptomology.
2. Non-concussed participants were matched to concussed participants by sex, age, height, weight, sport (and sport position when possible).
3. Participants were excluded if they had a concussion or musculoskeletal injury within the previous six months that required current physical rehabilitation.
4. Participants were limited to collegiate athletes and students at the University of Virginia.

LIMITATIONS

1. The OURA ring is a consumer wearable device that is limited in accurate measurement of sleep stages.
2. The sample size for evaluation of sleep stages as measured by OURA ring was unequal.
3. Symptomology is self-reported and thus assumed to be accurate and honest as reported by concussed individuals.
4. Peak translocator protein (TSPO) expression occurs 7-14 days post-injury in rodent models. However, this temporal expression is not known in humans.
5. Standard uptake volume (SUV) is a widely used metric for positron emission tomography (PET) imaging, however it cannot delineate blood pool contamination for tissue specific PET signal, which may be important in perfused brain regions such as the pituitary.

OPERATIONAL DEFINITIONS

1. Concussion: A traumatic brain injury induced by biomechanical forces. [1]
2. Symptom burden: The sum severity of all symptoms reported. [1]
3. Acute phase of injury: Initial 72 hours following diagnosed concussion. [1]
4. Nonrapid Eye Movement-1 (NREM1): Lightest stage of sleep, accounting for 5% of total sleep time. [2]
5. Nonrapid Eye Movement-2 (NREM2): Second stage of NREM sleep, accounting for approximately 45% of total sleep time. [2]
6. Nonrapid Eye Movement-3 (NREM3): Deepest stage of NREM sleep, accounting for 25% of total sleep time. [2]

7. Rapid Eye Movement (REM): Stage of sleep characterized by rapid eye movement and increased breathing and bodily movement. Accounts for approximately 25% of total sleep time. [2]
8. Sleep cycle: The alteration between nonrapid eye movement (NREM) and rapid eye movement (REM).[2]
9. Light sleep duration (as measured by OURA ring): Total amount of NREM1 and NREM2 sleep registered during time asleep. [3]
10. Deep sleep duration (as measured by OURA ring): Total amount of NREM3 sleep registered during time asleep. [3]
11. REM sleep duration (as measured by OURA ring): Total amount of REM sleep registered during time asleep. [3]
12. Process C: The circadian timing of when sleep occurs. [4]
13. Process S: The homeostatic drive for sleep. [4]
14. Ascending reticular activating system (ARAS): Found in the anterior segment of the brainstem. Responsible for the coordination of sleep-wake cycle via nuclei. [5]
15. Microglia: Resident immune cells of the central nervous system (CNS). [6]
16. Translocator Protein (TSPO): Transmembrane protein found in the outer membrane of mitochondria.[7]
17. Positron Emission Tomography (PET): Neuroimaging technique utilized to measure physiological and biochemical function of cells. [8]

SIGNIFICANCE OF THE STUDY

A multimodal approach for the management of concussion is recommended by several governing bodies. [1,9] This approach consists of clinical measures of neurocognitive

function, balance, and self-reported symptoms. [1,9] Approximately 80% of high school and collegiate athletes will experience resolution of symptoms within ten days. However, a small subset (20%) of athletes may experience symptoms which may last several weeks or months. [1] As such, there is significant interest in modifiable factors that may contribute to the length of recovery. Sleep-related symptoms, such as excessive drowsiness, difficulty falling or maintaining sleep, and overall changes in quality and/or quantity of sleep have been reported in up to 70% of individuals following a concussion.[10] Additionally, sleep-related symptoms have been shown to be associated with greater symptomology, longer recovery times and increased reports of symptoms of anxiety and depression. [11–14] Though there is growing empirical evidence that sleep is an important modifier of concussion recovery, most of this research has been performed with adolescent athletes (<18 years of age), with sleep-related symptoms being assessed after the acute phase of injury (>72 hours). The varying assessment time points reported in literature make it difficult to determine the degree to which sleep disturbances, particularly in the acute phase, impact clinical recovery. Provided that sleep is a necessary component of health, and that changes in sleep may negatively affect recovery timelines, it is imperative to examine how sleep is associated with recovery from injury. Our current study is the first step to establish characteristic of sleep in the acute phase of injury in a sample of collegiate athletes with and without a diagnosed concussion. Our study will evaluate biometric measures of sleep to evaluate changes in sleep architecture (i.e., stages of sleep) in the acute phase (<72 hours) which may provide insight regarding not yet examined factors of sleep that influence recovery. Additionally, we will utilize advanced neuroimaging to determine potential etiology for sleep-related changes

commonly reported following a concussion. Overall, our study will be the first to: 1. Utilize advanced statistics to determine the effect sleep symptom severity has on overall symptom severity in the acute phase of injury; 2. Utilize a novel wearable device to examine differences in sleep architecture (i.e., stages of sleep) in the acute phase of injury; and 3. Examine levels of neuroinflammation using advanced neuroimaging in regions of the brain that regulate sleep. Our findings will provide evidence of the role of sleep in recovery from concussion, as well as lead to future investigations that may result in an evidence-based protocol for the management of sleep following injury.

APPENDIX B: LITERATURE REVIEW

Pathophysiology of Concussion

A concussion can be defined as any biomechanical force to the head, neck, or spine that results in neuronal strain in the brain.[1,2] The neuronal strain that occurs as a result of the biomechanical forces may damage the structure of the neurons and glial cells within the brain, potentially causing diminished neuronal transmission. [3–5]Neuronal strain of the axonal membrane results in an immediate physiological response known as the neurometabolic cascade.[3,4] Immediately following the concussion-related neuronal strain, there is a marked increase in cerebral blood flow and the binding of the excitatory neurotransmitter glutamate to N-methyl-D-asparate receptors, creating a dysregulation of ionic levels. [3,4] This ionic flux results in the depolarization of neurons. In an effort to restore cellular homeostasis, voltage and ligand-activated channels are opened, causing the depletion of intracellular adenosine triphosphate (ATP), thus creating a hypermetabolic state.[3,4] Moreover, there is an influx of calcium ions, and efflux of potassium and sodium ions. This influx of calcium ions due to increased glutamate activity adversely affects cellular mitochondrial function that may impair mitochondrial function that may impair the generation of ATP needed to restore balance.[3,4] This dysregulation of ions and overall disruption of homeostasis typically lasts between 24 hours up to 4 daysafter a concussion.[3,4] Additionally, glucose metabolism and cerebral blood flow may remain impaired for up to 10 days following injury.[4,6]

Measurement

Evidence based recommendations for the management of a concussion includes a multimodal approach consisting of concussion-related signs and symptoms, cognitive function, and sensorimotor status to assess for recovery and safe return to play (RTP) following injury.[1,2] This multimodal approach is due to the absence of a gold standard for the diagnosis of concussion. However, sufficient evidence exists that when the multimodal battery of assessments are used, a sensitivity of up to 100% and specificity of 97.5% is achieved for concussion when used immediately following injury (i.e.≤24 hours).[7] The aforementioned domains are often assessed through the use of symptom checklists, computerized neurocognitive tests (e.g. ImPACT), and postural control tests (e.g. Balance Error Scoring System).[1,2,8]

Symptoms

As mentioned, following a concussion, individuals may experience a myriad of symptoms including but not limited to headache, dizziness, difficulty remembering, and changes in mood state. [1,2] Symptoms following a concussion are primarily assessed through symptom inventories, in which individuals may or may not endorse symptoms and then subsequently rate their severity and/or duration[1]. Since their first utilization in college athletics, a variety of symptom inventories have been created that still continue to be used today, such as the Rivermead Postconcussion Symptom Scale, the Postconcussion Symptom Scale (PCSS), the Revised Head Injury Scale (HIS-r), and the Graded Symptom Checklist (GSC).[9–14] Generally speaking, symptom inventories consist of similar symptoms (e.g. headache, dizziness, difficulty remembering), however they may differ in the time frame for which an individual is asked to rate the symptoms.[11,12]

For example, the PCSS asks individuals to rate symptoms as they currently feel them at that time, whereas the HIS-r instructs individuals to report if they have felt the listed symptoms at any point in the previous 24 hours.[10,12–14] Irrespective of the time frame, the symptom inventories are completed by indicating the presence of any symptoms and subsequently rating the severity of the symptoms. Severity is rated on a Likert scale with higher numbers indicating more severity. Total symptom severity is then calculated by summing the respective individual severity scores, resulting in a total score that may range from 0 to 132, depending on the symptom inventory used. Though the sensitivity and specificity of symptom inventories increases when used as part of a multimodal approach, they have demonstrated adequate sensitivity and specificity on their own, ranging from 53-89% and 89.4-100% respectively.[15–17]

Neurocognitive Measures

Neurocognitive deficits are commonly seen following concussion, including but not limited to working memory, processing speed, and attention.[9] These deficits may be assessed with traditional neuropsychological tests or computerized neurocognitive tests.[18] Traditional paper-and-pencil neuropsychological tests are comprehensive and may take anywhere from 4-6 hours to administer to a single patient. Common examples of tests used within a battery are the Trail Making Tests A and B, which assesses sustained as well as divided attention; the Digit Span Test, which assesses working memory; or the Symbol Digit Modalities test which assesses visual-spatial and motor speed and accuracy.[19] Though traditional paper and pencil tests are considered to be a comprehensive assessment of cognitive abilities, their time and cost intensiveness make their utility limited in a sports medicine setting. Abbreviated versions of paper-and-pencil

tests have been created and have been demonstrated to be sensitive to cognitive deficits following concussion, (e.g., lengthened reaction time). [9,20,21]

In an effort to overcome the practical limitations associated with traditional neuropsychological testing, computerized neurocognitive tests (CNTs) were created. Several advantages of CNTs exist, such as an ability to test multiple individuals at once, decreased time of overall assessment, and automated scoring.[22,23] Moreover, with specific cognitive constructs, such as reaction time, CNTs have been demonstrated to be more. Commonly used CNTs include the Immediate Post-Concussion Assessment and Cognitive Test (ImPACT), HeadMinder, CogSport, and the Automated Neurocognitive Assessment Metrics (ANAM). The ImPACT measures a variety of cognitive constructs, including visual and verbal memory, and reaction time, and is most commonly used in collegiate and high school athletes. When used within 24 hours of injury, ImPACT demonstrates high levels of sensitivity (79.2-91.4%) and specificity (69.1-89.4%). [23,24]

Balance Measures

Concussive injuries have been demonstrated to result in postural control deficits, which is an indirect measure of sensorimotor status.[8,25] The involvement of the central nervous system (CNS) to maintain an upright posture can be divided into two categories: sensory organization and muscle coordination. [25,26] The preferred impression for balance control comes from the somatosensory system, such as the feet in contact with support surface. [25–27] Whereas, muscle coordination refers to the processes that determine the sequencing and delivery of contractile activity within the muscles of the lower extremity and trunk that create supportive reactions for maintain balance. [25,26] In individuals with balance deficits, such as those seen following a concussion, it is thought that the

organization between the sensory systems (vestibular, visual, and somatosensory) is disrupted. [25,27,28]

The Sensory Organization Test (SOT) and Balance Error Scoring System (BESS) are two commonly used measures to assess postural stability following a concussion. The SOT quantifies balance through use of a force plate that measures changes in vertical projection of the center of mass of the body, while simultaneously altering sensory cues available to their somatosensory or visual systems.[29–31] The SOT is considered the gold standard measurement of balance, however its clinical utility is somewhat limited for sports medicine clinicians due to its high cost and required space to administer the test.[25,30] The BESS is a more time and cost effective measure of postural control when scompred to the SOT. The BESS was developed as a practical and inexpensive way to indirectly assess for balance deficits commonly observed with a concussion.[26,32] Unlike the SOT, the BESS is a static measurement of balance. The BESS is subjectively scored for errors by the professional administering the test, therefore validity of the BESS is based on the experience of those professionals at time of administration. [30]

Sleep

Sleep is an essential component of recovery following a concussion. [1] Of the multitude of symptoms that an individual may experience following the injury, sleep symptoms such as excessive drowsiness, difficulty maintaining sleep, or changes in the quantity and/or quality of sleep are endorsed by up to 70% of injured patients. [33] For this reason, sleep disturbances are being suggested as modifier of recovery following a diagnosed concussion. [34–39] To date, research has demonstrated that individuals who experience sleep-related symptoms take a greater number of days to report symptom free

and ultimately return to play following a diagnosed concussion. [36,38,40–42] However, the physiological mechanism(s) of why sleep disturbances occur and how they influence recovery remain poorly understood.

Though sleep symptoms are commonly reported following injury, the pattern and time frame in which they present may vary between individuals. For this reason, it is important that health care providers understand the physiological link between sleep and concussion, how to evaluate sleep disturbances, and how to manage injured athletes who are identified as having sleep symptom(s) to facilitate recovery. To address these items, this review will address; what is known about the physiological link between sleep and concussion, common measures of sleep, sleep throughout recovery from a concussion, and areas for future research.

Parallels between Concussion and Sleep Physiology

Sleep

The regulation of sleep is driven by the interaction of two separate biological mechanisms: the homeostatic need for sleep (Process S), and the circadian timing of when sleep happens (Process C).[43–45] The interaction between Process S and Process C is referred to as the 2 Process Model.[43,44] The homeostatic component, Process S, can be described as an individual's "need to sleep" during times in which there is sustained wakefulness.[43–45] During these periods of sustained wakefulness, glycogen stores are being depleted while resulting the amino acid adenosine is accumulating. The longer an individual is awake, the more accumulation of adenosine occurs.[43–45] Increased levels of

adenosine subsequently inhibit various regions of the brain responsible for wakefulness, ultimately resulting in the transition from wakefulness to sleep.

The circadian component, Process C, is described as an "internal clock", setting the timing at which sleep and wakefulness occur in a given 24 hour cycle. [43–45] This internal clock is located in the suprachiasmatic nucleus (SCN) which is located in the hypothalamus. Process C is largely influenced by light input from the surrounding environment (i.e., sun light), thus activating wake-promoting regions of the brain.[44,45] As light dissipates, Process C influences the pineal gland to release the sleep-promoting neuropeptide known as melatonin, thereby inhibiting wake-promoting regions of the brain which then facilitates Process S. [44,45]

As established, the 2 Process Model regulates sleep and wakefulness. This regulation of sleep and wakefulness is reflective of the inhibition or activation of various neurotransmitters.[43] The excitement of sleep-promoting neurons or inhibition of wake-promoting neurons may prompt sleep.[46] Conversely, waking can be promoted through the excitement of wake-promoting neurons, the inhibition of sleep-promoting neurons, or both. The excitation or inhibition of these neurons results in the release of specific neurotransmitters. The primary neurotransmitters involved with sleep include: gamma-aminobutyric acid (GABA), hypocretin, acetylcholine, dopamine, norepinephrine, serotonin, adenosine, and histamine.[46] This excitation or inhibition of neurons and subsequent release of neurotransmitters is initiated and maintained by the ascending reticular activating system(ARAS) which is located in the brain stem.[47] During sleep, the ARAS is inhibited, thus allowing the excitation of the sleep-promoting neurotransmitters (e.g.,GABA). Waking occurs when the inhibition of the ARAS is weakened, allowing it

to initiate a widespread network of signals to wake-promoting regions of the brain, resulting in the excitation of wake-promoting neurotransmitters including hypocretin, acetylcholine, dopamine, norepinephrine, serotonin, and histamine. This excitation transitions the body from a state of sleep to a state of waking.[46,47]

Importantly, this activation or inhibition of the ARAS is dependent on the extracellular ionic concentration of calcium, magnesium, and potassium in the brain. [48] When the ARAS is inhibited (i.e., during sleep), the extracellular concentration of calcium and magnesium is elevated and levels of potassium are low. Conversely, during ARAS activation (i.e., wakefulness), the extracellular calcium and magnesium are low, and potassium levels are high.[48] This complimentary ionic balance from sleep to wakefulness, and vice versa, has been demonstrated in animal models. In rodent models, researchers observed that the sleep-wake state can be manipulated by altering the concentrations of calcium, magnesium, and potassium, in the absence of neurotransmitters.[48] These findings suggest that the ionic concentration alone plays a crucial role in the sleep-wake cycle.

Concussion

A concussion can be defined as any biomechanical force to the head, neck, or spine that results in neuronal strain in the brain. [1] Neuronal strain of the axonal membrane results in an immediate physiological response known as the neurometabolic cascade. [3,4] Immediately following neuronal strain, there is a marked increase in cerebral blood flow, as well as the binding of the excitatory neurotransmitter glutamate to N-methyl-D-aspartate (NMDA) receptors, creating a dysregulation of ionic levels. [3,4] This ionic flux results in depolarization of neurons. In an effort to restore cellular homeostasis, voltage

and ligand-activated channels are opened, causing the depletion of intracellular adenosine triphosphate (ATP), thus creating a hypermetabolic state.[3,4] Moreover, there is an influx of calcium ions, and an efflux of potassium and sodium ions. This influx of calcium ions due to increased glutamate activity adversely affects cellular mitochondrial function that may impair the generation of ATP needed to restore balance. [3,4] This dysregulation of ions and overall disruption of homeostasis typically lasts between 24 hours up to four days after the insult..

A Tale of Two Physiologies

The relationship between sleep and concussion is likely complex and multifaceted. At the onset of sleep, activity of the hypothalamic-pituitary-adrenal (HPA) axis, an area within the brain that aids in the regulation of the sleep-wake cycle, is suppressed. Immediately following a concussion, the pathophysiological process of the neurometabolic cascade triggers an increased activation of the HPA axis and the sympathetic nervous system (SNS).[49] This activation results in an increased release of the neurotransmitter noradrenaline (NA), which stimulates arousal.[46,49] Relatively low levels of NA can exert neuroprotective properties, however in higher levels such as those seen in an inflammatory response, NA can be destructvive.[50] REM sleep specifically is crucial to maintaining low levels of NA as it allows for optimum immune response. During REM sleep neurotransmitters such as GABA and acetylcholine act as anti-inflammatory regulators in the sense that they have been shown to block NF-KB pathways, thereby contributing to the maintenance of inflammatory response during sleep.[51] However, if NA levels are too high, this inflammatory regulation is affected.[52] In this way, both acute and chronic inflammation may be modulated by NA, which in turn is modulated by sleep.

