

Pupillary Response as a Physiological Indication of Neural Resource Utilization in Those With and
Without a Concussion History

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ABSTRACT

KOU YANG: Pupillary Response as a Physiological Indication of Neural Resource Utilization in Those
With and Without a Concussion History
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Pupillary response to cognitive workloads is a reliable assessment of neural resource utilization – but has not been used to investigate post-concussion physiological impairment. This study investigated the effect of concussion history, sequence length, and task performance on average change in pupil size from baseline to retention periods during a backwards digit-span task. Recreationally active college students, (10 healthy, 10 with a concussion history; age = 21.85 ± 2.13 years; 50% male), completed a backwards digit-span task (66 trials) in a virtual reality environment while recording pupil size. A generalized linear mixed model analyzed and suggested no main effects for concussion history ($F_{1,18}=0.01$, $P=0.906$), or accuracy ($F_{1,17}=0.02$, $p=0.879$), but a main effect of sequence length ($F_{10,179}=3.34$, $p<0.001$). No significant interaction effect between concussion history and sequence length ($F_{10,179}=1.85$, $p=0.055$). Pupillary response to a cognitive load shows potential in assessing neural resource utilization but future investigations should consider concussion history variability.

To my amazing mentor and family.
Thank you for your support and guidance.

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CHAPTER 1: BACKGROUND

Introduction

Interest in proper concussion assessment and management has risen significantly over the past 10 years with the rise in concern regarding collision sports and head injuries. The 5th international conference on concussions in Berlin, defines sport-related concussions as functional brain injuries induced by biomechanical forces typically resulting in short-term neurological impairment, and not always involving a loss of consciousness.¹ The pathophysiological changes—often referred to as the neurometabolic cascade— following concussive injury involve an initial release of excitatory neurotransmitters and ionic imbalance, leading to increased metabolic demand during a time of decreased cerebral blood flow ultimately causing an energy crisis and secondary injury in the brain.² The result of these imbalances lead to various clinical neurological, motor, cognitive, and behavioral impairments that typically follow a gradual decrease in severity.^{3,1,4} The majority of patients demonstrate concussion symptom resolution within a 5 to 7 day period following injury.⁵

Currently, a diagnostic gold standard for concussion diagnosis does not exist , therefore clinicians rely on a comprehensive clinical assessment battery that enlists several tools for proper assessment and management.¹ The current recommended battery includes a symptom inventory, balance and neurocognitive assessments.¹ Common symptoms following concussion often include headaches, nausea, balance deficits, and dizziness.⁶ Decreased postural control and neurocognitive impairments are also common following injury, where athletes often exhibit deficits in attention, processing speed and memory.⁶ Clinical measures of balance and neurocognitive function have been shown to normalize between 3 and 5 days following injury,^{7,8} though advanced neuroimaging studies have shown that persistent physiological dysfunction extends beyond this timeframe; at a time when athletes often return to play which may lead prolonged recovery, persistent symptoms, or vulnerability to reinjury.

A variety of neuroimaging techniques have been used to investigate the physiological response and recovery following concussion (e.g., functional magnetic resonance imaging (fMRI) and electroencephalography (EEG), cerebral blood flow (CBF)) though there remains a dearth of evidence to fully characterize this response given inhibiting factor related to cost and applicability. A recent systematic review suggests that persistent physiological deficits extend beyond normalization of clinical assessments and represents an over-excitatory period during which the brain remains physiologically compromised and at an increased risk for re-injury.^{9,10} However, application and generalizability of these findings are limited due to extensive training, high cost, and time intensive factors. Recent studies using eye tracking technology—specifically pupillary responses to cognitive demands as an indicator of neural resource utilization— may provide a clinically feasible, valid, and sensitive mechanism by which researchers and clinicians can understand physiological aspects of concussive injury. Prior studies have revealed a relation between brain activity and pupil diameter with one study correlating pupil diameter to blood-oxygen-level-dependent fMRI activity in the locus coeruleus of the human brain (a nucleus in the dorsal pons that secretes neuromodulator noradrenaline).¹¹ A recent review have also suggest a strong link in pupil dilation and positive going even-related potentials seen in EEG studies, another marker of LC-NE activity.¹²

Pupillary responses to cognitive demands or mental activity is modulated by the noradrenergic locus coeruleus (LC) where increasing cognitive demands yield greater changes in pupil dilation until individuals reach their neural resource capacity for a specific demand.¹² The LC is a small nucleus in the brainstem, that propagates various brain functions through the synthesis and secretion of the neuromodulator noradrenaline and is important in various cognitive demands.¹¹ Many studies consistently indicate that as cognitive demands increase in difficulty—or present a greater cognitive demand— greater pupil dilation responses are exhibited.^{9,12,13} Kahneman and Beatty were the first to describe this relationship using a cognitive task called a digit-span task where participants were given phone numbers to recall. Unfamiliar numbers elicited a greater pupil response.¹³ Digit-span task have also been recently

utilized in concussion assessments as a form of working memory⁵ but no previous study has investigated digit-span task presentation and pupillary response in a concussion population.

Changes in pupillary responsivity have been validated as an index of neural resource utilization—via fMRI and EEG—and often examined in cognitive testing paradigms of attention, processing speed and working memory.¹⁴ Common neurocognitive testing paradigms used to examine these cognitive constructs include the stroop task and the digit-span task where during incongruent trials of the stroop and presentation of digit-spans of greater lengths are directly related to increases in pupil diameter in healthy individuals.¹⁵ The pupillary response to cognitive demands has also shown discriminatory ability beyond task performance or accuracy outcomes in those with and without neurocognitive impairment (e.g., Alzheimer's¹⁶ and Parkinsons¹⁷). Specifically, these studies have demonstrated that those with neurological impairment exhibit greater neural resource utilization in the form of greater pupillary dilation response, in order to produce the same level of task performance for a given task. Therefore, the true value of pupil responsivity as a physiological index of neural resource utilization during a cognitive task may be a cost effective, noninvasive, assessment of physiological response and recovery following concussion. Therefore, the purpose of this study was to examine pupillary responsivity to various levels of sequence lengths during a digit-span task, in those with a concussion history versus healthy recreationally active individuals with no prior concussion diagnoses. We hypothesized that those with a concussion history would exhibit greater average change in pupil diameter during the digit-span task compared to healthy participants, while controlling for behavioral response accuracy, indicating greater neural resource utilization to produce similar task performance.

Research Question

What is the influence of concussion history, digit-span length, and task performance on average change in pupil diameter during an overload digit-span task?

Research Hypothesis

We hypothesize that those with a concussion history would exhibit greater average change in pupil diameter throughout the digit-span task compared to healthy participants, while considering behavioral response accuracy, indicating greater neural resource utilization to produce similar task performance throughout the task.

Definition of Terms

1. Behavioral Response - Accuracy: single trial accuracy required the participant to correctly report all digits in exact reverse order. Accuracy for each sequence length (4-14) required at least 3/6 (50%) accurate trials.
2. Sequence length: the numbers of digits in a digit-span.
3. Neural Resource Utilization: brain activity used to accomplish a cognitive task.
4. Baseline Period: two seconds prior to each digit-span being presented to allow for pupils to rest and stabilize.
5. Loading Phase: the portion of the digit-span task in which participants are presented with a sequence of digits at the rate of 1 per second and asked to remember the number sequence.
6. Retention Period: three second period after each digit-span is presented when participants process/encode the information and prepare to recall.
7. Unloading Phase: the portion of the digit-span task during which participants recite the previous sequence of digits in the reverse order in which they were presented.
8. Average Change in Pupil Diameter: the difference between the average pupil diameter during the last 15 samples of the baseline period (before each span is presented) and the first 120 seconds of the retention period (after participants have been presented with all of the digits they are expected to recall for a given trial).

Operational Definitions

1. Healthy: individuals physically active at least 30 minutes a day, 3 times a week of moderate to vigorous activity between the ages of 18 and 30, with no previous concussion history.
2. History of Concussion: physically active individuals between the ages of 18 and 30, with at least one previous concussion diagnosed by a physician.
3. Behavioral response—Accuracy: response accuracy will be defined by trial and across digit-span lengths (loads).
 - a. Task performance—Accuracy within trial: requires 100% accurate digit-span recall (in reverse order as presented) in both content and position.
 - b. Task performance—Accuracy across sequence lengths: requires trial accuracy on 4 of the 6 trials for each sequence length.

Delimitations

1. Individuals with concussions within the past 6 months, or not diagnosed by a physician were excluded.
2. Individuals who were not physically activate at least 30 minutes of moderate to vigorous physical activity 3 times a week.
3. Individuals with permanent vision loss, strabismus, amblyopia, or eye surgery in the last 6 months were not included in this study.
4. Individuals who participate in visual or vestibular therapy were excluded to prevent confounding variables.

