Automated Pupillometry Following Sport-Related Concussion in National Level Rugby League Athletes

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Abstract

Background

Sport-related concussion accounts for 9% of all sporting injuries. Within elite rugby league athletes, 17% will suffer a concussion in a three-year period. Although there is increasing knowledge of incidence and risk of SRC in rugby league, gaps regarding the optimal diagnostic and return to play protocols remain. The National Rugby League requires teams to follow a strict concussion protocol, the Head Injury Assessment, which incorporates the Sport Concussion Assessment Tool (3rd ed). The effectiveness of the Sport Concussion Assessment Tool 3rd Edition protocol, along with existing cognitive assessment tools to diagnose and predict a return to play have been disputed, which has highlighted the need for objective biomarkers. The pupil light reflex is an autonomic nervous system function that occurs when there is a light stimulus upon the pupil. Emerging evidence has been suggested that pupil light reflex may be useful as a potential objective physiological biomarker for neuroanatomical pathway disruption. The aim of the present study was to determine whether there was a change in the pupil light reflex and anisocoria (pupil asymmetry) following a sport-related concussion in national level rugby league athletes.

Aims and Objectives

The study aimed to determine whether a change in the PLR and anisocoria is detectable following an acute sport-related concussion in national level rugby league athletes. To achieve this, comparisons of the PLR, anisocoria, variability and time-frame variations between concussed and non-concussed athletes were undertaken.
Methods

Fifty-five male volunteer athletes (age: 23 ± 4.5 years) were recruited from a local National Rugby League club. Eight pupil light reflex parameters were measured via a handheld monocular pupillometer during the rugby league pre-season. During the 2017 National Rugby League season, the pupil light reflex of nine athletes without a sport-related concussion diagnosis was monitored. Sixteen athletes were diagnosed with sport-related concussion and the pupil light reflex was recorded over a ten-day time frame, and broken down to three periods, 0 – 3 days, 4 – 6 days and 7 – 10 days, to measure recovery. Absolute change scores between the pupil light reflex parameter values were calculated for each athlete. Independent t-tests and Mann-Whitney U tests were used to test the study objectives.

Results

The neurological pupil index was significantly lower in the sport-related concussion group compared to the no-sport-related concussion (\(p = 0.0002\)). There was no statistical difference in the additional seven pupil light reflex parameters between the two groups. Given the variability within sport-related concussion and no-sport-related concussion athletes, absolute change scores were calculated to determine difference between groups. The absolute change score for resting pupil diameter (\(p = 0.001\)) and minimum pupil diameter (\(p < 0.0001\)) were statistically larger for athletes in the sport-related concussion group compared to no-sport-related concussion. No significant differences were found for the remaining six PLR parameters between groups. Throughout the acute phase of sport-related concussion, resting pupil diameter and minimum pupil diameter were found to be statistically different at 0 – 3 days, 4 – 6 days and 7 – 10 days following a sport-related concussion (\(p < 0.05\)).
Maximum constriction velocity was statistically different at 0 – 3 days \((p = 0.04)\) following a sport-related concussion, however no difference was observed at the remaining time frames \((p > 0.05)\).

**Conclusion**

The pupil light reflex has previously been observed to have a statistical difference between control groups and mild traumatic brain injury groups. The present study found scientifically significant changes in the pupil light reflex following acute sport-related concussion in national level rugby league athletes compared to non-concussed athletes. The neurological pupil index for athletes with sport-related concussion were found to be statistically smaller than non-concussed athletes. Similar to previous research investigating mild traumatic brain injuries, the resting and minimum pupil diameter were found to be significantly different between a sport-related concussion group and non-concussion group. Results of the present study suggests the pupil light reflex may provide an objective physiological biomarker for diagnosis and recovery monitoring of an acute sport-related concussion in national level rugby league athletes. The change in the pupil light reflex observed in the present study suggest the potential application within the diagnosis and recovery monitoring of an acute sport-related concussion in national level rugby league athletes.
List of Outputs from Work

Statement of Originality

This work has not previously been submitted for a degree or diploma in any university. To the best of my knowledge and belief, the report contains no material previously published or written by another person except where due reference is made in the report itself.

Daniel Brown
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Although only very brief, and somewhat sheltered, my master of medical research candidateship has been a great introduction into the world of scientific research. Firstly, I would like to thank my two supervisors, Dr Kerrie Evans and Dr Gary Grant. Without their patience, dedication and guidance my journey through this program would not have been possible. Their dedication and efforts to not only myself, but all students and research as a whole is something I greatly appreciate and aspire towards. Secondly, to the medical staff, athletes and Gold Coast Titans community for welcoming the project and supporting throughout. Thirdly, to the fellow research candidates, thank you for the support and encouragement throughout the program. Finally, to my supportive family and partner, without your reassurance, patience and unwavering support, this would not be possible.
List of Abbreviations

ANS  Autonomic nervous system
ATP  Adenosine triphosphate
CTE  Chronic traumatic encephalopathy
CISG Concussion in Sport Group
CNII Cranial nerve 2 (optic nerve)
CNIII Cranial nerve 3 (oculomotor nerve)
DAI  Diffuse axonal injury
DTI  Diffuse tensor imaging
EWN Edinger-Westphal nucleus
EEG  Electroencephalogram
fMRI Functional magnetic resonance imaging
ipRGCs Intrinsically photosensitive retinal ganglion cells
MRI  Magnetic resonance imaging
mTBI Mild traumatic brain injury
MDMA 3,4-methylenedioxymethamphetamine
NAA  N-acetylaspartate
NaSSA Noradrenergic and specific serotonergic antidepressants
NPi Neurological pupil index
NRL National Rugby League
PLR Pupil light reflex
PNS Parasympathetic nervous system
RTP Return to play
SNRI Serotonin and norepinephrine reuptake inhibitor
SSRI Selected serotonin reuptake inhibitor
SCAT Sport concussion assessment tool
SCAT3 Sport concussion assessment tool 3rd edition
SRC Sport-related concussion
SAC Standardised Assessment of Concussion
SNS Sympathetic nervous system
TBI Traumatic brain injury
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1. Introduction

Concussion accounts for 5 - 9% of all sport-related injuries (1). Despite the increased risk of concussive insult, 30% of athletes suspected of sustaining a concussion return to play (RTP) during the same game (1). Additionally, a history of a concussion increases the risk of recurrent concussions (2). The Concussion in Sport Group (CISG), comprised of international experts on sport-related concussion (SRC), convene every 4 to 6 years to discuss and update guidelines for concussion assessment and RTP protocols (3). The protocols are employed by major sporting bodies worldwide, such as the National Rugby League (NRL), the premier Rugby League competition in Australia and New Zealand.

This thesis first reviews current SRC diagnostic and RTP protocols within the NRL and then evaluates whether the assessment of the pupil light reflex (PLR) could serve as a physiological objective biomarker to aid in the diagnosis and monitoring of recovery following an acute SRC.

1.1 Background

In recent decades, there has been an increase in the awareness and concern surrounding the impact of SRC. The working definition of SRC differs between research groups, making it difficult to compare findings across different studies. To overcome this, the CISG regularly updates a SRC consensus and definition, most recently in 2016 (3). Often interchangeable with mild traumatic brain injury (mTBI), the CISG broadly describe SRC as immediate and transient symptoms of traumatic brain injuries (TBI) induced by
biomechanical forces (3). The CISG have published a list of common features frequently associated with SRC (Table 1). However, this definition does not provide insight into severity or presentation of symptoms (3). Nevertheless, the recent CISG definition will be used throughout this thesis.

Table 1: Common Features Associated with SRC (3)

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Direct blow to the head, face, neck or body with an impulsive force transmitted to the head.</td>
</tr>
<tr>
<td>2.</td>
<td>Rapid onset of short lived neurological function impairment, typically resolves spontaneously. However, signs and symptoms may manifest over minutes to hours.</td>
</tr>
<tr>
<td>3.</td>
<td>Presentation of neuropathological changes. Acute clinical symptoms suggest functional disturbances rather than structural injury. No abnormalities are detected on standard structural neuroimaging studies.</td>
</tr>
<tr>
<td>4.</td>
<td>There are a range of clinical signs and symptoms which may or may not include loss of consciousness. Although symptoms may be prolonged in certain cases, clinical and cognitive symptoms resolve following a sequential course.</td>
</tr>
<tr>
<td>5.</td>
<td>The presenting signs and symptoms cannot be explained by drug alcohol, medication or other injuries (e.g. cervical injury, peripheral vestibular dysfunction) or other comorbidities.</td>
</tr>
</tbody>
</table>

The mechanism of a SRC has been described as a low-velocity incident that is believed to result in rotational or linear acceleration forces (1, 4, 5). The traumatic biomechanical insult results in neuronal shearing within the brain, causing abnormal complex pathophysiological processes, subsequently presenting with clinical signs and symptoms (1, 4, 6). An issue was raised by the CISG in relation to the classification of SRC and whether it falls within the traumatic brain injury (TBI) spectrum (Table 2) or, alternatively, is a reversible physiological process (3). Understanding relationships between SRC and other brain injuries are important to distinguish in order to develop diagnostic and monitoring protocols for recovery.
### Table 2: Severity of TBI indices (7)

<table>
<thead>
<tr>
<th></th>
<th>Mild TBI</th>
<th>Moderate TBI</th>
<th>Severe TBI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glasgow coma scale score</td>
<td>13 - 15</td>
<td>9 - 12</td>
<td>4 - 6</td>
</tr>
<tr>
<td>Loss of consciousness</td>
<td>&lt; 30 min</td>
<td>30 min – 24 hours</td>
<td>&gt; 24 hours</td>
</tr>
<tr>
<td>Posttraumatic amnesia</td>
<td>0 – 1 day</td>
<td>&gt;1 - &lt; 7 days</td>
<td>&gt; 7 days</td>
</tr>
</tbody>
</table>

In addition to the aforementioned definition, several physical, cognitive and behavioural signs and symptoms have been suggested by numerous authors to aid health professionals and sport trainers in the diagnosis of a SRC (4, 8) (Table 3). Should any of the signs and symptoms listed in Table 3 be present during assessment, SRC should be suspected (3).

### Table 3: SRC signs and symptoms (3, 8-10)

<table>
<thead>
<tr>
<th>Physical Features</th>
<th>Headache</th>
<th>Decreased co-ordination</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dizziness</td>
<td>Blurred vision</td>
</tr>
<tr>
<td></td>
<td>Nausea</td>
<td>Photosensitivity</td>
</tr>
<tr>
<td></td>
<td>Impaired playing</td>
<td>Hyperacusis</td>
</tr>
<tr>
<td></td>
<td>Balance deficit</td>
<td>Seizures</td>
</tr>
<tr>
<td>Cognitive Features</td>
<td>Decrease processing</td>
<td>Decrease concentration</td>
</tr>
<tr>
<td></td>
<td>Awareness deficit</td>
<td>Decrease consciousness</td>
</tr>
<tr>
<td></td>
<td>Confusion</td>
<td>Foggy sensation</td>
</tr>
<tr>
<td></td>
<td>Amnesia</td>
<td>Slowed reaction time</td>
</tr>
<tr>
<td>Behavioural Features</td>
<td>Sleep disturbance</td>
<td>Fatigue</td>
</tr>
<tr>
<td></td>
<td>Irritability</td>
<td>Distracted</td>
</tr>
<tr>
<td></td>
<td>Anxiety</td>
<td>Emotional (lability)</td>
</tr>
<tr>
<td></td>
<td>Apathy</td>
<td>Slurred speech</td>
</tr>
</tbody>
</table>
It is imperative to recognise an athlete with SRC to ensure they are removed from play, thoroughly assessed, and allowed adequate time for rehabilitation and recovery (3). Without adequate understanding of the broad range of signs and symptoms that may present following a SRC, there is an increased risk of missed SRC diagnosis and inappropriate athletic participation. Failing to recognise SRC, may result in neglecting more sinister and potentially long-term brain injuries (Table 4), such as mental health concerns (3). Mental health concerns associated with SRC have been highlighted in recent literature. For example, it has been suggested that SRC leads to depression, cognitive deficits and chronic traumatic encephalopathy (CTE) (3, 11). Thought to be a process of long-term neurological damage, CTE may be a result of repetitive mTBI, whereby the risk is increased if a diagnosis of SRC is missed (9). Understanding the pathophysiological processes that occur following a SRC is required to develop potential strategies and protocols to aid in athlete wellbeing and health management.

Table 4: Pathologies related to SRC

<table>
<thead>
<tr>
<th>Structural (3, 4, 8, 9, 11)</th>
<th>Axonal injury</th>
<th>Cortical contusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intracerebral haemorrhage</td>
<td>Subarachnoid haemorrhage</td>
<td></td>
</tr>
<tr>
<td>Intraventricular haemorrhage</td>
<td>Subdural hygroma</td>
<td></td>
</tr>
<tr>
<td>Cavum septum pellucidum lengthening</td>
<td>Cortical thinning</td>
<td></td>
</tr>
<tr>
<td>Fracture</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Non-Structural (11, 12)</th>
<th>Neuropsychological changes</th>
<th>Cognitive deficits</th>
</tr>
</thead>
<tbody>
<tr>
<td>Depression – declining quality of life</td>
<td>Psychological health</td>
<td></td>
</tr>
</tbody>
</table>
1.1.1 Pathophysiology of Sport-Related Concussion

Developments in neuroimaging have improved the understanding of the metabolic, physiological and microstructural pathophysiological processes that occur following SRC (13, 14). However, despite these developments, further research is required to understand the underlying complex pathophysiological cascade that occurs following an acute SRC and throughout recovery (15). Understanding the pathophysiology following acute SRC may potentially lead to the development of detection and monitoring protocols within sporting environments.

A SRC is a result of direct or indirect linear and/or rotational forces to the brain that causes neuronal shearing (1, 6, 16). Neuronal shearing causes stretching of axons and neuronal cell membrane disruption resulting in changes to membrane permeability (6, 17), known as diffuse axonal injury (DAI) (6, 18). Damage to neuroanatomical structures may lead to changes in cerebral physiological responses and affect the autonomic nervous system (ANS) (16). The ANS is a combination and balance between two divisions – the parasympathetic nervous system (PNS) and the sympathetic nervous system (SNS) (19). The PNS is the primary division for energy conservation (16). Conversely, the SNS is the primary driver in times of stress (16). Results from a study using diffuse tensor imaging to investigate patients with post-concussive syndrome supported the idea that the primary ANS control centre is vulnerable to dysfunction or damage following a concussion (16, 20). For example, recent evidence has shown that following a concussion, the ability for the PNS and SNS to alternate in the control of cardiac function is diminished, supporting the notion that the ANS control centre is impacted (16, 21).
In addition to dysfunction in the ANS, changes in metabolic activity within the brain has also been observed following an acute SRC (13). For example, it has been shown that there is an increase in the neuronal depolarisation in the areas surrounding the insult, resulting in an intracellular influx of calcium and efflux of potassium (22). Numerous animal studies have shown ionic changes following a TBI using ion-sensitive electrodes and microdialysis (23, 24). As the sodium-potassium pump increases in activity to restore neuronal membrane potential, an increase in glucose metabolism is required (22, 23, 24). Animal studies have suggested the increase in cerebral cellular energy demand is met with a decrease in cerebral blood flow (CBF), resulting in a decrease in energy substrate delivery (22). The period of hyperglycolysis and excitability lasts up to a few hours (6, 22). Following the excitability phase, a period of neuronal suppression occurs, which is thought to be a result of decreased adenosine triphosphate (ATP) production (6, 22). This decrease in ATP may be a result of excess intracellular calcium within mitochondria leading to oxidative metabolism impairment (6, 22). Metabolic dysfunction has been shown to last for up to 30 days following a SRC, and 45 days if a secondary SRC occurs before recovery (16, 25). These findings were observed in a study investigating the metabolic brain vulnerability in concussed athletes by measuring N-acetylaspartate (NAA) via proton magnetic resonance spectroscopy (25). The metabolite, NAA, is a biomarker of neuronal integrity and may indicate damage to such structures (6). The authors concluded that resolution of metabolic imbalance does not linearly correlate with clinical symptom resolution and suggests that the addition of NAA in SRC protocols could serve as an objective physiological biomarker for SRC (25).
1.1.2 Epidemiology

Within sport, the risk of a SRC is high, particularly in contact sports such as rugby, ice hockey and American football (26). It has been estimated that in the USA alone there is 1.6 - 3.8 million concussive events per annum (27, 28). In Australia, precise data on SRC is limited. However, in the state of Victoria, hospitalisations for SRC increased by 60.5% between 2002/2003 to 2010/2011 (29). Currently, there is limited data on SRC available from other Australian states and territories.