The mediating effects of NA can further be appreciated through their interactions with microglia. Microglia are immune cells within the central nervous system (i.e.-brain and spinal cord) that perform immune surveillance and macrophage activities.[53,54] Such activities include production of cytokines and/or chemokines.[53] When an inflammatory process is triggered, microglia go from a state of surveillance to a state of activation.[54] Microglia possess two adrenergic receptors, β2 which is active in a resting state, and α2A which is active in an inflammatory state.[55] Interesting, NA mediates both; in a resting state, NA binds to β2 receptors and downregulates the expression of pro-inflammatory cytokines, whereas in an inflammatory state when the α2A receptor is active, NA will bind and cause an upregulation of cytokine production.[56] As previously mentioned in the context of concussion, immediately following injury, there is activation of the HPA axis and the sympathetic nervous system, which results in an increased release of NA.[49] Due to the activation of microglia in response to the injury, NA now binds to the α2A receptor, creating further cytokine production and release.

When increased activation of the central nervous system is chronic, lasting days to weeks to months, maladaptive sleep patterns occur, which are known as sleep disturbances.[57] These sleep disturbances can manifest in the timing and length of sleep stages such as increased duration of REM sleep and decreased or increased duration of slow wave sleep (NREM, stage 3).

As mentioned there are many cytokines that act on the sleep-wake cycle. These include: interleukines 1,2,4,6,8,10,13,15,18; tumor necrosis factor (TNF); growth hormone releasing hormone (GHRH), adenosine.[57] This provides strong evidence to suggest that cytokines are heavily involved in the regulation of sleep. Specifically, IL-1

and TNF have been found to be deemed sleep regulating substances (SRS) as they fulfill the criteria of being able to enhance or inhibit sleep if injected, their actions on sleep regulatory circuits, and that their levels have been shown to be altered in a pathological state that is associated with increased sleep.[58] Partial night-time sleep deprivation has been found to activate inflammatory pathways involving nuclear factor-KB (NF-KB). The activation of NF-KB results in an upregulation of the cytokines IL-1 and (TNF) and has been found to occur in areas such as the lateral hypothalamus.[58] Additionally, an increase in IL-1 may stimulate and increased release in NA, further disrupting sleep and the inflammatory process. The role of neurotransmitter, specifically that of NA can further be appreciated through its interaction with the glymphatic system.

The glymphatic system is a series of perivascular channels throughout the brain that serve to clear away and expel waste in the central nervous system (CNS).[59] The glymphatic system is most active during sleep, bringing an influx of cerebral spinal fluid (CSF) into the brain to clear away metabolic waste (e.g., amyloid beta) that has naturally accumulated while awake. [59] Disruption of glymphatic activity has been implicated in various pathologies, such as anxiety, depression, and moderate traumatic brain injury (TBI), in which deficient sleep was associated with perivascular space, indicating dysfunction with waste clearance.[60,61] The enhanced efficiency of the glymphatic system due to sleep may be intertwined with the actions of NA.[59] During wakefulness, the release of NA acts to suppress glymphatic activity by decreasing perivascular space. Conversely, interstitial space is increased when NA release is inhibited.[59] This poses the theory that that increased levels of NA as a result of the inflammatory response from

concussion may hinder the glymphatic system's ability to expel the cellular debris created from the injury.

Additional potential rationale for why sleep dysfunction following a concussion occurs may associate with the similarities the ionic concentration between the neurometabolic cascade and the sleep-wake cycle, ultimately affecting glymphatic function. The ionic concentration of the neurometabolic cascade is high in potassium and low in calcium. The same concentration has been observed during the initiation and maintenance of wakefulness. Theoretically, this may partially explain why sleep is disrupted following the injury. This theory may be further explained by the findings of Lundgaard and colleagues in which they observed the state of wakefulness or sleep is ionic dependent.[48] As such, it can be hypothesized that the resulting disruptions in sleep following a concussion may lead to decreased glymphatic clearance of the known metabolic waste associated with the pathophysiology of the injury. The multifaceted interaction of the physiology of sleep and concussion effectively creates a feedback loop in which sleep disturbances manifest. (Figure 1.)

Measures of Sleep

Questionnaires

Sleep diaries and questionnaires are the most cost-effective and simple way to assess sleep. Sleep diaries may vary in length and may include the self-reporting of bed and wake times, lights-out time, night time awakenings, or daytime naps.[62] Compared to a sleep questionnaire, diaries are particularly useful if they are used consistently for a period of time (i.e.-one to two weeks) as opposed to a singular point.[63] Sleep

questionnaires are often used as the first step in an initial assessment of sleep due to their time and cost-effectiveness. Questionnaires measure a number of aspects of sleep quality such as insomnia, sleep patterns, sleep apnea, or increased drowsiness.[63] Additionally, sleep questionnaires serve to quantitatively capture an individual's perception of their sleep over a given period of time, ranging from one week to one month.

Actigraphy

Actigraphy measures sleep by recording physical activity through the use of an accelerometer.[64] The recorded accelerometer data is then processed to determine an individual's sleep or waking states.[65] Such devices can be worn on the wrist, hip, or finger, and allow for the continuous monitoring of sleep over multiple nights outside a laboratory setting. The majority of consumer-available actigraphy devices used in measuring sleep began as fitness trackers. Consumer-available actigraphy devices offer limited utility in terms of which sleep metrics are recorded. The sleep metrics are limited to the binary classification of sleep or awake.[65] Some devices were developed for the purpose of measuring sleep and offer additional sleep metrics (i.e., sleep staging). Generally speaking, when compared to polysomnography (PSG), actigraphy tends to overestimate sleep and underestimate wakefulness. [65]

Polysomnography

PSG is considered the gold standard for assessing sleep. [66] Performed in a laboratory setting, PSG measures a number of bodily functions including sleep staging, respiratory rate and effort, heart rate, limb movement, and oxygen saturation.[66] As the

gold standard, it is both time and cost inducing, and as such is typically only used for assessment of sleep disorders after previous forms of assessments have been exhausted.

Natural History of Sleep and Concussion

Following a concussion, athletes may experience a myriad of symptoms, including but not limited to headache, dizziness, confusion, or sleep disturbances. Though approximately 80% of individuals will experience a resolution of these symptoms within 14 days, the remaining 20% may take longer to recover. [1] This has led to substantial research around which specific symptoms associate with a prolonged recovery. Sleep is a component of "rest." Rest is advised immediately following a concussion (<48 hours), however no evidence based recommendations currently exist for sleep following injury.[1] Presumably, this limitation may be due to sleep disturbances, such as difficulty falling asleep, staying asleep, and or excessive drowsiness, are considered to be symptoms of the injury, as opposed to potential modifier of recovery. To fully appreciate sleep's impact on the management of concussion, sleep can be looked at on the spectrum from prior to the injury (baseline) to throughout recovery.

Baseline

Evidence based recommendations for the management of concussion include a multimodal approach consisting of neurocognitive, balance, and symptom and symptom assessments to assess for recovery and safe return-to-play (RTP) following a diagnosed injury.[1] Baseline values provide a patient-specific reference point for what is "normal" for post-injury assessments. Provided that a safe return to sport is predicated on this

comparison between post-injury and baseline values, it is important for clinicians to understand potential modifiers of a baseline assessment performance.

As mentioned previously, computerized neurocognitive testing (CNT) is one clinical measure of the battery of tests used in the management of concussion. CNTs measure various cognitive constructs (e.g.- memory, processing speed) and most include a separate measure of concussion-related symptomology (e.g., sleep quality and quantity). In high school and collegiate athletes, it has been demonstrated that less than seven hours of sleep the night prior to CNT completion is associated with increased reports of cognitive, somatic, and sleep-related symptoms. [67,68] Moreover, deficient sleep has been demonstrated to associate with increased Reaction Time and decreased Visual Memory and Verbal Memory outcome scores on ImPACT, a commonly used CNT by athletic trainers. [67]

Post-Injury

The relationship between sleep and concussion has been implicated throughout the spectrum of injury and throughout recovery in varying samples.[33,34,36,38,40–42,69–71] Changes in sleep quality and/or quantity has been associated with greater concussion-related symptomology and a longer recovery following injury. As such, clinicians should be cognizant of changes in sleep following injury to better identify and manage athletes that may be at risk for prolonged recovery. Knowing the timing in which these changes in sleep may occur is highly variable and clinicians should be aware of how sleep patterns may evolve throughout recovery from a concussion.

Following a concussion, up to 70% of individuals report changes in sleep quality and quantity. [33] Changes in sleep during the acute phase of injury includes greater difficulties falling asleep, increased hours of sleep, and nightly variability of overall quality throughout recovery.[34,72] Reports of decreased quality of sleep and an increase in quantity, suggest individuals diagnosed with a concussion may demonstrate a greater need for sleep immediately following their injury. In one study of collegiate athletes in the first 48 hours , it was found that those with a concussion demonstrated longer time to fall asleep and greater variability in the number of minutes aware after having initially fallen asleep, when compared to healthy controls.[72] The findings of Hoffman et al., are one of the only studies that have examined sleep in the acute phase of injury. As such, there remains a gap in the research regarding sleep in the acute phase of injury, particularly in collegiate athletes, with the majority of research examining sleep in the sub-acute (i.e.-3-14 days) or chronic (14+ days) phase of injury. (Figure 2)

In the sub-acute phase of injury, changes in sleep begin to become more prominent. Adolescents between 11-18 years of age have been demonstrated to have up to a fourfold increase in recovery time if sleep symptoms (e.g., difficulty falling asleep or staying asleep) are reported when compared to those that did not endorse the same symptoms. [69] Similarly, poor sleep quality has also been reported to result in a higher number of post-concussion symptoms, greater generalized anxiety and poorer quality of life when re-examined three months after injury.[38] Moreover, when examining the relationship between self-reported sleep quantity and post-concussion symptoms in the first week following injury, an increase in cognitive and migraine symptoms has been observed with a reduced number of hours of sleep. . [36] Using wearables, quality and

quantity as measured by actigraphy has demonstrated that individuals who are awake longer throughout the night or are less efficient at sleeping, experience concussion-related symptoms for a greater time period following injury.

Continued sleep disturbances beyond the typical time of post-concussion symptom resolution are common and present similarly to trends seen in the acute and sub-acute phases. The endorsement of sleep disturbances is associated with a greater severity of post-concussion symptoms and decreased neurocognitive performance being associated with greater hours of sleep. [33,42] Additionally, decreased sleep efficiency and increased time spent awake have been found to be associated with poor sleep quality up to one year after injury.[40]

Conclusion

Following a concussion, and throughout recovery sleep disturbances are often reported.[33,34,36–38,69] These disturbances include but are not limited to delayed onset of sleep, frequent nighttime wakenings, daytime sleepiness, and insomnia.[33,36,38,40,42,69] Current literature surrounding sleep and concussion provides important rationale for examining why sleep disturbances are present immediately following injury and may persist years after. Thus far, research has demonstrated that both subjective as well as objective measures of sleep disturbances are associated with prolonged recovery. [33,36,38,41,42,69,72] Currently, an evidence-based approach does not exist for the management of sleep-related problems following concussion, however a better understanding of the physiological contributions of sleep may provide important first steps in the evaluation and management of sleep.

FIGURES

Figure 1. Feedback loop between the pathophysiology of concussion and sleep. **A**. Following a concussion, a neuroinflammatory response is initiated and microglia cells are activated. The α2A adrenergic receptor of the microglia becomes active, which subsequently creates an upregulation of pro-inflammatory cytokines. **B**. The pathophysiological process of a concussion triggers an increased activation of the sympathetic nervous system (SNS) and hypothalamic-pituitary-adrenal (HPA) axis, resulting in an increased release of noradrenaline (NA), which stimulates arousal. **C.** The extracellular ionic concentration that occurs as a result of the neurometabolic cascade is high in K^+ and low in Ca^{2+} and NA^+. The same ionic concentration has been observed during the initiation of wakefulness by the ascending reticular activating system (ARAS), thus potentially further stimulating wake-promoting neurotransmitters. **D.** The release of wake-promoting neurotransmitters, specifically NA, suppresses glymphatic activity by stimulating vasoconstriction, potentially hindering the glymphatic system's ability to expel cellular debris created from the injury.

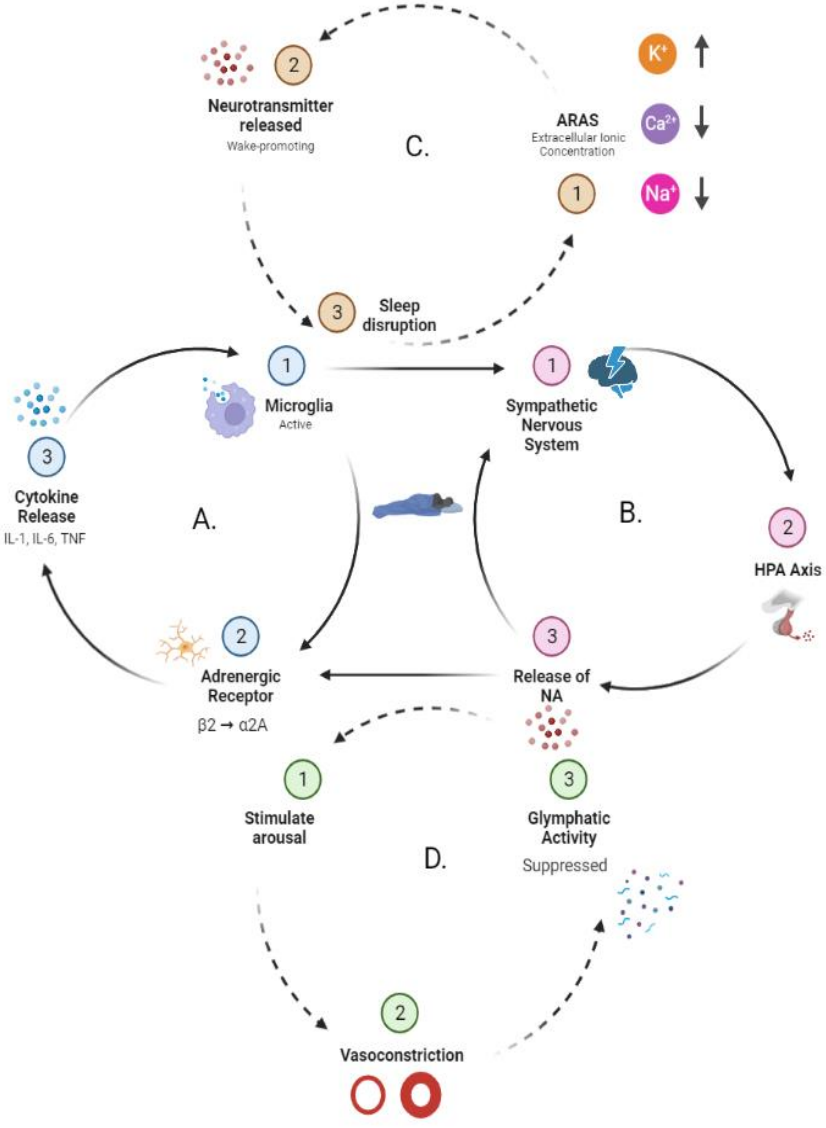
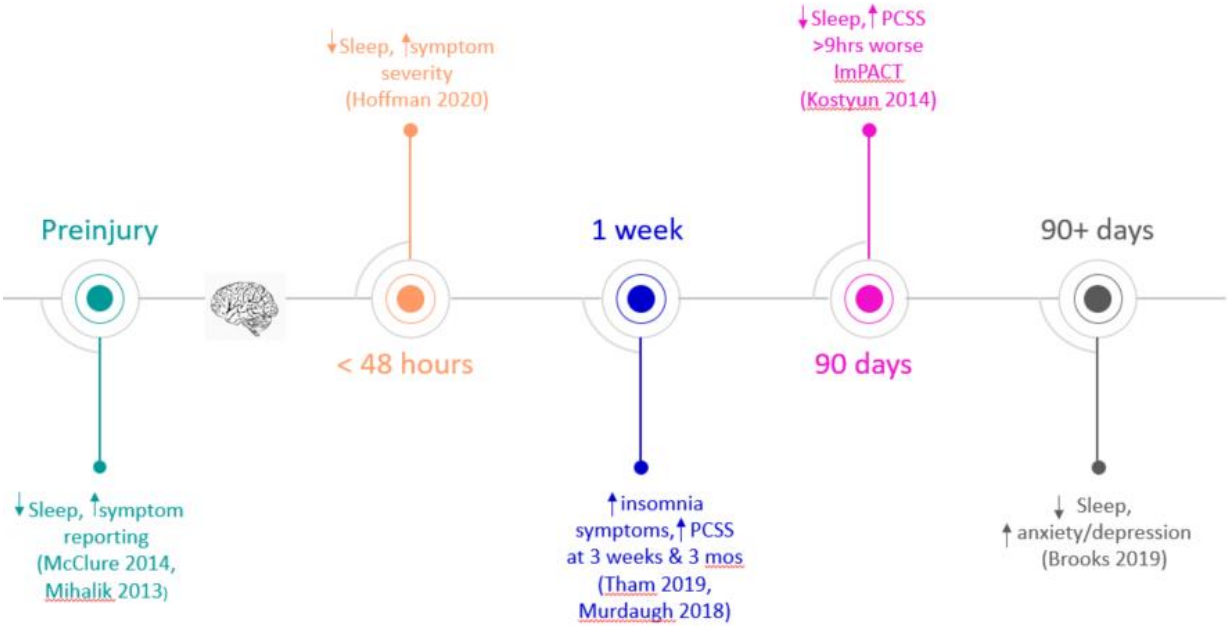


Figure 2. Assessment of sleep before and after a diagnosed concussion.