Limitations

1. Participants with a concussion history were responsible for reporting their own medical history. Being so, there is potential for participants to be included in the concussion history group who should not have been.

2. Participants were responsible for reporting their own activity level and thus some participants may have been included into the study that did not meet the physical activity criteria.

Assumptions

1. Participants accurately reported past medical history.
2. Participants accurately reported typical physical activity levels.
3. Participants remembered and accurately reported all concussion injuries with recurrent symptoms.
4. Participants remained engaged and gave full effort during the task.

Variables

1. Dependent
 - a. Average change in pupil diameter between the retention and baseline periods. Calculated by taking the difference between average pupil size during the first 120 samples of the retention period and the last 15 samples of the baseline period for each trial, then averaging across sequence lengths.
2. Independent
 - a. Group
 - i. Concussion history
 - ii. No prior concussions
 - b. Behavioral response
 - i. Accurate
 - ii. Inaccurate
 - c. Sequence Lengths
 - i. 4 Digit-span
 - ii. 5 Digit-span

- iii. 6 Digit-span
- iv. 7 Digit-span
- v. 8 Digit-span
- vi. 9 Digit-span
- vii. 10 Digit-span
- viii. 11 Digit-span
- ix. 12 Digit-span
- x. 13 Digit-span
- xi. 14 Digit-span

CHAPTER II: LITERATURE REVIEW

Concussion Prevalence

Sport related concussion are functional brain injuries that are typically induced by biomechanical forces (generally a direct or indirect blow to the head, neck, or body) resulting in an impulsive force to the head which results in a cascade of short-lived neurological and neuropathological changes.¹⁸ According to the CDC, sport related traumatic brain injury (TBI) that resulted in reported loss of consciousness (LOC) accounted for approximately 300,000 incidents annually. As sport related TBI/sport related concussions do not necessarily have to result in LOC (as LOC only accounts for 8-19.2% of TBI), a better estimate of sport related TBI/concussions is between 1.6 million to 3.8 million per year.¹⁹ This estimated number, however, is believed to be lower than the actual prevalence as many injuries go unrecognized due to concussion assessments being dependent on athlete reporting.¹⁹ The difficulty in this particular injury is the variability in symptomology resulting from concussion pathophysiology.

Concussion Pathophysiology

Concussion is a complex pathophysiological condition that results in various functional neurological impairments. Biomechanically induced head trauma via linear and rotational accelerations creates neuronal shear stress and strain that triggers what has been described by Giza et al. as the neurometabolic cascade. During the acute phase of concussion injury and start of the neurometabolic cascade, there is a release of excitatory neurotransmitters in the brain, resulting in neuronal depolarization. This depolarization causes an ionic imbalance with the efflux of potassium and influx of calcium ions. High levels of intracellular calcium ions cause upregulation of the sodium potassium pump to restore ionic homeostasis. A period of hypermetabolism results due to increased ATP and glucose demand. Additionally, the influx of calcium is appropriated into mitochondria for short-term relief but eventually leads to mitochondrial dysfunction and decreased oxidative capacity. This leads to

mitochondrial dysfunction with decreased glucose utilization and metabolism. Increased glucose demand with decreased in oxidation leads to an energy crisis.^{2,20} Reduced cerebral blood flow also contributes to secondary injury. These physiological deficits are reflected in athlete/patient presentation though have been shown to persist beyond clinical recovery and timeframe of the current neurometabolic cascade. These findings, outline by neuroimaging studies suggest what has been termed as a ‘window of neural vulnerability’ during which time the brain remains physiologically-compromised and at risk for repeated trauma which may be detrimental long term.²⁰

Concussion Assessment and Recovery

Clinical Recovery

The pathophysiological response to injury results in various signs and symptoms and therefore requires clinicians to use a comprehensive assessment battery that includes a symptom inventory, and balance and neurocognitive assessment.¹ A symptom check list is used to account for the variety of common symptoms following concussions including headache, balance deficits, dizziness, and fatigue.⁶ As balance and/or postural control is also commonly effected, clinicians commonly use the Balance Error Scoring System (BESS).^{7,21,22} Computerized neurocognitive tests assess common neurocognitive impairments following concussion of attention, processing speed, and memory following concussion and include CNS-Vital Signs, CogState, and Immediate Post-Concussion Assessment and Cognitive Test (ImPACT).²³ The Sports Concussion Assessment Tool 5 (SCAT5), and Standard Assessment of Concussion (SAC) also include some components of mental status assessment.¹ Normalization of these clinical measures of balance and computerized neurocognitive tests is typically seen between 3 to 5 days following injury.⁵ Advanced neuroimaging studies however, have shown that physiological markers of concussion persist beyond normalization of clinical measures suggesting that our battery of assessments may not be sensitive enough for determining injury recovery and making clinical decisions around return to play/learn.

Persistent physiological deficits

Physiological response and recovery from concussion remains poorly characterized²⁴ though, advanced neuroimaging technology has been used to expand our understanding. A recent systematic review by Kamins et al. suggests that the many physiological changes makes the development of a single ‘physiological time window for concussion recovery’ impossible. Rather, more research should use imaging techniques to link symptoms with physiological changes, and potential risk for subsequent injury.²⁴ Examples of these advanced imaging include: Diffusion tensor imaging (DTI), magnetic resonance spectroscopy (MRS), cerebral blood flow (CBF), electrophysiology (EEG), fluid biomarkers, and transcranial magnetic stimulation (TMS).²⁴ A recent systematic review on neuroimaging and physiological response to concussion investigated some of the most popular neuroimaging techniques²⁴ and revealed prolonged physiological deficits beyond clinical recovery time. The review of 18 fMRI studies (imaging technology used to measure brain activation at various regions of the brain), reported changes in cerebral activation from 3 to 23 months after return to play, and suggest that this may be due to ‘network modifications’ that are separate from the acute injury.²⁴ Neurocognitive testing paradigms that assess attention, processing speed and working memory are used during these neuroimaging studies to characterize regional brain activity and neural resource utilization as an indication of physiological response and recovery following concussion.

Neurocognitive Testing Paradigms

Neurocognitive assessments are extensively studied in psychological research and are often combined with neuroimaging techniques. These assessments of attention, processing speed, and working memory allow researchers to describe the cognitive demand each task presents and neural resources necessary to complete the task with an acceptable level of performance or accuracy. Past studies have utilized subjective, indirect measures of cognitive demand that included performance statistics,^{25,26} testing accuracy,²⁷ and through dual-task methodology (as the performance of the second task deteriorates the higher the cognitive demand)^{28,29}

Digit-span tasks are some of the most common assessments of working memory and are commonly examined in concussion patients requiring individuals to remember, process, and synthesize a collection of pieces of information (e.g., words, digits, letters) for recall.³⁰ Our working memory has a limit, known as working memory capacity (WMC) which is thought to hold around between 5 and 9 pieces of information at a time.³¹ A subset of digit-span tasks used to assess cognitive demand and neuro-resource utilization is the n-back test. Rondell et al. used this paradigm in relation to pupillary response to assess for resource utilization (the portion of the brain that is called upon during cognitive processing).¹⁴ The amount of resources that a person allocates in order to complete a complex task is suggested to be indicative of ability cognitive control.^{14,32}

Concussion Assessment Battery Limitations

A large limitation to the current concussion battery is the lack of clinically useful physiological evaluations/ biomarkers despite knowing a prolonged physiological time of recovery. These physiological alteration persist beyond typical return to play and research is limited on how these alterations could potentially correlate with injury vulnerability.²⁴ There is not enough evidence to suggest a change in the current return to play progression, but future studies should investigate the physiological biomarkers with longitudinal analysis (especially to account for multiple blows).²⁴ However, this suggestion is difficult due to expensive imaging equipment, extensive training necessary to run tools, and lack of portability of equipment. These factors can be challenging in conducting studies at secondary schools where there is large youth concussions occur and where it may be needed the most. One possible solution to this predicament is the utility of eye tracking techniques, specifically changes in pupil diameter, to obtain physiological changes in brain function.