In Australia, Rugby League is a popular contact sport with approximately 165,239 registered players (30). The sport is played from 4 years of age to adults, and although the majority of participants are male, in recent years there has been a growth in female participation across the lifespan (30). A game of Rugby League consists of two teams of 13 players and 4 reserves. The attacking team has possession of a ball and physical contact is required to stop the momentum of the ball-carrying player. A typical adult game is played over two 40 minute halves. The NRL, the professional Rugby League competition, consists of 16 teams usually with a roster of 25 players. Within the NRL, SRC has been reported to account for 9% of all injuries and 29% of injuries that occur during illegal play (31). The incidence within the NRL is reported to be one in every four games (14.8/1000 match hours) (32). A 2015 systematic review of concussion in Rugby League found there to be disproportionally higher rates of concussion as a result of illegal play compared to legal play (31, 32). A study using video analysis of concussive events in the NRL reported all SRC injuries had occurred via impact to an athlete’s head or face (33). The impact to the head or face causing concussion were due to a number of different types of collisions, including: 35% of concussions were as a result of collisions from an opposing athletes’ shoulder; 20%
impacting the knee of an opposing athlete; 20% from head to head impact; 10% impacting the torso of an opposing athlete and 10% from either the elbow or forearm of an opposition athlete (33). In addition, 25% of concussions were observed to have a secondary impact with either the playing surface, or a second athlete’s knee (33). The study shows that SRC in Rugby League occurs as a result of different impact mechanisms and, while important to be considered during the diagnosis protocol, may also influence the type of pathophysiological changes that occur following a SRC.

1.1.3 Sideline Concussion Assessment in the NRL

The term ‘sideline assessment’ refers to the acute evaluation of an athlete’s cognitive and physical function immediately after a concussion, generally occurring in a time restricted environment, such as the field sideline where rapid assessment is required (10). There has been much debate about sideline assessment protocols, particularly about which specific tests should be included (3, 10). Most frequently, and as recommended by the CISG, the sport concussion assessment tool (SCAT), now in its 5th iteration, is employed and utilised by major sporting bodies (3) and large international organisations such as the Federation of International Football Associations (FIFA) and the International Olympic Committee (IOC) (34). The NRL incorporates the 3rd edition of the SCAT (Appendix A) in its sideline SRC protocol which is termed the head injury assessment (HIA). In addition to the SCAT, the HIA incorporates a footage review system, where medical officials must review relevant footage of a suspected SRC incident. The HIA requires an athlete to be removed from the field for a 15-minute period whereby at the 5-minute mark, the SCAT assessment is commenced. If an athlete is diagnosed with a SRC, they are not permitted to RTP. All NRL athletes undergo
pre-season baseline testing, the results of which help determine whether an athlete’s sideline assessment indicates a decline in performance (10).

The SCAT involves assessing an athlete’s signs and symptoms, cognition evaluation, physical evaluation, cervical spine assessment and neurological presentation (3, 35, 36). For the cognition component, the SCAT incorporates Maddocks questions and the Standardised Assessment of Concussion (SAC). Maddocks questions are incorporated to assess sport-specific orientation and includes questions such as place and score of the game (37). The SAC has been designed for brief mental status screening when neurocognitive testing tools are not available or cannot be administered due to time constraints (3, 38). A SAC score is calculated by orientation, immediate memory, concentration and delayed recall measures (39). Additionally, the SCAT investigates level of consciousness, using the Glasgow Coma Scale (GCS). Balance assessment utilities the modified balance error scoring system (mBESS) or a tandem gait test (11). The mBESS tests balance in three stances (Figure 1) - double leg stance (feet together), single leg stance (non-dominate foot) and tandem stance (non-dominate foot at the back). For a more challenging balance assessment, athletes are required to stand on a foam mat (35). The SCAT has been designed for persons over the age of 13 years but there is also a child specific SCAT for ages 5-12 years (4).
Figure 1: BESS protocol positioning (38). a) double leg stance; b) single leg stance; c) tandem stance; d-f) stance position with medium density foam. For the mBESS, a-c is assessed. The addition of d-f may be incorporated by the physician to complete the BESS.

While the SCAT offers assessments of cognitive function, physical function and signs and symptoms, components of the SCAT protocol have well documented limitations (3, 40, 41, 42, 43). For example, while the SCAT has been reported to have a sensitivity of between 80 – 94% and a specificity of 76% immediately after a concussion (40, 41), the use of the SAC after more than 1-day post-concussion reduces the sensitivity to 31% (40, 42). The BESS has been reported to have a sensitivity of 34 - 64% immediately after a concussion, with a specificity of 91% (43), but performance on the BESS may be affected by multiple factors such as previous ankle injury, exertion and fatigue. Additionally, a learning or practice effect has been reported following the repeated administration of the BESS (43, 44). This learning effect should be considered when administering the BESS multiple times over short time periods which frequently occurs in NRL teams (43, 44). The effectiveness of the SCAT in SRC diagnosis has also been questioned (4). The CISG released a consensus
statement acknowledging that sideline protocols may be abbreviated by health care professionals and sport trainers for rapid screening in time sensitive situations, such as sporting events (3, 4). The CISG also made acknowledgement that signs and symptoms may evolve and present hours after a SRC, and this may be missed with immediate sideline assessment (3). These factors may result in assessment errors, leading to an inappropriate or premature RTP (4).

1.1.4 Investigations and RTP Protocol for SRC

Following the diagnosis of a SRC from an on-field incident, athletes may require further evaluation, such as neurological imaging, to exclude a more severe brain injury and determine an estimated recovery time (3). The staff involved in NRL teams are required to follow a stepwise protocol (Table 5) during rehabilitation following a SRC and there are protocols that athletes are required to pass before they are allowed to RTP.

Table 5: Return to play rehabilitation protocol suggested by the CISG in 2016 (3)

<table>
<thead>
<tr>
<th>Step-wise RTP Protocol</th>
</tr>
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<tbody>
<tr>
<td><strong>Stage</strong></td>
</tr>
<tr>
<td>1</td>
</tr>
<tr>
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<tr>
<td>3</td>
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<td>4</td>
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</tbody>
</table>
Following medical clearance, participate in normal training

Restore confidence and assess functional skills

Return to sport

Normal game play

NB: 24 – 48 hours of relative physical and cognitive rest has been recommended before beginning the RTP protocol. At least 24 hours should separate each stage of progression. Should symptoms reoccur, the athlete must return to the previous stage.

Following the stepwise protocol, in order for an athlete to be given clearance to RTP, they are required to pass a licence-to-use computerised neuropsychological assessment (Table 6). To assist in the diagnosis or the RTP decision, the NRL require athletes to undergo pre-season baseline testing for the computerised neuropsychological assessment which consists of eight tasks (6) that involve reaction time, identification, working memory and learning tests (6, 45). After a SRC, athletes must achieve the same scores on the neuropsychological assessment as they achieved at baseline/pre-season testing before they can RTP. While some studies have suggested computerised neuropsychological assessments to have a sensitivity of 94.6% (46), others have reported the sensitivity to be as low as 1%, which suggests a very low chance for detecting a true positive SRC (47). The specificity has been reported to be between 86 – 100% (46). Usefulness of the existing neuropsychological assessment has been questioned with concerns regarding intentional suboptimal performances at baseline (37, 48). Studies have reported that between 11 and 34% of athletes were able to successfully complete the assessment whilst providing a suboptimal effort (49, 50). Studies investigating high school and college athletes reported that 4 – 11% of participants reported completing the assessment below their best abilities (50-52). With respect to the computerised neuropsychological assessment adopted by the NRL, Chen et al. (53) found that mildly concussed athletes produced valid assessment scores despite abnormal findings being detected with functional magnetic resonance imaging (fMRI) (53). These results
suggest that the current RTP protocol may result in the premature RTP of athletes still in a vulnerable state following a SRC.

**Table 6: NRL SRC protocol summary.**

<table>
<thead>
<tr>
<th>Timeframe</th>
<th>Protocol</th>
<th>Test</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Suspected SRC</strong></td>
<td>Remove from play</td>
<td>Video review of incident</td>
</tr>
<tr>
<td></td>
<td>HIA</td>
<td>Signs/symptoms and GCS</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cognition - SAC, Maddocks questions</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Physical - mBESS or tandem gait, cervical assessment</td>
</tr>
<tr>
<td><strong>Failed HIA</strong></td>
<td>Not to RTP</td>
<td>Review of SCAT</td>
</tr>
<tr>
<td><strong>Rehabilitation</strong></td>
<td>CISG RTP protocol</td>
<td>Symptom limiting progression</td>
</tr>
<tr>
<td><strong>RTP</strong></td>
<td>Neuropsychological testing</td>
<td>Pay-per-use licenced computerised assessment</td>
</tr>
</tbody>
</table>

The use of conventional structural neuroimaging techniques, such as computed tomography (54) and magnetic resonance imaging (MRI) are generally unremarkable and contribute little to detection or grading of acute concussion (6). These imaging techniques are however useful when there is suspicion of intracerebral or structural lesions (4). Advanced neuroimaging techniques, such as fMRI, diffuse tensor imaging (DTI) and electroencephalogram (EEG), may provide greater insight into the physiological changes that occur in the brain post-concussion (55, 56). All of these techniques have demonstrated physiological processes are altered long after clinical recovery has been determined (55-57). Studies investigating the use of fMRI in individuals with concussion found that cerebral activation changes remained evident up to 23 months after clinical recovery and RTP had been achieved (55, 56). The fMRI studies have shown consistency in reporting decreased activity in mTBI cohorts in the anterior region of the brain (frontal lobe/anterior cingulate and temporal lobes), and an increased activity in the posterior regions (cerebellum, two insula regions and parietal lobes) (56). The use of DTI has been reported as a most promising
modality for categorising subtle changes following mTBI (56). Measures of DTI, such as fractional anisotropy, axial and mean diffusivity, assess the structure of brain fibres through water diffusion properties (55, 58). Studies have found changes to the orientation of white matter through fractional anisotropy, and axial and mean diffusivity, which persisted following clinical recovery for 5 – 180 days post-concussion (55, 58). Similar to fMRI findings, the anterior regions of the brain are reported to have an increased vulnerability to mTBI (56). Additionally, areas of the midbrain have been suggested to detect anisotropy abnormalities following mTBI (56). Electrophysiological measures assessed via EEG, have indicated a change in the electrical integrity and variations in brain electrical activity up to 45 days post-concussion (57). Metabolic alterations in the NAA/creatine ratio have been reported up to 1-month post-concussion in proton magnetic resonance spectroscopy (25, 55).

While cortical imaging techniques offer innovative advantages in detection and grading of SRC, these techniques are currently expensive and lack portability, and are therefore not practical or available for sideline assessment or SRC diagnosis, particularly when recurrent SRC are likely, nor for making RTP decisions in the NRL (8, 59). For these reasons, the use of routine neuroimaging is not appropriate in the NRL in SRC protocols. Currently, the HIA and computerised neuropsychological in the NRL risk false negative diagnosis and early RTP. Evidence has shown that NRL athletes that suffer a potential SRC incident, RTP before neurological imaging indicates recovery. In 2013, a study investigated potential SRC incident and subsequent athlete treatment within the NRL. A total of 20 SRCs were reported, 19 were attended on the field of play, with 50% removed from play (33). Of the athletes that were removed from play, 70% walked with assistance, 10% walked without assistance, and 20% were removed with a medicab or a stretcher (33). Of all the athletes removed from the play, only one athlete RTP during the same game. All athletes medically
diagnosed with SRC that remained in the game did not miss the following match (33). Eight of the nine athletes that did not return to the same game returned the following game, between 5 – 19 days following the incident (33). What can be ascertained from the review on SRC in the NRL, is that athletes return to play within a relatively short time-frame, well before physiological recovery has been met (57). Returning to play in a relatively short time-frame is not uncommon, with majority of athletes found to be cleared within 10 days as per the CISG consensus statement (3). The current NRL RTP time-frame may result in a potential premature RTP, leaving athletes at risk of potential catastrophic injury, such as repetitive neurotrauma (11). These findings indicate the need for an improvement in the sensitivity of the HIA and RTP protocols within the NRL.

The limitations of current testing protocols available for SRC diagnosis and RTP are well documented. Although clinical recovery may be met, there is well established literature to show that physiological dysfunction remains at RTP (4, 37). In 2016, the CISG reported the need for diagnostic objective biomarkers to assist in the clinical assessment for the presence and severity of SRC (3). Previous studies have investigated the use of pupillometry to assess the pupil light reflex (PLR) in a range of mTBI cohorts (18, 60, 61). All have suggested the potential for PLR assessment via pupillometry to be an objective biomarker for mTBI (18, 60, 61). There was no research found investigating the pupil light reflex (PLR) within athletes with SRC. The afferent pathway of the PLR travels from the anterior portion of the brain to the midbrain where it is processed and efferent fibers terminate at the iris (18). As discussed previously, advanced neuroimaging has shown abnormalities within areas within the midbrain and anterior regions (56). Thus, the aim of the present study was to investigate whether there is potential for the PLR to be used as an objective physiological biomarker in the assessment protocols of SRC for rugby league athletes.
1.2 Pupil Light Reflex

The importance of the clinical use of objective physiological biomarkers in relation to SRC detection and monitoring has been emphasized in the literature (3). As mentioned previous, abnormalities are observed throughout large portions of the brain following a SRC or mTBI. The PLR, measured via pupillometry, has previously been suggested as a potential objective physiological biomarker for brain injury, such as concussion (4, 18).

1.2.1 Pupil Light Reflex – Neuroanatomy Structure and Physiology

Light enters the anterior eye, the cornea, which travels through the pupil towards the lens (62). At the lens, light is refracted and focused on the retina (62). Pupil diameter is determined by the action of the posterior layer of the iris, which contains the dilator and sphincter musculature (62, 63, 64). The pupillary reflex occurs during a period of increased light intensity over the retina via passing through the pupil, resulting in pupillary constriction (62, 63, 64). The pupils’ reflex to a light stimulus is a function of the ANS and an important function for visual perception (60, 63, 64). This reflex is known as the PLR, and is controlled by the divisions of the ANS: the PNS and SNS pathways (Figure. 2) (63, 64). The PLR affects the diameter and movement of the pupils through constriction and dilation in relation to light stimulus (64).
Figure 2: PNS and SNS pathways involved with PLR (27). a) parasympathetic nervous system PLR pathway. Blue indicates the nasal retina input decussating to the contralateral side. Red indicates the lateral retina input. b) Sympathetic nervous system pupil dilation pathway. Beginning in the hypothalamus, travelling through the brainstem and synapsing at level C7-T2 within the spinal cord.

Pupil constriction is a function of the PNS (60, 64). Constriction occurs in response to an increase in the PNS activity with a corresponding decrease in SNS activity (60). The PNS neural pathway for PLR function has three divisions; afferent, interneuron and efferent (60, 64). The afferent division initiates PLR response when increased illumination stimulates the retina, where phototransduction takes place by photoreceptors (64). Originally, the photoreceptors, the rods and cones, mediated by the retinal ganglion cells, were believed to contribute differently to the PLR depending on the light conditions (64-66). When a low-intensity light (scotopic) stimulus illuminates the pupil, the PLR response is predominately an action of the rods, resulting in a low-amplitude constriction (66). Inversely, when there is a stronger, or brighter (photopic) light stimulus, the eye relies on the cones to instigate the pupil constriction (64, 66). More recently, research has shown that the retinal ganglionic cells
have input from an additional photoreceptor, the intrinsically photosensitive retinal ganglionic cells (ipRGCs) or melanopsin-containing retinal ganglion cells (64, 66-68). Along with the capability of light transduction independently under photopic conditions, the ipGRCs receive input by rods and cones, meaning the cells are sensitive to both photopic and scotopic light stimuli (64, 66).

The afferent pathway of the PNS pathway is innervated when a light stimulus which is emitted upon the cones, rods and the ipRGCs. These photoreceptors innervate the retinal ganglion cell and their axons (running along cranial nerve II (CNII)) which project towards the midbrain (64, 69). The nasal retina innervations of the CNII and axons of the retinal ganglionic cells decussate at the optic chiasm, which forms the two optic tracts (64, 69). Through the optic tract, the axons travel in the brachium of the superior colliculus, synapsing in the midbrain on the retinorecipient neurons located in the pretectal olivary nucleus (PON) (64, 66), also referred to as the interneuron division (64).

The interneuron division is situated within the midbrain. Efferent projections from the pretectal area travel to the Edinger-Westphal nucleus (EWN), with some fibres crossing through the posterior commissure to the contralateral EWN (62, 64, 66, 70). This process allows for even distribution of information to the efferent pathway. In humans, the decussation of the CNII and crossing of pretectal fibres are near equal to the ipsilateral fibres, allowing for a consensual light reflex to be achieved (62, 64). From the EWN, the efferent division of the PNS arises. The fibres that leave the EWN are known as preganglionic parasympathetic neurons (64, 66). The axons travel along cranial nerve III (CNIII), towards the orbital apex (64, 70). These fibres pass through the cavernous sinus towards the apex and synapse in the ciliary ganglion (64). From the ciliary ganglion, postganglionic
parasympathetic neurons project to the sphincter pupillae muscle of the iris by way of the short ciliary nerves, resulting in pupil constriction (64, 66, 70) (Figure 3).