APPENDIX C: ADDITIONAL METHODS

TABLE C1. UNIVERSITY OF VIRGINIA INSTITUTIONAL REVIEW BOARD APPROVED PROTOCOL (IRB-HSR 200425)

PROTOCOL

Background

1. Provide the scientific background, rationale and relevance of this project.

INSTRUCTIONS

- This should include a referenced systematic evidenced-based review when possible.
- If this study involves qualitative research explain the major constructs of your study.
- Do not state in this section what you plan to do in this study. This information should be entered later under "What will be done in this protocol?"
- Do not include the bibliography in this section.
- For studies submitted under the Expedited review criteria, this section need not be more than a few paragraphs.
- For those studies where data will be analyzed collaboratively by multiple sites doing a similar study for which there is no common protocol (Collaborative Site Analysis Study) include a description of the common scientific goals/ procedures/data points.
- If this is an update to current templates from Protocol Builder make sure the information throughout the protocol includes the most current information.

Answer/Response:

Sleep is an essential component of recovery following a sport concussion (SC). [1–3]Of the multitude of symptoms that a collegiate athlete is diagnosed with following a SC, sleep symptoms such as excessive drowsiness, difficulty maintaining sleep, or changes in the quality and/or quantity of sleep are endorsed by 70% of patients. [1,4,5]SC-related sleep disturbances are a known modifier of recovery following a diagnosed injury. [6–11] To date, research has demonstrated that individuals who experience sleep-related symptoms take a greater number of days to report symptom free and ultimately return-to-play following a diagnosed SC. [4,6–11] However, the physiological mechanism of why

sleep disturbances occur following a SC and how they influence recovery remain poorly understood. Alterations in the sleep-wake cycle, such as those experienced following SC, may hinder the glymphatic system's ability to effectively carry out its primary function of clearing away metabolites and proteins that have accumulated as a result of axonal injury, therefore creating an environment conducive to prolonged recovery. Identifying specific targets associated with sleep disturbances may allow clinicians to effectively treat sleep-related symptoms, restore glymphatic system function, in order to promote recovery.

The alternation between nonrapid eye movement (NREM) and rapid eye movement (REM) is referred to as a sleep cycle. A sleep cycle typically lasts 90-120 minutes and occurs approximately four to five times a night. [12] NREM and REM stages lengthen in time throughout the night from 10 minutes initially to 60 minutes in the final cycle. [12] To achieve the restorative benefits of sleep, the sleep cycle must occur. Sleep symptoms may disturb the sleep cycle by shortening the length of NREM and REM stages following a diagnosed SC. Though sleep symptoms have been associated with longer recovery times following a SC, research has not addressed the interplay between NREM and REM and their influence on recovery and following an unrestricted return to play. The absence of these data may potentially limit a clinician's ability to facilitate a more timely recovery following a diagnosed SC in collegiate athletes. Our study is an important first step to understand how NREM and REM contribute to SC recovery and may yield potential targets for intervention to reduce the number of days collegiate athletes experience SC-related symptoms. We will use a non-invasive, sensor-derived measure of sleep to understand the interplay of NREM and REM in collegiate athletes with and without a diagnosed SC following their diagnosis, throughout recovery, and upon making an unrestricted return-to-play following a diagnosed SC.

Objectives/Hypothesis

INSTRUCTIONS:
If this study involves biomedical research clearly state the objectives and hypotheses and clearly define the primary and any secondary outcome measures. If this study involves qualitative research clearly state your research hypothesis or question.

This section should not include information already included in other sections such as background information or information from the procedures section.

Answer/Response:

The objective of this study will be to address acute differences in sleep following Sport Concussion (SC) through the following aims:

AIM 1: To observe differences in sleep architecture (REM, NREM, sleep onset latency, wake after sleep onset, total sleep time) immediately following SC and throughout recovery compared to similarly-matched controls.
Hypothesis 1a: Individuals with SC will demonstrate significantly reduced time in REM and NREM in the first 72 hours of their diagnosis.
Hypothesis 1b: On the group level, individuals with SC will not demonstrate significantly different values in total sleep time when compared to similarly matched controls in first 72 hours of injury.
AIM 2: Determine if acute changes in sleep architecture in concussed individuals correlates with symptom recovery
Hypothesis for Aim 2: We hypothesize that changes in sleep architecture will be associated with days until symptom free.
Clinical Impact: Currently, an evidence-based approach does not exist for the management of sleep-related problems following SC. Research has placed an emphasis on the sleep monitoring, however, a better understanding of the contributions of sleep architecture to recovery is needed. Our findings will provide insight into new interventions to be implemented in the management of SC.

Study Design: Biomedical

1. Will controls be used?

Answer/Response:

Yes

►**IF YES, explain the kind of controls to be used.**

Answer/Response:

Demographic data collected via HSR IRB 20052 will be reviewed to match participants in the SC group to control participants without a SC based on height, weight, gender, age, and sport. Control participants will undergo the same study procedures as the injured group.

2. What is the study design?

Example: case series, case control study, cohort study, randomized control study, single-blind, double-blind, met-analysis, systematic reviews, other. You may also view the IRB-HSR Learning Shot on this topic to help you answer this question. https://hrpp.irb.virginia.edu/learningshots/Writing-a-Clinical-Research-Protocol/presentation.html

Answer/Response:

Cohort study

3. Does the study involve a placebo?

Answer/Response:

No

►**IF YES, provide a justification for the use of a placebo**

Answer/Response:

Human Participants

Ages: _18-25___
Sex: __M/F__
Race: __Any__

Subjects- see below

INSTRUCTIONS: For question 1-4 below insert an exact #. Ranges or OPEN is not allowed. This # should be the maximum # you expect to need to enroll (i.e. sign consent) If you are only collecting specimens the number of participants should equate to the # of specimens you need. If you are collecting only data from a chart review the number should designate the number of subjects whose medical records you plan to review. Age/ Sex/Race criteria should designate the demographics of participants from whom you will obtain the specimen/data.

1. **Provide target # of subjects (at all sites) needed to complete protocol.**

   INSTRUCTIONS: If this is NOT a database protocol, this number should be the same as the number of subjects needed to obtain statistically significant results.

   Answer/Response:
   20

2. **Describe expected rate of screen failure/ dropouts/withdrawals from all sites.**

   Answer/Response:
   We expect approximately 2 participants to withdraw from the current study.

3. **How many subjects will be enrolled at all sites?**

   INSTRUCTIONS: This number must be the same or higher than the # from question # 1 in order to account for the # of screen failures, dropouts, withdrawals described in question # 2.

   Answer/Response:
   A total of 20 participants will be enrolled at all sites with expected attrition to a final sample of 18 participants.

4. **How many subjects will sign a consent form under this UVA protocol?**

   INSTRUCTIONS: If the protocol does not have a consent form- the number listed here should reflect such things as the number of subjects from whom specimens will be obtained, the number of charts to be reviewed etc.

   Answer/Response:
   Up to 20 participants will sign a consent form under the current protocol.

Inclusion/Exclusion Criteria

INSTRUCTIONS:

- The inclusion and exclusion criteria should be written in bullet format.
- *This item applicable if the study will require consent (verbal or written).* Unless there is a scientific reason for not recruiting a certain type of vulnerable population(e.g. not enrolling fetuses, neonates or children in a study regarding Alzheimer's) list the following vulnerable populations under either Inclusion or Exclusion criteria below: pregnant women, fetuses, neonates, children, prisoners, cognitively impaired, educational or economically disadvantage, non-English speaking subjects .
- If you will not enroll subjects who do not speak English because certain procedures cannot be carried out if the subject does not speak English (e.g. a survey is not validated in other languages) insert the following as an Inclusion Criteria: Willingness and ability to comply with scheduled visits and study procedures.
- If this is a collection of only retrospective* specimens or data, the inclusion criteria must include a start and stop date for when specimens/ data will be collected.
- The stop date must be prior to the version date of this protocol.
- *Retrospective: all specimens are in a lab at the time this protocol is approved by the IRB. All data exists in medical records or records from previous studies at the time this protocol is approved by the IRB.

1. List the criteria for inclusion

Answer/Response:

There will be different eligibility for the test subjects and controls as detailed below.

Inclusion- Test subjects:

1. Are active in their respective sport
2. Present within 72 hours of injury
3. Meet criteria for concussion with 1 of the following after sustaining a force to head:
    a. Loss of consciousness
    b. Post traumatic amnesia
    c. Seizure
    d. Neurological deficit
    e. Vomiting
    f. Headache
    g. Dizziness
    h. Other mental status change (feeling dazed, stunned, confused, repetitive questioning, forgetful)
4. Enrollment in HSR20052

Inclusion- Control Subjects:

1. No acute medical complaints or active illness
2. Match injured participant based on previously mentioned demographic criteria.

2. List the criteria for exclusion

Exclusion- Subjects with Concussion:

- Glascow Coma Scale (GCS) score <13 on initial evaluation
- Traumatic injury requiring intensive care unit (ICU) monitoring or operative repair
- Structural abnormality on brain CT (if obtained)
- Non-English speaking
- Any prior traumatic brain injury (TBI) requiring hospitalization
- Brain tumor
- Prior self-reported concussion within the past 3 months (excluding present concussion)
- Neurologic or neurodevelopmental disorder (epilepsy, intellectual disability, dementia, autism, migraine, but not ADHD)
- Use of prescribed or over the counter sleep-promoting medication (e.g.-diphenhydramine, melatonin)
- Psychiatric disorder requiring hospitalization in last year

Exclusion- Control Subjects

- Non-English speaking
- Any prior self reported TBI requiring hospitalization, including prior intracranial surgery and prior intracranial hemorrhage (self-reported)
- Self-reported Brian tumor
- Self-reported use of prescribed or over the counter sleep-promoting medication (e.g.-diphenhydramine, melatonin)Prior self-reported concussion in the past 6 months
- Self-reported neurologic or neurodevelopmental disorder (epilepsy, intellectual disability, dementia, autism, migraine, but not ADHD)

3. List any restrictions on use of other drugs or treatments.

INSTRUCTIONS: List only those drugs or treatments that are prohibited while on study, not those listed as an exclusion criteria.

Answer/Response:

A participant will not be able to take any sleep-aid medication (prescribed or over the counter) such as melatonin or diphenhydramine during study participation.

Statistical Considerations

1. Is stratification/randomization involved?

Answer/Response:

No

►IF YES, describe the stratification/ randomization scheme.

INSTRUCTIONS:
The stratification factors and/or the randomization plan should be identified. If there is no randomization component or important patient characteristics that will be used in treatment allocation or data analysis, a statement to this effect should be included.

Stratification factors: These are pretreatment patient characteristics which could be balanced across treatment arms by design or may be used to determine starting dose or treatment allocation.

If randomization is going to be used, the details of the randomization plan should be described.

The description should include:
--the method and timing of randomization
--the type of randomization scheme that will be used in the study
--whether or not the randomization masked/blinded/if so, then to whom is it masked/blinded
--who has access to the randomization scheme

Answer/Response:

►IF YES, who will generate the randomization scheme?

_____ Sponsor
_____ UVA Statistician. Insert name Answer/Response:
_____ UVA Investigational Drug Service (IDS)
_____ Other: Specify Answer/Response:

2. What are the statistical considerations for the protocol?

The objectives section and the statistical section should correspond, and any objective for which analysis is unfeasible should be deleted. Also, the estimates and non-statistical assumptions of the statistical section should be supported by discussion in the background section.

The answer to this question should include:
--Study Design/Endpoints
--Recap of study objectives and endpoint definitions. An assessment of how study objectives will be assessed by identifying & defining which endpoints will be used to assess each component of the study objectives.

--The study design should include contingencies for early stopping, interim analyses, stratification factors (If applicable), and any characteristics to be incorporated in analyses.
--The power/precision of the study to address the major study endpoint(s), the assumptions involved in the determination of power/precision.
--If statistical hypothesis testing is included then specify the null and alternative hypotheses, the test statistic, and the type I and II error rates
--If precision of an estimate, then provide a definition for precision
--If other, then specify

Answer/Response:

To compare changes in sensor derived biometric measures of sleep and all patient-reported outcome measures as the primary outcome variables we will conduct 2x3 analyses of variance (ANOVA) to assess for an interaction between group (concussed and injured) and time (<72 hours, symptom free, 14 days after symptom free). Post hoc analyses will include independent and paired t-tests.

3. Provide a justification for the sample size used in this protocol.

Include sample size calculations or statistical power estimation. If not applicable, please provide explanation.
Also include the anticipated accrual rate, the accrual goal for the study, including accrual goals by strata if appropriate, adjustments for drop-outs etc. and study duration.

Answer/Response:

The current study is a pilot study. The current target sample (n=20) will allow for equal number of injured participants and control participants.

4. What is your plan for primary variable analysis?

Include primary outcome(s)/predictor variable(s), statistical methods/models/tests to be employed, or descriptive summaries as appropriate. If not applicable, please provide explanation.

Answer/Response:

Dependent and Independent Variables:

The independent variables that will be included for this study are group (injured vs. control) and time. The primary dependent variables will include the sensor-derived REM sleep and NREM sleep. Secondary variables will include total sleep time, sleep onset latency, wake after sleep onset as recorded by the OURA ring as well as the outcome scores of the Revised Head Injury Scale, Pittsburgh Sleep Quality Index, and sleep diary questionnaire, as well as the number of days an athlete reports SC-related symptoms.

Analytic Plan:

Aim 1: To compare changes in sensor derived biometric measures of sleep and all patient-reported outcome measures as the primary outcome variables we will conduct 2x3 analyses of variance (ANOVA) to assess for an interaction between group (concussed and injured) and time (<72 hours, symptom free, 14 days after symptom free). Post hoc analyses will include independent and paired t-tests.

Hypothesis 1a: Collegiate athletes diagnosed with a SC will exhibit a significantly reduced time in NREM and REM, and an increased sleep onset latency within 72 hours of injury when compared to healthy, similarly matched controls.
Hypothesis 1b: Collegiate athletes diagnosed with a SC will not demonstrate significantly different values in total sleep time when compared to similarly matched controls in the first 72 hours after injury.
Aim 2: To determine the association between sleep architecture (TST, WASO, SOL, REM, NREM sleep) and patient reported outcome scores in collegiate athletes with and without a diagnosed SC.
Hypothesis 1a: Changes in sleep architecture will be moderately correlated (0.50-0.70) with the total symptom severity of collegiate athletes diagnosed with a SC symptom burden.

5. What is your plan for secondary variable analysis?

Include the following:
--Secondary outcome(s)/predictor variables, statistical methods/models/tests to be employed, or descriptive summaries as appropriate. If not applicable, please provide explanation.
--For phase III studies, the power/precision of the study to address the secondary objective(s).

Answer/Response:
N/A

6. Have you been working with a statistician in designing this protocol?

Consultation with a professional statistician is highly recommended to ensure good science of the study and facilitate the review process.

Answer/Response:
No

IF YES, what is their name?
Answer/Response:

7. Will data from multiple sites be combined during analysis?
Answer/Response:
No

Study Procedures-Biomedical Research

1. What will be done in this protocol?

INSTRUCTIONS:

This should include everything that will be done as part of this protocol. Do not repeat information that is included in other sections such as Background or Hypothesis sections.
This section should include an indication of which research interventions if any offer a prospect for direct benefit and which interventions (invasive measurements, collection of blood, tissue, data, surveys, etc.) are being done solely to answer a research question and generate generalizable knowledge. If the interventions done solely for research purposes are associated with greater than minimal risk they need to be justified. Describe and justify any control and experimental arm and include method, dose, and duration of drug administration. Reference any claim of clinical equipoise if applicable.

If you are obtaining specimens or data, provide information regarding the type of specimen/data, amount of specimen needed and how the specimen/data will be obtained and what analysis will be done with the specimen/data.

Special note for studies with waiver of consent/waiver of documentation of consent: Include a statement regarding how subjects will be recruited. For other studies this information is captured in Recruitment does not need to be duplicated in this section.

Answer/Response:

Procedure: Upon being diagnosed with a SC by their team's athletic trainer (AT) or team physician, a potential participant will be informed of the study. If the potential participant expresses interest, a member of the research team (CD) will be provided the individual's contact information (i.e.-phone number) and arrange a time to review the study and conduct the consenting process. Participants will be asked to report to our research laboratory within 72 hours after injury, within 24 hours of symptom resolution (approximately 14 days after injury), and 14 days after symptom resolution. Healthy control subjects will complete the same procedures as injured participants at approximately the same time points as the injured participant to minimize the variability in sport demands based on the athlete's respective sport season.

VISIT 1:

- Participants will provide written consent for participation in study.
- Participants will then provide demographic information including date of birth, sex, previous history of concussion, and any current medications.
- Participants will be measured for their OURA ring size which will be based on their second, third, or fourth digit (on either hand).
- Additionally, participants will then download the OURA ring app on their personal smartphone. This will allow for the continuous collection of data from the ring while being encrypted using the OURA "blackout mode" in order to ensure participants will not focus on their sleep data.

- Between VISITS 1 and 2, all participants will complete a patient-reported outcome measure associated with symptomology via the Revised Head Injury Scale (HIS-r).
- Participants will also complete an electronically distributed, secure Sleep Diary survey via Qualtrics that asks them to enter hours of sleep of previous night, time they got into bed and out of bed, number and duration of awakenings. These Sleep Diary questions have been shown to be a reliable for evaluating sleep and wake periods, demonstrating high sensitivity (92.3%) and specificity (95.6%) when compared to PSG.