Pupillary Response to Cognitive Demand

Anatomy and Physiology of Pupillary Response

Two muscles, the dilator pupillae and the sphincter pupillae control changes in pupil size, via dilation and constriction respectively. They are both directly controlled by sympathetic and parasympathetic activity. The dilation response in particular is controlled by sympathetic activity and the

noradrenergic locus coeruleus (LC). The LC is a small nucleus in the brain stem that plays a role in physiological arousal, cognitive function, and is central for release of norepinephrine (NE also called noradrenaline) throughout the brain. NE is important for central and peripheral nervous system activity and for proper brain development. This LC-NE activity directly communicates with the parasympathetic Edinger-Westphal nucleus that controls the constricting fibers. Inhibition signals from LC activity will directly inhibit the Edinger-Westphal nucleus and induce the constricting fibers to inhibit, causing pupil dilation.¹² Various studies have confirmed the relationship linking the pupillary constriction/dilation system and LC-NE activity.¹²

Pupillary Response to Cognitive Demand

Pupillary response has been studied under various testing paradigms inducing cognitive demand. In Beatty and Kahneman's research, they examined the relationship between a number recall task and pupil dilation and found dilation was related to string length and level of difficulty. In their experiment, participants recalled phone numbers that they were familiar with compared with novel phone numbers that they provided. Their findings concluded that participants had greater dilation when they were required to remember a new phone number when compared to recalling a familiar phone number. This experiment was later expanded upon in Klinger's et al. research in which participant pupil responses were obtained while given either a visual or an aural presentation of a digit recall (digit-span) task. They concluded that visual presentation of the digit recall task had higher accuracy and auditory presentation led to larger pupillary dilations.⁹ In Piquado's research, younger and older populations recalled various digit-span lengths and also found that pupil sizes were "sensitive" to task difficulty (i.e., digit length) with older populations showing a larger effect. This indicated a greater effort for older populations during similar task difficulty levels.³³

Locus Coeruleus Norepinephrine Activity

The relationship between LC activity and pupil changes have well been research. In a study by Murphy et al., pupil diameter was shown to be correlated with activity in the locus coeruleus-noradrenergic neuromodulatory system.¹¹ Essentially, in his study Murphy tested 14 healthy participants

completing two class oddball paradigms and while at rest. Brain activity was recorded using pupillometry and blood-oxygen-level-dependent (BOLD) fMRI to compare changes in pupil diameter with LC activity. In summary, their results concluded that changes in pupil diameter was a traceable and non-invasive tool in determine LC-NA activity of the brain.¹¹ The utility in pupil diameter changes could potentially be used to evaluate brain activity in various concussion settings without expensive imaging techniques and provide consistent data points for longitudinal testing.

Joshi et al. research further expands and solidifies the pupillary response as a reliable tool for assessing cognitive demand. In the study, adult rhesus monkeys performed various task for varied amount of time while being probed with an electrical microstimulant in the LC and other brain regions connecting to it. During the first portion of the test, passive fixation, they found fluctuations in pupil dilations which they account to pupil noise, spontaneous pupil oscillations, and pupillary unrest.³⁴ Subjects were presented with a brief, loud, startling tone at varying times while observing their pupillary response and found that the sound caused dilation in all of the monkeys (showing responses to the tone) within the LC and other brain regions connecting to it. They concluded that changes in pupil diameter can reflect neural activity of the locus coeruleus.³⁴ To further solidify the connection between the LC activation and pupil response, Joshi et al. also used electrical microstimulation on the LC and found that the sites reliably evoked increases in pupil diameter within 1,000ms of stimulation.³⁴

The use of pupil measurements in assessing for cognitive brain function has also been successfully used in degenerative brain disorders like Parkinson and Alzheimer's. Kahya et al. was interested in the cognitive demand in Parkinson's patients using pupil response. The study utilized a letter-number sequence task, in which participants were to recall a sequence of random letter and numbers in various orders (ascending order and alphabetical). Measuring the pupil changes between healthy participants and Parkinson's patients, they concluded that pupillary response is able to detect cognitive demand of task in healthy and Parkinson's participants and acknowledges the potential for pupil changes to be utilized in Parkinson's diagnosis.¹⁷ When looking at Alzheimer's, Granholm et al. found that although there was normal clinical performance between single-domain mild-cognitive impaired

participants, non-amnestic single-domain mild-cognitive impaired, and cognitively normal participants, amnestic participants had greater compensatory effort to maintain consistent performance.¹⁶

Instrumentation and Measurement

Prior to the link between pupil response and LC-NE activity, pupil responses were used in measuring cognitive ability via various paradigms and tools. Cognitive ability has been assessed using various paradigms and tools. Past research have specifically targeted skills like engagement,³⁵ inhibition,³⁶ task switching ability,³⁶ cognitive effort,^{16,33} cognitive demand,^{9,17,37} and resource utilization.³² Testing paradigms are cognitive exertional test like a short-term memory task³³, reaction time task³², digit-span task^{9,16}, or an arithmetic problem^{9,37} which are completed under a pupil measuring device like eye trackers^{9,17,33,35–37} and pupillometers.^{16,32} Notably, the most popular cognitive task used with pupillometry is the digit-span task.^{9,13}

Beatty and Kahneman was the first to report the use of this paradigm with pupil recording and established the typical pupil response pattern to the task. In their study, participants were given unfamiliar telephone numbers (one digit at a time) to remember and asked to recall the information after a brief pause. 5 phases were described in the experiment, an early control phase (5 seconds where a pupil baseline diameter was taken), a listen phase (3 seconds where telephone numbers were presented), a pause phase (2 seconds of silence), a reporting phase (participants recited telephone numbers), followed by another control phase (an extended period where pupils were recorded). Their results first demonstrated pupil dilation/constriction pattern throughout the paradigm with pupil stabilization during the control phase, dilation during the listening phase, maximum dilation during the pause, and constriction during the report phase until it reaches baseline levels on the last digit reported as seen in **Figure 3**.¹³ Klinger et al. expanded this research by investigating auditory and visual digit presentation methods. **Figure 4** shows the resulting patterns for visual and verbal presentation which resembles Beatty and Kahneman's results. The data further establish the pupil response pattern for a digit recall paradigm while demonstrating how more difficult digits lengths caused greater pupil dilations.

Rationale for Current Study

Concussions are of great concern for the general population, especially those competing in sport activities. Concussion results in a cascade of physiological changes and clinical signs and symptoms that typically recover within 7-10 days following injury. Current concussion assessments may not be sensitive enough to persistent physiological deficits known to occur as reported by recent systematic reviews where these assessments look specifically at neurological resource utilization. Infrared eye tracking technology has the ability to track pupillary responses during common neurocognitive testing paradigms, as an indication of neural resource utilization and has been validated using neuroimaging techniques. The digit-span test, is commonly used to examine impairments in working memory following concussion and therefore may provide a cost effective, noninvasive and portable assessment substitute measuring and expanding our understanding of neurological resource utilization and physiological response and recovery post-concussion.

CHAPTER III: METHODS

Design and Participants

This quasi-experimental, cross-sectional study recruited physically active, college aged individuals with and without a concussion history. All participants were recruited from a single university, were between 18 and 30 years of age, and had normal or corrected-to-normal vision. Additionally, concussion history group participants were included if their most recent physician diagnosed concussion occurred more than 6 months prior to their participation date. Physically active was operationally defined as participating in at least 30 minutes of moderate to vigorous physical activity, 3 times per week. Concussion was defined using the Consensus Statement on Concussion in Sport from the 5th International Conference on Concussion in Sport as a functional brain injuries that are typically induced by biomechanical a cascade of short-lived neurological and neuropathological changes.¹⁸

All participants reported for one testing session and provided written informed consent prior to any study participation. The study was approved by the institution's Office of Human Research Ethics board. Upon study completion, participants were compensated \$10 for their time.

Laboratory Procedures

Participants completed a demographic and medical history questionnaire that included specific questions regarding their concussion injury history. Participants were then seated in a chair and fitted with the FOVE Virtual Reality (VR) head-mounted display (FOVE, Inc. Silicon Valley, San Mateo, CA) while wearing a disposable sanitary face cover mask. The positioning camera was vertically aligned parallel to top of the headset to ensure participants were vertically aligned and facing forward in the VR environment. Adjustments were made so 6 corneal glints were made visible participant pupils. Research assistants then started a 5-point calibration in which participants followed a highlighted point using only their eyes to accurately measure pupil size and gaze direction. Participants were then familiarized with the

task by completing practice trials (all a 4-digit sequence) until study staff had determined that the participant demonstrated adequate understanding and compliance with task requirements. Participants were instructed to keep their head still with their eyes focused on the testing wall in front of them, refrain from closing their eyes and/or looking around the room while trying to remember the digits.