Figure 3: PNS pathway for the PLR

The primary function of the SNS is to control pupil dilation in response to a light stimulus (60, 61). Two processes generally take place with pupil dilation; first, the sphincter pupillae muscle relaxes and secondly, the iris dilator contracts (Figure 4). These processes are a function of the supranuclear inhibition in the midbrain (61). The SNS input produces inhibition of the EWN, thus decreasing input to the sphincter pupillae, resulting in decreased pupil constriction.

Figure 4: SNS pathway for the PLR (60)
In animal studies, sympathetic neurons have been found to pass through the periaqueductal grey area, innervating pupil efferent neurons at the EWN and the synapse activates an $\alpha_2$-adrenergic receptor which innervates pupil dilation (64, 71). The process results in suppression of preganglionic parasympathetic neuronal innervation, thereby limiting innervation to the pupil sphincter muscles, suppressing pupil constriction (64).

Secondly, the SNS stimulates the iris dilator to contract, thereby increasing pupil dilation (61, 64). This process is achieved via ipsilateral SNS function. When increased illumination has been removed or adapted by the retina, sympathetic outflow takes place (64). Retinal ganglionic cells are innervated, and the axons travel through the optic chiasm, with approximately half decussating into two optic tracts. The optic tracts innervate the hypothalamus. From the hypothalamus, the central neuron descends through the brain stem on either side into the lateral column of the spinal cord, synapsing at the cervicothoracic level, C7-T2 (64). The preganglionic sympathetic neuron is innervated and travels over the apical pleura of the lung to the spinal rami, synapsing at the superior cervical ganglion at the carotid artery bifurcation (64). Finally, the postganglionic sympathetic neuron travels along the internal carotid artery into the head, passing through the cavernous sinus and finally into the orbit (via the long ciliary nerves), where the iris dilator muscles are innervated (64).

1.2.2 Factors Affecting a Healthy Individual’s Pupil Light Reflex

An individual’s PLR response may be affected by a number of factors which need to be considered when quantifying data for scientific and medical use. Factors influencing pupil reactivity to a light source include; cognitive function, pharmaceuticals, diurnal and circadian cycles and illumination (72). It is acknowledged that age is a factor that may contribute to
changes in the PLR, however age is not a consideration in the present study since all participants were < 40 years of age.

**Cognitive Function**

Pupil diameter and reactivity have been suggested to be modulated by cognitive state and processing such as emotion/depression, arousal, attention, effort and sleepiness (67, 73). Partala et al. (2003) investigated pupil diameter variations with response to auditory stimulation. The authors used neutral emotional sounds, along with negative and positive arousing sounds (74). Both female and male participants were included in the study and, although there were some differences between genders, there were significant increases in pupil diameter with both negative and positive auditory stimulation for both male and female (p < 0.05) (74). The results showed that pupil size was affected with non-illumination, emotional stimuli thought to be a result of the ANS response to emotional stimuli. A 2003 study investigated pupil dilation in depressed and non-depressed individuals (75). The authors used valence identification task stimuli and sentence rating tasks to assess pupil change within patients with depression. The valence identification task included 60 positive, negative and neutral words. Participants were required to select the emotional response to each word (75). The sentence rating task required participants to view 30 positive and negative sentences from the Automatic Thoughts Questionnaire and report if the sentences were personally relevant, somewhat relevant or not personally relevant (75). The authors observed a depressed individual sustained greater pupil dilation following valence identification task stimuli (p = 0.02) and the sentence rating task (p = 0.01) (75). The results indicate that cognitive and emotional processing factors may influence pupil diameter and may in turn result in PLR changes.
Pupillary reactivity has been shown to be altered in sleep deprived healthy young adults (76). Healthy participants (n = 30) were randomly assigned to either a sleep deprivation or control group (76). Pupil diameter was recorded using pupillometry with the light source pointed at the participant’s left eye to track change in size (76). The authors observed larger pupillary responses for negative pictures compared to positive and neutral pictures for sleep deprived participants ($p = 0.015$) (76). Differences between sleep deprived and non-sleep deprived groups were additionally reported (76). There were significant differences between negative ($p = 0.05$) and neutral ($p = 0.07$) stimuli, with a larger pupillary response recorded (76). From this, it was proposed the pupil dilation differences were a measure of sleep deprivation-related emotional reactivity (76). The current study did not measure cognitive function and effects of sleep deprivation. It has been acknowledged that participants within the study may be effected by these factors and should be taken into consideration when interpreting the results.

**Pharmacological Agents**

Pupil reactivity and size has been found to be affected by certain pharmacological agents (72). Studies have indicated drug intake can affect pupil diameter, amplitude and latencies (77). Some pharmacological classes that have been reported to incur direct effects on the pupil are sympathomimetics, sympatholytic agents, cholinergic and anticholinergic agents, opioid agonists and antidepressants medications (72, 78, 81, 84, 92, 97). The drug intake of individual participants of this study was not recorded, however it is important to consider pharmacological influence on the PLR throughout this thesis.
Sympathomimetics are a class of drugs that produces the effect that occurs with SNS stimulation (78). Drugs within this class are also known as adrenergic agonist drugs and produce an exaggerated mydriasis (pupil dilation) (64). Clinically, sympathomimetics are used for treating hypertension and withdrawal from drug or alcohol abuse, as well as the treatment of asthma (79, 80). Recreationally, drugs such as cocaine and 3,4 methylenedioxymethamphetamine (MDMA or ecstasy) elicit sympathomimetic effects (81, 82). Cocaine inhibits the reuptake of noradrenaline and dopamine at the preganglionic synaptic terminals which increases the monoamine concentration, enhancing the effect of noradrenaline (82, 83). Additionally, cocaine stimulates the release of noradrenaline and adrenaline from the adrenal medulla (82). An example of cocaine being used medically is in the diagnostic assessment of conditions such as Horner syndrome which is a condition that affects the sympathetic pathway (83). Many amphetamines produce indirect sympathetic activation by stimulating the release of noradrenaline and dopamine, additionally MDMA stimulates seratonergic activity (81). Many studies have investigated the effect of MDMA on pupil size. A study by Torre et al. (2000) investigated the pharmacological effect on physiological parameters, including pupil diameter. The authors found that with increased MDMA dosages there was greater mydriasis observed (81). Although recreational drug use was not recorded as part of this study, acknowledgement of the possible effects of drug usage needs to be considered in relation to pupil parameters as a possible limitation.

Conversely, sympatholytic agents, also known as adrenergic antagonist, block and oppose the effects of the postganglionic fibres of the SNS, producing a miosis (pupil constriction) presentation (84, 85). Sympatholytic drugs are used as a treatment option for hypertension, but have also been used to treat anxiety disorders (86, 87). Clonidine, a sympatholytic drug, has been found to elicit changes in the PLR (84). In comparison to a
control group, 0.2 mg of clonidine administered orally resulted in a decrease in resting pupil diameter ($p < 0.05$), reduced amplitude ($p < 0.05$) and a decrease in initial constriction velocity ($p < 0.05$) in healthy adult male volunteers (84).

Another class of drugs that effects pupil diameter is cholinergic and anticholinergic drugs. It is acknowledged that cholinergic agonist drugs resemble acetylcholine, increasing the PNS activity, and produces a miosis presentation (88). For example, Leavitt et al. (2002) investigated the effect of pilocarpine on the pupillary response in normal individuals and found that there was a decrease in pupil diameter with all concentrations of the drug tested ($p < 0.001$) (89). Anticholinergic drugs inhibit PNS activity by binding to muscarinic receptors, inhibiting the binding of acetylcholine (85). Anticholinergic drugs such as atropine have been used in investigations of pupil diameter. Mirakhur (1978) investigated the intramuscular and oral doses of atropine and hyoscine effect on pupil diameter (90). The intramuscular doses of both drugs resulted in marked increase in pupil diameter. Although requiring larger doses, oral administration of atropine elicited similar increase in pupil diameter (90).

Mental health concerns have been investigated among elite athletes in Australia, with anxiety and depression being found to be prevalent (91) and the use of antidepressant medication not uncommon. While the present study investigated athletes, mental health status or the use of anti-depressant medication was not evaluated. However, there are a number of anti-depressant drugs that may affect the PLR; selective serotonin reuptake inhibitors (SSRI), serotonin and noradrenaline reuptake inhibitors (SNRI), tricyclic antidepressants, monoamine oxidase inhibitors, reversible inhibitors of monoamine oxidase A, tetracyclic antidepressants, and noradrenergic and specific serotonergic antidepressants (NaSSA) (92). In 2004, Bär et al. investigated the use of SSRI and NaSSRI medication in depressed individuals and the effects
on the PLR. There were three phases incorporated, not medicated, medicated and recovered (93). During the first phase, there was no statistical difference in pupil diameter and latency, however relative amplitude ($p < 0.05$) was lower for the depressive group compared to the control (93). During the medicated phase, there were a significant difference observed in latency ($p < 0.05$) and relative amplitude ($p < 0.01$) between the two groups (93). It was suggested that a change in the parasympathetic system function was detected following treatment (93). Another study investigated the effect of venlafaxine (a SNRI medication), desipramine (a tricyclic antidepressant) and paroxetine (a SSRI) on the PLR. Compared to the control, there were significant increases in resting pupil diameter following venlafaxine administration ($p < 0.001$) (94). There was an increase in constriction latency ($p < 0.001$) and a decrease in amplitude ($p < 0.001$) with venlafaxine administration (94).

It is common for athletes to have long-term and persistent pain and of the medication used to manage pain, opioid medications, such as tramadol, codeine, morphine and oxycodone (95, 96) may have an effect on the PLR via the PNS (97). Opioid agonists, such as morphine and codeine, have a miotic effect on the human pupil (97, 98). A study investigated pupil diameter following the administration of three opioid medications, codeine, tramadol and morphine (97). In comparison to a control group, which received a 0.9% sodium chloride bolus, individuals in each of the three opioid medications groups had detectable decreases in pupil diameter (97). Additionally, there was a decrease observed in pupil diameter for 6 hours post-morphine administration ($p < 0.001$) (97). The tramadol group had a gradual reduction in pupil diameter, and the diameter remained significantly smaller than the control group at 150 minutes post-administration ($p < 0.01$) (97). Individuals in the codeine group had significantly smaller pupil diameters up to 210 minutes post-administration compared to the control group ($p < 0.001$) (97).
Athletes may require the administration of anaesthetic agents throughout a season, an example would be following injury. There is evidence that anaesthetic agents have an effect on the PLR. A study investigated commonly used anaesthetic agents, ketamine and nitrous oxide (N-methyl-D-aspartic acid antagonist) and the effect of its administration on the PLR. At 10 minutes following administration of ketamine, there was a significant depression (p < 0.05) of constriction latency, constriction amplitude and the 75% recovery time (99). Following the administration of nitrous oxide there was a depression in the constriction latency, constriction amplitude and 75% recovery time, however this resolved after 10 minutes following the cessation of administration (99). Although the recovery of the PLR was relatively fast, this shows the possible effect of anaesthetic agents on the PLR.

Finally, drugs that may have implications on results of this study is the methylxanthine group. Caffeine is a methylxanthine derivative alkaloid, commonly consumed in various drinks and health products (100, 101). Caffeine promotes sympathetic noradrenaline and inhibition of adrenaline A1 receptors, which results in an increase in resting pupil diameter (100, 102). Abokyi et al. (2017) investigated pupil dilation and accommodation in healthy individuals. The authors reported a significant pupil size change following caffeine consumption from 3.4 mm to 4.5 mm at 90 minutes post-consumption compared to a vehicle group (p < 0.001) (103). Another group observed an increase in pupil diameter following caffeine consumption, however this trend was not found to be statistically significant (100). However, the authors reported this finding to be justified by the low amount of caffeine administered (100). Athletes regularly consume products which contain caffeine, at varying concentrations. This must be considered when interpreting the results of this study.
Diurnal Cycle

Another potential factor effecting the PLR is an individual’s circadian rhythm (104). In 2000, Kraemer et al. used pupillometry to assess the effect of time of day on 15 adults as an indicator of attention (105). The authors found there were fluctuating significant differences in the pupil diameter throughout different periods of the day (p < 0.001) (105). The greatest pupil resting diameter was observed immediately after waking and at 9pm, with the lowest mean pupil diameter at 7pm (105). Another study found that maximum pupil diameter was significantly smaller in the afternoon compared to the morning (p = 0.022) (106). Another study investigated whether the PLR varies in relation to the time of day, and in turn affected by the circadian rhythm (107). Specifically, the authors investigated the intrinsic melanopsin-mediated pupil response (the ipRGCs) and 24-hour pupil response changes. With the use of blue light stimulus (463 ± 24 nm), the authors found a 24-hour modulation pattern of the post-stimulus pupil response to one second of stimulus. The response was greatest at times closest to habitual waking time, which was recorded after peak salivary melatonin concentration (107). It was suggested that the changes detected were due to circadian regulation of melanopsin-driven (ipRGCs) pupil response (107). These studies indicate that time-of-day may influence the PLR, and therefore, it is important to ensure the timing of scheduled data collection is standardised.

Retinal Illumination

The PLR response is affected by environmental lighting conditions that change retinal illumination (64). Resting pupil diameter is modulated by the environmental illumination (18, 64, 66) likely due to the differential effect on photoreceptor responsivity (18, 64). Under
scotopic conditions, or low light environments, the pupil response is mediated by the rod photoreceptors and the pupil is dilated for maximal retinal illumination (64, 66). Conversely, under photopic conditions, daylight, pupil diameter is decreased with the increased environmental illumination (64, 66). The pupil is reported to be more sensitive to light under scotopic or mesopic conditions. One study investigated pupil diameter under photopic and mesopic conditions in a population post-cataract surgery. The findings indicated that under mesopic conditions, pupil diameter was significantly greater than pupil diameter assessed under photopic conditions (p < 0.001) (108). Differences in pupil diameter were also reported by Zele & Cao (2014) (109) in relation to three different illumination conditions, scotopic, mesopic and photopic (109). The lowest illuminance, the scotopic condition, had the largest pupil diameter, 7.76 – 7.99 mm (109). In dim lighting, the mesopic condition, pupil diameter decreased to 5.00 – 6.99 mm (109). Finally, in bright conditions, photopic, the pupil diameter was smallest, 2.23 – 3.00 mm (109). For the purpose of this study, a standardised photopic condition was utilised for consistency during data collection.

1.2.3 PLR assessment - Pupillometry

Assessment of the PLR is often performed as part of a neurological examination following a brain injury and is traditionally performed using a penlight (60, 65). The two pathways of the ANS, the PNS and SNS, control pupillary function and pupillometry may be utilised to ascertain dysfunction or disruption of these neuroanatomical pathways (110). Following a brain injury, changes in the PLR have been noted (110, 111). Limitations of the manual assessment technique using a ‘swimming flashlight’ have been documented, particularly in relation to the ability of examiners to quantify subtle discrepancies in pupil reactivity (60, 65). Accuracy is dependent on the skill or competence of the examiner and
variations in the light source (65). Since 1989, in order to reduce the subjectivity of the penlight assessment, the use of a portable infrared pupillometer has increased in practice (98). Infrared pupillometry is a non-invasive technique used to measure the PLR via a small, portable device called a pupillometer (65, 98). The portable infrared pupillometer has been found to have greater accuracy, including between-examiner results and detectable change when compared with the manual penlight test (112). For example, Meeker et al. (112) found the median difference in error of manual detection was 0.27 mm greater than that of the portable pupillometer (p < 0.001). The use of infrared portable pupillometers allow for quantifiable measures of the PLR (64, 65, 98). Current pupillometers can assess values not available with subjective testing means (65). In quantifying pupil reactivity to light, pupillometers are able to detect a number of PLR parameters (Figure 5).

\[\text{Figure 5: Schematic diagram of the PLR (seven of eight parameters for this study). a) resting pupil diameter; b) constriction latency; c) maximum constriction velocity; d) average constriction velocity; e) percentage of pupil change; f) minimum pupil diameter; g) dilation velocity.}\]
The NeurOptics NPi-200™ (NeurOptics, Irvine, CA) is a frequently utilised commercial pupillometer device (65). The device measures eight parameters associated with the PLR all of which were investigated in the current study (Table 7). The parameters measure both SNS and PNS function (Figure 4). That is, the parameters that are indicative of SNS function are the resting pupil diameter and dilation velocity whereas minimum pupil diameter, average and maximum constriction velocity, latency and percentage of pupil change are functions of PNS activity (60, 65). In addition, the pupillometer device uses an algorithm to calculate a single value relating to the overall reactivity of the pupil, the Neurological Pupil Index (NPI). Finally, the NPi-200™ can be used to detect the presence of anisocoria. Anisocoria, an important clinical pupil presentation, is the term used to describe pupil diameter asymmetry, and is reported to represent disruption to the ANS along either the SNS and PNS (64).

