PHYSIOLOGICAL CORRELATES OF WORKING MEMORY BEHAVIOR AND COGNITIVE EFFICIENCY: IMPLICATIONS FOR CONCUSSION MANAGEMENT

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A dissertation submitted to the faculty at the University of North Carolina at Chapel Hill in partial fulfillment of the requirements for the degrees of Doctor of Philosophy in the Human Movement Science Curriculum in the School of Medicine.

Chapel Hill
2020

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ABSTRACT

Christina B. Vander Vegt: Physiological Correlates of Working Memory Behavior for Cognitive Efficiency: implications for concussion management
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Cognitive efficiency—characterized via robust behavioral and physiological response dynamics, may provide clinically meaningful information with respect to dynamic concussion injury response and return to play considerations. Moreover, a feasible and ecologically valid method to assess dynamic behavioral and physiological metrics is also needed to best characterize cognitive efficiency in athletes and soldiers at a greater risk for concussion. This project was designed to examine the clinical utility and feasibility of a cognitive efficiency assessment designed to be completed within a virtual reality environment, which may hold significant implications for improved concussion clinical management. As such, we aimed to first further understand the relationships between heart rate variability and pupillary response as physiological correlates of digit-span task behavior in the context of cognitive efficiency, then to examine the effects of prior concussion on these responses. Additionally, we applied innovative, reliably sound, and validated psychophysiological methods using a feasible instrumentation option (virtual reality headset with pupillometry and heart rate monitor watch and strap). We feel these methodological considerations allowed us to best address our study aims and inform future directions of this line of inquiry to have direct ecologically valid applications. Collegiate club sports athletes, (N=59; 40% with a concussion history; age = 20.48 ± 1.86 years; 58% male), completed a backwards digit-span task (20 trials) in a virtual reality environment while recording pupil size. Linear mixed effects models showed a significant effect of cognitive load (digit sequence-length) on pupillary response ($F_{4,232}=3.67$, $p=0.006$). Negligible effects were seen in task performance, heart rate variability or concussion history $p<0.05$ Pupillary response shows potential in informing cognitive load and efficiency in applied settings, using VR and eye tracking technology. Future investigations should consider participants' concussion history variability.

ACKNOWLEDGEMENTS

I would like to express my deepest appreciation and gratitude for the many people who helped to make this work possible. To my mentor and friend Johna Register-Mihalik: thank you for your unyielding support and guidance throughout this journey, both personally and professionally. Your devotion to your faith, family, and career is unparalleled, and balanced with such grace. I would also like to thank my committee members, Greg Appelbaum, Adam Kiefer, Jason Mihalik, and Kevin Guskiewicz, for sharing your time, wisdom, and expertise with me. Each of you contributed such unique and vital perspectives to support the success of this project, and I am grateful for your guidance.

I would like to thank my research assistants Kou Yang and Emily Barron for their time and dedication to the success of this project. I'd also like to thank Ryan MacPherson for his assistance modifying the digit-span task to the hardware and software platforms used in this study.

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ABBREVIATIONS

ANS	Autonomic Nervous System
CBF	Cerebral Blood Flow
DLPFC	Dorsolateral Prefrontal cortex
fMRI	Functional Magnetic Imaging
HR	Heart Rate
HRV	Hear Rate Variability
ImPACT	Immediate Post-concussion Assessment and Cognitive Test
PFC	Prefrontal cortex
RTP	Return to Play
SOT	Sensory Organization Test
SRC	Sport-Related Concussion
VR	Virtual Reality
WM	Working Memory

CHAPTER 1: INTRODUCTION

1.1 Overview of Concussion Injury and Associated Deficits

Repetitive head impact exposures and concussion are major athletic health concerns[1] for which the physiological response and recovery dynamics are poorly understood. Concussion among college aged athletes participating in National Collegiate Athletic Association (NCAA) sports is estimated around 10,560 nationally with the overall concussion rate of 4.47 per 10,000 athlete-exposures.[2] Recent literature regarding the effects associated with repetitive head impact exposures remains unclear. However, studies suggest greater functional impairments and potentially long-term structural changes in those who have experienced multiple prior concussions.[3–5] Contact and collision sport athletes (e.g., American football, rugby, men's lacrosse, etc.) are likely at the greatest risk of such deficits, as they experience relatively high numbers of head impact exposures and greater concussion rates over the course of a single season and their athletic careers.65 [5,6]Understanding the relationships between clinical and physiological response dynamics following repetitive head impact exposures and concussion is therefore important to ensure proper care and management.

Neuropathophysiological changes following concussion and repetitive head impacts—and the associated clinical impairment manifestations[7,8] do not adhere to fixed response and recovery timelines. As such, these changes are difficult to fully characterize with currently available clinical measures. The recommended concussion assessment paradigm[6,10,119] uses a variety of clinical measures (e.g., symptom inventory, motor control, neurocognitive screening, etc.) known to demonstrate ceiling effects[10] and rapidly lose signal detection[11,12] in the days following concussion injury.[13–15] The current battery functions to guide clinical decisions such as return to play (RTP), often made following clinical measure normalization. Clinical normalization for collegiate aged athletes typically occurs within 7 to 10 days following injury.[16,17] Recent neuroimaging studies however, report prolonged physiological impairment exhibited by increased spatial and temporal activity disproportional to task demands.[8,18,19] Moreover, the sole monitoring of performance deviations relative to baseline or normative values, further limits dynamic clinical and/or physiological response characterization.[8,9,18]

Prolonged post-concussion physiological impairment has been described as compensatory neural resource utilization to meet cognitive demands, which previous studies have described as neurophysiological cognitive inefficiency. [7,8,19] These compensatory mechanisms are posited to provide some explanation for early clinical normalization in performance-based outcomes.[8] Studies using advanced neuroimaging measures have contributed valuable evidence regarding compensatory physiological mechanisms, and suggest that they may result in prolonged neural vulnerability and associated negative consequences (e.g. increased risk for neurodegenerative disease, neuropsychiatric deficits, etc.).[8,18,19] However, the poor ecological validity (e.g., cost, time, availability) of these studies,[18] limit our ability to further elucidate neurophysiological responses and recovery associated with various head trauma exposure types (e.g., documented concussion injury[7,8], repeat concussions,[3,4,18] repetitive head impacts,[6,20,20] and participation in a single season of collision sport play[6,21]). Overall, these issues highlight the need for more ecologically valid assessment options to better capture concussion injury response dynamics.[8,11,12]

Recent studies concerning the clinical concussion assessment paradigm have suggested the need for critical modifications to include more robust, reliable, and validated measures that capture the dynamic nature of post-injury clinical and physiological responses. Adaptations to the current assessment battery that fulfill these needs may support clinical decision-making regarding athlete/soldier readiness for return to activity and/or duty, potentially mitigating negative and injurious consequences associated with premature return. [22,23]

1.2 Cognitive Efficiency Following Concussion

Current concussion assessments lack neurophysiologic and performance-based measurement options that are feasible and demonstrate adequate utility, to fully inform cognitive efficiency. Combining these two measurements and examining them in the context of task demands allows extends their meaning to that of efficiency—beyond effectiveness. Whereby, efficiency refers to the assessment of the dynamic interplay between elements of cognitive task demands (task difficulty), physiological characteristics associated with cognitive effort and capacities, and performance-based outcomes[24,25] is not represented in the current clinical battery. Current neurocognitive assessments within the concussion assessment battery statically capture two of these three elements—i.e., task demands (design and difficulty) and performance outcomes. However, important adaptations within these two elements may be necessary to improve our understanding of task performance dynamics as they relate to cognitive efficiency following concussion. For example, task performance on the SAC's digit-span task is one of the most sensitive to acute concussion.[11,12] Previous findings in healthy adult samples suggest that relatively minor (though necessary) task adaptations (i.e., adapted task demands, scoring, and administration parameters) elicit better dynamic working memory processes and should therefore be considered.[26,27] Additionally, feasible measurement options for the third element of cognitive efficiency—physiological characteristics of cognitive effort and capacities—have yet to be established.

An objective physiological marker for neural resource utilization and/or cognitive effort would need to demonstrate sensitivity, dynamic responsivity, and robustness, with discriminatory abilities across varying levels of cognitive efficiency associated with the concussion response acutely and in the longer-term. Specific consideration should also be given to the clinical feasibility of potential metric solutions to best examine their overall utility.

1.3 Physiological Correlates of Cognitive Efficiency

Pupillary responses have demonstrated significant associations with various cognitive and emotional constructs, to examine information processing. Research efforts in cognitive pupillometry specifically, have shown that pupillary responses reflect changes in cognitive processing load.[28,29] As such, pupillary response dynamics serve as an indication of the allocation of neural resources, relative to variations in cognitive load and information processing. Cognitive pupillometry metrics have further demonstrated associations with advanced spatial and temporal measures of neural activation using electroencephalography (EEG) and functional magnetic resonance imaging (fMRI).[29–33] Concurrent validation with advanced imaging concluded that cognitive pupillary response metrics are modulated by the noradrenergic Locus Coeruleus neuromodulatory system (LC-NE) with widespread projections that extend to nearly all cortical and subcortical regions.[30,33]

Autonomic Nervous System (ANS) contributes dual ciliary innervation of both sympathetic and parasympathetic branches, to regulate pupil dilation and constriction mechanisms respectively.[31,33] Pupillary response dynamics across cognitive constructs generally follow that of a within trial incremental pupil dilation (sympathetic activity) response relative to task demands and/or difficulty until the point of capacity, at which point pupil size plateaus or constricts.[34–36] As such, researchers often examine pupillary responses to the varying cognitive demands of digit-span tasks, with respect to working memory and working memory capacity.[28,35,70 28,32,35,37–39] Pupillary response dynamics to cognitive demands may therefore prove meaningful with respect to physiological and task performance dynamics following concussion, given its valid association with increasing cognitive demands across various cognitive processes.[31,32,37,40] including those often affected by concussion (e.g., attention, processing speed, and working memory) and ANS regulatory involvement considering known dysfunction in both sympathetic and parasympathetic activity following injury.