Overload Digit-span Task

Task Design

The backward digit-span task designed to overload working memory used in the present study was adapted from Johnson et al.¹³ to accommodate a university sample. Our adaptation of the task utilized a randomized blocked design containing 6 consecutive testing blocks of 11 random digit-sequence lengths between 4 and 14 digits long—each including the numbers 1 through 9—as depicted in **Figure 1**. These digit sequence lengths were chosen based on previous concussion literature, in order to overload individuals within this age range beyond they're working memory capacity sequences would need to be beyond 12 digits long.⁵ A Latin Square was used to complete task presentation randomization with respect to sequence presentation order and individual digit presentation within each sequence—this approach ensured appropriate counterbalancing and exclusion of duplicate and consecutive integers.³⁸ A custom random number generator determined initial sequences for each Latin Square model. All participants completed the same task as designed, which took approximately 40 minutes to complete, including task familiarization.

Task Performance

Participants responded verbally and study personnel entered participants' responses into a digital survey—back-up audio recordings were also collected. Task performance was determined for each trial and summarized across digit-sequence lengths; specifically, trial accuracy required participants to correctly report all digits in exact reverse order and summarized accuracy required at least 50% (3 of 6 trials) accuracy.

Task Presentation

FOVE VR Headset

The digit-span task and virtual reality (VR) environment were custom developed using Unity® software and presented using the FOVE VR head-mounted display (FOVE, Inc. Silicon Valley, San Mateo, CA) with imbedded infrared eye tracking. The head-mounted display simultaneously recorded pupillary responses in both eyes at 60Hz and a Logitech keyboard was used by participants to record when they were finished reporting all digits they remember from each trial and to initiate each trial. Two infrared illumination eye tracking sensors within the VR headset measured participant pupils by fitting an ellipse to the infrared pupil image—producing pupil radius measurements in millimeters. The headset display consisted of wide quad high definition organic light-emitting diode (WQHD OLED) 2560 x 1440, displaying up to 70 frames per second and having up to 100 degrees of viewing angle. To control for shifts in gaze, participants were asked to look straight ahead throughout testing. The task was displayed on a fixed wall within the VR environment to eliminate peripheral and distant distractions, reducing potential influence of the accommodation reflex.

Unity and Task Environment

The task presentation environment was a square shaped room (10x10x10 meters, with grey walls (R: 169, G: 169, B: 169, A: 255), built using Unity –a cross platform game development engine. To account for major confounding variables, with the largest being the pupillary light reflex, the scene utilized the “Default-Skybox” light, with an intensity multiplier of 1 and remained consistent to standardize luminance between participants. Participants’ vision within the VR environment was directed toward the forward-facing wall (3 meters away), on a blank canvas measuring 0.6m X 0.34m, at a scale of 0.083m. Stimulus cue text on the canvas was standardized across all aspects of the task using Arial text (size= 40, color= R:46 G:46 B:46 A: 255) and scaled in the x-axis by 0.0015, in the y-axis by 0.0015, and in the z-axis by 0.005 to ensure the best clarity. Data recording was initiated by study personnel prior to any stimulus presentation.

Stimulus Presentation

Participants initiated each trial by pressing the “right direction arrow.” Each digit-span trial began with a baseline/accommodation period that lasted 3 seconds, indicated by an “X”. Each digit was then displayed at a rate of 1 digit per second. Final digit presentation for each trial, was followed by a 3 second retention period before participants were prompted to verbally recall the numbers in the reverse order in which they were presented for that trial. Stimulus presentation is shown in sequence in **Figure 1**. When the participant finished their response, they pressed the “space bar” to notify that they finished their response and then they pressed the “right directional arrow” to proceed to the next trail. Participants continued through all 66 trials until reaching the ‘end of trial’ slide, without reinforcement from research assistants.

Data Reduction and Processing

The variables obtained from the raw data included left and right pupil radius (mm) and testing time (s) each sampled at 60Hz. Timestamp were recorded for: 1) trial number (1-66), 2) span length (4-14), 3) digit presentation (s), 4) retention X display, 5) response box display, and 6) participant finished response. Participant data files were directly exported into a spreadsheet and imported into Matlab (MATLAB and Statistic Toolbox Release 2017b, The MathWorks, Inc., Natick, MA, USA). The study team used a custom Matlab program adapted from the suggested procedures for processing pupil size data by Kret and colleagues in 2018³⁹ to complete all pupil size data processing and reduction. The adapted processing program employed 6 procedural levels as follows: 1) raw pupil radius measures for each eye were converted into diameter and averaged across both eyes; 2) raw data were prepared for filtering by removing identified blinks (recorded as .000mm or .0001mm); 3) additional artifacts were identified and removed (e.g., signal noise, longer eye closures, etc.) to identify valid samples (i.e., pupil measurements not interrupted by blinks or noise); 4) the remaining valid samples were then up-sampled and smoothed using linear interpolation; 5) pupil response regions of interest were segmented (i.e., the last 15 samples of each baseline period and the first 120 samples of each retention period) and individually averaged; and

6) segment averages were used to determine pupil size change for each trial (i.e., average pupil size during retention – average baseline pupil size).³⁹

Processed data (i.e., change in pupil diameter for each trial) were exported from Matlab and reduced using SAS 9.4 (Cary, NC). Trails were binned by sequence length (4-14) and averaged within each sequence length (6 trials per sequence length) to obtain 11 average change in pupil diameter per sequence length. Ensembled average changes in pupil diameter were calculated across all trials and served as our dependent variable (primary outcome) compared between healthy and participants with a concussion history summarized in **Table 1**.

Statistical Analysis

Continuous data were summarized using means and standard deviations and frequencies and associated percentages summarized our categorical variables (**Table 2**). A generalized linear mixed model examined average pupil response changes across digit-sequence lengths, while considering summarized accuracy. The model compared effects across testing groups (healthy to concussion history and accurate to inaccurate) and random intercepts, representing individual average pupillary response change for each digit-sequence length.

Power analysis

To our knowledge, this is the first study to compare pupillary responses to cognitive demands within an backwards digit-span task designed to overload working memory in those with and without a concussion history. Given the exploratory nature of the study, we anticipated that 20 participants (10 in each group) was sufficient to inform future investigations in this population.

Table 3. Statistical Analysis Overview

Aims	Dependent Variable	Independent Variable	Analysis
To examine the influence of concussion history, digit-span length, and task performance (accuracy) on average change in pupil diameter during an overload digit-span task.	Average change in pupil size between the retention and baseline periods.	Group • Concussion History Behavioral Response • Accurate • Inaccurate Sequence Length • Digit-span 4-14 Interaction • Concussion History by Load (Sequence Length)	Random Intercept General Linear Mixed Model

CHAPTER IV: MANUSCRIPT

Introduction

Concussion is a complex injury induced by biomechanical forces and characterized by a cascade of relatively short-lived neurological and neuropathological changes.¹⁸ These changes result in clinical presentation of a variety of symptoms and deficits that most individuals report recovery within 10 to 14 days.¹⁸ Previous research reported that mental status, balance, and symptoms typically return to baseline within 2 days,⁴⁰ 3-5 days,^{21,41} and 7 days,²¹ respectively using validated clinical test. However, neuroimaging studies show that physiological markers of concussion persist beyond clinical normalization^{18,24} and suggest that the current battery of assessments lack a clinically useful physiological component. A clinically useful physiological assessment would improve our understanding of physiologic function across the recovery continuum and help clinicians with more objective return to play decisions.

Pupillary dilation response to a cognitive demand is a relatively low resource physiologic measure that has not currently been examined post-concussion. The pupillary dilation response is modulated by the noradrenergic Locus Coeruleus neuromodulatory system (LC-NE). Measuring pupil dilation in response to a cognitive demand is a validated indirect physiological measure of neural resource utilization.^{11,34} Pupil dilation may provide a feasible and cost-effective physiological alternative to more expensive non-invasive counterparts like electroencephalography (EEG) and functional magnetic resonance imaging (fMRI). EEG is a tool that functionally measures electrical brain cortical activity;^{6,10,12,42} whereas, fMRI is indirectly measures brain activity by detecting and analyzing the Blood-Oxygenation Level Dependent (BOLD) signal.⁴³ Pupils diameter has been reported to increase in size with cognitive demands which has been previously studied across various cognitive domains including attention, processing speed, and working memory—with each domain inducing a different cognitive demand and resource utilization.⁶ The foundational study by Beatty and Kahneman in 1966 examined

this relationship between a number recall task/digit-span task and pupil dilation and concluded that dilation was related to string length.¹³ A more recent study in TBI by Hershaw comparing percent change in pupil diameter in a cued-attention task found that those with a TBI history had a higher cognitive load compared to controlled counterparts.⁴⁴ Despite the research support for the use of the pupillary response as a physiological indicator, it is limited clinically, as pupils are sensitive to the light reflex and testing must be conducted in a light controlled environment. However, recent advancements in eye tracking technology have introduced Virtual Reality (VR) headsets with pupil tracking capabilities that could overcome this limitation.