Table 7: PLR parameters measured by the NPi-200™

<table>
<thead>
<tr>
<th>Pupil Function</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maximum diameter (mm)</td>
<td>Diameter at rest, before light stimulus</td>
</tr>
<tr>
<td>Minimum diameter (mm)</td>
<td>Diameter at peak constriction amplitude</td>
</tr>
<tr>
<td>Percentage change (%)</td>
<td>Percentage of change in pupil size</td>
</tr>
<tr>
<td>Latency of constriction (mm/s)</td>
<td>Time elapsed before constriction occurs</td>
</tr>
<tr>
<td>Constriction velocity (mm/s)</td>
<td>Average constriction velocity of pupil</td>
</tr>
<tr>
<td>Maximum constriction velocity (mm/s)</td>
<td>Maximum constriction velocity of pupil</td>
</tr>
<tr>
<td>Dilation velocity (mm/s)</td>
<td>Pupil dilation velocity during recovery</td>
</tr>
<tr>
<td>Neurological pupil index (NPi)</td>
<td>Calculated value measuring the strength of pupil response</td>
</tr>
</tbody>
</table>

The NPi is a score provided by the NeurOptics NPi-200™ pupillometer and is based on an algorithm that takes into account pupil size, latency constriction velocity and dilation velocity (113). The calculation for the NPi has been published by Chen et al., (110) and Kim et al.,
(113). In brief, as described by Kim et al., (113) “Each variable from an individual pupil measurement taken by the pupillometer is compared against the mean of a reference distribution of healthy human subjects for the same variable, and the difference is taken and standardized with by the corresponding standard deviation. Finally, the set of all the standardized differences (or z-scores) is combined such that is falls into a scale set between 0 and 5” (p 477). The NPi has been developed to quantify pupil reactivity and remove subjectivity (65, 110). A limitation with portable infrared pupillometry is that the pupils are assessed individually rather than binocularly. Monocular measurements do not allow for the adequate detection of anisocoria (pupil size asymmetry), nor does it allow for the consensual reflex to be assessed (60, 65).

Anisocoria has been associated with a decline or deterioration in neurological function (114). Pupil inequality is believed to be a result of damage to the musculature which controls dilation and constriction or the disruption of innervation (64). Anisocoria is not expected where damage has occurred to the optic nerve or retina due to the crossing of neuronal paths at the optic chiasm (64). Although there are difficulties noted with monocular handheld pupillometer assessment, Taylor et al. (2003) utilised a handheld monocular pupillometer and reported normative data for healthy and brain-injured patients (Table 8) and found that pupillary asymmetry of up to 0.5 mm was common in healthy adults (111). Additional research has suggested normal physiological anisocoria to fall between 0.3 – 1 mm (72). Troung & Ciuffreda (2017) assessed and compared anisocoria of normal and mTBI populations. There was no difference reported, with an average of 0.26 mm asymmetry reported in both populations (72). For the purpose of this study, a difference of 0.26 mm will be used to define anisocoria.
Table 8: Pupillary measurements in healthy adults and patients with a head-injury (111)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Value (M ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy adults (n = 310; 2432 paired measures)</td>
<td></td>
</tr>
<tr>
<td>Resting pupil diameter (mm)</td>
<td>4.1 ± 0.34</td>
</tr>
<tr>
<td>Minimum pupil diameter (mm)</td>
<td>2.7 ± 0.21</td>
</tr>
<tr>
<td>Percentage of pupil diameter change (%)</td>
<td>34</td>
</tr>
<tr>
<td>Constriction velocity (mm/sec)</td>
<td>1.48 ± 0.33</td>
</tr>
<tr>
<td>Latency duration (seconds)</td>
<td>0.24 ± 0.4</td>
</tr>
<tr>
<td>Head-injured patients (n = 26; 168 paired measures)</td>
<td></td>
</tr>
<tr>
<td>Resting pupil diameter (mm)</td>
<td>2.10 ± 0.16</td>
</tr>
<tr>
<td>Minimum pupil diameter (mm)</td>
<td>1.7 ± 0.1</td>
</tr>
<tr>
<td>Percentage of pupil diameter change (%)</td>
<td>19</td>
</tr>
<tr>
<td>Constriction velocity (mm/sec)</td>
<td>1.18 ± 0.18</td>
</tr>
<tr>
<td>Latency duration (seconds)</td>
<td>0.26 ± 0.6</td>
</tr>
</tbody>
</table>

1.2.4 Pupillometry – Concussion and mTBI

Recent pupillometry research has investigated the effect of concussion and mTBI on the PLR (18, 60). The use of pupillometry to assess the PLR in recent research has suggested the potential for use as an objective biomarker for mTBI (Table 9) (18, 60, 61). Although both monocular and binocular testing protocols have been used within the literature (60, 61), monocular testing, particularly because of the cost and portability of the device was adopted in the present study was conducted in the present study.

In 2013, Capó-Aponte and colleagues investigated the use of PLR as an early biomarker for blast-induced mTBI (60). The study investigated forty military personnel who were divided into two groups – a blast-induced mTBI group and a non-TBI group (60). All
participants were age-matched, and the blast-induced mTBI group were recruited during the subacute phase (15 – 45 days) post-incident (60). To be classified as a subacute blast-induced mTBI, participants were required to be receiving treatment from an army medical centre, and meet a set criteria based on the American Congress of Rehabilitation Medicine, which included loss of consciousness of no more than 30 minutes, no more than 24 hours of post-traumatic amnesia, 13 – 15 on the Glasgow coma scale and alteration of mental stage (60). All participants underwent extensive medical history and eye examination to determine ocular health (60). Utilising a hand-held PLR-200™ (NeurOptics) pupillometer, no significant within-group differences for the left or right eye for any parameters were found and thus the data were combined for further analysis (60). Results showed that there were significant differences in four parameters detected significant differences between the blast-induced mTBI and non-mTBI groups (60). Specifically, the blast-induced mTBI group had significantly slower reaction time (constriction latency) \(( p < 0.001)\), average constriction velocity \(( p = 0.003)\), recovery to 75% \(( p < 0.001)\) and dilation velocity \(( p = 0.001)\) compared with the non-mTBI group (60).

*Table 9: PLR parameters using monocular testing protocols in participants with mTBI and concussion (18, 60).*

<table>
<thead>
<tr>
<th>Authors</th>
<th>PLR Parameter</th>
<th>mTBI</th>
<th>Non-Injured</th>
<th>( p )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Capó-Aponte et al. (2013)</td>
<td>Maximum diameter</td>
<td>5.50 (0.73)</td>
<td>5.63 (0.79)</td>
<td>0.29</td>
</tr>
<tr>
<td></td>
<td>Minimum diameter</td>
<td>3.62 (0.45)</td>
<td>3.78 (0.56)</td>
<td>0.14</td>
</tr>
<tr>
<td></td>
<td>Percentage change</td>
<td>34.16 (2.16)</td>
<td>32.90 (3.09)</td>
<td>0.10</td>
</tr>
<tr>
<td></td>
<td>Constriction latency</td>
<td>239.10 (24.58)</td>
<td>211.75 (9.51)</td>
<td>&lt; 0.001***</td>
</tr>
<tr>
<td></td>
<td>75% recovery time</td>
<td>4.47 (0.48)</td>
<td>1.77 (0.38)</td>
<td>&lt; 0.001***</td>
</tr>
<tr>
<td></td>
<td>Average constriction velocity</td>
<td>-3.58 (0.61)</td>
<td>-4.11 (0.44)</td>
<td>0.003**</td>
</tr>
<tr>
<td></td>
<td>Maximum constriction velocity</td>
<td>Dilation velocity</td>
<td>Thiagarajan et al. (2015)</td>
<td></td>
</tr>
<tr>
<td>------------------------------</td>
<td>-------------------------------</td>
<td>------------------</td>
<td>---------------------------</td>
<td></td>
</tr>
<tr>
<td>Maximum constriction velocity</td>
<td>-4.91 (0.62)</td>
<td>-5.15 (0.99)</td>
<td>4.8 (0.2)</td>
<td></td>
</tr>
<tr>
<td>Dilation velocity</td>
<td>0.80 (0.27)</td>
<td>1.02 (0.17)</td>
<td>219 (0.004)</td>
<td></td>
</tr>
<tr>
<td>Maximum constriction velocity</td>
<td>5.8 (0.1)</td>
<td></td>
<td>5.8 (0.1)</td>
<td></td>
</tr>
<tr>
<td>Average constriction velocity</td>
<td>3.6 (0.1)</td>
<td>4.4 (0.08)</td>
<td>3.6 (0.2)</td>
<td></td>
</tr>
<tr>
<td>Average dilation velocity</td>
<td>0.9 (0.03)</td>
<td>1.1 (0.03)</td>
<td>0.9 (0.03)</td>
<td></td>
</tr>
<tr>
<td>T75 dilation recovery</td>
<td>2.2 (0.2)</td>
<td>2.4 (0.1)</td>
<td>2.2 (0.2)</td>
<td></td>
</tr>
<tr>
<td>Maximum diameter</td>
<td>5.2 (0.2)</td>
<td>5.8 (0.2)</td>
<td>5.2 (0.2)</td>
<td></td>
</tr>
<tr>
<td>Minimum diameter</td>
<td>3.6 (0.2)</td>
<td>3.8 (0.1)</td>
<td>3.6 (0.2)</td>
<td></td>
</tr>
<tr>
<td>Amplitude of constriction</td>
<td>1.6 (0.08)</td>
<td>2.0 (0.006)</td>
<td>1.6 (0.08)</td>
<td></td>
</tr>
</tbody>
</table>

* significant at $p <0.05$; ** significant at $p <0.01$; *** significant at $p <0.001$

An extension of the work by Capó-Aponte et al. (2013) (60), Thiagarajan & Ciuffreda (2015) (18) investigated non-blast-induced mTBI in civilian adults in the chronic phase (> 45 days) post-injury with the same monocular hand-held pupillometer (60, 61, 115, 116).

Similar to Capó-Aponte et al. (2013), no significant difference was detected between left and right eyes of each group (18). The authors reported the non-blast-induced mTBI group had significantly slower dilation velocity ($p < 0.001$), average and maximum constriction velocity ($p < 0.001$) compared to the non-mTBI group (18). Additionally, there were smaller pupil diameter ($p = 0.04$) and percentage of change ($p < 0.001$) reported in the non-blast-induced mTBI group compared with the non-mTBI group (18). Although there were differences in the designs of the two studies, both found significant differences in the PLR when comparing affected and non-affected groups.

As discussed, pupil constriction and dilation is controlled by the PNS and SNS, respectively (60). The results from the monocular pupillometry based research indicates there
may be deficiencies in the PNS and SNS pathways following mTBI (18, 61). These deficits could be due to the DAI which involves the brainstem and anatomical structures relating to the control of the pupillary system (18). The increase in constriction latency indicates abnormal PNS function found in the sub-acute phase, however not detected in the chronic phase. Changes in the constriction velocities indicate possible PNS dysfunction (61). Dilation velocity reductions are considered to be related to abnormal SNS control (18, 60, 61). The reduction in resting pupil diameter is believed to be related to an efferent-based SNS abnormality (Figure 6) (18, 61). The midbrain supranuclear inhibition of the EWN controls pupil diameter (61). The decrease in resting pupil diameter suggests a decrease in EWN suppression resulting in stimulation of the sphincter pupillae (61). Decease in dilation velocity within mTBI groups may suggest not only is the iris dilator still innervated, but there is less stimulation to the iris dilator (61).

Figure 6: SNS activation pattern following mTBI (60).

There is limited research investigating mTBI or concussion using the NPi algorithm developed by NeurOptics®. The NPi algorithm compares the PLR parameters taken from a pupillometry assessment and compares these parameters against the mean of a reference distribution of healthy individuals for each parameter (110). The NPi is a calculated score between 0 and 5.0, with values above 3.0 considered normal (65, 114). A NPi score that is ≥ 3 is said to fall in the normative boundaries of pupil behaviour, and the pupillary reaction is
described as “brisk”, with 5 being more “brisk” than 3 (110). If a score is <3, the pupillary response is described as abnormal, or “sluggish” (110). Chen et al. (2014) reported the assessment of the NPi in a number of intensive care units (114). The NPi was calculated in five individuals who had sustained a severe brain injury with a unilateral pupillary dilation (114). All participants were assessed by neurosurgeons and underwent CT and MRI studies to determine diagnosis and aetiology of pupil abnormalities (114). While the presentation of a unilateral non-reactive pupil was found to not be indicative of increases in intracranial pressure or drug response, the NPi was a satisfactory means of tracking and predicting increasing intracranial pressure (110, 114). The findings were an expansion on previous work by Chen et al. in 2011 (60). The authors investigated 134 patients from eight different intensive care units who had sustained a severe brain injury and were monitored continuously for 72 hours (110). It was determined that NPi < 3 was suggestive of increasing intracranial pressure trends, significantly higher than those with 3-5 NPi scores (110). Currently, there is a lack of scientific evidence associating the NPi and PLR parameters in SRC. However, there is potential for the sensitivity of pupillometry to aid in diagnosing SRC.

In summary, the current SRC protocol utilised by the NRL has limited scientific evidence for accurate diagnostic. The use of an objective quantifiable tool, such as pupillometry may aid in the diagnosis and for monitoring recovery following SRC. Current research suggests that there is a change in the PLR following a mTBI injury, and potentially SRC. That is, while the previous literature investigated the PLR in the sub-acute and chronic phases of patients who had sustained a mTBI, there is no known literature that has investigated the PLR following an acute SRC. For the purpose of the present study, PLR parameters following a SRC will be assessed during the acute phase which has been defined
as between 0 to 10 days following the concussive event. The acute time frame was chosen due to the expectation that athletes will commonly RTP following this period (3).

1.3 Research Hypothesis

1.3.1 Hypothesis

Null hypothesis: There will be no difference in the parameters of the PLR, including anisocoria, in the acute (0 – 10 days) phase following a SRC.

Alternative hypothesis: A significant change will be detected in parameters of the PLR in the acute (0 – 10 days) phase following a SRC.

1.4 Aim

The aim of this study was to determine whether there was a significant change, either positive or negative, in PLR parameters including anisocoria following SRC in national level rugby league athletes.

1.4.1 Objectives

In order to meet the aim of this study, the following objectives were undertaken:
• To compare the PLR parameters and anisocoria measures between no-SRC and athletes diagnosed with SRC;

• To compare the variability of the PLR parameters within individual athletes following SRC;

• To determine any changes in PLR parameters between pre-season and during season measures and compare between athletes with and without acute SRC. The objective PLR parameters included in the study are maximum and minimum pupil diameter, percentage of change of diameter, latency of constriction, constriction velocity and maximum constriction velocity, dilation velocity and the NPi; and

• Investigate time-frame variations in measures of the PLR parameters between 0 – 3 days, 4 – 6 days and 7 – 10 days (or prior to RTP, whichever occurs first) following a SRC in national level rugby league athletes. Determine if baseline recovery has been achieved in these time-frames for a RTP.
2. Methods

2.1 Study Setting

The study was conducted at local NRL club facilities located on the Gold Coast, Queensland, Australia. Data was collected during standardised times, during general physiotherapy scheduled screening. Ethical approval was obtained by the Griffith University Human Research Ethics Committee (GU Ref No: 2016/818).

2.1.1 Participant recruitment

A total of 64 contracted athletes of a local NRL club originally volunteered to participate in the present study, which included athletes from the Under 20s National Youth Competition (NYC) and NRL squads. There were a number of athletes drop out of the study (n = 9) within the first 12 weeks of commencement of the study, the sole reason being their departure from the club, leaving the study with 55 volunteering participants (M = 23 ± 4.5 years; range 18 to 35 years). Athletes were provided with an information sheet and given time for questions to be answered by the investigators. Prior to data collection, athletes provided written informed consent indicating willingness to participate in the study. The study was approved by the club’s medical staff and administration team.

2.1.2 Inclusion/Exclusion Criteria

For inclusion, athletes were required to be over 18 years of age and be registered to the NRL club via a NRL or NYC contract. For an athlete to be included in the study
following a SRC, they must have been diagnosed by the club medical practitioner under the current NRL protocol, the HIA. Athletes not on an NRL or NYC registered contract were excluded from participation. Athletes with optical pathologies were not eligible to be included in the study. Pupillometry testing was delayed if there were any orbit structural damage, open lesions surrounding the eye or soft tissue oedema until these lesions had healed and been cleared by the medical practitioner.

2.2 Study Design and Testing Protocol

The study was an observational study conducted over nine months period which included the pre-season and NRL competition duration (Figure 7).
2.2.1 Baseline Testing

Demographic data and previous SRC history was collected with the support from the club’s medical staff. Previous SRC history was self-reported by the athletes and corroborated where possible with the team’s medical staff. Baseline pupillometry data was collected during the 2017 NRL and NYC pre-season, data was collected during scheduled screening sessions. Volunteer participants underwent three pupillometry testing sessions at one-week intervals, during the unchanged time of the day. During each testing session, three
pupillometry tests were completed with a 60 second interval between tests to account for pupil recovery following light stimulus. This method was based on previous research using pupillometry measurements (18). The mean ± standard deviation for the triplet measures of each PLR parameter were recorded and used in subsequent analyses.