Pupillary response dynamics to the backwards digit-span task (**Figure 1.1)** include incremental dilation as each digit is encoded, reaching maximum dilation following final digit presentation (while manipulating and reordering) or at the point of encoding capacity, whichever comes first.[28,32,35] Upon recall, pupils recover to pre-trial size.[28,32,37] Pupillary response magnitudes are often summarized for each trial as the average pupil size change following final digit presentation, while encoding and reordering numbers, compared to pre-trial size—where longer digit sequence lengths elicit greater dilation responses until the point of capacity.[28,34] Outcomes represent individual neural resource utilization for a given sequence-length—which may inform individuals' cognitive efficiency.[30,35] Current literature supports resultant decreases in performance with increased resource utilization via cognitive effort in response to higher levels of task difficulty[29], also seen in dual-task concussion literature.[3931,41] Therefore, incorporating simultaneous pupillary response recording during the digit-span task may be a meaningful physiologic metric for concussion assessment, with respect to the individual characteristic (capacity) of cognitive efficiency.[41,42]

Pupillary response dynamics may be useful to inform cognitive efficiency in isolation—though recent psychophysiological investigations suggests monitoring multiple metrics when examining associations between physiological and behavioral outcomes associated with complex cognitive constructs and processes.[29,34,41,42] Juxtaposed with concussion literature, recent systematic reviews regarding the physiological response to injury concludes that a single 'perfect metric' that accounts for the complexities associated with concussion is highly unlikely—rather, a combination of measures may be more appropriate.[8,18] Moreover, physiological metrics regulated by the ANS require careful consideration given the many latent variables that may contribute to variability in response dynamics (e.g., stress, emotional response, etc.).[41,43–45] Heart rate variability (HRV) may be a meaningful supplement to pupillary response during digit-span task completion to inform cognitive efficiency, as an index of neurocardiac function associated with the ANS.[44,46,47] Limited research is available to fully describe HRV and ANS dysfunction following concussion, though most studies report HRV response dynamics to physical activity/movement task demands.[46–49] The majority of HRV investigations in the concussion space focus on persistent cerebral metabolic deficiencies related to reduced cerebral blood flow at rest, and threshold determinants for exercise tolerance testing.[18,50,51] Applications of this measure to estimate potential thresholds for cognitive load are unclear.

Cognitive neuroscience data clearly acknowledges environmental factors that influence current pupil size monitoring. Specifically, environmental luminance and accommodation responses, have previously hindered scientific inquiry progression in this space and other physiologic outcomes, with respect to internal validity.[32] Recent advancements in virtual reality (VR) head mounted displays with embedded infrared eye tracking technology provide a controlled, portable, and cost-effective solution to this problem and improved ecological validity for pupillary response parameter assessment to inform cognitive efficiency. Examination of HRV as it relates to ANS activity may serve as a useful supplement to describe the resolution of post-concussion compensatory mechanisms and physiological impairment, combined with an ecologically valid marker for neural resource utilization.[46,52,53]

1.4 Problem Statement

Clinicians and researchers need objective measures to better characterize behavioral and physiological response dynamics associated with cognitive inefficiency following concussion. While cognitive efficiency can be described relative to various cognitive processes, working memory is most often examined with respect to relationships between task demands and available cognitive resources. Working memory is also one of the most common cognitive impairments following concussion—where associated post-injury clinical measures (digit-span task) demonstrate high diagnostic sensitivity.[11,12]

Clinical task performance and physiological response dynamics associated with cognitive efficiency are difficult to characterize following concussion due to rapidly deteriorating signal detection and poor ecological validity of current clinical assessments and advanced neuroimaging modalities.[8,18] Dynamic cognitive efficiency characterization via clinical task performance and physiological and metrics may better inform concussion recovery response dynamics. Results may therefore have important implications for improved concussion clinical assessment and management regarding readiness to return to athletic and or military activity.

The study aims were to first examine relationships between clinical task performance, heart rate variability, and cognitive load associated with a digit-span working memory task, on pupillary response dynamics, then examine potential concussion history effects.

1.5 Specific Aims

Specific Aim 1: To examine associations between task performance accuracy, heart rate variability, and pupillary response dynamics, across levels of task difficulty within a digit-span working memory, task in healthy collegiate club sports athletes.

***Hypothesis**: We anticipate significant associations to exist across participants between task performance accuracy, heart rate variability, and pupillary response dynamics with respect to the levels of task difficulty within a digit-span working memory task. Specifically, we anticipate individuals will demonstrate decreasing heart rate variability and task performance accuracy across increasing digit sequence-lengths—while pupillary responses will increase (dilation) to a point of working memory capacity, then plateau or decrease (constriction).*

Significance: The digit-span task within the current recommended concussion assessment battery is known to be one of the most sensitive to acute injury. Rapid loss in this signal detection is likely attributable to limitations associated with clinical task performance factors (e.g., task design, administration and interpretation, etc.) and lack of psychophysiological characterization of cognitive efficiency. If combined task performance and physiological (heart rate variability and pupillary response) assessments can objectively inform individual differences in cognitive efficiency, the potential exists to also provide insight regarding neurocognitive deficits. Dual physiological assessment monitoring during the digit-span task by both pupillary response and heart rate variability may provide a more robust picture of cognitive efficiency with respect to physiological resource availability.

Specific Aim 2: To examine the effect of prior concussion injury, and task performance accuracy and heart rate variability response dynamics on pupillary response dynamics, across levels of task difficulty within a digit-span working memory task in healthy collegiate club sport athletes.

Hypothesis: *We anticipate that those who report prior concussion, task performance accuracy, and heart rate variability dynamics will exhibit worse physiological outcomes of pupillary response (increased dilation responses) across digit-sequence lengths, though performance-wise they may not differ from those without a concussion history.*

Significance: Recent studies examining the individual ability of potential physiological biomarkers to discriminate between those who have a concussion history —with respect to neurocognitive deficits—have exhibited various threats to internal validity. Examination of the effects of prior injury as it relates to cognitive efficiency may provide insight regarding the potential utility of a more dynamic behavioral and physiological assessment for post-concussion assessment and monitoring.

1.6 Independent Variables

1. Digit sequence length: the number of digits within a given sequence. Discussed in terms of cognitive load as a representation of task difficulty level associated with longer sequences. (Aims 1 & 2)
2. Task performance—average percent of correctly identified digits (by serial position) with respect to sequence-length for each trial. (Aims 1 & 2)
3. Heart rate variability— Total HRV as the root mean square of successive differences (RMSSD) from baseline to baseline for each trial. [64] [52,54,55] (Aims 1 & 2)

4. Concussion history—Self-reported by first providing athletes with the definition for concussion from included in **Section 1.8**, then asking them to consider their concussion history following provision of a definition. Participants included in the history group were those who reported their most recent concussion occurring between the years in which they attended secondary school (grades 9-12) until 6 months prior to their study participation date. (Aim 2)

1.7 Dependent Variable:

1. Pupillary response: Pupillary Response represented as the baseline corrected pupil diameter in mm, during the retention period–measured by trial, whereby greater dilation response reflects greater neural resource utilization.[28,32,56]

1.8 Potential Co-variates:

1. Sex—male versus female
2. Prior contact/collision sport participation—examined via 2 variables using questions from the (Head Impact Exposure Index—HIEI[57])
    i. Total number of years participating in contact/collision sport
    ii. Total number of hours participating in contact/collision sport

1.9 Definition of Terms

1. Concussion: The definition provided in the Berlin Concussion Consensus statement will be applied throughout as follows: A change in brain function following a force to the head, which may be accompanied by temporary loss of consciousness and is identified in awake individuals with measures of neurologic and cognitive dysfunction. Common concussion symptoms include headache, feeling slowed down, difficulty concentrating or focusing,

dizziness, balance problems/loss of balance, fatigue/loss of energy, feeling in a fog, irritability, drowsiness, nausea, memory loss, sensitivity to light/noise, and blurred vision.

2. Sequence-length: the numbers of digits in a digit-sequence.
3. Cognitive load: cognitive demands relative to the task—i.e., task difficulty associated with longer sequences.
4. Neural Resource Utilization: brain activity used to accomplish a cognitive task.
5. Baseline Period: two seconds prior to each digit-span being presented to allow for pupils to rest and stabilize.
6. 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.
7. Retention Period: three second period after each digit-span is presented when participants process/encode the information and prepare to recall.
8. Task performance: percent correctly identified digits (by serial position) across trials for each sequence-length.
9. Pupillary Response: average pupil size (diameter) across trials for each sequence length.
10. Cognitive Efficiency: the ability to maximize neural resource utilization while maximizing task performance.
11. Heart Rate Variability: The root mean square of successive differences (RMSSD). A reliable estimate of vagally mediated changes in heart rate variability (i.e., beat-to-beat variance in heart rate), from ultra-short-term measurement durations—shown to capture acute mental stress during cognitive tasks.

1.10 Delimitations

1. Individuals who were not a collegiate club sport athlete were not included in this study.
2. Individuals with permanent vision loss, strabismus, amblyopia, or eye surgery in the last 6 months were not included in this study.
3. Individuals participating in visual or vestibular therapy were excluded to prevent confounding variables.

1.11 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 or vice versa.
2. Study sample may predominantly consist of males, and therefore, we may be unable to examine sex differences within the proposed study aims.

1.12 Assumptions

1. Participants accurately reported past medical and sport participation history.
2. Participants remembered and accurately reported all concussion injuries.
3. Participants remained engaged and gave full effort during the task.