The purpose of the study was to investigate the effect of concussion history, digit-span sequence length, and task performance on average change in pupil response during an overload digit-span task using infrared eye tracking technology embedded in a VR headset. The eye tracking technology will indirectly examine impairments in brain neural resource utilization in those with a concussion history to expand our understanding of the physiological response and post-concussion recovery. We hypothesize that those with a concussion history would exhibit greater average change in pupillary dilation response throughout the digit-span task compared to healthy participants, while considering behavioral response accuracy, indicating greater neural resource utilization to produce similar task performance throughout the task.

METHODS

Design and Participants

This quasi-experimental, cross-sectional study recruited physically active, college aged individuals with and without a concussion history. All participants were recruited from a single university, were between 18 and 30 years of age, and had normal or corrected-to-normal vision. Additionally, concussion history group participants were included if their most recent physician diagnosed concussion occurred more than 6 months prior to their participation date. Physically active was operationally defined as participating in at least 30 minutes of moderate to vigorous physical activity, 3 times per week. Concussion was defined using the Consensus Statement on Concussion in Sport from the 5th International Conference on Concussion in Sport as a functional brain injuries that are typically induced by biomechanical a cascade of short-lived neurological and neuropathological changes.¹⁸ All participants reported for one testing session and provided written informed consent prior to any study participation. The study was approved by the institution's Office of Human Research Ethics board. Upon study completion, participants were compensated \$10 for their time.

Laboratory Procedures

Participants completed a demographic and medical history questionnaire that included specific questions regarding their concussion injury history. Participants were then seated in a chair and fitted with the FOVE Virtual Reality (VR) head-mounted display (FOVE, Inc. Silicon Valley, San Mateo, CA) while wearing a disposable sanitary face cover mask. The positioning camera was vertically aligned parallel to top of the headset to ensure participants were vertically aligned and facing forward in the VR environment. Adjustments were made so 6 corneal glints were made visible participant pupils. Research assistants then started a 5-point calibration in which participants followed a highlighted point using only their eyes to accurately measure pupil size and gaze direction. Participants were then familiarized with the task by completing practice trials (all a 4-digit sequence) until study staff had determined that the participant demonstrated adequate understanding and compliance with task requirements. Participants

were instructed to keep their head still with their eyes focused on the testing wall in front of them, refrain from closing their eyes and/or looking around the room while trying to remember the digits.

Overload Digit-span Task

Task Design

The backward digit-span task designed to overload working memory used in the present study was adapted from Johnson et al.¹³ to accommodate a university sample. Our adaptation of the task utilized a randomized blocked design containing 6 consecutive testing blocks of 11 random digit-sequence lengths between 4 and 14 digits long—each including the numbers 1 through 9—as depicted in **Figure 1**. These digit sequence lengths were chosen based on previous concussion literature, in order to overload individuals within this age range beyond they're working memory capacity sequences would need to be beyond 12 digits long.⁵ A Latin Square was used to complete task presentation randomization with respect to sequence presentation order and individual digit presentation within each sequence—this approach ensured appropriate counterbalancing and exclusion of duplicate and consecutive integers.³⁸ A custom random number generator determined initial sequences for each Latin Square model. All participants completed the same task as designed, which took approximately 40 minutes to complete, including task familiarization.

Task Performance

Participants responded verbally and study personnel entered participants' responses into a digital survey—back-up audio recordings were also collected. Task performance was determined for each trial and summarized across digit-sequence lengths; specifically, trial accuracy required participants to correctly report all digits in exact reverse order and summarized accuracy required at least 50% (3 of 6 trials) accuracy.

Task Presentation

FOVE VR Headset

The digit-span task and virtual reality (VR) environment were custom developed using Unity® software and presented using the FOVE VR head-mounted display (FOVE, Inc. Silicon Valley, San

Mateo, CA) with imbedded infrared eye tracking. The head-mounted display simultaneously recorded pupillary responses in both eyes at 60Hz and a Logitech keyboard was used by participants to record when they were finished reporting all digits they remember from each trial and to initiate each trial. Two infrared illumination eye tracking sensors within the VR headset measured participant pupils by fitting an ellipse to the infrared pupil image—producing pupil radius measurements in millimeters. The headset display consisted of wide quad high definition organic light-emitting diode (WQHD OLED) 2560 x 1440, displaying up to 70 frames per second and having up to 100 degrees of viewing angle. To control for shifts in gaze, participants were asked to look straight ahead throughout testing. The task was displayed on a fixed wall within the VR environment to eliminate peripheral and distant distractions, reducing potential influence of the accommodation reflex.

Unity and Task Environment

The task presentation environment was a square shaped room (10x10x10 meters, with grey walls (R: 169, G: 169, B: 169, A: 255), built using Unity –a cross platform game development engine. To account for major confounding variables, with the largest being the pupillary light reflex, the scene utilized the “Default-Skybox” light, with an intensity multiplier of 1 and remained consistent to standardize luminance between participants. Participants’ vision within the VR environment was directed toward the forward-facing wall (3 meters away), on a blank canvas measuring 0.6m X 0.34m, at a scale of 0.083m. Stimulus cue text on the canvas was standardized across all aspects of the task using Arial text (size= 40, color= R:46 G:46 B:46 A: 255) and scaled in the x-axis by 0.0015, in the y-axis by 0.0015, and in the z-axis by 0.005 to ensure the best clarity. Data recording was initiated by study personnel prior to any stimulus presentation.

Stimulus Presentation

Participants initiated each trial by pressing the “right direction arrow.” Each digit-span trial began with a baseline/accommodation period that lasted 3 seconds, indicated by an “X”. Each digit was then displayed at a rate of 1 digit per second. Final digit presentation for each trial, was followed by a 3 second retention period before participants were prompted to verbally recall the numbers in the reverse order in

which they were presented for that trial. Stimulus presentation is shown in sequence in **Figure 1**. When the participant finished their response, they pressed the “space bar” to notify that they finished their response and then they pressed the “right directional arrow” to proceed to the next trail. Participants continued through all 66 trials until reaching the ‘end of trial’ slide, without reinforcement from research assistants.

Data Reduction and Processing

The variables obtained from the raw data included left and right pupil radius (mm) and testing time (s) each sampled at 60Hz. Timestamp were recorded for: 1) trial number (1-66), 2) span length (4-14), 3) digit presentation (s), 4) retention X display, 5) response box display, and 6) participant finished response. Participant data files were directly exported into a spreadsheet and imported into Matlab (MATLAB and Statistic Toolbox Release 2017b, The MathWorks, Inc., Natick, MA, USA). The study team used a custom Matlab program adapted from the suggested procedures for processing pupil size data by Kret and colleagues in 2018³⁹ to complete all pupil size data processing and reduction. The adapted processing program employed 6 procedural levels as follows: 1) raw pupil radius measures for each eye were converted into diameter and averaged across both eyes; 2) raw data were prepared for filtering by removing identified blinks (recorded as .000mm or .0001mm); 3) additional artifacts were identified and removed (e.g., signal noise, longer eye closures, etc.) to identify valid samples (i.e., pupil measurements not interrupted by blinks or noise); 4) the remaining valid samples were then up-sampled and smoothed using linear interpolation; 5) pupil response regions of interest were segmented (i.e., the last 15 samples of each baseline period and the first 120 samples of each retention period) and individually averaged; and 6) segment averages were used to determine pupil size change for each trial (i.e., average pupil size during retention – average baseline pupil size).³⁹

Processed data (i.e., change in pupil diameter for each trial) were exported from Matlab and reduced using SAS 9.4 (Cary, NC). Trails were binned by sequence length (4-14) and averaged within each sequence length (6 trials per sequence length) to obtain 11 average change in pupil diameter per sequence length. Ensembled average changes in pupil diameter were calculated across all trials and

served as our dependent variable (primary outcome) compared between healthy and participants with a concussion history summarized in **Table 3**.

Statistical Analysis

SAS 9.4 (Cary, NC) was used to analyze processed data. Change in pupillary diameter was quantified using the subtraction method whereby the averaged pupil size in the first 120 samples of the retention period was subtracted from the averaged 15 last samples from the baseline period to obtain our average change in pupil diameter per trial. Trails were binned by sequence length (4-14) and averaged within each sequence length (6 trials per sequence length) to obtain 11 average change in pupil diameter per sequence length. Ensembled average changes in pupil diameter were calculated across all trials and served as our dependent variable (primary outcome) compared between healthy and participants with a concussion history summarized in **Table 3**.