2.2.2 Within-Season Testing

Following a SRC diagnosis:

Data collection for participants with a diagnosis of SRC occurred during the 2017 NRL and NYC seasons (n = 16). To be considered as having sustained a SRC, diagnosis must have been made by the club’s medical practitioner via the HIA. Each participant who was diagnosed with SRC was required to attend three pupillometry testing sessions between 0 – 3 days, 4 – 6 days and 7 – 10 days and/or immediately prior to RTP. All three sessions were completed during the morning during rehabilitation grouped training the week following a SRC diagnosis. Three trials were collected at each test session with a 60 second interval incorporated as per the pre-season testing protocol. The mean ± standard deviation for the triplet measures of each PLR parameter were recorded.

Participants who did not sustain a SRC during season:

Following the 2017 pre-season baseline data collection, a group of participants (n = 9) were randomly selected to attend three pupillometry testing sessions during the 2017 season. The within-season testing sessions corresponded with identical time of day as per the pre-season baseline sessions and completed during the middle of the season. None of these
participants sustained a SRC during the season and were therefore classified as the no-SRC group. These participants were monitored to determine whether any changes in the PLR during a season of rugby league occurred. As for the baseline and SRC groups, participants underwent three pupillometry trials, with a 60 second interval incorporated and the mean ± standard deviation for the triplet measures of each PLR parameter were recorded.

2.3 Pupillometry

The present study utilised the NPi-200™ (NeurOptics®, Irvine, CA), a handheld monocular infrared pupillometer, to obtain quantifiable measures of the PLR (Table 7). The pupillometer emits a LED light stimulus for 800 milliseconds, at a sampling rate of 30 frames per second, over a 3 second period.

A standardised screening technique was used throughout the study. Each participant was seated with hands on knees, with a forward-facing head position. A headrest (SmartGaurd, NeurOptics®, CA) was attached to the end of the NPi-200™. The headrest was gently placed on the participant’s zygomatic bone, at a 90-degree angle to the face (Figure. 8). This ensured the distance and intensity was standardised for data collection (60).

Figure 8: Positioning of NPi-200™
Participants were encouraged to maintain a forward head posture during testing. Binocular viewing conditions were used, and participants were prompted to maintain this throughout. The non-test eye was fixated on a neutral wall opposite the participant. The right eye was recorded, followed by the left eye. This was completed three times, at 60 second intervals to allow recovery of the pupil following increased illumination (Figure 9). The NPi-200™ advised for a retest if the device was held incorrectly or blinking was detected.

![Figure 9: Single session schematic diagram. 60 second intervals between each trail was incorporated in accordance to previous pupillometry research (18).](image)

### 2.4 Data Analysis

All data were obtained using the NPi-200™. Following the triplet pupillometry trials, the data were then extracted manually and the mean of each session was recorded into an excel spreadsheet. For each participant, the data were separated into baseline (pre-season) and during season records. All data were deidentified and stored on a secure device. Descriptive statistics (Mean ± SD) were calculated and recorded. To assess normality, Shapiro-Wilks test was utilized, and to assess the assumption of homogeneity of variance,
Levene’s test was used. Independent t-tests were used to determine significant difference between SRC and no-SRC groups for PLR parameters which meet parametric assumptions. For non-parametric data, Mann-Whitney U tests were utilised to determine statistical difference between SRC and no-SRC groups. To determine change, comparison between no-SRC and SRC athletes were assessed by calculating absolute change scores between baseline and during season for participants within the SRC group and no-SRC group. Absolute change was also calculated between baseline and 0 – 3 days, 4 – 6 days and 7 – 10 days post-SRC to determine change in PLR prior to RTP. Variability between the groups were determined by analysing the descriptive statistics and the coefficient of variation for each PLR parameter. All data were transferred from excel, into GraphPad Prism 7.0c (GraphPad Software) where all statistical analyses were conducted. Statistical significance was set at $p < 0.05$ with confidence intervals of 95%.
3. Results

3.1 Athlete Characteristics

Sixty-four male athletes from two national level rugby league teams were originally volunteered for the present study. During the baseline data collection period, nine volunteers departed the club so subsequent analyses were performed on a total of 55 participants (age = 22 ± 4 years, height = 1.85 ± 0.1 m, weight = 96 ± 9 kg) volunteered for the study. The years of rugby league experience among athletes ranged from 9 – 28 years (M = 15 ± 5 years). Forty-one of these athletes (82%) self-reported a previous history of SRC. During the baseline data collection period, nine volunteers departed the club so subsequent analyses were performed on a total of 55 participants. No athlete reported optical pathology.

Comparison between the PLR parameters of the participants of this study were made against reported normative data for healthy adult persons (111). A significant difference between all commonly assessed parameters were observed. There was a statistically smaller resting pupil diameter in participants within the current study compared to reported normative values (t(343) = -2.379, p = 0.02). A statistically significant larger minimum pupil diameter among participants within this study compared to previously reported normative values (t(343) = 4.220, p < 0.0001) was observed. There was statistically significant slower dilation velocity in participants in the current study compared to the normative value group (t(343) = -8.726, p < 0.001). A statistically larger average constriction velocity was found in participants within the current study compared with reported normative values (t(343) = 11.67, p < 0.0001). There was no statistical difference between the two studies baseline groups for constriction latency (t(343) = -0.295, p = 0.77).
3.2 Sport-Related Concussion and No Sport-Related Concussion Groups

3.2.1 Number of Sport-Related Concussions for 2017

During the 2017 rugby league season, of the 55 participants, 16 were diagnosed with a SRC. All 16 participants presented with a GCS of 13 – 15 and no loss of consciousness was reported. Of the 16 participants diagnosed with SRC via the HIA, 14 were identified via the SAC score and two participants via the video review system.

3.2.2 Comparisons Between Sport-Related Concussion and No Sport-Related Concussion Groups

Nine participants who did not sustain a SRC formed the no-SRC group for PLR comparison with the SRC group. First, the characteristics of each group, SRC and no-SRC, from the 2017 season are reported in Table 10. All assumptions of normality and variance were met for this analysis. There was no statistical difference between the groups age, height, weight or previous history of SRC ($p > 0.05$). Additionally, there was no anisocoria detected in either the SRC (0.26 mm) or the no-SRC (0.24 mm). There was no statistical difference in pupil asymmetry between the two groups ($p > 0.05$). At baseline, there were no significant differences between groups for any of the PLR parameters ($p > 0.05$).
Table 10: Characteristics between no-SRC and SRC athletes.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>No-SRC (n = 9)</th>
<th>SRC (n = 16)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>21 ± 2</td>
<td>22 ± 3</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>92.2 ± 5.87</td>
<td>91.8 ± 10</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.81 ± 0.05</td>
<td>1.83 ± 0.05</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>Previous SRC (%)</td>
<td>88%</td>
<td>100%</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>Anisocoria (mm)</td>
<td>0.24</td>
<td>0.26</td>
<td>&gt; 0.05</td>
</tr>
</tbody>
</table>

3.3 Pupil Light Reflex Difference Between No-Sport-Related Concussion and
Sport-Related Concussion

3.3.1 Between Group Differences in the Pupil Light Reflex

The PLR parameters were compared between the SRC and no-SRC groups for the 2017 season. Results for parametric tests are displayed in Table 11. Given that minimum pupil diameter did not meet the assumption of homogeneity of variance, the Welch-Satterthwaite method was utilised for the independent t-test (Table 11).
Table 11: Independent t-test of PLR parameters between no-SRC and SRC groups.

<table>
<thead>
<tr>
<th>PLR Parameter</th>
<th>No-SRC (n = 9)</th>
<th>SRC (n = 16)</th>
<th>t-score</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean (SD)</td>
<td>Mean (SD)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Minimum pupil diameter (mm)</td>
<td>3.13 (0.23)</td>
<td>2.91 (0.62)</td>
<td>1.28</td>
<td>0.21</td>
</tr>
<tr>
<td>Percentage of pupil change (%)</td>
<td>28.30 (5.30)</td>
<td>26.64 (4.55)</td>
<td>0.83</td>
<td>0.42</td>
</tr>
<tr>
<td>Constriction velocity (mm/s)</td>
<td>2.74 (0.64)</td>
<td>2.42 (0.54)</td>
<td>1.31</td>
<td>0.20</td>
</tr>
<tr>
<td>Maximum constriction velocity (mm/s)</td>
<td>3.96 (0.89)</td>
<td>3.51 (0.60)</td>
<td>1.51</td>
<td>0.15</td>
</tr>
<tr>
<td>Constriction latency (s)</td>
<td>0.22 (0.02)</td>
<td>0.22 (0.01)</td>
<td>0.43</td>
<td>0.67</td>
</tr>
<tr>
<td>Dilation velocity (mm/s)</td>
<td>1.19 (0.20)</td>
<td>1.07 (0.22)</td>
<td>1.30</td>
<td>0.20</td>
</tr>
</tbody>
</table>

The assumption of normality was violated in the NPi and resting pupil diameter parameters. Mann-Whitney U tests were used to compare SRC and no-SRC groups for these parameters (Table 12). Of the eight parameters, only NPi was found to be statistically different between the SRC (Mdn = 2.95) and no-SRC (Mdn = 3.75) groups (U = 11, p = 0.0002) (Figure 10).

Table 12: Mann-Whitney U test of PLR parameters between no-SRC and SRC groups within-season.

<table>
<thead>
<tr>
<th>PLR Parameter</th>
<th>No-SRC (n = 9)</th>
<th>SRC (n = 16)</th>
<th>U-value</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Median (min, max)</td>
<td>Median (min, max)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NPi</td>
<td>3.75 (3.23, 4.38)</td>
<td>2.95 (2.22, 4.62)</td>
<td>11</td>
<td>0.0002***</td>
</tr>
<tr>
<td>Resting pupil diameter (mm)</td>
<td>4.51 (3.98, 4.76)</td>
<td>4.04 (2.82, 5.87)</td>
<td>46</td>
<td>0.15</td>
</tr>
</tbody>
</table>

***significant at p < 0.001
Figure 10: During season, individual mean NPi score post-SRC; blue = no-SRC; red = SRC. The black line indicates the separation between normal and abnormal pupil light reflex response.

3.3.2 Variability and Spread of Results Between Groups

The variability for parametric parameters were calculated by a coefficient of variation. The spread has been recorded for comparison of non-parametric parameters. The spread for NPi was slightly greater in the no-SRC group (IQR = 3.46 – 4.21) compared to the SRC group (IQR = 2.65 – 3.10) (Figure 9). For resting pupil diameter, there was an increased spread found in the SRC (IQR = 3.32 – 4.50) compared to no-SRC (IQR = 4.00 – 4.65) group. For minimum pupil diameter, there was a greater variability found between athletes with SRC (cv = 21%) compared to no-SRC (cv = 7%). For percentage of pupil change, there was minimal difference between the variability within SRC (cv = 17%) and no-SRC (cv = 18%) groups. For constriction velocity, there was high variability in both SRC (cv = 22%) and no-SRC (24%). For maximum constriction velocity, there was equal variability between the SRC (cv = 22%) and no-SRC (cv = 22%) groups. Constriction velocity had the least variability for SRC (cv = 5%) and no-SRC (cv = 8%) compared to the other eight parameters. Finally, there was greater variability for dilation velocity in the SRC group (cv = 21%) compared to the no-SRC group (cv = 17%).
3.3.3 Bidirectional Change of the Sport-Related Concussion Group

Given the range of variability within the PLR parameters of the SRC group, further investigation was conducted looking at direction of change (Figure 11). For NPi, all but one athlete had a decrease in NPi score following a SRC. For the remaining seven PLR parameters, there were observable differences between individuals within the SRC group. Given the bidirectional change following SRC, absolute change scores were calculated to determine if there were differences between SRC and no-SRC groups.
Figure 11: Inter-athlete comparison of pre-season (black) and SRC (red) PLR parameter values. (a) NPi; (b) resting pupil diameter; (c) minimum pupil diameter; (d) percentage of pupil change; (e) constriction velocity; (f) maximum constriction velocity; (g) constriction latency; (h) dilation velocity.
3.3.4 Difference in Absolute Change Between No-Sport-Related Concussion and Sport-Related Concussion Groups

To determine statistical difference between SRC and no-SRC groups, absolute change scores were calculated (Table 13). Violation of the homogeneity of variance was detected in resting pupil diameter and minimum pupil diameter, which was overcome by utilising the Welch-Satterthwaite method.

Table 13: Independent t-tests of absolute change scores (differences of pre-season and within season) for PLR parameters between no-SRC and SRC groups.

<table>
<thead>
<tr>
<th>PLR Parameter</th>
<th>No-SRC (n = 9)</th>
<th>SRC (n = 16)</th>
<th>t-score</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean (SD)</td>
<td>Mean (SD)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NPi</td>
<td>0.19 (0.15)</td>
<td>0.25 (0.19)</td>
<td>0.91</td>
<td>0.37</td>
</tr>
<tr>
<td>Resting pupil diameter (mm)</td>
<td>0.09 (0.07)</td>
<td>0.39 (0.30)</td>
<td>3.77</td>
<td>0.001***</td>
</tr>
<tr>
<td>Minimum pupil diameter (mm)</td>
<td>0.11 (0.04)</td>
<td>0.41 (0.17)</td>
<td>6.61</td>
<td>&lt;0.0001****</td>
</tr>
<tr>
<td>Constriction velocity (mm/s)</td>
<td>0.19 (0.11)</td>
<td>0.22 (0.16)</td>
<td>0.46</td>
<td>0.65</td>
</tr>
<tr>
<td>Maximum constriction velocity (mm/s)</td>
<td>0.21 (0.20)</td>
<td>0.27 (0.17)</td>
<td>0.75</td>
<td>0.46</td>
</tr>
</tbody>
</table>

*** significant at p < 0.001; **** significant at p < 0.0001

There was a violation of normality detected in percentage of pupil change, constriction latency and dilation velocity. Mann-Whitney U tests were conducted for statistical difference (Table 14). Results indicated that two of the eight PLR parameters were statistically different between SRC and no-SRC groups.
Table 14: Mann-Whitney U tests of absolute change scores for PLR parameters between no-SRC and SRC groups.

<table>
<thead>
<tr>
<th>PLR Parameter</th>
<th>No-SRC (n = 9)</th>
<th>SRC (n = 16)</th>
<th>U-value</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Percentage of pupil change (%)</td>
<td>Median (min, max)</td>
<td>Median (min, max)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Constriction latency (s)</td>
<td>2.50 (0.003 – 5.00)</td>
<td>1.50 (0.33, 5.83)</td>
<td>60</td>
<td>0.51</td>
</tr>
<tr>
<td>Dilation velocity (mm/s)</td>
<td>0.01 (0.00, 0.04)</td>
<td>0.006 (0.002, 0.03)</td>
<td>63.5</td>
<td>0.65</td>
</tr>
<tr>
<td></td>
<td>0.05 (0.004, 0.20)</td>
<td>0.06 (0.00, 1.94)</td>
<td>56.5</td>
<td>0.53</td>
</tr>
</tbody>
</table>

First, for resting pupil diameter, the results indicated a significantly greater absolute change for the SRC group (M = 0.39, SD = 0.30) compared to the no-SRC group (M = 0.09, SD = 0.07) \((t(17.98) = 3.77, p = 0.001).\) Secondly, there was statistically greater change in the minimal pupil diameter for the SRC group (M = 0.41, SD = 0.17) compared to the no-SRC groups (M = 0.11, SD = 0.04) \((t(17.42) = 6.61, p < 0.0001).\) The remaining six PLR parameters were not found to be statistically different between the SRC and no-SRC groups \((p > 0.05).\)

### 3.3.5 Pupil Light Reflex Throughout the Acute Phase of Sport-Related Concussion

Additional analysis was conducted to determine the progression of the PLR parameters throughout the acute recovery phase at 0 – 3 days, 4 – 6 days and 7 – 10 days post-SRC. Absolute change scores were used to measure progression within the SRC group. At each three points in time, there was no anisocoria detected \((p > 0.05).\)
At 0 – 3 days post-SRC, the assumption of normality and homogeneity of variation was met for NPi, percentage of pupil change, constriction velocity and maximum constriction velocity (Table 15).

Table 15: Independent t-tests of PLR parameters between no-SRC and 0 - 3 days post-SRC.

<table>
<thead>
<tr>
<th>PLR Parameter</th>
<th>No-SRC (n = 9)</th>
<th>SRC (n = 16)</th>
<th>t-score</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>NPi</td>
<td>0.19 (0.15)</td>
<td>0.24 (0.20)</td>
<td>0.63</td>
<td>0.53</td>
</tr>
<tr>
<td>Percentage of pupil change (%)</td>
<td>2.30 (1.50)</td>
<td>2.26 (1.38)</td>
<td>0.07</td>
<td>0.95</td>
</tr>
<tr>
<td>Constriction velocity (mm/s)</td>
<td>0.19 (0.11)</td>
<td>0.24 (0.20)</td>
<td>0.58</td>
<td>0.57</td>
</tr>
<tr>
<td>Maximum constriction velocity (mm/s)</td>
<td>0.21 (0.20)</td>
<td>0.39 (0.20)</td>
<td>2.14</td>
<td>0.04*</td>
</tr>
</tbody>
</table>

* significant at p < 0.05

There were violations of normality for resting pupil diameter, minimal pupil diameter, constriction latency and dilation velocity and Mann-Whitney U tests were utilised to compare groups (Table 16). Three out of eight PLR parameters were determined to be significantly different between the two groups. There were no statistical differences for NPi, percentage of pupil change, constriction velocity, constriction latency and dilation velocity (p > 0.05).
Table 16: Mann-Whitney U tests of PLR parameters between no-SRC and 0 - 3 days post-SRC.