VISIT 2: In line with IRB-HSR 20052, and University Athletics Department Concussion Protocol , upon reporting symptom free, (approximately 14 days after injury) injured participants will complete asymptomatic assessment for return to play. This will include computerized neurocognitive testing, balance assessment, and patient-reported outcome associate with symptomology, mood state and quality of life. These data will be used to inform progression through the return-to-play protocol. The assessment results will be collected from the research records for HSR20052. Following this visit they will continue to wear the OURA ring for an additional 14 days.

VISIT 3: After the two-week period, participants will return to our lab, return the OURA ring and will be instructed to uninstall app from their phone. Participants will then answer questions regarding their sleep over the previous month as indicated on the Pittsburgh Sleep Quality Index (PSQI).

Instrumentation to Assess Primary Outcome Measures- Validity and Reliability:

OURA ring: Previous studies have demonstrated a sensitivity (ability to detect sleep) of 0.96 and a specificity (ability to detect wake) of 0.48 in the OURA ring when compared to PSG. [13] Additionally, OURA ring demonstrated 65% agreement in detecting NREM stages 1 and 2, 51% agreement in detecting NREM stage 3, and 61% agreement in detecting REM sleep. These outcomes make the OURA ring better when compared to other actigraphy devices[13] and thus support its use to track changes in sleep in participants of this study.

Pittsburgh Sleep Quality Index: The Pittsburgh Sleep Quality Index (PSQI) is a 19-item questionnaire that has been utilized and validated across research for assessing sleep quality and habits that may be associated with depression-related symptoms, over a one month interval. The PSQI has been demonstrated to have a sensitivity of 0.89 and specificity of 0.87 (kappa = 0.75, $p<0.001$) when diagnosing "poor" sleep as indicated by a global score of <5.[14]

Revised Head Injury Scale: The revised Head Injury Scale (HIS-r) consists of 22 symptoms related to SC, and has demonstrated sensitivity of 77.5% and specificity of 100%.[15] For any symptom experienced, symptom severity and duration are subsequently asked. Duration is indicated on a Likert scale ranging from 1 ("briefly"[i.e.- 15 minutes]) to 6 ("always"). Severity is scored on a Likert scale ranging from 0 ("not severe") to 6 ("as severe as possible").

2. If this protocol involves study treatment, explain how a subject will be transitioned from study treatment when they have completed their participation in the study.

Example: If the subject will be taking an investigational drug, will they need to be put back on an approved drug when they have completed the study? If yes, explain how this will be accomplished and who will cover the cost. If the subject has a device implanted will it be removed? Again- who will cover the cost of the removal?
Instructions: Answer NA if this study does not involve a study treatment.

Answer/Response: NA

Bibliography

INSTRUCTIONS: Provide a current bibliography supporting the hypothesis, background and methodology including references to papers and abstracts that have resulted from previous work by the investigator and references to the work of others.

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14. The Pittsburgh Sleep Quality Index: a new instrument for psychiatric practice and research - PubMed. Accessed February 1, 2021. https://pubmed.ncbi.nlm.nih.gov/2748771/

15. Resch JE, Brown CN, Schmidt J, et al. The sensitivity and specificity of clinical measures of sport concussion: three tests are better than one. *BMJ Open Sport Exerc Med*. 2016;2(1):e000012. doi:10.1136/bmjsem-2015-000012

TABLE C2. UNIVERSITY OF VIRGINIA INSTITUTIONAL REVIEW BOARD APPROVED CONSENT FORM (IRB HSR 200425)

Consent of an Adult to Be in a Research Study

In this form "you" means a person 18 years of age or older who is being asked to volunteer to participate in this study.

Participant's Name_____

What is the purpose of this form?

This form will provide you with information about this research study. You do not have to be in the study if you do not want to. You should have all your questions answered before you agree to be in this study.

Please read this form carefully. If you want to be in the study, you will need to sign this form. You will be given a copy of this form.

Key Information About This Research Study

Principal Investigator:	Dr. Jacob Resch University of Virginia 210 Emmett St South Charlottesville VA 22904 Telephone: (434)243-6525 Email: jer6x@virginia.edu

You are being asked to take part in a research study. You do not have to take part in this study.
You should only agree to take part in this study after reading this consent form and discussing it with the study team.
You may also discuss this with your family, friends, health care providers or others before you make a decision.

What problem is this study trying to solve?
We want to find out how concussions affect sleep, and how those changes to sleep impact concussion symptoms. This study is looking at sleep data from college athletes, with and without concussions.

You are being asked to take part in this study because either you are a collegiate athlete who has recently had a concussion, or you are a healthy collegiate athlete without a concussion in the past 6 months.

Why would you want to take part in this study?
You will not be helped by being in this study, but the information gained by doing this study may help others in the future.

Why would you NOT want to take part in this study?
You might not want to take part in this study because you will be asked to wear a sleep-monitoring device as well as answer questions each day throughout entire study.

What will I have to do if I take part in this study?
If you participate in this study,

- Athletes with concussion will have 3 research visits during one month with the study team
- Athletes without a concussion will have 2 visits during one month.

Each visit will last about 10 minutes.

ALL subjects will:

- Answer some health questions
- Download and install an app for the sleep monitoring called Oura Ring on your smartphone
- Wear the Oura ring on your hand every day and night
- Answer daily questions about previous night's sleep
- Answer questions about the last month of sleep
- Answer questions about concussion symptoms

What is the difference between being in this study and getting usual care?
If you take part in this study, the following things will be done differently than if you do not take part in this study:

- You will wear a sleep-tracking ring, as well as answer daily sleep diary questions until 2 weeks after you are symptom free

What will happen if you are in the study?

If you agree to be in this study, you will sign this consent form before any study related procedures take place.

For research purposes you will:

- Complete a health history form, which will ask about your medications, previous concussions, and sleep habits
- Allow us to access test results from study HSR20052, including the Revised Head Injury Scale, which asks about the severity and duration of a variety of symptoms

- Complete daily questions about your sleep habits and how you are feeling
- Wear a sleep-tracking ring, called the Oura ring, every day and night
- Complete a survey regarding sleep quality in the past month, Pittsburgh Sleep Quality Index, (PSQI)

	Visit 0 **(Screening)**	**Visit 1** **(Baseline)**	**Every day after head injury**	**Visit 2** **(Symptom-free)**	**Visit 3** **(14 days after symptom free)**
Study Day	-1 day	0-3 days	All	About 14 days after injury	About 29 days after injury
Phone call	X				
Review study eligibility	X				
Informed Consent		X			
Medical History		X			
Symptom Questions		X	X	X	X
Daily Sleep diary		X	X	X	X
Install Oura app/ pair ring to your smartphone		X			
Wear Oura ring		X	X	X	X
Sleep Survey					X
Return Oura ring					X
Uninstall Oura app from your smartphone/ unpair ring					X

What are the risks of being in this study?

- The main risk of allowing us to collect information about you is a potential loss of privacy. The University of Virginia will do its best to protect your records so that facts about you and your health will be kept private. The chance that information identifying you will be given to someone else is very small. However, we cannot *guarantee* it will be safe.

- Some of the questions asked may be upsetting, or you may feel uncomfortable answering them. If you do not wish to answer a question, you may skip it and go to the next question

What are your other choices if you do not join this study?

The only choice is not to be in this study. If you are an employee of UVA your job will not be affected if you decide not to participate in this study. If you are a student at UVA, your grades will not be affected if you decide not to participate in this study.

Will you be paid for being in this study?

You will be paid $50 in an Amazon gift card for finishing the study. You will receive payment at the completion of the final visit.

Will being in this study cost you any money?

All of the procedures in this study will be provided at no cost to you or your health insurance. You will be responsible for the cost of travel to come to any study visit and for any parking costs.

What if you are hurt in this study?

If you are hurt as a result of being in this study, there are no plans to pay you for medical expenses, lost wages, disability, or discomfort. The charges for any medical treatment you receive will be billed to your insurance. You will be responsible for any amount your insurance does not cover. You do not give up any legal rights, such as seeking compensation for injury, by signing this form.

What happens if you leave the study early?

You can change your mind about being in the study any time. You can agree to be in the study now and change your mind later. If you decide to stop, please tell us right away. You do not have to be in this study to get services you can normally get at the University of Virginia.

How will your personal information be shared?

The UVA researchers are asking for your permission to gather, use and share information about you for this study. If you decide not to give your permission, you cannot be in this study, but you can continue to receive regular medical care at UVA.

If you sign this form, we may collect any or all of the following information about you:

- Personal information such as name, address and date of birth
- Social Security number ONLY IF you are being paid to be in this study
- Your health information if required for this study. This may include a review of your medical records and test results from before, during and after the study from any of your doctors or health care providers. This may include mental health care records, substance abuse records, and/or HIV/AIDS records.

Who will see your private information?

- The researchers to make sure they can conduct the study the right way, observe the effects of the study and understand its results
- People or groups that oversee the study to make sure it is done correctly
- The sponsor(s) of this study, and the people or groups it hires to help perform or review this research
- Insurance companies or other organizations that may need the information in order to pay your medical bills or other costs of your participation in the study
- Tax reporting offices (if you are paid for being in the study)
- People who evaluate study results, which can include sponsors and other companies that make the drug or device being studied, researchers at other sites conducting the same study, and government agencies that provide oversight such as the Food and Drug Administration (FDA) if the study is regulated by the FDA.
- If you tell us that someone is hurting you, or that you might hurt yourself or someone else, the law may require us to let people in authority know so they can protect you and others.

  The information collected from you might be published in a medical journal. This would be done in a way that protects your privacy. No one will be able to find out from the article that you were in the study.

What if you sign the form but then decide you don't want your private information shared?

You can change your mind at any time. Your permission does not end unless you cancel it. To cancel it, please send a letter to the researchers listed on this form or complete the "Leaving the Study Early" part of this form and return it to the researchers. Then you will no longer be in the study. The researchers will still use information about you that was collected before you ended your participation.

Please contact the Principal Investigator listed earlier in this form to:

- Obtain more information about the study
- Ask a question about the study procedures or treatments
- Report an illness, injury, or other problem (you may also need to tell your regular doctors)
- Leave the study before it is finished
- Express a concern about the study

What if you have a concern about this study?

You may also report a concern about this study or ask questions about your rights as a research subject by contacting the Institutional Review Board listed below.

University of Virginia Institutional Review Board for Health Sciences Research
PO Box 800483
Charlottesville, Virginia 22908

Telephone: 434-924-9634

When you call or write about a concern, please give as much information as you can. Include the name of the study leader, the UVA Study Tracking Number (at the bottom of this form), and details about the problem. This will help officials look into your concern. When reporting a concern, you do not have to give your name.

You may also report a concern anonymously by calling the UVA Compliance Hotline phone number at 1-800-235-8700.

Signatures

What does your signature mean?

Before you sign this form, please ask questions about any part of this study that is not clear to you. Your signature below means that you have received this information and all your questions have been answered. If you sign the form it means that you agree to join the study. You will receive a copy of this signed document.

Consent From Adult

_____	_____	_____
PARTICIPANT (SIGNATURE)	PARTICIPANT (PRINT)	DATE

To be completed by participant if 18 years of age or older.

Person Obtaining Consent

By signing below you confirm that you have fully explained this study to the potential subject, allowed them time to read the consent or have the consent read to them, and have answered all their questions.

_____	_____	_____
PERSON OBTAINING CONSENT (SIGNATURE)	PERSON OBTAINING CONSENT (PRINT)	DATE

Leaving the Study Early

Signatures should be obtained in this section if the subject decides to leave the study early.

If you leave the study early the study leader will keep the data collected about you up until the time you leave the study to help determine the results of the study.

Check one option below:

____ I am withdrawing my consent from the intervention or treatment part of this study but agree to continue to have follow up information about me collected by the study team.

____ I am withdrawing my consent for this study. No additional information may be collected about me including follow up information from my medical records.

Consent From Adult

_____	_____	_____
PARTICIPANT (SIGNATURE)	PARTICIPANT (PRINT)	DATE

To be completed by participant if 18 years of age or older.

Person Obtaining Consent

By signing below you confirm that you have fully explained the implications of withdrawing from the study to the subject and have answered all their questions.

_____	_____	_____
PERSON OBTAINING CONSENT (SIGNATURE)	PERSON OBTAINING CONSENT (PRINT)	DATE

TABLE C3. HEALTH HISTORY FORM

Please answer the following questions as accurately and thoroughly as possible:
Last name:_____
First name:_____
Date of birth:_____
Sex: (CIRCLE ONE) FEMALE MALE

How many times have you had a concussion diagnosed by a medical professional (e.g., athletic trainer or physician)?:
_____
Starting with the most recent, please list the approximate month and year of each concussion. (If you have never been diagnosed with a concussion please leave blank)

Month	Year
_____	_____
_____	_____
_____	_____
_____	_____
_____	_____

Have you ever been diagnosed (by a physician) with any of the following?
Please circle Yes or No for each question:

Epilepsy	YES	NO
Intellectual disability	YES	NO
Dementia	YES	NO
Autism	YES	NO
Migraine	YES	NO
Other (not ADHD)	YES	NO

Do you take sleep-promoting medicine (prescribed or over the counter)? YES NO
If yes, which one? _____

Do you share a room with another person (i.e.- a roommate) or do you sleep in your own room:
_____
_____

TABLE C4. SLEEP DIARY

Please enter your first and last name:

_____

At what time did you go to bed last night:

_____

After you went to bed, (approximately) how long did it take for you to fall asleep?

_____

After falling asleep, how many times did you wake up last night?

_____

At what time did you wake up this morning?

_____

TABLE C5. REVISED HEAD INJURY SCALE

Revised Head Injury Scale

Name_____ **Sport** _____

Symptom Checklist; *Circle "YES" if you have experienced the symptom within the last 24 hours or "NO" if you have not experienced the symptom over the last 24 hours.*

1. Have you had a headache in the last 24 hours?	YES/NO
2. Have you experienced nausea in the last 24 hours?	YES/NO
3. Have you had difficulty balancing in the last 24 hours?	YES/NO
4. Have you experienced fatigue in the last 24 hours?	YES/NO
5. Have you experienced drowsiness in the last 24 hours?	YES/NO
6. Have you experienced sleep disturbances in the last 24 hours?	YES/NO
7. Have you had difficulty concentrating in the last 24 hours?	YES/NO
8. In the last 24 hours have you felt like you are "in a fog"?	YES/NO
9. In the last 24 hours have you felt "slowed down"?	YES/NO
10. Have your eyes been sensitive to light in the last 24 hours?	YES/NO
11. Have you felt sadness in the last 24 hours?	YES/NO
12. Have you experienced vomiting in the last 24 hours?	YES/NO
13. Have your ears been sensitive to noise in the last 24 hours?	YES/NO
14. Have you experienced nervousness in the last 24 hours?	YES/NO
15. Have you had difficulty remembering things in the last 24 hours?	YES/NO
16. Have you experienced numbness in the last 24 hours?	YES/NO
17. Have you experienced tingling sensations in the last 24 hours?	YES/NO
18. Have you experienced dizziness in the last 24 hours?	YES/NO
19. Have you experienced any neck pain in the last 24 hours?	YES/NO
20. Have you been irritable in the last 24 hours?	YES/NO
21. Have you experienced feelings of depression in the last 24 hours?	YES/NO
22. Have you experienced blurred vision in the last 24 hours?	YES/NO

For each item that you responded "yes" please rank the symptom in terms of duration (1 – 6) and severity (0 – 6) in terms of the last 24 hours on the next page

For each item that you responded "yes" to on the preceding page please rank the symptom in terms of duration (1 – 6) and severity (0 – 6) in terms of the last 24 hours.

	DURATION						SEVERITY						
	Briefly			Sometimes		Always	Not Severe						As Severe As possible
1) Headache	1	2	3	4	5	6	0	1	2	3	4	5	6
2) Nausea	1	2	3	4	5	6	0	1	2	3	4	5	6
3) Difficulty balancing	1	2	3	4	5	6	0	1	2	3	4	5	6
4) Fatigue	1	2	3	4	5	6	0	1	2	3	4	5	6
5) Drowsiness	1	2	3	4	5	6	0	1	2	3	4	5	6
6) Sleep Disturbances	1	2	3	4	5	6	0	1	2	3	4	5	6
7) Difficulty Concentrating	1	2	3	4	5	6	0	1	2	3	4	5	6
8) Feeling "in a fog"	1	2	3	4	5	6	0	1	2	3	4	5	6
9) Feeling "slowed down"	1	2	3	4	5	6	0	1	2	3	4	5	6
10) Sensitive to light	1	2	3	4	5	6	0	1	2	3	4	5	6
11) Sadness	1	2	3	4	5	6	0	1	2	3	4	5	6
12) Vomiting	1	2	3	4	5	6	0	1	2	3	4	5	6
13) Sensitive to noise	1	2	3	4	5	6	0	1	2	3	4	5	6
14) Nervousness	1	2	3	4	5	6	0	1	2	3	4	5	6
15) Difficulty Remembering	1	2	3	4	5	6	0	1	2	3	4	5	6
16) Numbness	1	2	3	4	5	6	0	1	2	3	4	5	6
17) Tingling	1	2	3	4	5	6	0	1	2	3	4	5	6
18) Dizziness	1	2	3	4	5	6	0	1	2	3	4	5	6
19) Neck Pain	1	2	3	4	5	6	0	1	2	3	4	5	6
20) Irritable	1	2	3	4	5	6	0	1	2	3	4	5	6
21) Depression	1	2	3	4	5	6	0	1	2	3	4	5	6
22) Blurred vision	1	2	3	4	5	6	0	1	2	3	4	5	6

The student-athlete understands that the annual baseline symptom assessment is to check for symptoms related to a concussion. By signing this form, the student-athlete acknowledges he/she has no questions and no additional concussion related symptoms.