Continuous data were summarized using means and standard deviations for all as described in **Table 1**. Generalized linear mixed model examined average pupil response changes during the retention period compared to baseline, across digit-spans, considering for accuracy. The model compared effects across testing groups (healthy to concussion history and accurate to inaccurate) by sequence length with random intercepts representing individual average pupillary response change during the retention period for each digit-span

RESULTS

A total of 20 individuals participated in data collection (mean age = 21.9 ± 2.1 years and 50% male). **Table 2** provides group demographic data. In the healthy participants, 90% achieved test accuracy at 4 digit-spans, 60% achieved test accuracy at 5 digit-spans, 50% achieved test accuracy at 6 digit-spans, 20% achieved test accuracy at 7 digit-spans, 10% achieved test accuracy at 8 digit-spans, and none achieved test accuracy between the 9 to 14 digit-spans. In the concussion participants, 80% achieved test accuracy at 4 digit-spans, 80% achieved test accuracy at 5 digit-spans, 50% achieved test accuracy at 6 digit-spans, and none achieved test accuracy between 7 and 14 digit. Average change in pupil diameter

for both concussion history and accuracy groups across digit-span sequence length are summarized in **Table 2**. The average numbers of years since the last concussion incident for concussion history participants was 5.08 ± 3.67 years, summarized in **Table 2**. Test statistics for the generalized linear mixed model are summarized in **Table 4**. These data suggest no main effects for concussion history ($F_{1,18}=0.01$, $P=0.906$) or accuracy ($F_{1,17}=0.02$, $p=0.879$). There was a main effect of sequence length ($F_{10,179}=3.34$, $p<0.001$). This main effect was driven by significant differences between spans 5 and 13, 5 and 14, 7 and 14, as well as 10 and 14; whereby, spans at the higher load had smaller average change in pupil diameter. We did not observe a significant interaction effect between concussion history and digit-span load (length); however, this omnibus finding was trending towards clinical significance ($F_{10,179}=1.85$, $p=0.056$).

DISCUSSION

The purpose of the study was to investigate the effect of concussion history, digit-sequence length, and task performance on average change in pupil response during a digit-span task designed to overload working memory. Concussion history did not significantly affect average change in pupil diameter across all cognitive demands (i.e., digit-sequence lengths) of the task, while considering summarized task performance. These results may suggest that having a concussion history does not influence long term cognitive efficiency as determined by pupillary response changes to varying cognitive demands. However, in some instances where the cognitive demand was greater, the average change in pupil diameter decreased due to cognitive overload and task disengagement. Task accuracy also had no significant effect on average change in pupil diameter, signifying that when a participant is accurate vs. inaccurate for a span length, there is no change in average pupil diameter and no difference in neural resource utilization. Finally, concussion history and cognitive demand were trending towards clinically significant, highlighting potential interaction given sufficient sample size. These findings overall do share similar findings to previous literature concerning a drop in change in pupil diameter at higher sequence lengths, suggesting participants reached cognitive capacity and disengaged with the task. Findings suggest that this type of task may be useful in indirectly measuring neural resource utilization.

Concussion history had no significant effect on average change in pupil diameter, which suggests proper brain resource utilization recovery and efficiency within our concussion history participants. Physiologically, Kamins et al. summarized that physiological changes after a concussion have been reported in EEG studies up to 4 years post-concussion, up to 9 months post-concussion in transcranial magnetic stimulation, and up to 23 months post-concussion in fMRI.²⁴ As many of our concussion history participants reported their most recent concussion being 5.08 ± 3.67 years ago, which exceed previously proposed time frames of reported changes and may account for the lack of significant effect. Additionally, our study was limited in the number of recruited participants and a larger sample would lead to a more concrete conclusion.

Our current findings contradict Hershaw's conclusions in which he investigated pupillary response of 76 participants to a cognitive demand (cued attention paradigm) in individuals with a concussion history (median years since previous concussion was 6.91 years) and concluded that those with a prior history of a traumatic brain injury had a greater cognitive demand during a controlled attention task.⁴⁴ Although our results differ in findings, Hershaw's study investigated a different dependent variable (baseline pupil diameter and variability) and a different cognitive demand (cued attention task). These differences in task design, outcome measure, and time since previous concussion could account for discrepancies between our results. Future studies should investigate outcomes reported in Hershaw's study.

Our results also concluded that sequence lengths did not elicit a general increase in average pupil diameter as summarized in **Table 4**. This finding contradicts previous studies that reported general increases pupil diameters up until reaching their memory capacity.⁴⁵ Studies by Granholm, Rondeel, Klingner, and Kahya investigated pupil response in various populations using varying cognitive demands. Despite differences, they all reported that with increased task difficulty, there was an incremental increase in pupil size.^{9,16,17,36,45} Despite not finding the same incremental increase, longer sequence lengths did have a decreased average change in pupil diameter compared to shorter sequence lengths. **Table 1** summarizes the results and show a significant decrease in overall average pupil diameter from sequence

lengths of 5 to 13, 5 to 14, 7 to 14, and from 10 to 14. Similar findings were reported by Johnson et al. who found that children tasked with a forward digit-span test from the Wechsler Intelligence Scale for Children had constricting pupil sizes after the 6 sequences. He suggested that the children had reached their cognitive limits and began to disengage with the task.⁴⁵ Likewise, the drop in average change in pupil diameter reported could be due to participants reaching their cognitive limits and disengaging with the task at higher sequence lengths. Future studies could encourage more task engagement by decreasing overall task difficulty.

Accuracy was found to have no significant effect with average change in pupil diameter, further suggesting no effect on neural resource utilization. This implied that our participants, regardless of whether they were able to meet our accuracy standards at a specific span length, utilized the same amount of resources to accomplish the task. Additionally, the healthy group outperformed the concussion history group in relation to task accuracy; however, these results were not significantly different and both groups decreased in accuracy as task difficulty increased. Further examinations including larger sample sizes may provide additional information with respect to the influence of task performance on pupil response changes. Additionally, researchers should consider accounting for accuracy by trial to further describe this relationship.

Despite concussion history having no significant main effect, there is a trending interaction between concussion history and sequence length. Understanding of this interaction is still preliminary, but may suggest that group differences could be isolated at certain sequence lengths or cognitive demands. Granholm demonstrated this when concluded that amnesic single domain-mild cognitive impairment participants had similar cognitive task performance but greater pupil dilation when compared to cognitively normal participants at lower cognitive demands. This suggest a greater compensatory effort and decreased neural system efficiency at low cognitive demands as opposed to high cognitive demands.¹⁶ More participants beyond the current 10 concussion participants are needed to fully understand this interaction.

Clinical Significance: Why does this matter?

Ultimately, pupillary response to a digit-span inside a VR environment may add viable knowledge to the current literature on the physiological considerations post-concussion injury. McCrory and colleagues list the current measures of physiological change after concussions into 9 different categories: 1) functional MRI (fMRI), 2) diffusion tensor imaging (DTI), 3) magnetic resonance spectroscopy (MRS), 4) cerebral blood flow (CBF), 5) electrophysiology, 6) heart rate, 7) exercise performance, 8) fluid biomarkers, and 9) transcranial magnetic stimulation.¹⁸ These physiological assessment tools commonly used in concussion literature are essential tools in exploring concussion recovery. However, many are limited in the availability and cost. The pupillary dilation response as an indirect measure of neural resource utilization has been reliably used in psychophysiology research and this study serves as one of the first studies to control for the pupillary light reflex. Because the constricting sphincter muscles of the pupil are influenced from light stimulus, cognitive, and autonomic activity, controlling for the light reflex is crucial in controlling environmental confounds. This would make the application more versatile for clinicians who work in various sport settings. The current study support the use of pupil response as an indirect measure of brain resource utilization and help in understanding physiological recovery in concussion populations.

Previous research suggests pupil diameter is a reliable measure of brain utilization as first described by Beatty who investigated pupillary response to recalling phone numbers.¹³ Murphy et al. contributed to this finding and confirmed that pupil dilation as a non-invasive index of LC activity by comparing it to BOLD response in fMRI. Joshi et al. then expanded on this relationship between resource utilization and pupillary response through the use of electrical microstimulation across different brain regions in monkeys which resulted in reliable increases in pupil diameter.³⁴ This supports how pupil diameter can reflect neural activity of the (LC) in monkeys³⁴ and that the pupillary response is a reliable tool for assessing cognitive demand.