<table>
<thead>
<tr>
<th>PLR Parameter</th>
<th>No-SRC (n = 9)</th>
<th>SRC (n = 16)</th>
<th>U-value</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Median (min, max)</td>
<td>Median (min, max)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Resting pupil diameter (mm)</td>
<td>0.07 (0.02, 0.23)</td>
<td>0.22 (0.02, 2.67)</td>
<td>32</td>
<td>0.02*</td>
</tr>
<tr>
<td>Minimum pupil diameter (mm)</td>
<td>0.11 (0.04, 0.18)</td>
<td>0.38 (0.50, 2.12)</td>
<td>37</td>
<td>0.047*</td>
</tr>
<tr>
<td>Constriction latency (s)</td>
<td>0.01 (0, 0.04)</td>
<td>0.01 (0, 0.02)</td>
<td>61.5</td>
<td>0.55</td>
</tr>
<tr>
<td>Dilation velocity (mm/s)</td>
<td>0.05 (0.004, 0.20)</td>
<td>0.09 (0, 2.06)</td>
<td>38.5</td>
<td>0.13</td>
</tr>
</tbody>
</table>

* significant at p < 0.05

Firstly, the change in the maximum constriction velocity was statistically greater at 0 – 3 days post-SRC (M = 0.39, SD = 0.20) compared to no-SRC (M = 0.21, SD = 0.20) (t(23) = 2.14, p = 0.04). The change in the resting pupil diameter was statistically greater in the SRC group at 0 – 3 days (Mdn = 0.22) compared to no-SRC (Mdn = 0.07) (U = 32, p = 0.02). Finally, there was a statistically greater change in the minimum pupil size at 0 – 3 days post-SRC (Mdn = 0.38) compared to no-SRC (Mdn = 0.11) (U = 37, p = 0.047).

4 – 6 Days Post-Sport-Related Concussion

At 4 – 6 days post-SRC, although the assumption of normality was met for resting pupil diameter, minimal pupil diameter and constriction velocity there was violation of homogeneity of variance. Thus, analysis was conducted using the Welch-Satterthwaite method (Table 17).
Table 17: Independent t-tests of PLR parameters for comparison between no-SRC and 4 - 6 days post-SRC.

<table>
<thead>
<tr>
<th>PLR Parameter</th>
<th>No-SRC (n = 9)</th>
<th>SRC (n = 16)</th>
<th>t-score</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean (SD)</td>
<td>Mean (SD)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Resting pupil diameter (mm)</td>
<td>0.09 (0.07)</td>
<td>0.43 (0.30)</td>
<td>4.42</td>
<td>0.0003***</td>
</tr>
<tr>
<td>Minimal pupil diameter (mm)</td>
<td>0.11 (0.04)</td>
<td>0.39 (0.21)</td>
<td>5.13</td>
<td>&lt;0.0001****</td>
</tr>
<tr>
<td>Constriction velocity (mm/s)</td>
<td>0.19 (0.11)</td>
<td>0.30 (0.26)</td>
<td>1.46</td>
<td>0.16</td>
</tr>
</tbody>
</table>

*** significant at p < 0.001; **** significant at p < 0.0001

There was a violation of normality for NPi, percentage of pupil change, maximum constriction velocity, constriction latency and dilation velocity therefore Mann-Whitney U tests were conducted (Table 18). There was a statistical difference between the SRC and no-SRC groups in two of the eight PLR parameters, resting pupil diameter and minimum pupil diameter. There was no statistical difference for NPi, percentage of pupil change, constriction velocity, maximum constriction velocity, constriction latency and dilation velocity (p > 0.05).

Table 18: Mann-Whitney U test of PLR parameters for comparison between no-SRC and 4 - 6 days post-SRC.

<table>
<thead>
<tr>
<th>PLR Parameter</th>
<th>No-SRC (n = 9)</th>
<th>SRC (n = 16)</th>
<th>U-value</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Median (min, max)</td>
<td>Median (min, max)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NPi</td>
<td>0.15 (0.02, 0.47)</td>
<td>0.22 (0.04, 0.82)</td>
<td>51</td>
<td>0.34</td>
</tr>
<tr>
<td>Percentage of pupil change (%)</td>
<td>2.50 (0.003, 5)</td>
<td>1.17 (0.16, 13.5)</td>
<td>52</td>
<td>0.37</td>
</tr>
<tr>
<td>Maximum constriction velocity (mm/s)</td>
<td>0.16 (0.02, 0.63)</td>
<td>0.26 (0.04, 0.96)</td>
<td>53</td>
<td>0.30</td>
</tr>
<tr>
<td>Constriction latency (s)</td>
<td>0.01 (0, 0.04)</td>
<td>0.01 (0, 0.03)</td>
<td>65</td>
<td>0.70</td>
</tr>
<tr>
<td>Dilation velocity (mm/s)</td>
<td>0.05 (0.004, 0.20)</td>
<td>0.14 (0.02, 1.82)</td>
<td>39.5</td>
<td>0.06</td>
</tr>
</tbody>
</table>
Firstly, there was a statistically significant greater change for the resting pupil diameter at 4 – 6 days post-SRC (M = 0.43, SD = 0.30) compared with no-SRC (M = 0.09, SD = 0.07) (t(18.13) = -4.42, p = 0.002). Secondly, there was a statistically significant greater change for the minimum pupil diameter at 4 – 6 days (M = 0.39, SD = 0.21) post-SRC compared to no-SRC (M = 0.11, SD = 0.04) (t(16.79) = 5.13, p < 0.0001).

**7 – 10 Days Post-Sport-Related Concussion**

At 7 – 10 days post-SRC, the assumption of normality was met for resting pupil diameter and minimal pupil diameter, however both violated the assumption of homogeneity of variance. The Welch-Satterthwaite method were utilised to compare groups for these parameters (Table 19).

<table>
<thead>
<tr>
<th>PLR Parameter</th>
<th>No-SRC (n = 9)</th>
<th>SRC (n = 16)</th>
<th>t-score</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resting pupil diameter (mm)</td>
<td>0.09 (0.07)</td>
<td>0.34 (0.27)</td>
<td>3.45</td>
<td>0.003**</td>
</tr>
<tr>
<td>Minimal pupil diameter (mm/s)</td>
<td>0.11 (0.04)</td>
<td>0.24 (0.19)</td>
<td>2.64</td>
<td>0.02*</td>
</tr>
</tbody>
</table>

* significant at p < 0.05; ** significant at p < 0.01

The assumption of normality was violated for NPi, percentage of pupil change, constriction velocity, maximum constriction velocity, constriction latency and dilation velocity. Mann-Whitney U tests were utilised to compare groups (Table 20). There was a statistical difference between the SRC and no-SRC groups in two of the eight PLR
parameters. There was no statistical difference for NPi, percentage of pupil change, constriction velocity, maximum constriction velocity, constriction latency and dilation velocity ($p > 0.05$).

Table 20: Mann-Whitney U tests of PLR parameters for comparison between no-SRC and 7 - 10 days post-SRC.

<table>
<thead>
<tr>
<th>PLR Parameter</th>
<th>No-SRC (n = 9)</th>
<th>SRC (n = 16)</th>
<th>U-value</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Median (min, max)</td>
<td>Median (min, max)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NPi</td>
<td>0.15 (0.02, 0.47)</td>
<td>0.18 (0.01, 0.77)</td>
<td>65</td>
<td>0.89</td>
</tr>
<tr>
<td>Percentage of pupil change (%)</td>
<td>2.50 (0.003, 5)</td>
<td>1.83 (0.67, 11)</td>
<td>62</td>
<td>0.98</td>
</tr>
<tr>
<td>Constriction velocity (mm/s)</td>
<td>0.15 (0.08, 0.38)</td>
<td>0.20 (0.01, 2.54)</td>
<td>65</td>
<td>0.71</td>
</tr>
<tr>
<td>Maximum constriction velocity (mm/s)</td>
<td>0.16 (0.02, 0.63)</td>
<td>0.42 (0.01, 0.84)</td>
<td>48.5</td>
<td>0.27</td>
</tr>
<tr>
<td>Constriction latency (s)</td>
<td>0.01 (0, 0.04)</td>
<td>0.01 (0, 0.03)</td>
<td>60.5</td>
<td>0.70</td>
</tr>
<tr>
<td>Dilation velocity (mm/s)</td>
<td>0.05 (0.004, 0.20)</td>
<td>0.10 (0.03, 1.26)</td>
<td>38.5</td>
<td>0.13</td>
</tr>
</tbody>
</table>

Firstly, a statistically significant change in the resting pupil diameter was observed at 7 – 10 days post-SRC ($M = 0.09$, $SD = 0.07$) compared to no-SRC ($M = 0.34$, $SD = 0.27$) ($t(17.22) = 3.45$, $p = 0.003$). Secondly, a statistically significant change in the minimal pupil diameter 7 – 10 days post-SRC ($M = 0.24$, $SD = 0.27$) compared to no-SRC ($M = 0.11$, $SD = 0.04$) minimal pupil diameter ($t(15.92) = 2.64$, $p = 0.02$) was found.

The greatest statistical difference for the resting pupil diameter and minimum pupil diameter was 4 – 6 days post-SRC. The maximum constriction velocity was found to be statistically different at 0 – 3 days post-SRC. However, no statistical difference was determined between the groups at the 4 – 6 and 7 – 10 days post-SRC. Similarly, no
statistically significant differences were found in any of the other parameters following a SRC in the first 0 – 10 days.
4. Discussion

The assessment of pupil size and dynamics via the PLR provides information about the structural integrity and potential dysfunction or damage of several different neuroanatomical pathways (110, 114). The PLR has been proposed to be a potential objective biomarker for the diagnosis and monitoring of mTBI (18, 60, 61). Previous research has demonstrated that, in participants with a mTBI, there is a change in parameters of the PLR in the subacute and chronic phase following the injury (18, 60, 61). The aim of the present study was to assess parameters of the PLR and anisocoria in national level rugby league athletes in the acute phase following a SRC and compare these measures to baseline measures. For the assessment of the PLR, a handheld automated pupillometer, the NPi-200™ was utilised. Pre-season data was collected and used as baseline measures for all participants. Two rugby league squads were recruited for the current study. Those athletes who were diagnosed by the club’s medical practitioner as having sustained a SRC during the following season were classified as the SRC group. For comparison, a group of randomly selected participants were monitored throughout the season with the view of having a no-SRC group. Fortunately, none of these randomly selected participants sustained a SRC during the season.

The current study found significant differences between the resting and minimum pupil diameter, average constriction velocity and dilation velocity between baseline (pre-season) measures for the current participant cohort, and previously investigated normative, healthy adult measures (111). The observed significant differences may be attributed to participant groups, previous and repeated SRC resulting in ongoing deficits, or undiagnosed acute SRC injuries. These findings suggest that it is important that baseline or pre-season measures of national rugby league athletes are taken rather than relying on previously published
normative data for healthy adults, particularly to allow comparison of any changes that may occur in these measures post-SRC. Within the current study, there was no difference in characteristics between the SRC and no-SRC groups. There were changes in the PLR when comparing the SRC group to the no-SRC group.

### 4.1 Sport-Related Concussion and No Sport-Related Concussion Group

**Difference**

There were no statistical significant differences found in athlete characteristics of age, height, weight and previous concussion history between the SRC and no-SRC groups. For this, comparison between the two groups were able to be completed.

#### 4.1.1 Anisocoria

Anisocoria has been associated with neurological deterioration following severe TBI (110). Studies utilising pupillometry have demonstrated that there is detectable pupil asymmetry following a mTBI (72). However, the present study found no anisocoria in either the SRC or no-SRC group suggesting that, in this group of national level rugby league athletes SRC does not result in unilateral pupil damage (72). Insult to the retina and/or optic nerve may be ruled out due to the observation of pupil symmetry (60, 64). Due to the monocular testing protocol, a consensual reflex (an observed PLR in the contralateral eye) was unable to be obtained. The consensual reflex may provide further information regarding the afferent pathway integrity (60).
4.1.2 Pupil Light Reflex

The results of the present study suggest that the NPi is significantly different between the SRC and no-SRC groups. No previous research has investigated the NPi as a potential measure to detect mTBI or SRC. Previously, the NPi has been used to provide a quantitative means of tracking intracranial pressure changes and pupillary function in individuals who have sustained a severe TBI (110, 114). A NPi of three or less, described as ‘weak’, indicates neurological impairment, with a score of greater than three described as normal, or ‘brisk’. In the current study, the mean NPi for the SRC group was below three, suggesting a detectable neurological impairment during acute SRC in national level rugby league athletes (110). These findings support the alternative hypothesis and indicates that pupillometry may be a useful quantitative test in the diagnostic assessment of SRC. Future studies should continue pupillometry assessment for greater than 10 days to determine when the NPi returns to baseline. Additionally, studies should be conducted to determine whether there is any relationship between NPi and neurological changes on imaging.

In contrast to previous investigations of the PLR in patients with mTBI, the findings of the present study suggested there were no statistically significant differences in pupil size or dynamics between those athletes who sustained a SRC and the no-SRC group. The findings of Capó-Aponte et al. (2013) (60) and Thiagarajan & Ciuffreda (2015) (18) indicated that mTBI results in decreases in the resting pupil diameter (18, 60), the average and maximum constriction velocities (18, 60, 61), amplitude of constriction (18) and the dilation velocity (18, 60, 61) in subacute and chronic cohorts. The authors suggested that these findings may be due to disruption and alterations within the ANS, with possible deficits in the SNS and/or PNS (18, 60). The fact that there were no differences in pupil size and
dynamics in the current national level rugby league cohort may be due to the phase of recovery where the measurements were taken, that is, in the acute phase in the present study compared with the subacute and chronic phases in the previous study. Alternatively, the differences may be explained by the controversy surrounding the complexity of the pathophysiological changes that occur in a mTBI versus a SRC. The contrast in results may suggest that the two injuries are different, resulting in different metabolic, electrical and neuroanatomical changes within the brain. Further studies are warranted to investigate these hypotheses.

Interestingly, the variability in the PLR measures between individual athletes in the SRC group is a novel finding. That is, the current study observed large variabilities in the PLR parameters between athletes within the SRC group. In contrast, previous research investigating a group of chronic mTBI patients found very small variabilities in PLR parameter measures (61). The increased PLR variability found between athletes with acute SRC may be explained by differences in athlete recovery time frames. Additionally, the variability may be suggestive of different mechanism of injury and responses to a SRC between athletes.

There are a number of possible factors that may contribute to the findings of the current study. First, the variability of measures for individual athletes may explain the absence of significant difference between SRC and no-SRC groups, there was a bidirectional change observed in each PLR parameter besides the NPi. The bidirectional changes observed may reflect differing deficits to the neuronal pathways of the SNS and PNS following a SRC (60). Secondly, there are a number of mechanisms which may result in a SRC, two examples are impact with the ground versus impact with an opposing player’s shoulder, and it is likely that individuals respond differently to a SRC. The differences in mechanism of injury and
differences in the individual athletes may be a factor for the observed bidirectional changes in the PLR parameters. Finally, 41 athletes reported a history of SRC – the results of the present study may be a representation of previous concussive history. Previously, research has investigated patients with subacute (15 – 45 days) (60) and chronic (>45 days) (18, 61) mTBI histories and compared to control groups with no mTBI histories. The results from these studies indicated scientific significant difference between those with and without mTBI histories. The present study had a control group, however, 88% of the group had a SRC history. For this, it is possible that the results obtained are affected by previous SRC that have not obtained physiological recovery. To account for the bidirectional shift and factors that influence the use of the PLR parameters’ value, absolute change scores were used to determine a difference in the PLR parameters following a SRC in national level rugby league athletes.

4.3 Difference in Absolute Change Between Sport-Related and No-Sport Related Concussion Groups

In the present study, resting pupil diameter and minimum pupil diameter were found to be statistically different between the SRC and no-SRC groups. There was a greater absolute change observed in the SRC group compared with the no-SRC group. The significant change difference may suggest abnormalities in the neuroanatomical and neurological processing of the PLR by the ANS following acute SRC (18, 64). The abnormalities of the ANS may represent dysfunction or deficits in the SNS and/or the PNS.

4.3.1 The Sympathetic Nervous System
Normal resting pupil diameter is a function of the SNS (64). Along with stimulating the pupil dilator musculature, the SNS provides active inhibition to the EWN at rest. That being said, a change in the resting pupil diameter following a SRC may be suggestive of a change in the SNS. Prior research has suggested that patients suffering from mTBI have abnormalities in the SNS function (18, 60, 61, 118). Resting pupil diameter differences between chronic mTBI and no-TBI groups have been reported in three previous studies (18, 61, 72). Authors in all three studies suggested that the observed decrease in the resting pupil diameter was a result of abnormal efferent-based sympathetic control.