1.13 Summary of Study Significance

This study is the first to examine an assessment for cognitive efficiency that accounts for both task performance and physiological response dynamics, which may provide meaningful insight for concussion injury response and recovery. Consideration for necessary adaptations to the digit-span task within the clinical battery to better elicit dynamic working memory processes, while also overlaying two physiological response dynamics known to be associated with digit-span task performance outcomes, will allow us to better describe efficiency.(**Figure 1.1**) We will also examine the effect of concussion history on these measures as a preliminary step towards improving our ability to capture the dynamic clinical and physiological aspects of cognitive efficiency.

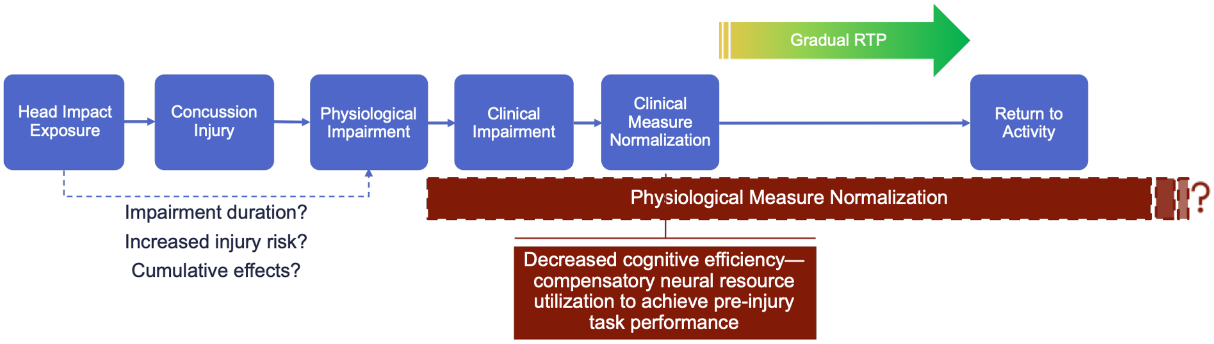


Figure 1.1 Conceptual Model for the current study highlighting the need for better characterization of physiological response and recovery dynamics following concussion

CHAPTER 2: LITERATURE REVIEW

2.1 Overview of Concussive Injury

Repetitive head impact exposures and concussion are major athletic health concerns[1] for which the physiological response and recovery dynamics are poorly understood. The 5th International Conference on Concussion in Sport defines concussion as: "A change in brain function following a force to the head, which may be accompanied by temporary loss of consciousness and is identified in awake individuals with measures of neurologic and cognitive dysfunction. Common concussion symptoms include: headache, feeling slowed down, difficulty concentrating or focusing, dizziness, balance problems/loss of balance, fatigue/loss of energy, feeling in a fog, irritability, drowsiness, nausea, memory loss, sensitivity to light/noise, and blurred vision."[9] Prolonged neurophysiological abnormalities in concussed individuals assessed using advanced imaging techniques suggest a prolonged over-excitatory period, when the brain remains physiologically-compromised requiring greater neural resource allocation and metabolic energy to balance task demands with available cognitive resources.[8,19] Consequences of these persistent deficits suggest decreased cognitive efficiency which may leave the brain at an increased risk for repeat injury, new musculoskeletal injury, prolong recovery, neurocognitive impairment, and persistent symptom presence.[4,22,50]

Despite growing evidence of persistent compensatory mechanisms and associated neurophysiological cost—dynamic clinical and physiological response dynamics for cognitive efficiency following concussion remains poorly characterized. Limited generalizability stems from the underlying issue of clinical feasibility and poor ecological validity. Below we review relevant literature supporting the need for a physiological measure associated with task performance response dynamics of working memory, and sensitive to potential deleterious repetitive head impact exposures and concussion to better inform cognitive efficiency. We then propose pupillary response dynamics to cognitive task demands as a physiological index for neural resource utilization to meet these assessment needs and inform cognitive efficiency. We also suggest the simultaneous measurement and inclusion of heart rate variability to characterize cognitive efficiency as a secondary physiological measure in order to best account for cognitive effort input relative to task demands whist still accounting for task performance outcomes. Finally, we review relevant literature to support the methodological considerations associated with the proposed study in the context of design, instrumentation, and overall ecological validity to allow for interpretation of future directions in this space.

2.1.1 Epidemiology

Estimated prevalence for recreational and sport-related concussion (SRC) in the United States from the national injury databases report prevalence between 1.1 to 1.9 million, in pediatric and adolescent populations.[12] Concussion injury rates for this population are 2.5 per 10,000 athlete exposures and are higher in collision/high-contact sport athletes. Concussion among college aged athletes participating in National Collegiate Athletic Association (NCAA) sports is estimated around 10,560 nationally with the overall concussion rate of 4.47 per 10,000 athlete-exposures.[2] Recent literature regarding the effects associated with repetitive head impact exposures remains unclear—though studies suggesting greater functional impairments in those who have experienced multiple prior concussions and potentially greater long-term structural changes, relative to cumulative exposure provide sufficient evidence for further pathophysiological characterization.[4,20,58] Contact and collision sport athletes (e.g., American football, rugby, men's lacrosse, etc.) are at the greatest risk, as they experience relatively high numbers of head impact exposures over the course of a single season and athletic career.[5,6,21] Many research- and clinically-based challenges limit the understanding for the true SRC epidemiology. Injury definitions lack consensus across disciplines and may contribute to decreased concussion injury-based knowledge among patient populations, and therefore athlete self-report/disclosure.[2,59]

Annual participation rates for collegiate club sports is currently unknown though estimated to make up a large percentage of competitive athletes at risk for concussion given the discrepancy between high school varsity and college varsity level athletes.[60,61] The majority of these athletes have prior experience participating in their respective sports and maintain a relatively high level of competition, though lack the medical coverage and clinical resources available to those rostered on varsity teams. These teams are also often larger than varsity collegiate sports and therefore still represent a large at-risk population.

2.1.2 Injury Mechanics and Response

Concussion is theorized to result from linear and/or rotational biomechanical forces (direct or indirect) to the head, neck, or body resulting in an impulsive force to the head and brain, causing axonal shearing and increased pressure gradients, resulting in diffuse axonal injury and subsequent altered neuronal function.[7,62] The neuropathophysiological process that follows is described as a neurometabolic cascade, posited to drive clinical deficits and dysfunction. Moreover, there are growing concerns regarding the elevated risk of repeat injury following concussion and associations with slower recovery, prolonged symptoms, etc.[63–65]

Additional concerns exist regarding potential effects of repetitive head impact exposure associated with contact and collision sport participation, in the absence of diagnosed injury.[6,18,64]

Studies examining these effects suggest greater functional impairments in those who have experienced multiple prior concussions and potentially greater long-term structural changes.[3,58] Attempts to better understand the cumulative effects between repetitive head impacts and long-term neurological consequences (e.g., increased risk for neurodegenerative disease, neuropsychiatric deficits, etc.) demonstrate limited correlational associations and causal relationships have yet to be established.[4,20,58] Continued investigation regarding physiological and clinical response dynamics following concussion with respect to symptoms, neuro-cognitive functioning, and motor control/postural stability, is needed to further inform these concerns.

2.1.3 Physiological Response

2.1.3.1 Neurometabolic Cascade

The physiological response to concussion injury is multifactorial—primarily informed by animal models, and a few recent human studies.[4,27,29] The neurometabolic cascade of events following the biomechanical insult mentioned above, has been described in detail by Giza et al. relative to associated clinical impairments.[27] Initial potassium efflux causes a dramatic release of excitatory neurotransmitter glutamate causing neuronal depolarization and further ionic disruption. Initial hyperglycolysis supports sodium potassium pumps as they respond to homeostatic disruption, requiring additional adenosine triphosphate to restore ionic balance. Calcium influx is appropriated into mitochondria for short-term relief but eventually leads to mitochondrial dysfunction and decreased oxidative capacity. Glucose metabolism then shifts to support this energy demand, resulting in a state of hyperglycolysis. Concurrent reduction in cerebral blood flow during this time of high energy demands is posited to result in the ‘energy crisis’ associated with concussion injury. Mitochondrial dysfunction and impaired oxidative metabolism further contribute to the crisis as neurons attempt to restore homeostatic intracellular calcium levels.[27]

Post-concussion pathophysiological recovery is such that potassium and glutamate stabilization occur within 24 hours, and calcium levels within the first 3-4 days, and glucose levels and disruptions in cerebral blood flow within 7 to 10 days. Recent advanced imaging studies, however, suggest these physiological deficits indicating increased neural activity may persist beyond these timeframes. Implications of these prolonged physiological deficits indicate a window of cerebral vulnerability that extends beyond clinical measure normalization when the brain remains physiologically-compromised and at a greater risk of repeat injury.[7,8]

2.1.3.2 Role of the Autonomic Nervous System

Autonomic nervous system (ANS) function following concussion has been posited to play a role in the above-mentioned cerebrovascular-related alterations, with respect to sympathetic and parasympathetic activity balance.[46,55] Balanced ANS function is essential from a neurocardiac perspective with respect to its role in regulating cerebral blood flow to meet neurometabolic demands. Dysfunction within this system following concussion may significantly influence the prolonged energy crisis and subsequent secondary injury—though the mechanism behind these alterations are unknown.[7,8,50] Centers within the brain responsible for ANS regulation of neurocardiac function are suggested to be uncoupled following concussion, though more evidence is need to further elucidate its role in physiological injury response and recovery.[18,49,53,55,66]