Limitations

The study was limited in the number of participants. The average time since the last previous concussion was 5.08 ± 3.67 years which would increase the potential recovery time and decrease potential differences between groups. Future studies should examine the effects of multiple concussions and control for recovery time. The protocol was also number oriented, which may have given an advantage to students with an arithmetic background. Testing length may have led to a decrease in effort across the protocol due to disengagement; however, we aimed to control for this by allowing participant initiated adequate breaks in between trials. Although some studies that have found significant differences between healthy and concussion groups using pupillary response, these studies also looked at a drastically different cognitive populations, like Alzheimer's¹⁶ that had significant known differences in cognitive function.¹⁶

Conclusions

Pupillary response to a cognitive task is a relatively new tool in the concussion literature. Our findings conclude that concussion history has no significant effect on the average change in pupil diameter during an overload digit-span task. However, digit-span length, at specific loads, had significant effects on average change in pupil diameter during an overload digit-span task, consistent with findings from other sources. These findings contradict one recent article by Hershaw⁴⁴, but are consistent with some other previous literature on cognitively impaired states and future studies could potentially utilize this tool in better understanding the physiological recovery post concussions.

This study provides some preliminary support for the potential for VR headset imbedded with infrared tracking in assessing changes in pupil size and indirectly measuring cognitive function, consistent with previous literature utilizing more sophisticated techniques. Future studies should continue the use of this tool in concussion protocols and investigate participants with more recently recovered concussion participants, controlling for concussion time of recovery. Additionally, other variables like working memory capacity and dilation rate have been investigated in other literature and should also be considered as well. Future studies should also investigate this resource utilization using more common cognitive task like the SAC to see if these assessments are sensitive in assessing cognitive ability. Longitudinal studies

should also investigate changes in utilization between age groups (high school to college), and changes from baseline throughout concussion recovery.

Acknowledgements

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MANUSCRIPT TABLES AND FIGURES

Table 1. Average change in pupil diameter (mm) between healthy and previously concussed participants and across accurate and inaccurate responses at each sequence lengths

	Mean (SD) Change in Pupil Diameter				
	Group		Accuracy		
Sequence Length ¹	Healthy	Concussion History	Accurate ²	Inaccurate	Total Sample
4 digits	0.12 (0.21)	0.16 (0.18)	0.13 (0.20)	0.19 (0.12)	0.14 (0.19)
5 digits	0.33 (0.34)	0.19 (0.13)	0.31 (0.25)	0.13 (0.24)	0.26 (0.26)
6 digits	0.23 (0.32)	0.11 (0.11)	0.16 (0.25)	0.18 (0.24)	0.17 (0.24)
7 digits	0.26 (0.32)	0.18 (0.15)	0.37 (0.39)	0.20 (0.24)	0.22 (0.25)
8 digits	0.10 (0.51)	0.20 (0.24)	1.08 (no SD, n=1)	0.09 (0.33)	0.15 (0.39)
9 digits	0.14 (0.36)	0.15 (0.19)	n/a – n=0	0.14 (0.28)	0.15 (0.28)
10 digits	0.23 (0.40)	0.14 (0.15)	n/a – n=0	0.18 (0.30)	0.18 (0.30)
11 digits	0.17 (0.40)	0.11 (0.17)	n/a – n=0	0.14 (0.30)	0.14 (0.30)
12 digits	-0.04 (0.25)	0.17 (0.15)	n/a – n=0	0.06 (0.22)	0.06 (0.22)
13 digits	0.09 (0.34)	0.00 (0.19)	n/a – n=0	0.04 (0.27)	0.04 (0.27)
14 digits	-0.04 (0.31)	0.04 (0.25)	n/a – n=0	0.003 (0.28)	0.003 (0.28)
Overall	0.14 (0.35)	0.13 (0.18)	0.23 (0.28)	0.12 (0.28)	0.14 (0.28)

¹Sequence length refers to the number of digits at each provided at each level.

²Participants were considered accurate for a digit-span length if they reported all digits in the correct sequence for at least 3 of the 6 trials per digit-span. Percent of participants who were considered accurate at each sequence length is presented in the results text. No participants met the definition for accuracy for any sequence length beyond 8 digits.

Table 2. Demographics from the Study Sample

	Sex		Time Reported Since Last Previous Concussion (Years)	Number of Concussions	Age (years)
	Male	Female	Mean (S.D.)	Mean (S.D)	Mean (S.D.)
Healthy Controls (n=10)	4	6	n/a	n/a	21.9 (2.1)
Previously Concussed (n=10)	6	4	5.08 (3.67)	2.2 (1.41)	
Overall (n=10)	10	10			

Table 3. Statistical Analysis Overview

Aims	Dependent Variable	Independent Variable	Analysis
To examine the influence of concussion history, digit-span length, and task performance (accuracy) on average change in pupil diameter during an overload digit-span task.	Average change in pupil size between the retention and baseline periods.	Group - Concussion History Behavioral Response - Accurate - Inaccurate Sequence Length - Digit-span 4-14 Interaction - Concussion History by Load (Sequence Length)	Random Intercept General Linear Mixed Model

Table 4. Statistical results for concussion history group, sequence length, digit-span accuracy, and the Interaction effect of concussion history group* digit-span sequence length

Effect	<i>d</i>	df	F-Value	P-Value
Concussion history group	-.038	1,18	0.01	0.906
Digit-span sequence length		10,179	3.34	<0.001*
Digit-span accuracy	-.393	1,17	0.02	0.879
Concussion history group & digit-span sequence length interaction		1,179	1.85	0.055

* Indicates a significant main effect

Figure 1. Task design

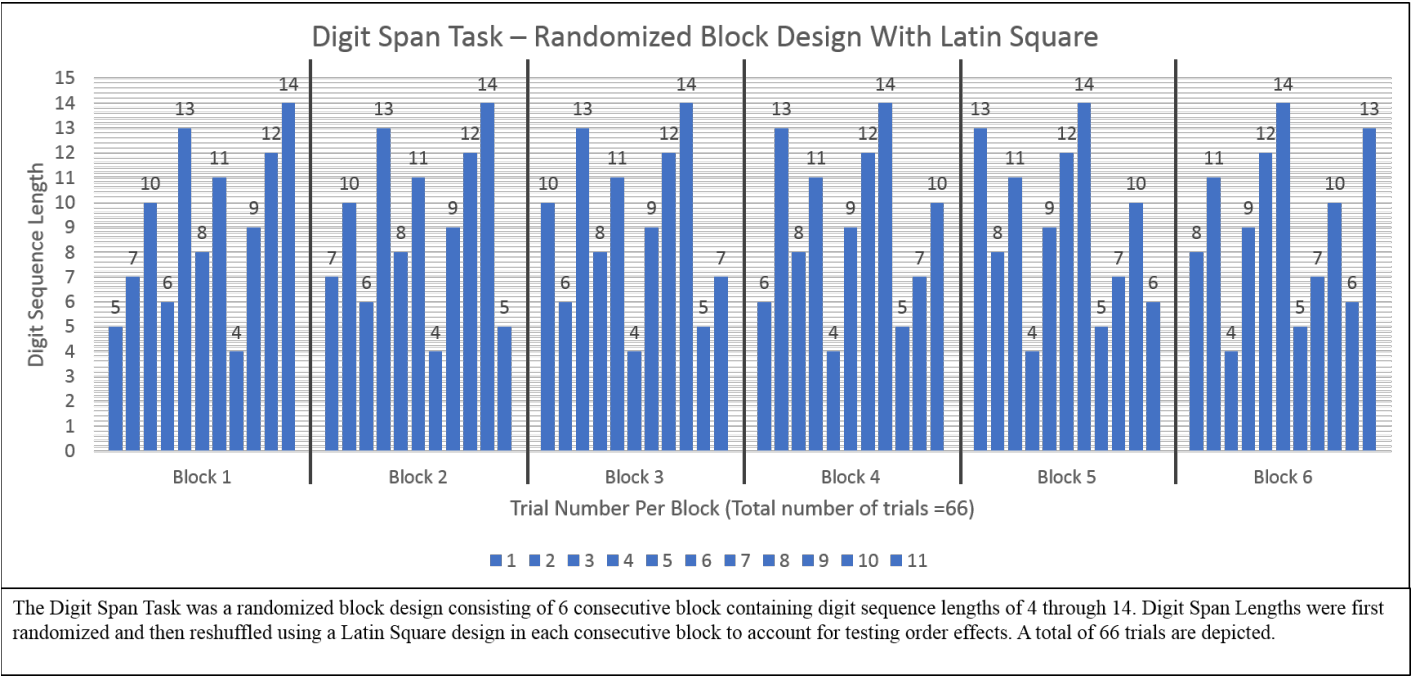
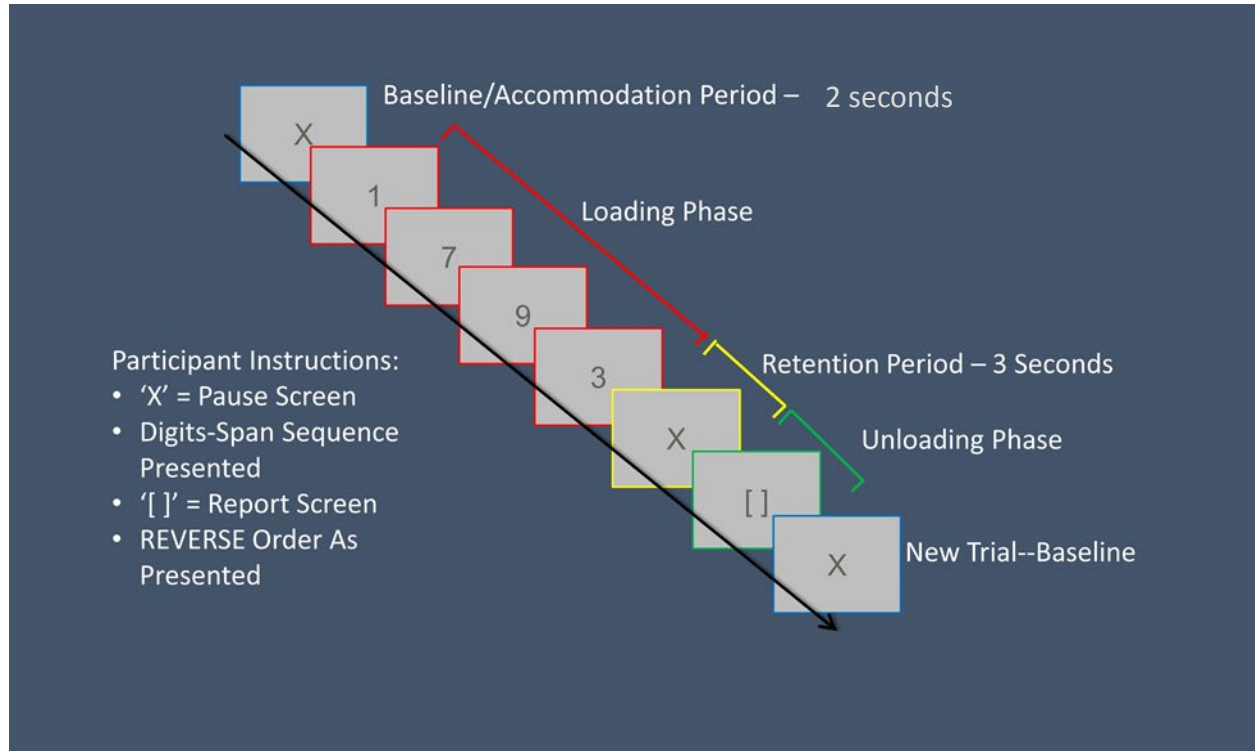