The current study cannot speculate on the direction of observed change following a SRC. Therefore, either a reduction or increase in the SNS function and activity may be used to explain the possible abnormal observations. Firstly, a reduction in the SNS function may be present. At rest, the SNS inhibits the EWN in the midbrain. A reduction in SNS function would result in a decrease in EWN inhibition, therefore increasing the innervation to the pupil sphincter musculature. The resting pupil diameter would be expected to be reduced compared to normal. Secondly, a reduced SNS function would result in less innervation to the pupil dilator musculature. In contrast, an increase in SNS function would have opposing effects. The EWN would have greater inhibition, therefore decreasing the innervation to the pupil sphincter musculature, resulting in increased resting pupil diameter. With an increase in SNS function, there would be greater innervation to the pupil dilator musculature, again, resulting in an increase in resting pupil diameter. Future studies may benefit from the addition of neurological imaging to determine if there are correlations between the observed changes in the PLR and changes in the brain.
The hypothesised abnormalities in the SNS does not seem to have an effect on the
dynamic parameters of the PLR. Dilation velocity is a function of the SNS during dynamic
response to light (18, 60, 61). The current study did not observe a change in the dilation
velocity which was in contrast to previous research investigating patients with chronic mTBI
(18, 61). During the chronic phase of a mTBI, significant differences in the resting pupil
diameter has been accompanied by significant changes in the dilation velocity (18, 61). The
results of the current study suggest that the potential abnormality in the SNS function is
present at rest.

4.3.2 The Parasympathetic Nervous System

The observations in the current study may be a result of abnormalities in the PNS.
The minimum pupil diameter is driven by the PNS and is a result of the constriction phase of
the PLR (18). Additionally, although the resting pupil diameter is controlled by SNS
function, abnormalities in the PNS at rest may influence the resting pupil diameter. A
reduction or increase in the PNS may explain the observations within the study.
Abnormalities in the midbrain may reflect PNS control dysfunction and deficit (18). With a
reduction in the PNS, a decrease in EWN innervation would be present. This would result in
a decreased innervation to the pupil sphincter musculature and larger pupil diameters would
be observed. An increase in PNS function would result in an increased innervation to the
EWN, increasing the innervation of the pupil sphincter musculature, resulting in smaller
pupil diameters.

The PNS predominately controls the dynamic PLR parameters involved with pupil
constriction (18, 64). The results observed in the present study indicate there was no
significant difference between the PLR constriction based parameters following a SRC,
beside minimum pupil diameter. As no other constriction-based PLR parameters were found to be different, the results suggest that following a SRC, there is no deficit in the dynamic PNS function (18). The significant difference in the minimum pupil diameter may be a representation of resting PNS and/or SNS function, rather than due to the pupillary reactivity from change in light stimulus. However, in relation to the present study, a change in the PLR was detected in acute SRC in national level rugby league athletes, indicating dysfunction or disruption within the ANS, and an altered balance between the PNS and SNS (119). Future studies investigating whether pupillometry measures of the PLR reflect changes in the integrity and variations in brain electrical activity following a SRC are warranted.

4.4 Pupil Light Reflex Throughout the Acute Phase of Sport-Related Concussion

This is the first study to assess the PLR during acute SRC. As there were statistically significant differences in the PLR during acute SRC observed between the SRC and no-SRC group, further analysis was completed to determine changes in the PLR within the acute time frame. Previous research suggested the use of the PLR as a potential objective physiological biomarker for the monitoring recovery following a mTBI (60, 61). To further investigate the effect of an acute SRC on the PLR, the present study investigated three time periods; 0 – 3 days, 4 – 6 days and 7 – 10 days following an acute SRC. Significant differences were observed in resting pupil diameter, minimum pupil diameter and maximum constriction velocity.

In the SRC group, the resting pupil diameter and minimum pupil diameter parameter were found to be statistically significantly different at baseline for all three time frames monitored during recovery. During the 0 – 10 day time frame, the change in PLR was
significantly different from baseline at 0 – 3 days post-SRC, which increased continued to increase in difference at 4 – 6 days. However, at 7 – 10 days post-SRC, there was a decrease in the absolute change, trending towards the no-SRC group. The continued increase in absolute change score up to 6-days following a SRC suggests abnormal ANS function continues to manifest days after the incident. Additionally, although there was a trend towards baseline the 7 – 10 day time frame, there was still abnormal ANS function present.

Along with the pupil diameter parameters, maximum constriction velocity was found to have statistically significant difference in absolute change at 0 – 3 days post-SRC compared with the no-SRC group. However, there were no statistical difference between SRC and no-SRC groups at 4 – 6 and 7 – 10 days post injury. Maximum constriction velocity is a function of the efferent-based PNS (18). The observations of the current study suggest there may be abnormal PNS function immediately following a SRC, however, the abnormality may resolve by 4 – 6 days following the incident. As the present study cannot determine where a deficit may be occurring, all explanations must be explored. Alternative to a dysfunction or change in the PNS function, if there was an abnormal SNS function present following a SRC, maximum constriction velocity would be effected. The change in maximum constriction velocity was unexpected after no difference was observe in the grouped 0 – 10-day analysis. Previous research by Thiagarajan & Ciuffreda (2015) (18) reported a significant decrease in the maximum constriction velocity in a group of patients with chronic mTBI. The authors suggested that, due to the significant changes in pupil diameter and dilation velocity, the observed decrease was a representation of abnormal sympathetic control rather than a PNS abnormality (18). This hypothesis is not supported by the results of the present study, as no dynamic SNS abnormalities were detected. An explanation may be possible distinct differences in the presentation of a SRC and the mTBI,
although there is limited scientific evidence on the differences between the two pathologies. However, an alternative hypothesis may explain the differences in maximum constriction velocity – that is photosensitivity immediately following a SRC. An observed change in maximum constriction velocity, coupled with a change in the pupil diameter, may be a result of photosensitivity following a SRC (120, 121). Previous research has suggested that photosensitivity following a mTBI is a result of a decrease in the effective innervation of the PNS (121). The hypothesis may explain the observations within the current study relating to the change in the maximum constriction velocity in the first 72 hours following a SRC.

In summary, by deconstructing the acute phase of a SRC in to three time frames, the progression and recovery of neurological function may be monitored via pupillometry assessment of the PLR. Although the current study suggests that there is a trend towards physiological recovery of the PLR, the specific time to physiological recovery for each individual athlete cannot be determined from the results of the present study although future studies could be conducted longitudinally to determine a return to baseline. All athletes had RTP at 7 – 10 days following a SRC diagnosis after successfully completing the RTP protocol. If the PLR assessment is a reflection of altered brain function and physiological change, the results of the current study may be indicative of premature RTP, before athlete baseline has been achieved.

4.5 Implications

The PLR has previously been suggested to be a potential objective physiological biomarker in the detection and monitoring of mTBI (18, 60, 61). To the best of our
knowledge, the present study is the first study to investigate changes in the PLR during acute SRC in national level rugby league athletes. Through the results of the present study, specific differences in the PLR were found to exist between national level rugby league athletes with and without a SRC throughout a rugby league season. These differences were able to be detected by the use of a handheld monocular pupillometer in the assessment of the PLR.

The current study suggests there is scope for pupillometry to be used to assess the PLR in the diagnosis and monitoring of recovery for SRC. First, due to the variability observed between athletes, the use of normative data methods in PLR assessment does not appear to be an applicable method. As with existing SRC protocols, the use of baseline data methods are suggested to be more appropriate in the development of SRC protocols which incorporate the PLR (45). By incorporating observed changes in the resting pupil diameters and maximum constriction velocity and the NPi into SRC assessment protocols, such as the HIA, may help with clinical decision making regarding diagnosis and injury recovery.

Anisocoria was not detected in any analysis within the current study. These findings are supported by previous research which concluded no difference in pupil symmetry between healthy adults and mTBI patients (72). For this, anisocoria assessment does not appear to be valuable in the assessment of SRC. The detection of anisocoria in athletes may be indicative of an alternative pathology rather than SRC itself.

Finally, from the present study, the use of a handheld monocular pupillometer has been demonstrated to be practical in a sport-related clinical setting for the assessment of SRC. Although further investigation is required, the handheld pupillometer is relatively cheap and portable making it a viable option of many sporting environments. The assessment of the
PLR is an involuntary physiology process and therefore potentially remove the intentional skewing of results as found in current testing protocols.

4.6 Study Limitations

There are a number of limitations of the current study that warrant consideration. First, the study was conducted with a single rugby league club, resulting in a relatively small sample size not dissimilar to previous PLR studies (18, 60). Similarly, the study was conducted over only one NRL season and repeated measures over several seasons may help the assessment and monitoring of SRC in individual athletes. Secondly, this is the first known study to investigate the use of pupillometry in acute SRC in national level rugby league athletes. For this reason, results of the current study could not be compared to previous SRC studies but instead were compared to previous research involved subacute blast-induced mTBI and chronic non-blast induced mTBI in military and civilian cohorts (18, 60, 61). The differences in results of the present and previous studies may indicate a difference in injury, and that may explain the contrast in results. Secondly, the present study relied on the HIA for a SRC diagnosis. The sensitivity and specificity of current testing protocols have noted limitations, meaning there is a risk of false negative and positive diagnoses of SRC within the current study. Thirdly, despite efforts to standardise lighting by ensuring all measures were taken to assess in the same room at the same time of day, at times, different rooms were required to be used due to game day limitations or club scheduling. To improve this, the use of a light monitoring device would be used to ensure standardised lighting conditions are used. Due to time restraints and club scheduling, the time of day was a factor that was unable to be controlled. Due to this the diurnal and circadian cycle influence on the PLR may affect the consistency in the data collection. The differences between athletes may be attributable to
the altered time of day testing. The impact of pharmacological intervention may have attributed to results obtained in the present study. The ingestion of caffeine may have contributed to the change in pupil diameter. Although not recorded, athletes regularly ingested caffeine supplementation during training and scheduled matches. Measures of athlete fatigue, hydration, psychological health, and iris colouring were not obtained during data collection and may have implications on the results of the study. The study design limits the ability to determine whether the PLR can be used to monitor recovery in national level rugby league athletes. Pupillometry assessment was terminated at 10 days following the SRC. Previous research indicates that there are changes in the PLR greater than 45 days following a mTBI. Finally, the NPi-200™ is a monocular pupillometer, limiting the capabilities of assessing a consensual reflex. The consensual reflex would provide additional information about the afferent pathways of the PLR (60).

4.7 Future Direction

The present study indicates the potential for the use of the PLR to be used as an objective biomarker for the assessment of SRC. Future research is warranted to determine the practical and clinical use of PLR in the NRL and sporting environments for SRC diagnosis and monitoring recovery for RTP protocol development. Furthermore, investigation into SRC at subacute and chronic phase of recovery would provide insight into long term effect of SRC. Investigations incorporating larger sample groups are warranted to improve the power of the study. Further research incorporating a larger sample population will allow for calculation of the sensitivity and specificity for pupillometry in the assessment of SRC. Additionally, to determine the positive and negative predictive values of pupillometry to diagnose SRC. Assessing the PLR throughout the athlete lifecycle and expertise level would
be beneficial to determine the reliability and potential for the PLR to be used in SRC assessment in children, youth and non-professional adults. To determine whether the addition of PLR assessment to current testing protocols would improve current testing protocols, comparative studies are required against the HIA. Comparison against aetiology and neurological imaging may be useful to correlate significant PLR parameter change with structural insult. If parameters of the PLR can be correlated with different mechanisms of injury to determine region of damage, then there is potential for the PLR to be used for interventional assessment. Finally, investigating the effect of common pharmacological agents and exercise supplements on the PLR would provide insight into usefulness following ingestion.
5.0 Conclusion

In summary, the present study was a detectable difference between national level rugby league athletes acutely diagnosed with SRC compared to athletes with no-SRC. There was a significant difference between athletes diagnosed with SRC compared to no-SRC for the NPi parameter. There were large variabilities in the measures of the PLR parameter measure among national level rugby league athletes. Further investigation determined a bidirectional change is detectable following a SRC. Using absolute change scores based off individual baseline measures, significant change was determined in resting and minimum pupil diameter during the first 10 days following a SRC compared to no-SRC athletes. Furthermore, the study suggests the potential for the PLR to be used to monitor the progression of SRC into recovery. The maximum constriction velocity was found to be statically different at 0 – 3 days following SRC, however was found to be non-significant for the remainder of the monitoring period. Further research is necessary to evaluate the practicality and clinical reliability of the PLR to be used for the diagnosis and monitoring of SRC. Additionally, for the use not only in national level rugby league athletes, but across all relevant sporting environments.
Reference

multicentre, proton magnetic resonance spectroscopic study in concussed patients. Brain 2010; 133(11):3232-42.


Appendix

Appendix A – Sport Concussion Assessment Tool – 3rd Edition

SCAT3™

Sport Concussion Assessment Tool – 3rd Edition
For use by medical professionals only

What is the SCAT3?1

The SCAT3 is a standardized tool for evaluating injured athletes for concussion and can be used in athletes aged from 13 years and older. It superseded the original SCAT and the SCAT2 published in 2003 and 2008, respectively.2 For younger persons, ages 12 and under, please use the Child SCAT3. The SCAT3 is designed for use by medical professionals. If you are not qualified, please use the Sport Concussion Recognition Tool. Forewarn baseline testing with the SCAT3 can be helpful for identifying post-injury test scores.

Specific instructions for use of the SCAT3 are provided on page 3. If you are not familiar with the SCAT3, please read through these instructions carefully. This tool may be freely copied in its current form for distribution to individual teams, groups and organizations. Any revision or adaptation in a digital form requires approval by the Concussion in Sport Group.

NOTE: The diagnosis of a concussion is a clinical judgment, ideally made by a medical professional. The SCAT3 should not be used solely to make, or exclude, the diagnosis of concussion as the absence of clinical judgement. An athlete may have a concussion even if their SCAT3 is “normal”.

What is a concussion?2

A concussion is a disturbance in brain function caused by a direct or indirect force to the head. It results in a variety of non-specific signs and symptoms (some examples listed below) and most often does not involve loss of consciousness. Concussion should be suspected in the presence of any one or more of the following:

- Symptoms (e.g. headache), or
- Physical signs (e.g. vomiting, diarrhoea), or
- Impaired brain function (e.g. confusion) or
- Abnormal behaviour (e.g., change in personality).

SIDELINE ASSESSMENT

Indications for Emergency Management

NOTE: A hit to the head can sometimes be associated with a more serious brain injury. Any of the following warrants consideration of activating emergency procedures and urgent transportation to the nearest hospital:

- Glasgow Coma score less than 15
- Deteriorating mental status
- Potentially open injury
- Progressive, worsening symptoms or new neurologic signs.

Potential signs of concussion?

If any of the following signs are observed after a direct or indirect blow to the head, the athlete should stop participation, be evaluated by a medical professional and should not be permitted to return to sport the same day if a concussion is suspected:

- Any loss of consciousness; [Y N]
  - If so, how long?
- Balance or motor incoordination (stumbles, slow/laboured movements, etc.); [Y N]
- Documentation or confusion (difficulty in reporting appropriately to questions); [Y N]
- Loss of memory; [Y N]
  - If so, how long?
- “Before or after the injury?” [Y N]
- Blank or vacant look.
- Visible focal injury in combination with any of the above; [Y N]

Glasgow coma scale (GCS)

Best eye response (E)

<table>
<thead>
<tr>
<th>Eye opening</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>4</td>
<td>4</td>
</tr>
</tbody>
</table>

Best verbal response (V)

<table>
<thead>
<tr>
<th>Verbal response</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>3</td>
<td>3</td>
</tr>
</tbody>
</table>

Glasgow Coma score (E + V + M) of 15

Maddocks Score3

“I am going to ask you a few questions, please listen carefully and try your best to answer:”

Wellotel: “I want to know about your injuries.”

What were you doing at the time?

[0 1]

What part of your body was injured?

[0 1]

Who was with you?

[0 1]

What time is it now?

[0 1]

Were you awake?

[0 1]

What went wrong?

[0 1]

Maddocks score of 5

Maddocks score is validated for sideline diagnosis of concussion only and is not used for serial testing.

Notes: Mechanism of injury “Tell me what happened?”

Any athlete with a suspected concussion should be REMOVED FROM PLAY, medically assessed, monitored for deterioration (i.e., should not be left alone) and should not drive a motor vehicle until cleared to do so by a medical professional. No athlete diagnosed with concussion should be returned to sports participation on the day of injury.
BACKGROUND

If you are having any problems or are not sure that you have had a concussion, please let your doctor know.

COGNITIVE & PHYSICAL EVALUATION

Cognitive assessment

Standardized Assessment of Concussion (SAC)

Orientation: 1 point for each correct answer.