2.1.4 Clinical Response

Clinical impairments are widely variable and often reflected in a multitude of subjective symptom reports, along with impaired motor control/balance, neurocognitive function, visual/oculomotor, and vestibular function—all associated with the pathophysiological response to concussion injury.[62,66] Given the complexities in clinical presentation, clinicians rely on a comprehensive assessment battery for injury identification and recovery monitoring (i.e., clinical measure normalization).[9] The clinical recovery time course (e.g., symptoms, neurocognitive, balance) has been well documented in large prospective studies over the last 2 decades with current available measures. Specifically, balance deficits and neurocognitive function often recover to baseline levels within 3-5 days.[11,12] Symptom severity scores are known to be the longest lasting clinical deficit, recovering around 7-10 days in most uncomplicated cases.[7,65]

The prolonged energy crisis (i.e., decreased glucose levels and cerebral blood flow), reportedly lasting up to 10 days in the neurometabolic cascade is theorized to drive secondary injury in the brain and associated symptom reports and neurocognitive deficits.[7] Subjective symptom reports post-concussion often include (e.g., headache, dizziness, fatigue, sensitivity to light, and difficulty with memory or concentration) though are widely varied. Symptom checklists including the most commonly reported post-concussion symptoms—and associated Likert scales for severity, are heavily relied upon by clinicians for recovery monitoring (i.e., clinical normalization).[63,65] In those experiencing prolonged symptoms (beyond 7 to 10 days post-injury), recent studies suggest closer clinical examination to identify potential neurological sub-system involvement (i.e., visual/vestibular, cervicogenic, and physiologic/metabolic) and alternated management strategies.[66] Additionally, concussion-like symptoms, in the absence of diagnosed injury, are common.[67] This complicates clinical decision making around the recommended graded exertion protocol and eventual return to play (RTP), given protocol progression is contingent on symptom stabilization and resolution.[9] This underscores the need for objective physiological markers to aid in the further elucidation of the relationship between clinical and physiological response dynamics post-concussion.

Visual and vestibular impairments are also common following concussion given axonal injury and impaired neurotransmission, or direct damage to their respective special sensory organs.[68,69] Specifically, impairments associated with binocular visual skills such as accommodation, convergence, smooth pursuits, and saccadic eye movement, are common following concussion.[69–71] Symptoms associated with these deficits specifically include blurred or double vision, headache, eyestrain, dizziness, nausea, and difficulty concentrating; which contribute to functional impairments such as difficulty reading and tracking, and trouble with near tasks.[72,73] Given the impact post-concussion visual and vestibular impairments can have on daily activity levels and quality of life, and their association with prolonged recovery, clinicians are encouraged to incorporate earlier screening and management for these specific deficits.[68] Common assessments used to capture these impairments (e.g., King Devick, Vestibular Ocular Motor Screen, etc.) have demonstrated clinical utility as physiological measures for dysfunction within their respective sensory sub-systems—though provide little insight with respect to global neurophysiological dysfunction following concussion.[69,74,75]

Neurocognitive deficits arise from initial axonal injury and impaired neurotransmission—where common deficits involve information processing, attention and reaction time, often captured using neuropsychological assessments.[16,76,77] These pathophysiologic factors may also affect the regulatory central and peripheral neural networks that contribute to motor control and balance.[7,63,79] Slowed information processing and reaction time, along with attention deficits play major roles in the maintenance of balance and postural control whereby specific cognitive processes within the frontal lobe and dorsolateral prefrontal cortex are generally responsible.[9,11,78] Neurocognitive deficits are often identified using clinical measures such as the Standard Assessment for Concussion (SAC) or many of the different computerized neurocognitive testing platforms such as the Immediate Post-Concussion Assessment and Cognitive Test.[9]

Motor control and balance deficits are most often assessed clinically using the balance error scoring system.[9,78] Of noteworthy concern, these neurocognitive and balance assessments are known to lose signal detection as quickly as within the first 24 hours post-concussion and demonstrate ceiling effects.[11,12] More advanced assessments using the Sensory Organization Test (SOT) or dual-task gait assessment protocols aim to examine more complex motor control/balance impairments with respect to higher order integration of sensory-motor information.[79–82] Feasibility and ecological validity concerns associated with advanced balance assessments such as the SOT are limiting for further clinical consideration. Clinical utility of dual-task 'cost' outcomes as they relate to post-concussion physiological response dynamics continue to be examined though may be useful in later stages of concussion recovery with respect to functional testing.[82,83] Overall, clinical response dynamics following concussion are complex and may not follow a fixed recovery time frame or be appropriately captured using the current assessment battery components. Combined dynamic clinical and physiological assessments may provide a better characterization of the neurophysiological concussion injury response and recovery to aid in improved concussion management paradigms.

2.2 Clinical Versus Physiological Considerations

While most individuals recover from concussion within 2-4 weeks, a substantial number may experience a prolonged recovery and persistent symptoms.[65,66,84] Physiological deficits following concussion have also been shown to persist beyond normalization of clinical measures suggesting that the current battery of assessments may not be sensitive enough to identify injury recovery.[16,19] The clinical and physiological response to concussive injury, is best described in the context of the integrated recovery model proposed by Dr. Mike McCrea first in 2009 and revised in 2015.[85] This model proposes a progression of recovery, characterized by an acute period of clinical signs and concurrent physiological dysfunction, followed by persistent physiological dysfunction in the sub-acute period, and finally, complete clinical and physiological recovery. This model has been further supported in a recent systematic review highlighting key physiological considerations in the context of concussion injury response and recovery.[8] Persistent physiological deficits cultivate a neural environment in which the brain is susceptible to injury, during a time in which most athletes are actually beginning an RTP. Too much or too little physical and/or cognitive activity during this time may further delay the recovery process)[51], therefore it is imperative that we understand the time course of the physiological response to injury in order to continue the development of appropriate management paradigms.

2.2.1 Advanced Physiologic Measures

2.2.1.1 Advanced Neuroimaging Techniques

Numerous advanced assessment techniques have been examined to improve our understanding regarding the time course of clinical and physiological recovery following concussion. Further characterization of the relationships between physiological disturbance and clinical outcomes continue to be pursued by many researchers to identify more objective diagnostic and recovery criteria for improved concussion management paradigms. Neuroimaging techniques in particular have been used to examine widespread neural systems that cross multiple functions, with the intent to characterize the pathophysiology following concussion—

though the level of evidence to support their clinical utility is low and therefore not recommended at this time.[18] Results from a recent systematic review summarizes the contributions from neuroimaging modalities (e.g., functional magnetic resonance imaging (fMRI), diffusion tensor imaging (DTI), and electroencephalography (EEG) to our current understanding regarding physiological and clinical responses following concussion. Specific results from fMRI studies report varied results regarding blood oxygen level dependent (BOLD) responses during resting state and task-based examinations following concussion.[18] Task-based fMRI studies often examine this measure to describe neural activity in task related networks of working memory (e.g., dorsolateral prefrontal cortex)—as one of the most commonly affected neurocognitive processes following concussion.[11,16,76] Varied results have been reported showing both lower and higher levels of activity have been exhibited in concussed individuals.[18] Prefrontal cortex (PFC) related activity is modulated by working memory cognitive loading,[31,77] where fMRI studies using a digit-span task show that higher digit sequence-lengths, representing a higher cognitive load is consistently associated with greater cortical activation, including critical PFC regions.[8,18,19] Results from these fMRI studies provide support for compensatory neural resource utilization associated with prolonged physiological impairment as brain activity extends beyond regions of interest in the dorsolateral prefrontal cortex and inferior parietal areas in these studies. Resting state fMRI is currently the most extensively studied network in SRC – though similar varied results have been reported where both increased and decreased connectivity between default mode network regions are observed following injury.[8]

Similarly, the use of fluid biomarkers has advanced our understanding of concussion pathophysiology, though the validation of these markers is in the preliminary stages.[18,86,87] Continued research in this area attempt to aid in concussion diagnosis and recovery monitoring by examining serum and blood biomarkers indicative of axonal injury.[18,87] Additional investigations using genetic testing has sought to inform prognostic factors associated with concussion injury risk, prolonged recovery and long-term neurological heath with respect to potential life-long consequences of injury and repetitive head impact exposure.[39,53] [8,18]The majority of these investigations have been completed in more traumatic brain injury cases, though increasing in athletic populations. The major limitations affecting fluid biomarkers pertain to the time needed for analysis and results and access to a basic science laboratory—thus currently not a feasible option for sports medicine clinical settings.[18] Future studies with longitudinal designs to further elucidate the dynamic recovery of these compensatory neural-mechanisms following concussion are not a viable option as fMRIs are very expensive.

Overall, authors of a recent and comprehensive systematic review including biomarkers (e.g., blood serum and plasma markers, salivary cortisol, cerebrospinal fluid, etc.)[8,18] highlight the current limitations challenging this research initiative to generalizability of findings including: small homogenous sample sizes across studies (primarily male participants), varied study designs, limited number of studies overall, differences in outcome measures and analytic methods, and lack of consistency post-injury data collection time points. Recommendations from this review emphasize continued research efforts to further characterize the pathophysiological response to concussion and repetitive head impact exposure in the absence of diagnosed concussion that include larger samples sizes inclusive of both sexes, standardized protocols, more stringent study designs that allow for baseline comparisons, appropriate controls, blinded analyses that include clinically applicable outcome measures.[8,18] Moreover, limited generalizability in neuroimaging outcomes stems from the underlying issue of clinical feasibility where many of the measures used to identify physiological abnormalities lack ecological validity. Investigations using outcomes known to be indirect measures associated with these advanced techniques such as heart rate variability and pupillary response may provide a more clinically feasible option that allows for the dynamic characterization of post-concussion physiological response and recovery.