Hours of sleep you got last night: ____ hours

Overall Quality of Sleep: 0 1 2 3 4 5 6 7 8 9 10
Very Poor **Excellent**

Signature of student-athlete: _____ Date: _____

Signature of Athletic Trainer: _____ Date: _____

TABLE C6. PITTSBURGH SLEEP QUALITY INDEX

INSTRUCTIONS:
The following questions relate to your usual sleep habits during the past month only. Your answers should indicate the most accurate reply for the majority of days and nights in the past month. Please answer all questions.

1. During the past month, what time have you usually gone to bed at night?

BED TIME

2. During the past month, how long (in minutes) has it usually taken you to fall asleep each night?

NUMBER OF MINUTES

3. During the past month, what time have you usually gotten up in the morning?

GETTING UP TIME

4. During the past month, how many hours of actual sleep did you get at night? (This may be different than the number of hours you spent in bed.)

HOURS OF SLEEP PER NIGHT

For each of the remaining questions, check the one best response. Please answer all questions.

5. During the past month, how often have you had trouble sleeping because you

a) Cannot get to sleep within 30

Not during past month_____ Less than once a week_____ Once or twice a week_____ Three or more times a week _____

b) Wake up in the middle of the night or early

Not during past month_____ Less than once a week_____ Once or twice a week_____ Three or more times a week_____

c) Have to get up to use the

Not during past month_____ Less than once a week_____ Once or twice a week_____ Three or more times a week_____

d) Cannot breathe comfortably

Not during the past month_____ Less than once a week_____ Once or twice a week_____ Three or more times a week_____

e) Cough or snore loudly

Not during the past month_____ Less than once a week_____ Once or twice a week_____ Three or more times a week_____

f) Feel too cold

Not during the past month_____ Less than once a week_____ Once or twice a week_____ Three or more times a week_____

g) Feel too hot

Not during the past month_____ Less than once a week_____ Once or twice a week_____ Three or more times a week_____

h) Had bad dreams

Not during the past month_____ Less than once a week_____ Once or twice a week_____ Three or more times a week_____

i) Have pain

Not during the past month_____ Less than once a week_____ Once or twice a week_____ Three or more times a week_____

j) Other reason(s), please describe:

_____

How often during the past month have you had trouble sleeping because of this?

Not during the past month_____ Less than once a week_____ Once or twice a week_____ Three or more times a week_____

6. During the past month, how would you rate your sleep quality overall?

Very good

Fairly good

Fairly bad

Very bad

7. During the past month, how often have you taken medicine to help you sleep (prescribed or "over the counter")?

Not during the past month_____ Less than once a week_____ Once or twice a week_____ Three or times a week_____

8. During the past month, how often have you had trouble staying awake while driving, eating meals, or engaging in social activity?

Not during the past month_____ Less than once a week_____ Once or twice a week_____ Three or more times a week_____

9. During the past month, how of a problem has it been for you to keep up much enthusiasm to get things done?

No problem at all _____

Only a very slight problem _____

Somewhat of a problem _____

A very big problem _____

10. Do you have a bed partner or room mate?

No bed partner or room mate _____

Partner/room mate in other room _____

Partner in same room, but not same bed _____

Partner in same bed _____

If you have a room mate or bed partner, ask him/her how often in the past month you have had . . .

a) Loud snoring

Not during the past month_____ Less than once a week_____ Once or twice a week_____ Three or more times a week_____

b) Long pauses between breaths while asleep

Not during the past month_____ Less than once a week_____ Once or twice a week_____ Three or more times a week_____

c) Legs twitching or jerking while you sleep

Not during the past month_____ Less than once a week_____ Once or twice a week_____ Three or more times a week_____

d) Episodes of disorientation or confusion during sleep

Not during the past month_____ Less than once a week_____ Once or twice a week_____ Three or more times a week_____

e) Other restlessness while you sleep; please describe_____

Not during the past month_____ Less than once a week_____ Once or twice a week_____ Three or more times a week_____

TABLE C7. UNIVERSITY OF VIRGINIA INSTITUTIONAL REVIEW BOARD APPROVED PROTOCOL (IRB-HSR 20817)

PROTOCOL

Background

2. Provide the scientific background, rationale and relevance of this project.

Experimental animal models show that brain inflammation follows mild traumatic brain injury (mTBI) (Gill et al.,2017; Giza et al., 2017; Johnson et al., 2013; Myer et al., 2006). Moreover, targeting aspects of the immune response can improve clinical outcomes post-mTBI or concussion in experimental animals (Chen et al., 2007; Churchill et al., 2017; Coughlin et al., 2015; Coughlin et al., 2017). During an inflammatory response, leukocytes and neutrophils are recruited to the site of injury, and activated microglia and astrocytes also mediate a local inflammatory response (Devoto et al., 2017; Echlin et al., 2012).

Until recently, no imaging methods were available to visualize inflammation from activated microglia in mTBI patients; however, development of selective ligands for the 18-kDa translocator protein (TSPO) allows visualization of inflammation through positron emission tomography and computed tomography (PET/CT) scanning. TSPO is a part of a protein complex located in glial cells, including astrocytes and microglia. It is minimally expressed in brain neuropil under normal, healthy conditions, and its basal expression rises in neuroinflammatory brain diseases (Venneti et al., 2006). Thus, TSPO is a promising target for the early imaging of microglial activation.

A recent pilot study of TSPO quantification in retired NFL players and healthy controls noted a significant increase ($p < 0.0036$) in imaging agent DPA-713 (a TSPO selective ligand) accumulation in the brains of retired player cohort (Coughlin et al., 2015). Another study in active and retired players also found evidence of higher brain inflammation in NFL players compared to matched controls in 8 of the 12 brain regions examined, however the authors concluded that “Further study is needed to confirm these findings...” (Coughlin, et al., 2017).

Another imaging agent selective to TSPO, DPA-714 ((N,N-diethyl-2-(2-(4-(2-18F-fluoroethoxy)phenyl)-5,7-dimethylpyrazolo[1,5-a]pyrimidin-3-yl)acetamide)), has been used in a number of studies to image inflammation. The development of DPA-714 occurred after the first TSPO radioligand [11C]-(R)-PK11195 was developed, and showed low bioavailability, poor signal-to-noise ratio, and high lipophilicity. DPA-714 has been shown to exhibit higher affinity for the TSPO in viro than PK11195 (James et al., 2008). Arlicot et al. (2012) first estimated the radiation dosimetry of [^{18}F]DPA-714 in humans, and evaluated its ability to quantify TSPO in the human brain based on biodstribution data in mice. This study demonstrated that DPA-714 exhibited a radiation exposure similar to other fluorine-radiolabeled tracers, with an effective dose of 17.2 μSv/MBq, and regional distribution in agreement with previous TSPO ligands in PET studies in humans. Since this initial study in humans, numerous studies have used DPA-714 PET

imaging to quantify and compare neuroinflammation in clinical populations and controls, and have found significant DPA-714 increased uptake in Alzheimer's disease (AD) (Hamelin et al., 2016; Golla et al., 2015), amyotrophic lateral sclerosis (ALS) (Corcia et al., 2012), and stroke subjects (Ribeiro et al., 2014), compared to controls.

One limitation in TSPO imaging is that 10% of the human population lack specific binding to TSPO (Kreisl et al., 2010) due to the Ala147Thr polymorphism of TSPO, and high-affinity binders and mixed-affinity binders can show approximately 50% difference in VT (mL/cm3) DPA-714 uptake in healthy individuals. Human studies have concluded that "TSPO genotyping of subjects is a prerequisite for a reliable quantification of [^{18}F]DPA-714 images," especially for neuroinflammatory studies of neuropathologies (Lavisse et al., 2015).

While small, pilot studies have shown inflammation in the brains of NFL players with TSPO PET Imaging, no studies have directly imaged brain inflammation during the acute phase of mTBI. Moreover, the relationship between clinical outcomes and inflammation has not been assessed. Brain inflammation may persist explaining the lag between physiological and clinical recovery (Halford et al., 2017; Hinds et al., 2013; Johnson et al., 2013). Finally, the molecular mechanisms driving brain inflammation are unclear.

This proposed pilot study will be conducted using the currently available whole-body research-dedicated PET/CT scanner at Fontaine to measure inflammation through TSPO in mTBI subjects and TSPO-genotype controls. We will measure neuroinflammation with unmatched capabilities in high spatial resolution with mathematical analysis techniques that provide information in network dynamics. Our overall hypothesis is that a high volume of inflamed brain tissue as measured through TSPO PET dynamic imaging will be measured in mTBI subjects vs. controls, and inflamed brain tissue volume will also be correlated with injury severity and symptoms.

Objectives/Hypothesis

The primary hypothesis follows:

Hypothesis 1: Subjects with acute mTBI, compared to controls, will have larger volumes of inflamed brain tissue as measured by DPA-714 PET/CT imaging.

In addition, the volume of inflamed brain tissue as measured by TSPO PET/CT will be correlated with other measures of recovery including neurocognitive testing and return to play assessments.

Study Design: Biomedical

1. Will controls be used?

Yes

►IF YES, explain the kind of controls to be used.

TSPO-genotype matched controls will be used.

4. **What is the study design?**

   Prospective, pilot cohort study to establish feasibility of PET/CT measured inflammation following mild traumatic brain injury using a matched-subjects design.

5. **Does the study involve a placebo?**

   No

   ►IF YES, provide a justification for the use of a placebo

   Answer/Response:

Human Participants

Ages: 18-30
Sex: Male and Female
Race: Any

1. **Provide target # of subjects (at all sites) needed to complete protocol.**

   15 subjects and 15 controls

2. **Describe expected rate of screen failure/ dropouts/withdrawals from all sites.**

   Because approximately 10% of the population does not show a TSPO binding signal (Kriesl et al., 2010), all subjects will have a blood draw and lab testing for affinity to the TSPO ligand. Those with low affinity will be excluded from further participation in the study. We have seen about a 30% screen failure/dropout/withdrawal rate in the early phase of the study.

3. **How many subjects will be enrolled at all sites?**

   Up to 40

4. **How many subjects will sign a consent form under this UVa protocol?**

   Up to 40

Inclusion/Exclusion Criteria

1. **List the criteria for inclusion**

There will be different eligibility for the test subjects and controls as detailed below.

<u>Inclusion- Test Subjects</u>

- Adult male or female (18-30 years old)
- Present within 96 hours of injury
- Meet WHO criteria for concussion with 1 of following after sustaining a force to head:
  - Loss of consciousness

  - Post traumatic amnesia
  - Seizure
  - Neurologic deficit
  - Vomiting
  - Headache
  - Dizziness
  - Other mental status change (feeling dazed, stunned, confused, repetitive questioning, forgetful)
- Willingness and ability to comply with scheduled visits and study procedures.
- Able to have an MRI (passes MRI screening form used for imaging at the Snyder facility at Fontaine Research Park)

Inclusion- Control Subjects

- Adult male or female (18-30 years old)
- No acute medical complaints or active illness
- Willingness and ability to comply with scheduled visits and study procedures.
- Able to have an MRI (passes MRI screening form used for imaging at the Snyder facility at Fontaine Research Park)
- Recreationally active (minimum of 30 minutes per day, 3 days per week) – assessed using Godin Leisure-Time Exercise Questionnaire.

2. List the criteria for exclusion

All Subjects:

- Age <18 or >30.
- Prisoners.
- Lack capacity to consent as determined by assessment of capacity to consent in IRB application Safeguards for Cognitively Impaired
- Unable to have an MRI (does not pass MRI screening form used for imaging at the Snyder facility at Fontaine Research Park)
- Pregnancy (verified via urine pregnancy test within 24hrs before any imaging), lactating or breastfeeding

Exclusion - Test Subjects

- Glasgow Coma Scale (GCS) score <13 on initial evaluation
- Traumatic injury requiring intensive care unit (ICU) monitoring or operative repair
- Structural abnormality on brain CT (if obtained)
- Non-English speaking
- Any prior TBI requiring hospitalization
- Brain tumor
- Prior self-reported concussion within the past 3 months (excluding present concussion)

- Neurologic or neurodevelopmental disorder (epilepsy, intellectual disability, dementia, autism, migraine, but not ADHD)
- Psychiatric disorder requiring hospitalization in last year
- Injury from physical abuse, with police involvement
- No working telephone number
- Prior adverse reaction to radiotracer
- Low affinity binder for TSPO ligand as measured by PCR
- Inability to follow protocol

Exclusion - Control Subjects

- Non-English speaking
- Any prior TBI requiring hospitalization, including prior intracranial surgery and prior intracranial hemorrhage
- Brain tumor
- Prior self-reported concussion within the past 6 months
- Neurologic or neurodevelopmental disorder (epilepsy, intellectual disability, dementia, autism, migraine, but not ADHD)
- Diagnosed psychiatric disorder currently undergoing treatment (excluding anxiety)
- No working telephone number
- Prior adverse reaction to radiotracer
- Low affinity binder for TSPO ligand as measured by PCR
- Inability to follow protocol

3. List any restrictions on use of other drugs or treatments.
Alcohol or marijuana cannot be consumed within 24 hours prior to Visit 2. Participants will not be able to complete Visit 2 if they consume alcohol or marijuana within 24 hours prior to Visit 2.

Statistical Considerations

2. Is stratification/randomization involved?

No

►IF YES, describe the stratification/ randomization scheme.
Answer/Response:

►IF YES, who will generate the randomization scheme?

_____ Sponsor
_____ UVa Statistician. Insert name Answer/Response:
_____ UVa Investigational Drug Service (IDS)
_____ Other: Specify Answer/Response:

2. What are the statistical considerations for the protocol?

This is a pilot study to establish preliminary data for DPA-714 PET/CT imaging of neuroinflammation following acute mTBI. A matched-subjects design is being employed to control for the influence of specific variables (TSPO genotype) on the primary measure of the study (inflammation), to most effectively compare inflammation between control subjects and those with acute mTBI. Because this is a pilot study with a small sample size, one consideration in the statistical design of the sample is a homogenous, well-controlled sample. This research will be informative for future larger studies.

3. Provide a justification for the sample size used in this protocol.
The sample size is based on an estimate of the subjects needed to establish the feasibility of the imaging technique and to account for attrition after the first visit.

4. What is your plan for primary variable analysis?
Neuroinflammation as measured by DPA-714 PET/CT imaging will be analyzed as follows. The reconstructed PET images will be first corrected for motion following methods described by Coughlin et al. (2015) and then co-registered with the high-resolution structural MRI images to facilitate anatomical delineation of regions of interest (ROI), and time activity curves generated. Pre-processing and ROI definition procedures include: for each subject, co-registration of the PET mean image and all 30 motion-corrected PET images using rigid body transformations, spatial normalization of each subject's MRI to the corresponding Montreal Neurological Institute (MNI) template and co-registered dynamic PET frames, and ROI definition using the Automated Anatomical Labeling brain template (Coughlin et al., 2015). The blood collected will be used to validate and correct the image derived blood input function (IDIF), for contamination due to metabolites from the reconstructed time-resolved PET images. This correction will be performed by spinning the blood using a centrifuge, isolate the plasma and radioactivity concentration in the plasma measured. The correction factor will then modeled by an exponential function and multiplied to the IDIF. The metabolite corrected IDIF will be then used in a tracer kinetic model with spill over and partial volume corrections to compute rate of uptake, Ki (1/min) (Zhong et al., 2013; Li et al., 2018) and other parameters including total distribution volume (VT) (Coughlin et al., 2015), defined as the ratio of the concentration of the radioligand in the brain tissue to that in plasma at equilibrium. VT will be obtained using graphical Logan method (Logan et al., 1990). In cases, where it is difficult to sample blood, reference tissue model will be used to compute the above mentioned parameters (Lammertsma and Hume, 1996). Standardized uptake values (SUV), obtained by normalizing the concentration of the radioligand in the brain tissue to the injected dose, will be computed from the static image.

The reconstructed PET/CT images will be first corrected for motion following methods described by Coughlin et al. (2015) and then regions of interest will be defined and time activity curves generated.

The resulting ligand uptake distribution maps will be averaged by group; differences between groups will be statistically evaluated with the use of z-score maps. We anticipate that there may be regional or diffuse changes in ligand uptake between groups, and therefore will divide brain maps by regions of interest as guided by the greatest z-score differences. We will also use the two-sample Wilcoxon rank sum test to assess if there is a significant difference in the volumes of inflamed brain tissues between the two groups.

5. What is your plan for secondary variable analysis?

Neuropsychological, neurocognitive, and kinesiology test scores will be compared between subjects and controls using the matched-study design to assess symptom severity and relationship between neuroinflammation and cognitive abilities.

We will correlate regional ligand uptake densities with neurocognitive measures within the TBI group with the use of nonparametric Spearman correlations.

6. Have you been working with a statistician in designing this protocol?

Yes

IF YES, what is their name?

Xin Qun Wang

7. Will data from multiple sites be combined during analysis?

No

INSTRUCTIONS: IF YES, answer the following questions

7(a). Does the study involve randomization?

Answer/Response:

IF YES, will randomization be done at each site or among sites?

Answer/Response:

7(b). Has the sample size calculation considered the variation among sites?

Answer/Response:

7(c). When combining the data from multiple sites to assess the study results, is the effect of the treatment to be tested (or the association to be tested) assumed to be the same across sites or vary among sites? What is the modelling strategy?

Answer/Response:

7(d). Is there a common protocol used in all sites?