- What month is it? 0 1
- What is the date today? 0 1
- What is the day of the week? 0 1
- What year is it? 0 1
- What time is it right now? (within 1 hour) 0 1

Immediate memory

Number of digits: 3

Concentration: Digits Forward

Concentration: Digits Backward

Concentration: Month In Reverse Order

Balance examination

Modified Balance Error Scoring System (BESS) testing

Condition

And/or

Tandem gait

Coordination examination

Upper limb coordination

Coordination score

SAC Delayed Recall

Delayed recall score
INSTRUCTIONS

Words in italics throughout the SCAT3 are the instructions given to the athlete by the tester.

Symptom Scale

"You should rate yourself according to the following symptoms, based on how you feel now".

To be completed by the athlete. In situations where the symptom scale is being completed after exercise, it should still be done in a resting state, at least 10 minutes post-exercise.

For total number of symptoms, maximum possible is 22.

For Symptom Severity score, add all scores in state, maximum possible is 2(4+4—16).

SAC*

Immediate Memory

"I am going to test your memory. I will read you a list of words and when I am done, repeat back as many words as you can remember, in any order."

Triage 2 & 3:

"I am going to test the same list again. Repeat back as many words as you can remember in any order, or if you said the word before."

Everyone: All trials regardless of score are told 1.8. Read the words at a rate of one per second.

Score 1 pt. for each correct response. Total score equals sum across all 3 trials. Do not inform the athlete that delayed recall is not tested.

Concentration

DigiToes backward

"I am going to test you again a string of numbers and when I am done, you repeat them back to me backward, in reverse order of how I read them to you. For example, if I say 7-3-0, you would say 1-2-7."

If correct, go to next string length. If incorrect, read list 1. One point possible for each string length. Stop after incorrect in both trials. The digits should be read at the rate of one per second.

Months in reverse order

"Now tell me the months of the year in reverse order. Start with the last month and go backward. So you’ll say December, November... Go ahead."

1 pt. for entire sequence correct.

Delayed Recall

The delayed recall should be performed after completion of the Balance and Coordination Exam.

("Do you remember that list of words I read a few times earlier? Tell me as many words from the list as you can remember in any order."

Score 1 pt. for each correct response.

Balance Examination

Modified Balance Error Scoring System (BESS) testing**

This balance testing is based on a modified version of the Balance Error Scoring System (BESS)®. A stopwatch will be used and 10 seconds will be required for this testing.

"I am going to test you balance. Please take your shoes off, roll up your pant legs above ankle (if applicable), and remove any ankle taping (if applicable). This test will consist of these two second walks with different scores."

(a) Double leg stance:

"The first stance is standing with your feet together and your hands on your hips and your eyes closed. You should try to maintain stability in that position for 20 seconds. I will be counting the number of times you move out of this position. I will start timing when you are set and have closed your eyes.

(b) Single leg stance:

"If you do it with both feet, correct? This will be the dominant foot. Now stand on your non-dominant foot. The dominant leg should be used in approximately 30 degrees of hip flexion and 40 degrees of knee flexion. Again, you should try to maintain stability for 20 seconds with your hand on your hip and your eyes closed. I will be counting the number of times you move out of this position. If you move out of this position, open your eyes and return to the start position and continue counting. I will start timing when you are set and have closed your eyes.

(c) Tandem stance:

"Now stand back-to-face with your non-dominant foot in back. Your weight should be evenly distributed across both feet. Again, you should try to maintain stability for 20 seconds with your hands on your hips and your eyes closed. I will be counting the number of times you move out of this position. If you move out of this position, open your eyes and return to the start position and continue counting. I will start timing when you are set and have closed your eyes.

Balance testing—types of errors

1. Hands/feet off
2. Opening eyes
3. Stumble, stumble, or fall
4. Moving hip into 30-degrees abduction
5. Lifting bonnet or heel
6. Remaining out of test position > 5 sec

Each of the 20-second trials is scored by counting the errors, or deviations from the proper stance, accumulated by the athlete. The examiner will begin counting errors only after the individual has assumed the proper start position. The modified BESS is calculated by adding one error point for each error during the three 20-second tests. The maximum total number of errors for any single condition is 10. If an athlete commits multiple errors simultaneously, only one error is recorded but the athlete should quickly return to the testing position, and counting should resume once subject is set. Subjects that are unable to maintain the testing procedure for a minimum of five seconds at the start are assigned the highest possible score, ten, for that testing condition.

OPTION: For further assessment, the same 3 stances can be performed on a surface of medium density foam (e.g., approximately 50cm x 40cm x 4cm).

Tandem Gait**

Participants are instructed to stand with their feet together behind a starting line (the test is back to back with foot removal). They will walk forward as quickly and as accurately as possible along a 30cm-wide strips taped, 3 meter line with an alternate foot behind the other. This task requires them to appreciate their feet and toe on each step. Once they cross the line, they turn, walk back and return to the starting line pursuing the same gait. A total of 4 trials are done and the best time is retained. Athletes should complete the task within 20 seconds. Athletes fail the test if they step off the line, have a separation between their heel and toe, or if they touch or grab the examiner or any object. In this case, the time is not recorded and the task repeated, if appropriate.

Coordination Examination

Upper limb coordination

Finger-to-nose (FTN) task

"I am going to test your coordination now. Please sit comfortably on the chair with your eyes open and your arm either right or left and extended but without shoulder flexion to 90 degrees and elbow and fingers extended, palm facing in front of you. When I give a start signal, I would like you to perform five successive finger-to-nose repetitions using your index finger to touch the tip of the nose, and then return to the starting position, as quickly and accurately as possible.

Scoring: 5 correct repetitions in 4 sec = 1

Note for testers: athletes fail the test if they lose touch with their nose, or if they touch or grab the examiner or any object. In this case, the time is not recorded and the task repeated, if appropriate.

References & Footnotes

1. This tool has been developed by a group of international experts at the 4th International Consortium meeting on Concussion in Sport held in Zurich, Switzerland in November 2012. The full details of the conference outcomes and the authors of the tool are published in "The 4th International Consensus on Concussion in Sport" (2014).


ATHLETE INFORMATION

Any athlete suspected of having a concussion should be removed from play, and then seek medical evaluation.

Signs to watch for
Problems could arise over the first 24–48 hours. The athlete should not be left alone and must be taken to a hospital if any of these signs of concussion are present:
- Have a headache that gets worse
- Are very drowsy or can’t be awakened
- Can’t recognize people or places
- Have repeated vomiting
- Behave unusually or seem confused; are very irritable
- Have seizures (arms and legs jerk uncontrollably)
- Have weak or numb arms or legs
- Are unsteady on their feet; have slurred speech

Remember: it is better to be safe. Consult your doctor after a suspected concussion.

Return to play
Athletes should not be returned to play the same day of injury, when returning athletes to play, they should be medically cleared and then follow a stepwise supervised program, with stages of progression.

For example:

<table>
<thead>
<tr>
<th>Rehabilitation stage</th>
<th>Functional exercises of each stage of rehabilitation</th>
<th>Objective of each stage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial phase</td>
<td>Physical and cognitive tests</td>
<td>Recovery</td>
</tr>
<tr>
<td>Locomotor exercise</td>
<td>Walking, step and balance</td>
<td>Increase stamina and balance</td>
</tr>
<tr>
<td>Movement specific</td>
<td>Striking a bat or hitting a ball in sports or physical activity</td>
<td>Improve movement</td>
</tr>
<tr>
<td>Non-contact training</td>
<td>Proprioceptive and balance training</td>
<td>Improve coordination and balance</td>
</tr>
<tr>
<td>Full contact practice</td>
<td>Following medical clearance in normal training activities</td>
<td>Return to normal gameplay</td>
</tr>
</tbody>
</table>

There should be at least 24 hours for each stage and if symptoms recur the athlete should rest until they resolve once again and then resume the program at the previous asymptomatic stage. Resistance training should only be added in the later stages.

If the athlete is asymptomatic for more than 10 days, then consultation by a medical practitioner who is expert in the management of concussion, is recommended.

Medical clearance should be given before return to play.

CONCUSSION INJURY ADVICE

To be given to the person monitoring the concussed athlete

This patient has received an injury to the head. A careful medical examination has been carried out and no sign of any serious complications has been found. Recovery time is variable across individuals and the patient will need monitoring for a further period by a responsible adult. Your treating physcian will provide guidance as to this timeframe.

If you notice any change in behaviour, vomiting, dizziness, worsening headache, double vision or excessive drowsiness, please contact your doctor or the nearest hospital emergency department immediately.

Other important points:
- Rest (physically and mentally), including training or playing sports until symptoms resolve and you are medically cleared
- No alcohol
- No prescription or non-prescription drugs without medical supervision
- No sleeping tablets
- Do not use aspirin, anti-inflammatory medication or sedating pain killers
- Do not drive until medically cleared
- Do not drive until medically cleared

Clinic phone number

SCAT3 SPORT CONCUSSION ASSESSMENT TOOL 3 | PAGE 4 © 2018 Concussion in Sport Group
Appendix B – Participant Information Sheet

Participant Information Sheet

Project Title:  Association between oculomotor function, brain excitability, heart rate variability and sport related concussion

GU Ref No:   2016/818

Investigator:

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What research is being conducted?

Concussion within sport has been within the sporting headlines for many years now, with many unknowns still surrounding the topic focus. The long term effects of sports related concussion are being researched, but the acute detection and rehabilitation to return to play are still limited in understanding. The aim of this study is to evaluate whether additional specific testing; pupillometry, heart rate variability, visual tracking and facial twitch, to the existing protocol incorporated by the National Rugby League (SCAT3, Sideline Concussion Assessment) can assist in developing a more accurate screening tool as well as return to play targets for players.

This research makes up a component of a Master of Medical Research program, that is currently being undertaken by Mr Daniel Brown.

What will you be invited to do:

During the preseason, a series of tests will be conducted to determine baseline measures for selected neurocognitive outcomes. All tests are quick to conduct and can be completed during normal screening schedule times during preseason, and regular season training.

The testing will include; pupillometry, visual tracking, facial twitch response and heart rate variability.

Pupillometry testing involves the use of a NeurOptics NPi-100 Pupillometer which will assess the response to the left and right pupils. Testing is conducted sitting stationary looking straight ahead. This is predicted to take 2 minutes. This will be completed once a week before training.

Visual tracking will involve the use of Gazepoint GP3 HD 150Hz Eye Tracker and Gazepoint Analysis UX Edition Software. Testing will be conducted sitting stationary looking straight ahead. This will require the participant to track certain patterns on a screen, as it records eye
movements. This is expected to take 5 minutes, and will be completed once a week before training.

Facial twitch will involve the use of FaceReader™. Testing will be conducted sitting stationary looking straight ahead at a set computer screen. This is expected to take 3 minutes and will be conducted before training once a week.

Heart rate variability will be tested via the use of a wristband by Empatica. This will be conducted by attaching the wristband to the wrist during the beginning of testing, then require 10 minutes of physical activity (to be completed during a training warm up). This will provide 20 minutes of data, with 10 minutes at a resting state, and 10 minutes in a physical activity state. This will be conducted once per week.

Within the duration of the testing period, if you sustain a concussion, the above tests will be conducted within the first 24-72 hours, then again throughout the rehabilitation phase of standard NRL requirements to return to play. Testing will continue until baselines are met. At no point will the results from the study be a determinant in your return to play.

With permission, if given on the consent form, during the testing process, there may be photos and/or videos taken of your participation. Should you wish to not have photo/video participation, this will not affect your eligibility to partake in the study.

The selection criteria:

- Elite rugby league player, contracted to the NRL;
- Over 18 years of age.

Exclusion

- Lacerations around eye/s means exclusion from pupillometry testing;
- Under 18 years of age.

Expected benefits of research:

To determine and develop additional testing options with greater sensitivity for sports concussions within rugby league. The benefits of this research will aid in player welfare and long term health. Additionally, the methods found beneficial from the research may be incorporated throughout the wider public, and not just elite level rugby league players.

Risk to you

There are no foreseeable risks to you by participating in the proposed study. All testing will be completed within the club setting. With exception to the balance testing protocol, all testing will be completed in a sitting position. To mitigate falls risks during balance testing, the researcher will be in close vicinity to ensure safety is kept.

Your Participation is Voluntary
Participation in the study is voluntary, and by no means are you required to participate. Deciding to not participate will not affect your position in the team and your decision will not prejudice you in any way. You can withdraw from participation at any time, your data will also be withdrawn.

Data Storage and Deletion

All research data collected for the purpose of this study will be retained in a locked cabinet on an encrypted drive at Griffith University for a period of five years, at this point, it will be destroyed. Note, all identifying information will be removed immediately.

Funding

There is no financial gain as a result of this study for any of the research investigators.

Questions/Additional Information

For any general questions or concerns with the study, we are happy to answer at any time. Please do not hesitate to contact Daniel Brown or Kerrie Evans (details provided on first page under investigators) for any questions or information.

The Ethical Conduct of this research

Griffith University conducts research in accordance with the National Statement on Ethical Conduct in Human Research. If potential participants have any concerns or complaints about the ethical conduct of the research project they should contact the Manager, Research Ethics on (07) 3735 4375 or research-ethics@griffith.edu.au.

Feedback to you

Participants will have the option of being informed of the results of the research either in the form of a summary of their results and/or copies of eventual publications.

Privacy statement

The conduct of this research involves the collection, access and/or use of your identified personal information. The information collected is confidential and will not be disclosed to third parties without your consent, except to meet government, legal or other regulatory authority requirements. A de-identified copy of this data may be used for other research purposes. However, your anonymity will at all times be safeguarded. For further information consult the University’s Privacy Plan at http://www.griffith.edu.au/about-griffith/plans-publications/griffith-university-privacy-plan or telephone (07) 3735 4375.

Thank you for your interest in this research project.
Appendix C – Consent Form

Consent Form

Project Title: Association between oculomotor function, brain excitability, heart rate variability and sport related concussion

GU Ref No: 2016/818

Investigator:

Dr Kerrie Evans
Dr Gary Grant
Mr Daniel Brown

Chief Investigator
Co-investigator
Co-Investigator

School of Allied Health Sciences
School of Pharmacy
Griffith University, Gold Coast

Ph: (07) 5552 7724
Ph: 0410 616 040

Email: kerrie.evans@griffith.edu.au
Email: gary.grant@griffith.edu.au
Email: daniel.brown3@griffithuni.edu.au

By signing below, I confirm that I have read and understood the information package and in particular have noted that:

- I understand my involvement in this research will include potential weekly testing during normal screening scheduled times. Testing will take approximately 10-15 minutes;
- I understand that testing involves; pupillometry, visual tracking, facial twitch and heart rate variability;
- I understand that my visual tracking, pupil measures, facial twitch and heart rate variability measures will be assessed;
- I understand that I can choose whether I give permission for photos or videos to be taken of me during the research project for the use in the write up of the paper, for conference presentations or for educational purposes (such as demonstrations of protocol set up, and uses to additional medical professionals) by ticking the check box below. I understand that if I choose NOT to have photos/videos taken that I am still eligible to participate in the study;
- I have had any questions answered to my satisfaction;
- I understand that there is no foreseeable risk to my involvement;
- I understand there will be no direct benefit to me from my participation in this research but that I will receive my results and summary of my outcome measures should I wish to do so;
- I understand my participation in this research is voluntary, and in no instance will it affect my return to play;
- I understand that if I have any additional questions I can contact the research team;
- I understand that I am free to withdraw at any time, without comment or penalty;
- I understand that I can contact the Manager, Research Ethics, at Griffith University Human Research Ethics Committee on (07) 3735 4375 or research-ethics@griffith.edu.au if I have any concerns about the ethical conduct of the project; and
- I agree to participate in the project.

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<thead>
<tr>
<th>Name</th>
<th>I agree to allow the researchers to take photo/videos of me during the research process. □ YES □ NO</th>
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<td>Signature</td>
<td>I would like to be informed of the results? □ Summary of findings</td>
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<td><strong>Date</strong></td>
<td>If so, please include contact email or address below:</td>
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<td><strong>Contact details:</strong></td>
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Appendix D – Letter of support from Gold Coast Titans NRL

30 September 2016

To Whom It May Concern:

As head Physiotherapist for the Gold Coast Titans, I am aware of and fully support the study being conducted by Dr. Kerrie Evans, Dr. Gary Grant and Mr. Daniel Brown. I understand the study is entitled *Association between oculomotor function, brain excitability, heart rate variability and sport related concussion* (GU Ref No: 2016/818).

I understand that the research will call for volunteers from both the NRL and Under 20 squads and we are happy to assist in explaining the recruitment process, and what the study entails. I understand that testing will take be conducted three times per week during the pre-season period, and will form the baseline data for each individual athlete. I understand that data will be collected during participants’ normal training sessions and will take approximately 10 minutes in total, with a random sample of 15 players measured repeatedly throughout the season. Additionally, data will be collected post any concussive event within 24-72hrs of the event, depending on availability of the player, and then three times during the first week, and weekly after that until return to baseline has been met.

Each player will be provided with the information package and consent form.

Should you have any further queries, please contact me on 0438 981 418.

Kind Regards,

Greg Condon
Head Physiotherapist – Gold Coast Titans