2.2.1.2 Heart Rate Variability

Heart Rate Variability (HRV) measures function as indices of neurocardiac function used to inform the dynamic responses associated with ANS dysfunction following concussion.[55] These measures represent the fluctuation in the time intervals between adjacent heartbeats with respect these subsystem dynamics as individuals adapt to environmental and psychological challenges.[54] Recent investigations have examined these measures at rest and in stressed states (both physically and psychologically), where specific outcomes have been linked to performance of executive functions like attention and emotional processing by the prefrontal cortex.[41,45] Common outcomes for HRV in the concussion literature space include heart rate and time- and frequency-domain indices of HRV. Heart rate (HR) is also a common metric used in concussion literature with respect to persistent cerebral metabolic deficiencies related to reduced cerebral blood flow at rest, and threshold determinants for exercise tolerance testing.[47,48,53,66] Specifically, HR is represented as the average difference between the highest and lowest HRs during each respiratory cycle (HR Max − HR Min). Examination of HRV measures is less common in this population. Time domain measures are used to quantify the amount of variability in measurements of the inter-beat interval (IBI) (i.e., the time period between successive heartbeats); and Frequency-domain measurements estimate the distribution of absolute or relative power into four frequency bands.[52] Overall, current understanding of the effects of concussion on ANS function as assessed with neurocardiac metrics (HR and HRV) are varied, though generally suggest post-concussion increases in sympathetic activity and lower parasympathetic compared to controls.[18,46] Individuals with concussion have been found to have higher rates of sympathetic nervous system output than controls, as exemplified by higher resting heart rates and higher heart rates during cognitive activity. [8,18,55]

Response dynamics associated with HRV measures may provide meaningful information regarding the concussion physiological response characterization given associations with clinical recovery and symptom resolution due to resolved metabolic impairment. Altered autonomic nervous system regulation is evident as individuals recover following concussion though when HRV measures are examined in isolation during task-based paradigms associated with working memory results are limited given the potential influence of the stress response associated with increasing cognitive demands.[14] Therefore, examination of HRV as it relates to ANS activity may serve as a useful supplement to describe the resolution of compensatory mechanisms and physiological impairment, combined with an ecologically valid marker for neural resource utilization.[6]

2.2.1.3 Current Visual Metrics

Visual impairments are among the most prevalent following concussion occurring in up to 60% of children and adolescents.[68,73] Visual disturbances following concussion are most often reported in oculomotor, and visual processing contexts,[4,72,75] given the widespread, neural architecture of the visual system within the brain, with over half of neural pathways related to vision.[71,75] This network widely expands fronto-parietal circuits and subcortical nuclei, cranial nerves, and interconnections between these areas, all of which are particularly vulnerable to head injury.[26,33] [88]

Oculomotor deficits in accommodation, convergence, smooth pursuits, and saccadic eye movement, are commonly affected following concussion. Symptoms of these deficits include blurred or double vision, headache, eyestrain, dizziness, nausea, and difficulty concentrating.[68,88,89] Moreover, recent studies identifying predominant visual impairments following concussion highlight their relationships with neurocognitive processes of memory and attention—also often impaired following concussion.[68,73]

Additionally, deficits in the pupillary light reflex have been reported following concussion due to diffuse axonal injury resulting in abnormal static and dynamic responsivity.[90] Specifically, the pupillary light reflex following concussion has been showed to be symmetric, though delayed, slowed, and reduced; additionally smaller initial baseline pupil diameters have been reported following concussion, compared to uninjured controls.[90,91] While there are limited studies published in this space, findings suggest dysfunction within afferent pupillary pathways, and the parasympathetic and sympathetic efferent pathways of the ANS. These findings underscore the role of the ANS in post-concussion physiological impairments and need for further response characterization.[90] Overall, this review echoes results from two of the most recent and comprehensive systematic reviews regarding the concussion pathophysiological response in that clinical utility of a physiological marker is more likely to derive from measurement combinations rather than by any one in isolation. [18,43]

2.2.2 Considerations for Advanced Clinical and Physiological Assessments

Task performance and physiological responses following concussion do not adhere to a fixed recovery time course, therefore assessments for impairment within these areas should match response dynamics to best monitor recovery trajectories post-injury.

2.2.2.1 Behavior Specific Considerations

Neurocognitive impairment following concussion describes deficits in memory, attention and processing speed as a result of diffuse axonal injury and the energy crisis that results in secondary neuronal injury. Neurocognitive assessments are used to capture task performance responses associated with each of these cognitive constructs following concussion. Longitudinal examination of working memory (WM) task performance outcomes are theorized to provide insight regarding cognitive efficiency given their representation of task performance across varying levels of difficulty.[25,77] Deficits in WM are common following concussion and are typically assessed using recall tasks in the Standard Assessment for Concussion (SAC).[9,76] The digit-span task in the SAC in particular is sensitive to injury as a performance-based representation of working memory capacity and/or the relationship between task demands and available cognitive resources. [11,12]

The digit-span is one of the most commonly used tests of working memory in clinical research and practice and can include forward and backwards administration. Backwards digit-span administration typically involves digit-sequence presentation (verbal or visual), a brief retention period, followed by a recall period during which participants are asked to recite the presented digits in exact reverse order (e.g., 3-5-8 correctly recalled is 8-5-3). Task difficulty is characterized by digit-sequence length (i.e., number of 'to be recalled' digits) and increases, every 1 to 2 trials depending on recall accuracy. Individuals who fail to demonstrate perfect recall on the first trial attempt for a given sequence-length are typically permitted a second attempt—with a new digit sequence. Traditional task administration implements a 'discontinue' rule following sequence length at which both trials are inaccurately recalled. This task requires updating, reordering, and/or dual- processing to engage working memory—critical to its clinical and theoretical utility.[31,77] However, recent evidence suggests that current administration of the backwards digit-span task, using all or nothing scoring and discontinuing when behavior is less than 100% limits our ability to understand how working memory may be affected when demands exceed capacity. All-or-nothing scoring assigns credit only for perfectly recalled sequences, whereas partial-credit scoring counts each digit recalled in the correct serial position. These adaptations to this task are suggested is suggested as a more robust examination of task performance responses during the digit-span task with more scoring variability and increased ability to detect individual differences.[27,92] Overall, this new evidence highlights the fluidity of working memory and cognitive efficiency, rather than having a fixed capacity)—worthy of examination in concussion populations with respect to known compensatory neural resource utilization post injury that demonstrate dynamic changes across recovery.[27]

2.2.2.2 Physiologic Specific Measures

Post-concussion physiological response and recovery remains poorly characterized by current clinical and advanced imaging techniques. Recent systematic reviews emphasize the dynamic nature of physiological changes following concussion injury that cannot be constrained within a single window of 'physiological recovery'.[8] Neurocognitive assessment paradigms that elicit a behavioral response are often used under advanced imaging to functionally characterize regional brain activity and neural resource utilization—though not feasible with respect to cost effectiveness for long term monitoring. Clinically based physiological measures such as heart rate variability have also been described in concussion literature to describe dynamic aspects of ANS function, though when used in isolation may not be adequate to give a full picture of the dynamic physiological and psychological processes.

2.3. Pupillary Response as a Potential Solution

Examination of pupillary response during a cognitive control task of working memory may provide clinicians with a more sensitive assessment of functional neurophysiological impairment and recovery following concussion, ultimately bridging the gap between clinical and physiological recovery. While pupillary response to cognitive load has been described in healthy and other cognitively impaired populations,[28,32,36] little is known about this measure in concussion populations. Clinical and advanced physiological evidence has described higher order cognitive impairment in attention, processing speed, and working memory utilization following concussion.[9,12] Many of the common testing paradigms used to examine these cognitive constructs in concussion populations (e.g., digit-span)— both clinically and with fMRI— have also been examined using pupillometry to reflect pupillary response changes to cognitive load in healthy and diseased states.[28,31,38,39]

2.3.1 Neurophysiological Underpinnings

The neurophysiological underpinnings of this response suggest that pupil dilation response in particular, is modulated by the noradrenergic Locus Coeruleus neuromodulatory system (LC-NE).[30,33,93] This system has widespread projections that extend to nearly all cortical and subcortical regions.[94] The LC-NE is a small collection of nuclei in the brainstem's pontomedullary reticular formation and plays a central role in behavioral adaptation, task performance, attention, functional reorganization of cortical activity when environmental contingencies change that allow for cognitive and behavioral adaptation, and working memory.[33,95] Current evidence validates pupillary response to cognitive load across various cognitive processes –including those mentioned above—with brain activity and these studies conclude that pupil dilation response to cognitive load is correlated with activity in brain regions engaged by current task demands.[29,30,33,93]

2.3.2 Pupillary Response Correlates with Behavioral Outcomes

Previous literature has shown that pupil dilation is associated with a broad range of cognitive processes (e.g. attention, memory, etc.) in healthy individuals. In this context, pupil dilation refers to a stimulus-induced increase in pupil diameter relative to a pre-stimulus baseline period, or a task-evoked pupillary response.[96] Pupillary responses during cognitive control tasks have been extensively examined in healthy populations ages 10-83 years, where pupil dilation increases with increasing tasks demands.[29,31,37,38] Previous studies have further supported pupil dilation as a valid physiological marker of cognitive load via comparison to EEG and fMRI measures of brain activity. [30,97]

2.3.3 Pupillary Response in Clinical Populations

More recently, pupil dilation and cognitive processes have been examined across varying diseases states such as Alzheimer's and Parkinson's disease.[38,39] One study examined pupillary response during a digit-span task in adults who are cognitively normal and those across varying levels of mild cognitive impairment (MCI), both single domain (S-MCI) and multiple domain(M-MCI). The results of this study suggested that pupillary responses during a digit-span task reflect compensatory effort —exhibited by greater pupil dilation—to achieve equal task performance in those with lower levels of MCI based on working memory capacity.[38] Additionally, those with M-MCI exhibited no significant changes in cognitive effort across varying levels of cognitive load suggesting that in later stages of this degenerative disease individuals lack the ability to appropriately adapt cognitive effort.[38] One study has examined pupillary response to increasing cognitive load in non-demented individuals with Parkinson's disease with the intent to identify a valid measure of cognitive dysfunction in this population.[39] No significant differences were seen in cognitive load between healthy and non-demented individuals with Parkinson's. This study was however underpowered, and the researchers reported that Parkinson's patients exhibited greater cognitive workload compared to healthy controls throughout testing. Future research is needed to determine the utility of pupillary response during a working memory task in the assessment of cognitive load and cognitive impairment detection in non-demented individuals with Parkinson's disease. [38,39]