Answer/Response:

IF NO, how will differences among sites, such as those related to the implementation, inclusion criteria, patient characteristics, or other sites characteristics, be considered to assess the study results?
Answer/Response:

Study Procedures-Biomedical Research

1. What will be done in this protocol?

All study procedures are being done for research purposes only with no direct therapeutic benefit to the subject.

Study Procedures:

- Visit 0 (Clinical visit following Injury. Subjects not contacted yet, not associated with the study):
  - Subjects are referred to Athletics or Emergency Department following injury
  - Data from injury and data from tests performed as usual part of clinical care at clinic visit will be collected for subjects who are enrolled at Visit 1.

- Visit 1
  - Informed Consent Process
  - Determination of Eligibility Screening
    - Health History Form (only assessing for eligibility criteria listed above)
    - Blood draw for TSPO genotyping – up to 4ml of blood will be drawn to be tested for the subject's affinity of the TSPO ligand. This information is necessary to determine if the subject is eligible for the research PET/CT scan in this study, individuals with the low-affinity TSPO genotype will not be included.
    - Neurocognitive and Kinesiology Testing (paper and computer tests and physical tests):
      - SCAT-5
      - ImPACT
      - Sensory Organization Test

- Visit 2 – anticipated to be 7 – 15 days after Visit 0, will vary. Will occur when subjects have clinically recovered but have not returned to contact sport/physical activity.
    - Re-confirm consent and eligibility
      - Confirm subjects have not experienced another concussion between Visit 1 and 2
    - Neuropsychological and Kinesiology Testing:
      - NIH Toolbox Reading Recognition
      - Trail Making Test A & B
      - NIH Toolbox Flanker Inhibitory Control and Attention Test
      - NIH Toolbox Picture Sequence Memory Test
      - Rey Auditory Verbal Learning Test

        - Word Choice Test
        - Sensory Organization Test
    - Questionnaires:
        - Pittsburgh Sleep Quality Index
        - Satisfaction with Life Scale
        - Patient Health-Questionnaire-9
        - Generalized Anxiety Disorder-7 item

- Visit 3 – anticipated to be 7 – 15 days after Visit 0, will vary. Will occur within 24 hours of Visit 2.
    - **Female Subjects ONLY**
        - Written attestation that they have not had sexual intercourse within 6 days prior to the pregnancy test
        - Urine Pregnancy Test
    - Magnetic Resonance Imaging (MRI) scan without contrast. Imaging will last about 1 hour.
    - Up to 30 minute break to allow participants to move around, and eat or drink, before PET/CT scan.
    - IV placed for DPA-714 administration
    - DPA-714 administration and PET/CT Scan
        - [^{18}F]DPA-714 will be delivered via intravenous bolus injection at the onset of a 90 min dynamic list mode PET acquisition.

Four blood draws (4ml, about 1/3 tablespoons each), total of 16ml of blood will be collected through the IV which is placed for DPA-714 administration during the PET/CT scan, between 15 – 30 minutes apart. This blood will be used to assay total activity in the separated plasma for the PET analysis input function.

- mTBI subjects only:
    - Interval of time from concussion to return to sport/physical activity, in days, will be collected and recorded.

Assessment Tools:

- **Health History form:** Includes medications, allergies, chronic illnesses, previous concussive injuries, as described in eligibility criteria.
- **SCAT-5** is a standardized tool used by medical professionals for evaluating athletes suspected of having sustained a concussion.
- **ImPACT** (Immediate Post-concussion Assessment and Cognitive Test) measures player attention span, working memory, problem solving, and reaction time. See https://www.impacttest.com/about/
- **Sensory Organization Test** is administered on the NATUS Smart Balance Master and the Bertec Computerized Dynamic Posturography system. The Sensory Organization Test is considered the “gold standard” of computerized posturography. Each unit (Natus Smart Balance Master or Bertec CDP) allows for balance perturbation via adjustments to the “walls” of the unit and/or the

"floor" based on anterior/posterior sway. The floor consists of two force plates which account for center of gravity. In order to complete the Sensory Organization Test, participants are asked to maintain their balance with their eyes open or closed with a) neither the floor or wall responding to postural sway, b) the "wall" moves in response to postural sway while the "floor" does not, c) the "floor" moves in response to postural sway while the "walls" do not, or d) both the "walls" and "floor" move in response to postural sway. There are a total of six difference conditions and each condition is repeated three times for a total of 18 trials. Each trial is 20 seconds. During each trial, participants will be spotted by a trained spotter in the rare occurrence of a fall.

- **Trail Making Test** is a brief test of visual attention and task switching in which the subject is instructed to connect a set of 25 dots.
- **The NIH Toolbox Flanker Inhibitory Control and Attention Test** is a test of attention and inhibitory control. The participant focuses on a given stimulus while inhibiting attention to stimuli flanking it.
- **The NIH Toolbox Picture Sequence Memory Test** is a test of episodic memory in which participants reproduce the order of an arbitrarily ordered sequence of pictures presented on a computer.
- **Rey Auditory Verbal Learning Test** is a test of short-term auditory-verbal memory, rate of learning, learning strategies, retroactive, and proactive interference, presence of confabulation of confusion in memory processes, retention of information, and differences between learning and retrieval.
- **Pittsburgh Sleep Quality Index** is a short questionnaire that measures the quality and pattern of sleep in adults.
- **Satisfaction with Life Scale** is a short questionnaire measuring global judgments of one's life satisfaction.
- **PHQ-9** is a brief measure of depression severity.
- **Generalized Anxiety Disorder-7 item** is a brief measure of anxiety severity.
- **Godin Leisure-Time Exercise Questionnaire** is used for self-reporting leisure-time exercise activity over the course of a week. This will be used in prescreening control subjects.
- **Word Choice Test is a verbal memory performance validity test.**

3. **If this protocol involves study treatment, explain how a subject will be transitioned from study treatment when they have completed their participation in the study.**
   N/A

Genetic Research

Risks of Genetic Research

The main risk of this genetic research is the potential loss of privacy. The sample will only have the subject's research ID and date of collection on it, remaining samples after genetic research is done will be destroyed, and the results of the genetic test will only be associated with the subject ID in order to minimize risk to privacy. It is difficult now to anticipate and predict the confidentiality risks that participants may face one, five, or ten years from now by being in this study at this time because the technology is not yet developed.

1. Risks of Accidental Disclosure/Loss of Confidentiality

1.a. If there were an accidental disclosure of this research data, could it affect either the subject or the subject's family member(s)?

No.

►**IF YES, explain how:**

Answer/Response:

1.b. What data will you be keeping about a subject?

The results of the genetic research in this study, the results of the procedures performed in this research, and health history as outlined in the Health History Form.

1.c. Where are you keeping this data and/or specimens?

Data will be kept in a RedCap database as outlined in the Data Security Plan.

1.d. Are there any documented correlations or associations that might be outside the study?

No.

2. Social risks

There are no associated or anticipated social risks associated with this genetic research.

2.a. If there were a deliberate or accidental disclosure of the results or associated data from this research, could an insurer or employer think that it would affect the insurability or employability of the subject?

No.

2.b. If there were a deliberate or an accidental disclosure of the results or associated data from this research, could it affect reproductive plans of the subject or the subject's family member(s)?

No.

►**IF YES, explain how:**

Answer/Response:

2.c. If there were a deliberate or an accidental disclosure of the results or associated data from this research, could it stigmatize the subject?
No.

►IF YES, explain how:
Answer/Response:

3. Psychological risks
There are no associated or anticipated psychological risks associated with this genetic research.

3.a. If there were a deliberate or an accidental disclosure of the results or associated data from this research, could this cause psychological stress to the subject or a family member (include rational and irrational reactions)?
No.

4. Harm to the community
There is no associated or anticipated harm to the community associated with this genetic research.

4.a. If your study involves an ethnic or cultural group, will the results affect that community?
No.

►IF YES, explain the risks:
Answer/Response:

4.b. How will the risks listed above be minimized?
N/A

4.c. How will these risks be communicated to participants?
N/A

Information Accompanying Specimens or Data to be Used for Genetic Research

- **What information will be on the label of the specimen to be used for genetic research?**
  Subject ID number (not MRN) and date of collection, date of collection is important for the personnel processing the sample to have on the tube in order to process the samples in the right amount of time for this research.

1. **What information will be "linked to" or will accompany the specimen to be used for genetic research?**

No information will be directly linked to or accompany the specimen to be used for genetic research, only the subject ID will be directly linked to the genetic research results.

2. **If identifiers will be linked to specimens/ data, when will the identifiers be destroyed?**
   _____ NA- No identifiers
   _____ After specimen is linked to clinical data but before genetic analysis is completed.
   _____ After specimen is linked to clinical data and immediately after the genetic analysis for the individual participant is completed.
   _____ After specimen is linked to clinical data and immediately after the genetic analysis for all participants is completed
   _____ Link to identifiers will not be destroyed
   ___X__ Other :Specify Answer/Response: Immediately after the genetic analysis for the individual participant is completed.

Confidentiality and Collection/Storage of Specimens to be Used for Genetic Research

1. **Will any information/ the consent form/ results regarding genetic research be placed in the participant's medical record?**
   The consent form will be placed in the participant's medical record in order to document participation in the study because it is important to document PET/CT imaging, but genetic research results will not be documented in the participant's medical record.

2. **How much material (e.g. blood, tissue) will be collected for genetic research and how will it be collected?**
   4ml of blood will be collected intravenously

3. **Who will be responsible for storing the specimens for genetic research?**
   Samples will be processed with the Biorepository and Tissue Research Facility (IBC # 078-99)

4. **If stored at UVa-where will the specimens to be used for genetic research be stored?**
   Samples will be processed immediately and not stored.

5. **Will another research institution or entity outside of UVa ever have control over the specimens?**
   No.
   ►**IF YES, list the name of outside institution/entity.**
   Answer/Response:

6. **List the information that will be on the specimen label when the specimen is sent outside of UVa.**

   N/A

7. **Will any additional information be sent with the sample?**

   N/A

   ►IF YES, list what will be sent.

   Answer/Response:

> **Note to Study Team**: If you plan to ship specimens outside of UVa, personnel performing this function must have taken the appropriate training from the Department of Transportation. Contact SOM CTO for training information.
>
> **Note to IRB Staff:** If the information sent outside of UVa with the specimen meets the criteria of "Identifiable" and the study does not have a consent form- the disclosure would require Tracking under HIPAA regulations. If it meets the criteria of a Limited Data Set, a Data Use Agreement will be required.

8. **Can participants withdraw their specimens or request that they be destroyed?**

   Yes.

Third Party Concerns

1. **Will family members be directly involved in the research?**

   No.

   ►IF YES, how and by whom (*e.g., an investigator, the participant, a support group)* will those family members be recruited?

   Answer/Response:

   ►IF NO, does this study have any implications for the participant's family members?

   No.

   ►IF YES, will that information be shared with relevant family members?

   Answer/Response:

Disclosure of Genetic Research Results to Subjects

- **Explain why it is appropriate to provide subjects with results.**

The results of the genetic research (whether or not the subject has a low affinity binding TSPO genotype) will be disclosed to the subject because the results determine whether the subject can participate in Visit 2. It will be emphasized and made sure that subjects understand that the results of this genetic testing done for the research are not used to diagnose or determine a treatment for anything and do not have clinical or medical significance and will not be placed in their medical record nor affect their medical care.

- **What information will study subjects receive?**

The study subjects will be told whether or not they have a low affinity binding TSPO-genotype. They will be told whether the results make them eligible to continue in the study because the radioligand used for PET/CT imaging binds to TSPO. It will be emphasized that the results of this test are only useful for this research.

- **At what point during the research will the results be disclosed?**

Results will be disclosed after Visit 1 and before Visit 2.

- **Who will disclose the results to subjects?**

The Clinical Research Coordinator for the study.

- **Will anyone other than the subject be informed of the results?**

No.

- **Will genetic counseling be provided?**

No. The results do not have significance outside of this research.

►IF YES, will genetic counseling also be provided before testing?
Answer/Response:

- **If future research yields results that are clinically meaningful or significant, would those results be disclosed to the subject? Why or why not?**
  Results will be disclosed to the subject as indicated in #2 and 3. Results of future genetic research will not be disclosed; results of future research are not anticipated to be clinically meaningful or significant.

- **Even if results may not be clinically valid,** (*recognized by FDA or generally recognized by practitioners in the field as established)* **might they affect a subjects clinical care?**
  No.

Subject Compliance with Study Procedures

3. **Explain how the study team will monitor the subject for compliance with the study procedures.**
   Subject compliance with the study procedures is not expected to be a concern given the design of the study. During the informed consent process, each of the visits and assessments will be well explained to maximize retention in the study for the 2 planned visits. Furthermore, the study team will be present with the subject for all study procedures.

4. **Describe criteria for when a subject is considered to be non-compliant with study procedures.**
   N/A. The study team will be present with the subject for all study procedures.

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TABLE C8. UNIVERSITY OF VIRGINIA INSTITUTIONAL REVIEW BOARD APPROVED CONSENT (IRB-HSR 20817)

Consent of an Adult to Be in a Research Study

In this form "you" means a person 18 years of age or older who is being asked to volunteer to participate in this study.

Participant's Name_____

Principal Investigator:	*Joshua Easter, MD* University of Virginia Department of Neurology 1215 Lee St *Charlottesville, VA*
Sponsor:	*UVA Brain Institute*

What is the purpose of this form?

This form will provide you with information about this research study. You do not have to be in the study if you do not want to. You should have all your questions answered before you agree to be in this study.

Please read this form carefully. If you want to be in the study, you will need to sign this form. You will be given a signed copy of this form.

Who is funding this study?

This study is being funded by the UVA Brain Institute.

Why is this research being done?

The purpose of this study is to measure the amount of inflammation that happens in the brain after a concussion. Inflammation is a natural immune response of your body to an injury and occurs in all tissues. The other purpose is to measure how brain inflammation relates to recovery from a concussion. This study is being done to see if subjects who have had a recent concussion have a higher amount of inflammation in the brain compared to those who have not had a recent concussion. Many of the current ways that health care providers use to take pictures of the brain are not able to visualize inflammation, but it is possible that inflammation in the brain can be seen better by a positron emission tomography and computed tomography (PET/CT) scan.

A PET/CT scan uses small amounts of radioactive material called a radioligand, a special camera, and a computer to create images of how a tissue is working. In this study, we are using an experimental radioligand called [18F]DPA-714 for the PET/CT scan. [18F]DPA-714 is a ligand (something that binds to) the translocator protein (TSPO), which is a marker of inflammation. When the radioligand attaches to the TSPO, it is expected to make the inflammation easier to see on the PET/CT images. The only part of your body that will be imaged with the PET/CT is the brain.

[18F]DPA-714 has not been proven to be safe or helpful, and is not approved by the U.S. Food and Drug Administration (FDA). So far, [18F]DPA-714 has been given to 171 people in total, 78 people who are healthy, 74 people who have Alzheimer's, 10 people who have ALS, and 9 who have had a stroke.

You are being asked to be in this study because you have recently had a concussion or you fit the criteria to be a control subject. Up to 40 people will be in this study at UVA.

How long will this study take?

Your participation in this study will require 3 study visits over 1-2 weeks.
Visit 1 will last about 2 hours.

- Visit 2 will last about 1.5 hours.
- Visit 3 will last about 3 hours.

What will happen if you are in the study?

VISIT 1 (Day 1 – visit will last about 2 hours):

Screening:

If you agree to participate, you will sign this consent form before any study related procedures take place. Before you can start in the study, there will be a screening period. You will have tests and procedures during this time to make sure you are eligible to participate. These include the following:

- Review of your medical history, including: prior concussions, neurologic or neurodevelopmental disorder. If you are a subject that has recently been diagnosed with a concussion, we will collect information about your concussion from your athletic trainer, such as your symptoms and results from tests performed as a part of your care for your concussion (see below for details).
- Blood test (1/3 tablespoon of blood) to test your TSPO genotype. This test will tell us whether the radioligand DPA-714 will bind to the TSPO. In some people, the radioligand does not attach to the protein very well. Because the study is trying to understand if you can see inflammation when the radioligand binds to the TSPO, people who have the type of gene that makes the binding not work very well will not continue to participate in the study for visit 2.

Neurocognitive and Kinesiology Tests:

For research purposes, we will ask you to complete the following tests proctored by a study peronnel:

- The ImPACT scale, a computerized assessment which measures memory, attention, reaction time, and problem solving.
- The SCAT-5, a test that was designed to evaluate athletes after a concussion.
- Sensory Organization Test, a test that measures your balance and how stable you are in reaction to sensory cues. An example of this is measuring your balance when your eyes are closed or when you are on a surface that sways.

If you are a subject that has had a recent concussion, the following tests/procedures may have been done during your clinic or hospital visit, and we will collect information from your medical records for these tests:

- Brain or spine imaging (like MRI or CT scan) or electroencephalography (EEG) results
- Physical exam
- Diagnosis(es) and disposition (how you are doing after your concussion)
- Medications taken with last 48 hours (including during visit)
- Demographics
- Vital signs (like heart rate, blood pressure, etc.)
- Medical history or other illnesses
- Recommended treatment – medications, rest, return to work
- The interval of time, in days, from your concussion to the time you return to play sports/physical activities will be collected and recorded.

VISIT 2 (Up to 14 days after Visit 1. Will last about 1.5 hours)

All tests and procedures this day are done for research purposes.

Neuropsychological Tests:

We will ask you to complete the following tests of your cognitive abilities (or how you are able to understand) which takes about 40 minutes to complete:

- NIH Toolbox Reading Recognition
- Trail Making Test A & B
- NIH Toolbox Flanker Inhibitory Control and Attention Test
- NIH Toolbox Picture Sequence Memory Test
- Rey Auditory Verbal Learning Test
- Word Choice Test

Kinesiology Test:

We will ask you to complete the following tests of your sensory abilities which takes about 15 minutes to complete:

- Sensory Organization Test, a test that measures your balance and how stable you are in reaction to sensory cues. An example of this is measuring your balance when your eyes are closed or when you are on a surface that sways.