Figure 2. Stimulus Presentation For the Digit-Span Task



APPENDIX A

Questionnaire

Subject ID: _____

Date: _____

Start Time: _____ AM/PM

End Time: _____ AM/PM

Demographics	
Age	
Sex	

Demographics	
Height	
Weight	

Medical History		
Have you been diagnosed with a concussion?	Yes	No
If yes, how many?		
Have you been diagnosed with ADHD?	Yes	No
If yes, list medications you are taking:		
Have you been diagnosed with any other learning disabilities?	Yes	No
If Yes, please elaborate:		
Do you have a history of consistent migraines?	Yes	No
How many hours of sleep do you typically get?	Sun- Thurs? hrs.	Fri-Sat? _____ hrs.
Have you been diagnosed with Anxiety?	Yes	No
If yes, list medications you are taking:		
Have you been diagnosed with Depression?	Yes	No
If yes, list medications you are taking:		

Concussion History				
	Date of prior concussion (mm/yyyy)	Time to recovery	Loss of consciousness?	Mechanism of injury
1		☐ Within 2 weeks ☐ Within a month ☐ > One month ☐ > One year	☐ Yes ☐ No	☐ Sports? _____ ☐ Other? _____
2		☐ Within 2 weeks ☐ Within a month ☐ > One month ☐ > One year	☐ Yes ☐ No	☐ Sports? _____ ☐ Other? _____

Sport History

From birth indicated, have you participated in any of the following organized sports?

(Check all that apply)

Sport	Level of sport Circle all that apply	How many years did you play?
Football	High School Club College	
Hockey	High School Club College	

Soccer	High School	Club	College	
Lacrosse	High School	Club	College	
Baseball	High School	Club	College	
Basketball	High School	Club	College	
Other: _____	High School	Club	College	

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CHAPTER V: SUMMARY & REFLECTION

Purpose

The purpose of this study was to examine the effect of concussion history, digit sequence length and task performance pupillary response magnitudes during a backward digit-span task. A total of 20 participants were included in the study, 10 healthy and 10 with a concussion history, and completed a digit-span task in a virtual reality environment. We will continue with this line of work until we meet 50 participants (25 in each concussion history group), beyond this Honor's Thesis project.

Summary Aim

Aim: To examine the influence of concussion history, digit-span length, and task performance (accuracy) on average change in pupil diameter during an overload digit-span task.

Hypothesis: We anticipate that those with a concussion history would exhibit greater average change in pupil diameter during the digit-span task compared to healthy participants, while considering for behavioral response accuracy, indicating greater neural resource utilization to produce similar task performance.

Results: Our results concluded that concussion history had no effect on average change in pupil diameter, but that digit-span load did have a significant effect and there was a trending interaction between concussion history and load. As many of our concussion history participants exceed previously proposed time frames of reported changes, this may account for the lack of significant effect. Additionally, our study was limited in the number of recruited participants and future work will continue to recruit up to 50 participants to have a solid conclusion and to fully determine what interaction effects may exist.

Lessons Learned

Reflecting on this past year, my honor's thesis has been a long and enduring process that challenged more than just my knowledge, but my patience, resilience, and understanding of what research

looks like. I was challenged and learned so many things about myself personally, in my struggle to manage so many responsibilities this entire year. I had learn to humble myself and accept the fact that letting go was sometimes better than holding onto my worries, and in those times of difficulty, I learned how to take a breath and push forward the best I could. This was a hard lesson to learn, as there were so many times where the project was not progressing and it was frustrating to handle every setback that occurred. Every obstacle tested my patience and I often contemplated on giving up on the project. However, it was because of the hardships and struggles that we encountered with this project that made it worthwhile and satisfying. In fact, it's quite remarkable how in less than a year, an unknown headset with little instructions, would become a full functioning assessment, and I'm excited to continue the project beyond the end of my thesis. Ultimately, this experience not only pushed my time management abilities, but honed my mentality and resilience to the difficulties faced before me.

Teamwork was also one of the greatest virtues that made this project successful. None of this would be possible without the hard work and effort from everyone who helped in troubleshooting our development problems, our recruiting and task management, statistical analysis, and especially in encouraging us to keep moving forward despite the setbacks. I learned that working cohesively to a common goal was difficult to manage but essential as research is a team effort that cannot be accomplished alone.

Pertaining to the research process itself, I gained a stronger understanding of the diligence, rigor, but also the reward that comes with it. Learning how to cipher through an area of research of interest, create an assessment based on past literature, develop a research question, submit a grant, write an IRB submission, run a pilot study, and finally organizing and managing data collection was a blur of deadlines and challenges. In the process of it all, some of the lessons that I took out of it was that nothing ever goes as planned and I should be prepared to handle it. One of the biggest hurdles of the project was getting the company to give us access to reading the pupil size. It took us months to get access and to develop a system that gave us the measurements we wanted, which pushed back our project much farther than what we were hoping for. I learned that sometimes, things will fail and you have to sometimes just try again.

During the calibration process, we had to constantly troubleshoot how to get good readings as we struggled to get the system to work sometimes. Finally, I learned that it is important to review my own work time and time again. When we were running the first pilot test for the system, I had to build the code to run the system, and I forgot to double check my work and make sure that it would run. Unfortunately, the pilot testing went on without me and crashed, leaving us delayed in the development process. Had I double checked my work, I would have not wasted time with our pilots.

Future Research

Future studies should continue the use of this tool or similar in concussion protocols and investigate participants with more recently recovered concussion participants, controlling for concussion time of recovery. A follow-up test-retest reliability study would also help to confirm the reliability of the outcome. Additionally, other variables like working memory capacity and dilation rate have been investigated in other literature and should also be considered as well. Future studies should also investigate this resource utilization using more common cognitive task like the SAC to see if these assessments are sensitive in assessing cognitive ability. Longitudinal studies should also investigate changes in utilization between age groups (high school to college), and changes from baseline throughout concussion recovery.