Pupillary responses in those with mild traumatic brain injury (mTBI) is currently limited to examination of the light reflex. To our knowledge, the effect of concussion history on pupillary responses during cognitive control tasks such as working memory have not been examined or reported. Additionally, this physiological pupillary response has not been examined across concussion recovery or repeated exposure to head impacts. Pupillary response during cognitive control tasks of working memory has however been associated with activity in long range frontal norepinephrine networks, which we know to be commonly affected following SRC[20,98] and following seasons of repeated head impact bouts.[6]

Clinicians are currently limited in the ability to longitudinally assess neurophysiological measures to determine the course of physiological recovery. Examination of pupillary response during a cognitive control task of working memory may provide clinicians with a more sensitive assessment of functional neurophysiological impairment and recovery following concussion, ultimately bridging the gap between clinical and physiological recovery. Moreover, pupillometric assessment provides the most clinically viable option for monitoring physiological recovery following concussion compared to measures currently being investigated with respect to cost, time, space, and training.

2.4 Methodological Considerations

Methodological considerations relative to the proposed study are essential in order to optimally meet the study aims. Relevant literature that provides support for each consideration is summarized below with respect to overall study design, digit-span task design, and response measures of interest. Additionally, we have provided preliminary analyses from our pilot project to further support the methodological considerations in the proposed study.

2.4.1 Study Design and Participants

Recent investigations in concussion research involving physiological biomarkers for concussion injury and recovery response have stressed the importance for providing 'real-world' significance of these measures relative to known clinical impairments.[8,18] Therefore, we felt it most appropriate to pursue a cross-sectional design and first examine relationships between our task performance and physiological outcomes of interest, then probe potential effects of other biological and historical variables (e.g., sex, concussion history, etc.). This will allow us to better characterize the potential clinical utility of our proposed assessment paradigm along with future clinical considerations and implications.

Club sport athletes represent a large population of college aged recreational and competitively active individuals who may be at risk for concussion.[61] These athletes also have similar academic cognitive demands as those who play at the varsity level, and thus, the impact of concussion on their daily activities and requirements is also important.[61,99] We propose to examine our response dynamics of interest in this competitive athletic sample as it closely represents a widely studied population in concussion literature (college-aged athletes) for which generalization of the our study may be possible.

2.4.2 Digit-Span Task Design and Administration Considerations

Working memory related cognitive impairments are among the most common deficits following concussion in athletic and military populations.[9,12] The backwards digit-span working memory task within the SAC has demonstrated the greatest sensitivity to acute concussion compared to other clinic-based tests—however, continued signal detection is insufficient beyond the first 3-5 days post injury.[11,12,100] Recent evidence suggests that task administration practices of the SAC's backwards digit-span task, fail to elicit meaningful working memory processes.[25,76,92] Minor administration adaptations have shown to engage adequate working memory processes and improve task performance response measurement precision.[92]

Standardized task design and administration considerations for the proposed study's backwards digit-span task are therefore critical in order to elicit adequate working memory processes and characterize task performance and physiological response dynamics of cognitive efficiency—above and beyond our current clinical tools. [27,92] Specific adaptations include elimination of the 'discontinue' rule—instead requiring participants to complete an equal number of trials at all digit-sequence lengths included in the task, regardless of performance accuracy. Additionally, the digit-spans in the proposed study will include longer sequence-lengths beyond average short-term memory capacity (7 +/- 2) in order to engage sufficient working memory processes in the absence of additional processing demands.

2.4.3 Task Performance and Physiological Response Considerations

2.4.3.1 Task performance

Adaptations to the scoring method of the digit-span task is also important in order to improve task performance response reliability. Various reliable and valid partial credit scoring approaches for task performance and clinical measures that generally assess item response/recall accuracy during working memory tasks have been established in healthy and clinical populations.[35,77,92] These approaches are typically pursued relative to their task-related/evoked design in order to capture improved response variability and precision.[25,27,92]

A previously validated approach for partial credit scoring using a digit-span task that eliminates the discontinue rule and overloads working memory beyond the average short-term memory capacity (7 ± 2 items) will be assigned based accuracy by serial position will be used in the proposed study.[92] This important adaptation allows for greater response variability and a more robust evaluation of working memory behavioral responses as they relate to cognitive efficiency.

2.4.3.2 Physiological Responses

Pupillary response data quality, reliability and precision of measure is dependent upon factors associated with task design, testing environment, and eye tracking instrumentation. Previous literature in this space stresses the importance of controlled environmental luminance and participant setup relative to the eye tracking technology (e.g., distance between participant and display, head fixation/movement during testing, etc.). This concept is further applied to task design elements such as background and stimuli contrast and brightness attributes.[37,40,101] Various instrumentation and eye tracking methods further complicate the issue (e.g., cumbersome and intrusive equipment and restricted head movement, etc.). Combined, the design, environmental and instrumentation related issues contribute to less scientific control, portability, and cost-effectiveness—and another poor ecologically valid option for physiological response characterization following concussion.

Virtual reality (VR) headsets with embedded infrared eye tracking technology may provide an optimal method to assess pupillary response dynamics in a highly scientifically controlled and portable environment that is relatively cost effective. Unlike desktop-based eye trackers, the head mounted display allows for freedom of head movement and a constant testing distance between the participants eyes and the display. Tasks design using Unity 3D® engine software further allows for complete control over environmental luminance and contrast elements within the headset.

Recent psychophysiological investigations suggests monitoring multiple metrics when examining associations between physiological and task performance-based outcomes associated with complex cognitive constructs and processes.[29,34,41,42] Dual physiological measurement monitoring has been suggested for future concussion related studies and posited to be better suited for capturing complexities associated with concussion.[8,18] This is especially important when examining measures regulated by the ANS due to the many latent variables that may contribute to variability in response dynamics (e.g., stress, emotional response, etc.).[41,43–45] Therefore, we decided to examine pupillary response correlates with an established index of neurocardiac function (HRV) within concussion literature.[44,46,47] Moreover, a validated and more feasible set-up for HRV measurement— using chest strap and watch—preserves the ecological validity of our overall proposed approach.

2.5 Summary—Study Rationale

Current concussion management practices emphasize post-injury clinical comparison to baseline measures for symptom resolution and neurocognitive and balance deficit normalization; though neurophysiological abnormalities in concussed individuals assessed via fMRI and EEG have been shown to persist beyond clinical recovery[7,8] Moreover, clinical neuropsychological performance measures do not match neurophysiological response, therefore compromising clinical decision making with respect to return to play. This is concerning given current literature suggesting the influence of repetitive concussive injury may result in neurodegenerative changes later in life.

Clinicians are currently limited in the ability to longitudinally assess neurophysiological measures to determine the course of physiological recovery. Examination of pupillary response during a cognitive control task of working memory may provide clinicians with a more sensitive assessment of functional neurophysiological impairment and recovery following concussion, ultimately bridging the gap between clinical and physiological recovery. Moreover, combined examination of HRV as it relates to ANS activity may serve as a useful supplement to describe cognitive efficiency combined with pupillary response as an ecologically valid marker for neural resource utilization.[28,29,31,32]

CHAPTER 3: METHODS

3.1 Experimental Design and Participants

3.1.1 Experimental Design and Study Setting

This was a quasi-experimental, cross-sectional study, conducted in a clinical laboratory setting, that included healthy club sport athletes at The University of North Carolina-at Chapel Hill. We examined within and between subjects group comparisons of associations between task performance- and physiological-based outcomes, across levels of digit-span task difficulty. This study was approved by the University's Institutional Review Board, and participants provided written informed consent prior to any data collection. Upon study completion, participants were compensated $10 for their time.

3.1.2 Participants

3.1.2.1 Inclusion and Exclusion Criteria

Study participation was available to all UNC club sport athletes, regardless of sport type and position within their respective competitive calendar (i.e., pre-, in-, and post-season). Specific inclusion criteria for study participation required all individuals to be between the ages of 18 and 30 and a rostered UNC club sport athlete. Individuals were excluded if they did not meet the above inclusion requirements and/or if they were unable to complete vision testing for whatever reason, had permanent vision loss in one or both eyes, had any visual surgery in the last year that would inhibit testing completion, were currently being treated to address balance or vision problems, and/or had strabismus or amblyopia. To support appropriate group designation using participants' self-reported concussion history, we employed methods similar to those reported by the NCAA–DOD CARE Consortium[102]—whereby all participants reviewed a definition for concussion and common concussion signs and symptoms prior to reporting their concussion injury history. The concussion definition and associated signs and symptoms were informed by evidence-based guidelines and the latest international consensus statement on concussion in sport[9] and read as follows:

"A complex pathophysiological process resulting from traumatic biomechanical forces imparted to the head, face, neck, or elsewhere on the body, resulting in the onset of signs and/or symptoms of a concussion and/or changes in neurocognitive function." Common concussion symptoms may include headache, feeling slowed down, difficulty concentrating or focusing, dizziness, balance problems/loss of balance, fatigue/loss of energy, feeling in a fog, irritability, drowsiness, nausea, memory loss, sensitivity to light/noise, and blurred vision. Participants were also informed that a concussion can occur without being "knocked out" or unconsciousness; getting your "bell rung" or "clearing the cobwebs" is a concussion.