Questionnaires:

During this study, you will be asked to fill out some questionnaires. These questionnaires will take about 15 minutes to complete. These questionnaires ask about:

- how you are feeling
- your lifestyle habits
- medicine use
- diet
- daily activities
- family history

During the research, if we learn you are having thoughts about suicide or hurting yourself or others, the research staff will ask you more questions about your thoughts. Based on your response, the staff may provide you with help to get treatment. This may include: working with you to contact your doctor or therapist, referral to a therapist to discuss your thoughts, contact a trusted family member, significant other or clergy or work with you on a plan that may include getting you to a hospital for safety and treatment.

VISIT 3 (Within 24 hours of Visit 2. Will last about 3 hours)

All tests and procedures this day are done for research purposes.

For Female Subjects ONLY:

3. For your own safety, you will be asked to complete a urine pregnancy test at Visit 3 BEFORE the PET/CT scan. You will also be excluded from the study if you produce a positive test for safety purposes. You will also be excluded if you are currently lactating.
4. At Visit 3, you need to attest in writing that you have not had sexual intercourse within 6 days prior to the pregnancy test (see below).

Magnetic Resonance Imaging (MRI) – 60 minutes:

- The MRI allows the researchers to see a sharp and finely detailed picture of your brain structure.
- The MRI will take about 60 minutes. During the MRI, you will be asked to lie on a table in a small space inside a tube-shaped machine. This machine is very noisy and uses magnetic fields and radio waves to take pictures of your brain.

Positron Emission Tomography and Computed Tomography (PET/CT) Scan and [^{18}F]DPA-714 Administration – 90 minutes:

- You will have an intravenous (in your vein) catheter placed. This will be used to administer the dose of radioactive material called [^{18}F]DPA-714 and salt water (saline).
- After the [^{18}F]DPA-714 is administered, PET scanning will begin immediately.
- The PET/CT scan will take about 90 minutes. During the scan, you will be required to lie flat on your back, without moving. Using a special nuclear medicine camera, pictures of your brain will be obtained by recording the distribution of radioactive material in your body.
- During the scan, 4ml (about 1/3 tablespoon) of blood will be collected through the catheter which is placed for [^{18}F]DPA-714 administration four times, every 15 – 30 minutes. A total of 16ml of blood will be collected. No additional venipuncture (needle sticks in your arm) will occur. This blood sample will be used to calculate radioactivity in the plasma, the liquid, in your blood.

Study Schedule

	Visit 1	**Visit 2**	**Visit 3**
Study Week	1	1-2	1-2
Informed Consent	x		
Review study eligibility	x	x	
Medical History	x		
Blood draw (for laboratory testing)	x		x
Neurocognitive testing	x		
Neuropsychological and kinesiology testing		x	
MRI			x
Study Drug Dispensed			x
PET/CT Scan			x
Questionnaires		x	
Pregnancy Test (Female Subjects)			x

What are your responsibilities in the study?

You have certain responsibilities to help ensure your safety. These responsibilities are listed below:

5. You must be completely truthful about your health history.
6. Follow all instructions given.
7. You or your parent/legal guardian should tell the study doctor or study staff about any changes in your health or the way you feel.
8. Answer all of the study-related questions completely.

9. Inform the study doctor or study staff as soon as possible if you have to take any new medications, including anything prescribed by a doctor or those that you can buy without a prescription (over-the-counter), including herbal supplements and vitamins.
10. Refrain from consuming alcohol or marijuana within 24 hours prior to Visit 2. You will not be able to complete Visit 2 if you consume alcohol or marijuana within 24 hours of Visit 2.
11. **Females subjects only**- You will be required to take a urine pregnancy test at Visit 3, prior to the PET/CT scan. You attest that you have not had sexual intercourse within 6 days of the PET/CT imaging (Visit 3). You will be excluded from the study if you produce a positive test for safety purposes. You will also be excluded if you are currently lactating.

Blood Testing

We will take (or "draw") up to 1/3 tablespoon of blood on Visit 1, and ½ teaspoon on Visit 2*.*

We plan to do genetic research on the DNA in your blood sample. DNA is the material that makes up your genes. All living things are made of cells. Genes are the part of cells that contain the instructions which tell our bodies how to grow and work, and determine physical characteristics such as hair and eye color. Genes are passed from parent to child.

Your blood sample on Visit 1 will be tested to find out which gene type you have for the translocator protein (TSPO) gene. The ligand DPA-714 used in this study binds/attaches to TSPO to help make the inflammation easier to see on the images. Your TSPO gene type will tell us whether the study drug will bind well to your TSPO. If the test tells us that the study drug will not bind well to your TSPO, your participation will end before Visit 2 and you will not be scanned since we do not think the image would help us identify inflammation.

Your blood sample on Visit 2 will be tested for radioactivity in the plasma of your blood, this information will be helpful in the analysis of the brain images obtained during your PET/CT scan.

When these tests are done, any left-over blood sample will be thrown away or they will be de-identified. This means there is no information that could be used by anyone to determine who the sample came from.

If you want to know about the results before the study is done:

During the study you are having an investigational test done. The purpose of the test is NOT to diagnose any disease or abnormality you may have. Because the test is investigational there is no way for the study leader to understand if the results are "normal" or "abnormal". However, IF any test results are concerning, your study leader will let you know. In addition, as the research moves forward, your study leader will keep you informed of any new findings about the research itself that may be important for your health or may help you decide if you want to continue in the study. The final results of the research will not be known until all the information from everyone is combined and reviewed. At that time you can ask for more information about the study results.

Collection of Samples for Genetic Research

What Sort of Research Will Be Done On Your Sample(s)?

You are being asked to provide two samples of your blood. The first sample, given during Visit 1, will be used for screening purposes only for this research, to determine your TSPO gene type as described above under "Blood Testing." The second sample will be drawn at Visit 2 and will be used to measure metabolites, or break down products of DPA-714, in your blood, these measurements will help in the analysis of the PET/CT scan brain images.

These research results will not be related to your clinical care.

What will you have to do to give samples for research?

During Visit 1, a phlebotomist (someone certified to draw blood) or a nurse or doctor will draw 1/3 tablespoon of blood as part of your screening for this study. The draw will take a minute of the total Visit 1 time which is about 2 hours.

During Visit 3, the imaging technician who is certified to draw blood, will draw ½ teaspoons of blood through the needle in your arm that is already placed for research for the drug administration. This draw will take less than a minute of the total Visit 2 time which is about 3 hours.

How Will Your Samples Be Labeled?

Your samples will not be labeled with your name or other information that would identify you directly. Instead, it will have a unique code that allows for the sample to be linked to some of your health information. This link means that your specimen can be identified but only indirectly. We can find out if we need to know which sample is yours in the event you wish the sample to be removed.

Dr. Joshua Easter will be responsible for protecting your privacy.

Which researchers can use your samples and what information about you can they have?

Your sample will not be shared with other researchers not involved in this study or organizations at or outside of UVA.

What Are the Benefits To Donating Your Sample(s) For Genetic Research?

The genetic research that is done with your sample is not meant to help you. But, doctors hope that in the future it will help people who have brain injuries or other brain conditions.

What Are The Risks of Donating Your Sample(s) For This Study?

Risks to Privacy from Genetic Research and/or Specimen Banking:

The main risk of allowing us to use your samples and certain limited health information for research is a potential loss of privacy. One of the risks to you is the release of information from your health records. The University of Virginia will do its best to protect your records so that facts about you and your health will be kept private. The chance that information identifying you will be given to someone else is very small. However, we cannot *guarantee* it will be safe. To further safeguard your privacy, information obtained from future research will not be placed in your medical record.

There are certain risks of having health information given to other people by mistake. In the unlikely event that this happens, it could cause discrimination or mental harm to you or your family members if others were to see this information. The results could be that you may not be able to get or keep certain kinds of insurance. It could also hurt family relationships.

Because everyone has unique DNA, it is also possible, although very unlikely, that someone could identify you through your DNA if they have another sample of your DNA.

Information about your genetic make-up could mean that you and your family members could face problems that could lead to getting or keeping some kinds of insurance or affect your ability to get or keep a job. To keep this from happening, the results of the genetic test will not be given to anyone outside of the study staff. There is no way to predict all the possible risks of this research.

Will You Find Out the Results of the Research on Your Sample for Genetic Research?

You will receive the results of the research done on your sample after Visit 1 when the study coordinator tells you whether you can participate in Visit 2 of the study. Neither your health care provider nor anyone in your family will receive the results of any research done on your sample. The results will not be put in your health records and have no known medical or clinical significance. Therefore, results from any research done on your sample will not affect your medical care. This helps protect you and other members of your family from harm that might be caused by this information.

What If You Change Your Mind About Donating Your Sample for Genetic Research?

If you decide now that your sample can be used for genetic research, and later change your mind, you can simply withdraw the sample at that time. To withdraw you will need to write to the Principal Investigator listed on the first page of this form. We will then destroy any of your tissue that has not already been used. However, if your sample has been used in genetic research, the information that we have learned will remain in the study, even if you withdraw. Unless you withdraw from the study, permission for researchers to use your tissue and to use and share your private health information for this study will never end.

Will You Be Paid For Donating Your Sample for Genetic Research?

You will not be paid to donate your sample for genetic research.

Will Donating Your Sample Cost You Any Money?

There is no cost to you to have your samples collected or used for genetic research.

Genetic Testing Options:

Because the genetic testing of your sample will determine whether you are eligible to participate in the study, you do have to participate and agree for specimens to be collected for genetic research in order to be in the main part of this study. No matter what you decide to do, your decision will not affect your medical care. You can tell us your choice by placing your initials in one of the options below:

GENETIC RESEARCH:

Please indicate your choice by placing your initials below (if applicable):

___ YES **Your sample(s) may be used for genetic research**

___ NO **Your sample(s) may not be used for genetic research**

What are the risks of being in this study?

Risks and side effects related to the procedures include:

Likely

- Temporary soreness or redness where the IV was placed in your arm
- Pain or bruising from the blood draw
- You may feel upset or frustrated while completing the cognitive tests.

Rare

- You might fall while performing the balance tests. A member of the study team will be at your side during this test as a preventive measure.

Rare but serious

- Allergic reaction to the radiotracer
- Violation of your privacy and confidentiality

Risks of having an MRI:

MRI scanning is a painless procedure that only requires that you lie quietly on a padded table that gently glides you into a large magnet. While the scanner is performing your scan, you will hear some humming and thumping sounds. These are normal and should not worry you. Because of the magnetic field and radio frequencies, people with a heart pacemaker, brain, aneurysm clips, and some implanted metallic or electrical devices should NOT have an MRI. It is important that you inform the technologist if you have any of these metallic appliances. Please inform the technologist if you are pregnant or think that you may be pregnant.

There is a low risk of experiencing symptoms of anxiety or claustrophobia while lying in the scanner. Should you experience these symptoms or otherwise become uncomfortable you can voluntarily stop your participation in this study. There will be NO consequences to your clinical care or to your participation in the study should you choose to stop your participation.

You will not be given any additional drugs with this MRI. This would include any drugs to help reduce your anxiety or any drug, such as a contrast dye, to help the tissues show up better on the scan.

Radiation Risks:

This study involves radiation exposure from 1 injection of a drug labeled with 9 millicuries of Flourine-18. This drug will travel throughout your body and will allow us to

image your brain with special cameras called a PET scanner. Use of this drug will allow us to collect a great deal of imaging information for this study.

Using the standard way of describing radiation dose, from participating in this study, you will receive a total of 48 millisieverts to your bladder. All other organs will receive smaller amounts of radiation.

For this study, you will also receive a small dose of radiation from 1 CT scan during your PET scan of your head.

The total effective radiation dose you will receive from this study is approximately 6.4 millisieverts. For comparison this dose is about 13% of the annual radiation dose safely allowed for a radiation worker such as the person performing your scans.

Finally, the goal of this study is to obtain basic information regarding the metabolism of the radioactive drug. The precise risk from the radiation dose is not known but is thought to be small. Furthermore, the radiation dose from this drug appears to be about the same as from other imaging agents used for similar diagnostic imaging studies. This radiation dose is what you will receive from this study only and does not include any exposure you may have received or will receive from other tests using radiation. This radiation exposure is not necessary for your medical care but is necessary to obtain the research information desired.

Blood Donation

If you participate in this study it may affect your ability to donate blood. If you have any questions call the organization where you donate blood and talk to one of their nurses.

Risks from Completing Questionnaires

Some of the questions asked may be upsetting, or you may feel uncomfortable answering them. If you do not wish to answer a question, you may skip it and go to the next question.

Risks of having your blood drawn:

Having blood drawn may cause:

- pain (common),
- a bruise (sometimes),
- fainting or passing out (not very often), and
- infection (rare).

If the people doing the study are exposed to your blood or body fluids in a way that could give them a disease, your blood may be tested. The tests might check for:

- hepatitis,
- HIV (Human Immunodeficiency Virus), or
- other infections.

You and the person exposed would be told the test results. However, your name would be kept private. If your test is positive for hepatitis or HIV or any other infection that may affect your clinical care, we will tell you the results and help you understand what the results mean for you.

Risks for men:
We do not know the effects of the drug on male sperm. If you are a male, you should not father a baby for 24 hours after your last dose of the drug. You should also not donate to a sperm bank during this time. To do so may hurt your unborn baby. You must use an effective method of birth control during this time.

If your partner becomes pregnant during the 24 hours after the study ends you must tell the study team right away.

Risks for women:
We do not know the effects of the drug on the female reproductive system. You must have a negative serum pregnancy test and not engage in sexual intercourse within 6 days of completing PET/CT imaging. You should not conceive a child for 24 hours after your last dose of the drug. To do so may hurt your unborn baby. You must use an effective method of birth control during this time.

If you become pregnant during the 24 hours after the study ends you must tell the study team right away.

Other unexpected risks:
You may have side effects that we do not expect or know to watch for now. Call the study leader if you have any symptoms or problems.

Could you be helped by being in this study?
You will not benefit from being in this study. However the information researchers get from this study may help others in the future.

What are your other choices if you do not join this study?
If you are an athlete or patient being treated for your concussion at UVa, you do not have to be in this study to be treated for your injury. You can get the usual treatment whether you choose to be in this study or not. The usual treatment and care would include:

- A neurological examination
- Cognitive testing
- Imaging tests (e.g. CT, MRI)
- Medications
- Resting

If you are not a patient or athlete at UVa, the only choice is not to be in this study, you will not be affected in any way if you choose not to be in this study.

If you are an employee of UVa your job will not be affected if you decide not to participate in this study. If you are a student at UVa, your grades will not be affected if you decide not to participate in this study. If you are an athlete, your decision not to participate will not affect your participation in your sport, your coach's decision to play you, nor your place on the team.

Will you be paid for being in this study?

You will be paid $150 for finishing this study through gift cards. You will receive $25 for participation in Visit 1, $75 for participation in Visit 2 and $50 for participation in Visit 3. You will receive your payment after each visit. The income may be reported to the IRS as income.

If you owe money to any Virginia state agency, the state can use the money you earn in this study to pay those debts. These state agencies include the UVa Medical Center, VCU Medical Center or a college or university. The money may be withheld to pay back debt for such things as unpaid medical bills, taxes, fines, child support. Even if this happens, the money you earn may be reported to the IRS as taxable income.

By agreeing to be in this study, you are donating your blood for research, and giving up any property rights you may have in them.

Will being in this study cost you any money?

For all subjects: All of the procedures in this study will be provided at no cost to you or your health insurance. You will be responsible for the cost of travel to come to any study visit and for any parking costs.

If you are an athlete or patient at UVa being treated for your concussion: You and/or your insurance company must pay for any tests or care given beyond what is required in this study. In addition, you and/or your health insurance may also have to pay for other drugs or treatments that are given to help you control any side effects. You will have to pay for any costs not covered by your health plan. You may be responsible for any co-payments or deductibles. You may wish to ask your insurance company for an estimate of what these costs might be or if pre-approval is required.

What if you are hurt in this study?

You do not give up any legal rights, such as seeking compensation for injury, by signing this form. If you feel you have been injured as a result of this study you may contact the Principal Investigator or the IRB (phone numbers are located near the end of this form). If you are hurt as a result of being in this study, there are no plans to pay you for medical expenses, lost wages, disability, or discomfort. The charges for any medical treatment you

receive will be billed to your insurance. You will be responsible for any amount your insurance does not cover.

What happens if you leave the study early?

You can change your mind about being in the study any time. You can agree to be in the study now and change your mind later. If you decide to stop, please tell us right away. You do not have to be in this study to get services you can normally get at the University of Virginia.

Even if you do not change your mind, the study leader can take you out of the study. Some of the reasons for doing so may include

a) Your study physician is concerned about your health
b) Your condition gets worse
c) You do not follow your doctor's instructions

Any data collected about you up until the time you leave the study must be kept in order to determine the results of the study.

How will your personal information be shared?

The UVa researchers are asking for your permission to gather, use and share information about you for this study. If you decide not to give your permission, you cannot be in this study, but you can continue to receive regular medical care at UVA.

If you sign this form, we may collect any or all of the following information about you:

- Personal information such as name, address and date of birth
- Social Security number ONLY IF you are being paid to be in this study
- Your health information if required for this study. This may include a review of your medical records and test results from before, during and after the study from any of your doctors or health care providers. This may include mental health care records, substance abuse records, and/or HIV/AIDS records.
- Blood samples if you agree to provide them for genetic testing for this study

Who will see your private information?