Following provision of the above definition the concussion history group included those who reported having sustained at least one concussion—via any mechanism (i.e., sports-related or not). Additionally, participants were also asked to report the amount of time since their most recent concussion—whereby study participation was limited to those who reported recency time frames during or since high school, but not in the past 6 months.

3.1.2.2 Recruitment Strategy

Various recruitment strategies were employed to achieve total participant enrollment of approximately 70 individuals (~21-28 with concussion history). Strategies included on-campus flyer distribution (e.g., postings near sporting venues and sports medicine facilities, within student common areas, etc.), campus-wide mass email distribution, and in person (e.g., pre/post practice announcements, classroom announcements, word of mouth, etc.). Emails with study information were also distributed through the UNC club sports listserv to provide initial study information.

3.2 Digit-span Working Memory Task

3.2.1 Task Design

The backwards digit-span task was developed for the proposed study in order to examine task performance and physiological response dynamics associated with working memory processes. Traditional task design and administration parameters were adapted to coincide with recent applications in healthy and clinical samples within psychophysiological and concussion-based research domains.[35,37,38,92] Specifically, these adaptations demonstrated the ability to elicit better working memory processes when cognitive and neural resources are limited.[92,103] Moreover, these adaptations provide a more robust examination of task performance given increased scoring variability and ability to detect individual differences.[27,92] Preliminary study data **(Appendix B)** were also used to refine digit-span task design and administration parameters, in order to accommodate a university sample and future clinical applications, with respect task difficulty and duration. The preliminary study was conducted by the Principal Investigator.

3.2.1.1 Traditional Design and Administration Parameters

A backwards digit-span task typically involves auditory or visual presentation of digit-sequences and requires individuals to recall digits in reverse serial order. Traditional design and administration parameters described by Wechsler et al.[104] are clinically implemented within the digit-span task in the SAC—a mental status screening tool used in concussion assessment. Task difficulty typically (i.e., sequence-length) increases by one digit every 1 to 2 trials—where perfect recall is required for progression to longer sequence-length trials, otherwise a discontinue rule is applied. The digit-span task performance outcome is then recorded as the 'longest sequence-length completed with perfect recall) –and has demonstrated sensitivity to concussion immediately following injury.[11,12] Importantly, this injury sensitivity rapidly declines between 3-5 days following injury—which might be explained by the inability of the task to capture compensatory neurophysiological resource allocation to achieve task performance normalization. The digit-span task design parameters employed within the SAC are likely responsible for the inability to fully elicit and capture working memory cognitive processes by limiting the task to perfectly recalled trials.[25,92,103] Recent studies instead support digit-span task administration parameters that include longer sequence-lengths and eliminate the discontinue rule —improving tasks reliability and construct validity for examination of working memory processes. [25,92,103]

3.2.1.2 Present Study

The backwards digit-span task used in the present study was refined to incorporate these administration parameters. **Figure 3.1** depicts the overall task design—i.e., randomized blocked, containing 4 consecutive testing blocks of 5 randomized digit sequence-lengths (i.e., 3, 5, 7, 9,

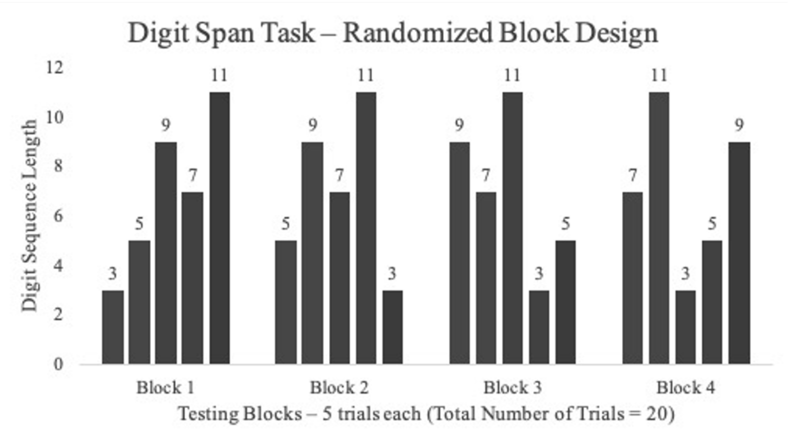


Figure 3.1 Digit-span randomized blocked design: Each block randomly presents a single trial at each level of difficulty. A random number generator was used to determine testing order for the first block and a Latin square was used to counterbalance trial order for each subsequent block.

and 11)—20 total trials. Sequence-length presentation order within the first testing block was determined using a random number generator followed by a Latin Square to counter-balance sequence-length presentation order for the remaining 3 blocks. The individual digits presented within each digit-sequence were randomly generated—consistent with previous literature that excludes within-trial immediate duplicates and consecutive integers.[35–37] 3.2.2 Instrumentation – VR Integration The custom developed digit-span task was developed using Unity 3D® engine software, to be visually presented within the HTC VIVE™ VR head-mounted display (HMD) integrated with Tobii Eye Tracking retrofit hardware technology (Tobii Technology, Inc.). The HMD uses 10 infrared illuminators per eye and allows for tracking accuracy of 0.5%, with a trackable view of 110 degrees. Pupil size (diameter in mm) was continuously recorded in both eyes at 90Hz—equal to the display refresh rate. Tobii Pro VR Solution software, Unity 3D®, and infrared illuminators worked together to recorded event marker timestamps to ensure appropriate response time-locking for key digit-span task components including 1) trial number (1-20), 2) sequence-length (3, 5, 7, 9, and 11), 3) digits presented, 4) baseline and retention X displays, 5) response box display, and 6) recall completion and trial advancement.

Previous studies have suggested that inaccuracy in camera-pupil distance measures may result in pupil size measurement error up to 5% though sample to sample changes in pupil size are more accurate. The use of the HMD also afforded us a solution to strict head fixation, implemented in other studies using remoted/desktop trackers in order to minimize pupil size measurement error.[56,105] Based on previously published criteria for appropriate proper identification of participants' eyes by the eye-tracker and gaze position, and consistent with other cognitive pupillometry studies, we ensured successful five-point calibration prior to each testing session for.[40,101,106]

3.2.1.3 Control Elements of Task Design

Additional task design parameters were applied—consistent with previous cognitive pupillometry literature— in order to mitigate potential pupil size measurement error.[101] These included standardized and equiluminant stimuli presentation, luminance considerations, and the use of a baseline corrected response outcome.[37,101,107]

In order to further minimize the influence of non-intended effects associated with saccades, accommodation, and blinks on pupil size—all stimuli were centrally presented within participants' field of view, and the user vision was fixated to imitate a 2D display.[40,106] Baseline corrcted changes in pupil size have been reported as a reliable load-dependent measure in cognitive pupillommetery studies, and less vulnerable to signal noise as they serve as a trial-by-trial pseudo-calibration. [40,101,106] Therefore, all stimuli were centrally presented and custom designed to ensure equiluminance (size= 200pixels, color= R:46 G:46 B:46 A: 255) throughout the duration of the task. Each trial began with a 5-second pre-stimulus baseline period. Digit-sequences were then presented at the rate of one digit per second, followed by a 3-second retention pause. A fixation 'X' was displayed for each trial baseline and retention periods (display time = 5 and 3 seconds respectively) to stimulus consistency during baseline corrected response intervals of interest. Participants were then prompted to verbally recall as many digits as they could remember in the exact reverse order. Recall was self-paced, and all trials were participant initiated. **Figure 3.2** outlines presentation parameters for a single trial of a 5-digit sequence.[37,40,101] Participants completed all trials until reaching an 'end of trial' slide, without reinforcement or feedback from study personnel.[40, 102]

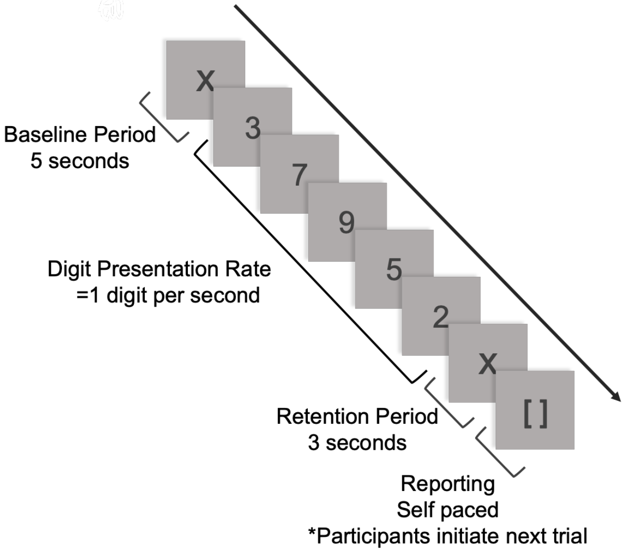


Figure 3.2 Sample five-digit-sequence presentation. Sample illustrates the blocked design for a single trial within the digit-span task. Digits were displayed at a rate of one digit per second. Shaded areas represent response regions of interest within baseline and retention period, used to calculate pupil size change for each trial.