- The researchers to make sure they can conduct the study the right way, observe the effects of the study and understand its results
- People or groups that oversee the study to make sure it is done correctly
- Insurance companies or other organizations that may need the information in order to pay your medical bills or other costs of your participation in the study
- Tax reporting offices (if you are paid for being in the study)
- People who evaluate study results and government agencies that provide oversight such as the Food and Drug Administration (FDA) if the study is regulated by the FDA.

- If you tell us that someone is hurting you, or that you might hurt yourself or someone else, the law may require us to let people in authority know so they can protect you and others.

Some of the people outside of UVa who will see your information may not have to follow the same privacy laws that we follow. They may release your information to others, and it may no longer be protected by those laws.

The information collected from you might be published in a medical journal. This would be done in a way that protects your privacy. No one will be able to find out from the article that you were in the study.

What if you sign the form but then decide you don't want your private information shared?

You can change your mind at any time. Your permission does not end unless you cancel it. To cancel it, please send a letter to the researchers listed on this form or complete the "Leaving the Study Early" part of this form and return it to the researchers. Then you will no longer be in the study. The researchers will still use information about you that was collected before you ended your participation.

A copy of this consent form will be put in your medical record. (This is not the same as the record of this research study.) This means that everyone who is allowed to see your medical records will be able to find out that you are in this study. This is done so your regular doctors will know what you receive as part of this study. If you have other health problems during the study, they will be able to treat you properly.

Please contact the researchers listed below to:

- Obtain more information about the study
- Ask a question about the study procedures or treatments
- Report an illness, injury, or other problem (you may also need to tell your regular doctors)
- Leave the study before it is finished
- Express a concern about the study

Principal Investigator: Joshua Easter, MD
PO Box 800699
Charlottesville, VA 22908-0699
Telephone: (434) 924-8485

What if you have a concern about this study?

You may also report a concern about this study or ask questions about your rights as a research subject by contacting the Institutional Review Board listed below.

University of Virginia Institutional Review Board for Health Sciences Research

PO Box 800483
Charlottesville, Virginia 22908
Telephone: 434-924-9634

When you call or write about a concern, please give as much information as you can. Include the name of the study leader, the IRB-HSR Number (at the top of this form), and details about the problem. This will help officials look into your concern. When reporting a concern, you do not have to give your name.

For Female Subjects ONLY:
You attest that have not had sexual intercourse within 6 days prior to the urine pregnancy test (Visit 3).

Date of Urine Pregnancy Test _____

Female Subject's initial _____

Signatures

What does your signature mean?
Before you sign this form, please ask questions about any part of this study that is not clear to you. Your signature below means that you have received this information and all your questions have been answered. If you sign the form it means that you agree to join the study. You will receive a copy of this signed document.

Consent From Adult

_____	_____	_____
PARTICIPANT (SIGNATURE)	PARTICIPANT (PRINT)	DATE

To be completed by participant if 18 years of age or older.

Person Obtaining Consent
By signing below you confirm that you have fully explained this study to the potential subject, allowed them time to read the consent or have the consent read to them, and have answered all their questions.

_____	_____	_____
PERSON OBTAINING CONSENT (SIGNATURE)	PERSON OBTAINING CONSENT (PRINT)	DATE

Notification of My Health Care Provider

If you are a patient within the UVA Health System: Your health care provider will be notified of your participation in this study.

If you are not a patient within the UVA Health System:
Please indicate below whether you want us to notify your health care provider that you have agreed to take part in this study.

_____ Yes, I want the study doctor to notify my health care provider that I have agreed to take part in this study.

Health Care Provider Name:
Health Care Provider Address:
Study team will send a copy of the consent form to the health care provider.

_____ No, I do not want the study doctor to notify my health care provider that I have agreed to take part in this study or I do not have a health care provider.

Leaving the Study Early

Signatures should be obtained in this section if the subject decides to leave the study early.

If you leave the study early the study leader will keep the data collected about you up until the time you leave the study to help determine the results of the study.

Consent From Adult

_____	_____	_____
PARTICIPANT (SIGNATURE)	PARTICIPANT (PRINT)	DATE

To be completed by participant if 18 years of age or older.

Person Obtaining Consent
By signing below you confirm that you have fully explained the implications of withdrawing from the study to the subject and have answered all their questions.

_____	_____	_____
PERSON OBTAINING CONSENT (SIGNATURE)	PERSON OBTAINING CONSENT (PRINT)	DATE

APPENDIX D

Figure D1. Demographic variables for age at time of injury (years), sex (male, female), days to self reported asymptomatic (SRA), and symptom burden, in concussed individuals. (Manuscript 1)

Descriptives

Descriptive Statistics

	N	Minimum	Maximum	Mean	Std. Deviation	Skewness		Kurtosis	
	Statistic	Statistic	Statistic	Statistic	Statistic	Statistic	Std. Error	Statistic	Std. Error
Sex	196	0	1	.37	.483	.555	.174	-1.710	.346
Age_at_injury	190	18	24	20.13	1.368	.562	.176	-.337	.351
Days to SRA	196	1	43	8.24	6.573	1.969	.174	5.522	.346
Symp_Burd	196	.0	74.0	19.227	15.6679	1.049	.174	1.043	.346
Valid N (listwise)	190								

Sex

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Male	124	63.3	63.3	63.3
	Female	72	36.7	36.7	100.0
	Total	196	100.0	100.0	

Figure D2. Demographic variable for Sport in concussed individuals. (Manuscript 1)

Sport_code

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	MLAX	10	5.1	5.1	5.1
	WLAX	6	3.1	3.1	8.2
	Football	77	39.3	39.3	47.4
	MSOC	8	4.1	4.1	51.5
	WSOC	8	4.1	4.1	55.6
	FH	4	2.0	2.0	57.7
	VB	13	6.6	6.6	64.3
	WBKB	6	3.1	3.1	67.3
	BSBL	4	2.0	2.0	69.4
	MSQ	1	.5	.5	69.9
	CHR	11	5.6	5.6	75.5
	DNC	1	.5	.5	76.0
	Dive	2	1.0	1.0	77.0
	ROW	10	5.1	5.1	82.1
	WRS	15	7.7	7.7	89.8
	MSWM	5	2.6	2.6	92.3
	SFTB	5	2.6	2.6	94.9
	WTN	3	1.5	1.5	96.4
	WTF/XC	3	1.5	1.5	98.0
	WSWM	3	1.5	1.5	99.5
	MGLF	1	.5	.5	100.0
	Total	196	100.0	100.0	

Figure D3. Group (control,concussed) differences in demographic variables of duration in Light sleep (minutes), REM sleep (minutes), Deep sleep (minutes), and awake time (minutes). (Manuscript 2)

Group Statistics

	Group	N	Mean	Std. Deviation	Std. Error Mean
light_sleep_duration	0	9	12496.67	4681.829	1560.610
	1	12	14920.00	4451.784	1285.119
rem_sleep_duration	0	9	2606.67	2010.025	670.008
	1	12	3415.00	2232.966	644.602
awake_time	0	9	2956.67	733.638	244.546
	1	12	4667.50	2114.229	610.325
deep_sleep_duration	0	9	8066.67	3252.353	1084.118
	1	12	6482.50	2088.375	602.862

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means							
						Significance				95% Confidence Interval of the Difference	
		F	Sig.	t	df	One-Sided p	Two-Sided p	Mean Difference	Std. Error Difference	Lower	Upper
light_sleep_duration	Equal variances assumed	.143	.709	-1.208	19	.121	.242	-2423.333	2006.389	-6622.754	1776.088
	Equal variances not assumed			-1.199	16.882	.124	.247	-2423.333	2021.642	-6690.887	1844.220
rem_sleep_duration	Equal variances assumed	.002	.967	-.856	19	.201	.403	-808.333	944.501	-2785.196	1168.529
	Equal variances not assumed			-.869	18.276	.198	.396	-808.333	929.743	-2759.539	1142.872
awake_time	Equal variances assumed	6.386	.021	-2.313	19	.016	.032	-1710.833	739.772	-3259.195	-162.472
	Equal variances not assumed			-2.602	14.308	.010	.021	-1710.833	657.495	-3118.174	-303.493
deep_sleep_duration	Equal variances assumed	5.779	.027	1.360	19	.095	.190	1584.167	1164.897	-853.991	4022.324
	Equal variances not assumed			1.277	12.821	.112	.224	1584.167	1240.465	-1099.502	4267.836

Independent Samples Effect Sizes

			95% Confidence Interval	
		Standardizer[a]	Point Estimate	Lower	Upper
light_sleep_duration	Cohen's d	4550.063	-.533	-1.406	.355
	Hedges' correction	4740.092	-.511	-1.350	.340
	Glass's delta	4451.784	-.544	-1.426	.360
rem_sleep_duration	Cohen's d	2141.926	-.377	-1.245	.500
	Hedges' correction	2231.381	-.362	-1.195	.480
	Glass's delta	2232.966	-.362	-1.231	.523
awake_time	Cohen's d	1677.646	-1.020	-1.930	-.086
	Hedges' correction	1747.711	-.979	-1.853	-.082
	Glass's delta	2114.229	-.809	-1.720	.133
deep_sleep_duration	Cohen's d	2641.738	.600	-.293	1.477
	Hedges' correction	2752.068	.576	-.281	1.418
	Glass's delta	2088.375	.759	-.176	1.663

a. The denominator used in estimating the effect sizes.
Cohen's d uses the pooled standard deviation.
Hedges' correction uses the pooled standard deviation, plus a correction factor.
Glass's delta uses the sample standard deviation of the control group.

Figure D4. Chi-square analysis for Group (control,concussed)*Genotype (mixed affinity binder [MAB], high affinity binder [HAB]). (Manuscript 3)

Group * TYPE

Crosstab

Count

		TYPE		
		MAB	HAB	Total
Group	Control	6	4	10
	Concussed	6	2	8
Total		12	6	18

Chi-Square Tests

	Value	df	Asymptotic Significance (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	.450[a]	1	.502		
Continuity Correction[b]	.028	1	.867		
Likelihood Ratio	.457	1	.499		
Fisher's Exact Test				.638	.437
Linear-by-Linear Association	.425	1	.514		
N of Valid Cases	18				

a. 2 cells (50.0%) have expected count less than 5. The minimum expected count is 2.67.

b. Computed only for a 2x2 table

Figure D5. Chi-square analysis for Group (control,concussed)*Sex (male, female). (Manuscript 3)

Group * Sex

Crosstab

Count

		Sex		
		Male	Female	Total
Group	Control	6	4	10
	Concussed	5	3	8
Total		11	7	18

Chi-Square Tests

	Value	df	Asymptotic Significance (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	.012[a]	1	.914		
Continuity Correction[b]	.000	1	1.000		
Likelihood Ratio	.012	1	.914		
Fisher's Exact Test				1.000	.648
Linear-by-Linear Association	.011	1	.916		
N of Valid Cases	18				

a. 3 cells (75.0%) have expected count less than 5. The minimum expected count is 3.11.

b. Computed only for a 2x2 table

Figure D6. Group (control,concussed) differences in demographic variables of maximum standard uptake volume (SUV_{max}) in Hypothalamus (Hypo_2) and Pituitary (Pit_2). (Manuscript 3)

➔ **T-Test**

Group Statistics

	Group	N	Mean	Std. Deviation	Std. Error Mean
Hypo_2	Control	10	.61261881813	.18886835463	.05972541786
	Concussed	8	.84193890622	.27718015089	.09799798215
Pit_2	Control	10	1.6554877629	.62195540454	.19667956814
	Concussed	8	2.1145029532	.71944961720	.25436385152

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means							
						Significance		Mean	Std. Error	95% Confidence Interval of the Difference	
		F	Sig.	t	df	One-Sided p	Two-Sided p	Difference	Difference	Lower	Upper
Hypo_2	Equal variances assumed	.764	.395	-2.087	16	.027	.053	-.2293200881	.10989759691	-.4622925862	.00365240998
	Equal variances not assumed			-1.998	11.890	.035	.069	-.2293200881	.11476380110	-.4796257583	.02098558210
Pit_2	Equal variances assumed	.201	.660	-1.452	16	.083	.166	-.4590151903	.31608542877	-1.129086366	.21105598518
	Equal variances not assumed			-1.428	13.984	.088	.175	-.4590151903	.32153354644	-1.148708053	.23067767200

Independent Samples Effect Sizes

		Standardizer[a]	Point Estimate	95% Confidence Interval Lower	Upper
Hypo_2	Cohen's d	.23168447708	-.990	-1.966	.014
	Hedges' correction	.24330117519	-.943	-1.872	.013
	Glass's delta	.27718015089	-.827	-1.826	.219
Pit_2	Cohen's d	.66636659341	-.689	-1.638	.281
	Hedges' correction	.69977832491	-.656	-1.560	.267
	Glass's delta	.71944961720	-.638	-1.604	.368

a. The denominator used in estimating the effect sizes.
Cohen's d uses the pooled standard deviation.
Hedges' correction uses the pooled standard deviation, plus a correction factor.
Glass's delta uses the sample standard deviation of the control group.

Figure D7. Group (control,concussed) differences in demographic variables of maximum standard uptake volume (SUV_{max}) in Hypothalamus (Hypo_2) and Pituitary (Pit_2) stratified by mixed affinity binder (MAB) Genotype. (Manuscript 3)

T-Test

Group Statistics

	Group	N	Mean	Std. Deviation	Std. Error Mean
Hypo_2	Control	6	.59519331815	.17612399989	.07190232186
	Concussed	6	.86238849433	.32224606441	.13155640491
Pit_2	Control	6	1.5674803997	.40458213584	.16516996531
	Concussed	6	1.9817059995	.73181651649	.29876284179

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
						Significance				95% Confidence Interval of the Difference
		F	Sig.	t	df	One-Sided p	Two-Sided p	Mean Difference	Std. Error Difference	Lower	Upper
Hypo_2	Equal variances assumed	2.098	.178	-1.782	10	.053	.105	-.2671951762	.14992341899	-.6012453709	.06685501849
	Equal variances not assumed			-1.782	7.742	.057	.114	-.2671951762	.14992341899	-.6149311389	.08054078649
Pit_2	Equal variances assumed	1.900	.198	-1.213	10	.126	.253	-.4142255998	.34138007129	-1.174867800	.34641660032
	Equal variances not assumed			-1.213	7.795	.130	.260	-.4142255998	.34138007129	-1.205062795	.37661159534

Independent Samples Effect Sizes

		Standardizer[a]	Point Estimate	95% Confidence Interval Lower	Upper
Hypo_2	Cohen's d	.25967497893	-1.029	-2.223	.209
	Hedges' correction	.28141556748	-.949	-2.051	.193
	Glass's delta	.32224606441	-.829	-2.033	.441
Pit_2	Cohen's d	.59128762817	-.701	-1.856	.487
	Hedges' correction	.64079159305	-.646	-1.713	.449
	Glass's delta	.73181651649	-.566	-1.723	.641

a. The denominator used in estimating the effect sizes.
Cohen's d uses the pooled standard deviation.
Hedges' correction uses the pooled standard deviation, plus a correction factor.
Glass's delta uses the sample standard deviation of the control group.

Figure D8. Group (control,concussed) differences in demographic variables of maximum standard uptake volume (SUV_{max}) in Hypothalamus (Hypo_2) and Pituitary (Pit_2) stratified by high affinity binder (HAB) Genotype. (Manuscript 3)

➔ **T-Test**

Group Statistics

	Group	N	Mean	Std. Deviation	Std. Error Mean
Hypo_2	Control	4	.63875706810	.23193983487	.11596991744
	Concussed	2	.78059014187	.09248116816	.06539406114
Pit_2	Control	4	1.7874988078	.92138369934	.46069184967
	Concussed	2	2.5128938142	.72265929115	.51099728526

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means							
						Significance		Mean	Std. Error	95% Confidence Interval of the Difference	
		F	Sig.	t	df	One-Sided p	Two-Sided p	Difference	Difference	Lower	Upper
Hypo_2	Equal variances assumed	1.348	.310	-.795	4	.236	.471	-.1418330738	.17850474125	-.6374416889	.35377554134
	Equal variances not assumed			-1.065	3.998	.173	.347	-.1418330738	.13313679049	-.5115394999	.22787335232
Pit_2	Equal variances assumed	.822	.416	-.956	4	.197	.393	-.7253950065	.75858588200	-2.831567065	1.3807770525
	Equal variances not assumed			-1.054	2.693	.189	.377	-.7253950065	.68800814377	-3.063058647	1.6122686339

Independent Samples Effect Sizes

				95% Confidence Interval	
		Standardizer[a]	Point Estimate	Lower	Upper
Hypo_2	Cohen's d	.20611952083	-.688	-2.409	1.109
	Hedges' correction	.25833250943	-.549	-1.922	.885
	Glass's delta	.09248116816	-1.534	-3.948	1.023
Pit_2	Cohen's d	.87593952636	-.828	-2.569	1.002
	Hedges' correction	1.0978273918	-.661	-2.050	.799
	Glass's delta	.72265929115	-1.004	-2.970	1.179

a. The denominator used in estimating the effect sizes.
Cohen's d uses the pooled standard deviation.
Hedges' correction uses the pooled standard deviation, plus a correction factor.
Glass's delta uses the sample standard deviation of the control group.

APPENDIX E: BACK MATTER

Recommendations for Future Research

- Compare time in stages of sleep throughout recovery in collegiate athletes following a diagnosed concussion.
- Development of evidence-based protocol for management of sleep disturbances following a diagnosed concussion.
- Incorporate baseline measure of time in sleep stages to evaluate for differences following a diagnosed concussion.
- Explore the role of therapeutic interventions (e.g., sleep hygiene education) in management of sleep symptoms and throughout clinical recovery.
- Explore the temporal expression of TSPO tracer to determine optimal time for measurement in human subjects.
- Compare actigraphy measurements of sleep when administered to high school athletes following a diagnosed concussion.

REFERENCES

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