3.3 Task Performance

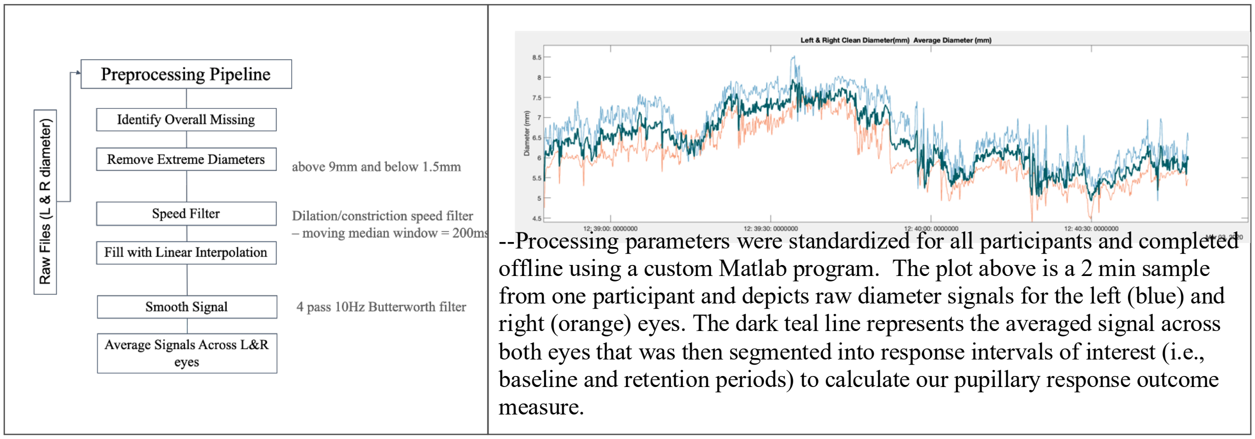
Participants' verbal recall for each trail was manually recorded in real-time by study personnel and compared with digits presented, to determine accuracy for each trial. Recorded trial responses were entered into separate spreadsheets for each participant. A custom Matlab script (MATLAB and Statistic Toolbox Release 2017b, The MathWorks, Inc., Natick, MA, USA) was used to extract trial level information (i.e., trial number, digit sequence length, and digit presented) from the pupillary response export, and compare with participant responses to calculate an accuracy score. Credit was assigned for each digit recalled in the correct serial position, and final trial accuracy measures were recorded as a percent correct i.e., total digits correct divided by digit-sequence length.[92] These scoring methods are consistent with previous literature—and findings from our pilot project **(Appendix B)**.

3.4 Heart Rate Variability

Raw inter-beat interval data (RR interval) were continuously sampled during the digit-span task at 1000Hz using a Polar H10 chest strap and exported for processing using Kubios HRV software, version 3.3 software (Biosignal Analysis and Medical Group, Kupio, Finland). A custom Matlab script was used to extract trial level information (i.e., trial start and end times) from the pupillary response export for each participant. Trial start and end times were then entered into a spreadsheet provided by Kubios—to time-lock RR interval raw data by trial (i.e., from baseline to baseline). The root mean square of successive differences (RMSSD), was calculated by trial for each participant, as a measure of beat-to-beat variance in HR. This measure has been shown to reliably estimate vagally mediated changes in HRV, capturing acute mental stress during other cognitive tasks (e.g., Stroop), from ultra-short-term recorded durations between 10-30 s.[54] Whereby, lower RMSSD measures are correlated with higher acute mental stress. [54]

3.5 Pupillary Response Measures

Participants' raw pupil data were directly exported into a spreadsheet and imported into Matlab (MATLAB and Statistic Toolbox Release 2019b, The MathWorks, Inc., Natick, MA, USA). A custom Matlab program was used for all pupil data processing and reduction in accordance with previously reported methods for response locked baseline-corrected pupillary responses.[56] The adapted processing program followed 5 procedural levels: 1) removal of extreme (out of range) pupil diameters and signal noise from blinks, to identify valid samples 2) blinks and missingness <400ms were filled with linear interpolation, 3) pupil responses were averaged across left and right eyes, 4) response intervals of interest were segmented (i.e., the last 40 samples of each baseline period and the first 200 samples of each retention period); and



--Processing parameters were standardized for all participants and completed offline using a custom Matlab program. The plot above is a 2 min sample from one participant and depicts raw diameter signals for the left (blue) and right (orange) eyes. The dark teal line represents the averaged signal across both eyes that was then segmented into response intervals of interest (i.e., baseline and retention periods) to calculate our pupillary response outcome measure.

Figure 3.3 Pupil Data Preprocessing

5) baseline correction was performed for each trial (i.e., average pupil size during retention – average baseline pupil size).[28,36,37]

Unilateral measures were used if/when there were instances of missingness in the other eye (e.g., eyelash blocking pupil, etc.).[28,36,37] Segmented response intervals of interest with greater than 20% missing data due to signal loss and or long eye closure durations >400ms were excluded. Following data processing and reduction, participants must exhibit at least 2 valid pupillary response trials for each sequence-length to be included in analyses. Finally, processed data were exported from Matlab, and SAS 9.4 (Cary, NC) was used for all descriptive statistics and inferential analyses.

3.6 General Testing Session Procedures

Participants reported for a single testing session, that lasted approximately one hour. Written informed consent was completed first, followed by a demographics and health history questionnaire that was completed using an online Qualtrics survey—see **Appendix A.** Specifically, participants were asked to provide general demographics (age, sex, etc.), along with information regarding prior sport participation (type, duration, and competition level), general medical history and concussion history. Additional questions regarding various factors that may influence study variables of interest were also be included—though not considered key outcome variables. These questions addressed prior contact/collision sport participation using questions from the estimated head impact exposure (Head Impact Exposure Index—HIEI[57]), self-perceived stress (Perceived Stress Scale—PSS-4[108]), anxiety/depression (Generalized Anxiety Disorder—GAD[109]), and self-reported task performance.

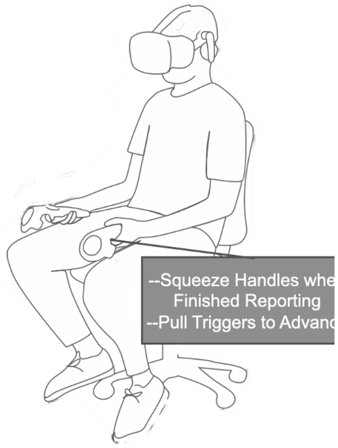


Figure 3.4 Participant set up

Participants were seated and fitted with the heart rate monitor and VR headset—as depicted in **Figure 3.4.** Task familiarization and practice procedures were consistent with previous literature examining pupillary responses during a backwards working memory digit-span task.[35,37,38,92] In order to ensure appropriate top-down processing[25], the task was explained to participants followed by 4 practice trials (all 5-digits in length) in order to familiarize them with task presentation and sequencing—to ensure understanding of task demands and required responses.[25,37,77,110] Participants were not informed of the digit sequence-length for each trial, though they were told that the task includes sequences between 3- and 11-digits long—and that they would be asked to recall as many digits as they could possibly remember (in exact reverse order), for each sequence. Recall periods were self-paced, and all trials were participant initiated using HTC VIVE handheld controller triggers. Participants were given compliance feedback (e.g., appropriate response timing, trial initiation accuracy, etc.) during practice, and encouraged to ask any questions they may have about the testing procedures prior to beginning the experimental trials. No feedback was given during experimental trials. All participants completed the same task as designed and task duration lasted approximately 20 minutes including calibration and task familiarization.

3.7 Statistical Approach

Continuous variables were summarized using means and standard deviations, and categorical variables were summarized using frequencies and associated percentages. Our main variables of interest were further summarized across sequence-lengths.

To address Aim 1, bivariate correlations were used to first examine relationships among numeric variables such as age, our main variables of interest (i.e., pupillary response, accuracy, and RMSSD). Associations between continuous descriptors considered for additional covariate inclusion (contact/collision sport participation) and pupillary response greater than or equal to $r=0.3$ were considered for inclusion in the mixed effects model for Aim 2 (see below). Response were then summarized for each participant across levels of task difficulty, plotted and reviewed prior to inclusion in the mixed effects model.

In order to examine the relationships among task related variables, with respect to cognitive efficiency, individuals must have demonstrated credible performance on the digit-span task. Participants' average accuracy scores were plotted as a function of sequence-length and inspected for appropriate load dependent response dynamics, demonstrating credible performance. Average accuracy <80% on the 3-digit sequence-length served as the indicator for identifying participants demonstrating non-credible performance, and subsequent exclusion from analyses. Linear mixed effects models were employed to examine how individuals' task performance and HRV outcomes, together may explain pupillary response changes across digit-span sequence lengths. To account for latent heterogeneity between subjects and their responses to levels of task difficulty, the model included two random effects—one 'by-subject' effect of participant, and sequence-length as a grouping variable. Task performance and HRV were included as fixed effects and pupillary response served as our outcome variable.

Aim 2 employed a similar model, with an added fixed effect for concussion history groups. Additional demographic variables demonstrating adequate significance in preliminary bivariate correlations ($r>0.03$) were considered in the model and removed if they did not demonstrate significance.

Distributive properties of mean pupillary response as a function of sequence length was examined for curvilinear relationships and the potential need for model adjustment to account for quadratic or cubic mean structure. An alpha (α) level of $p < 0.05$ was established a priori. A summary outline for the statistical analyses including dependent and independent variables of interest are provided in **Table 3.1** by aim.

3.7.1 Power Analysis

Considering our planned statistical approach for each aim using linear mixed models, formal power analysis was not appropriate at this time. However, based on the traditional "rule of thumb" for determining sample size in multivariable models, of 10-15 participants for each candidate variable (i.e., concussion history, trial difficulty, task performance, HRV and 1-2 additional covariates), our target sample size was considered sufficient to conduct the analyses for the proposed predictors/covariates for each aim. As such, we tested 62 total participants and we estimated approximately 30-40% of participants would report a prior concussion.

Table 3.1 Statistical Analysis Plan by Aim

<table>
<tr><td colspan="3">**Aim 1:** To examine associations between task performance accuracy, heart rate variability, and pupillary response dynamics, across levels of task difficulty within a digit-span working memory task in healthy collegiate club sports athletes</td></tr>
<tr><td>**DV**</td><td>**IV**</td><td>**Analysis Plan**</td></tr>
<tr><td>Pupillary Response: Change in pupil size from baseline to retention response periods</td><td>Digit sequence length (5 levels; 3, 5, 7, 9, 11)
Included Co-variates:
Task performance
Heart rate variability</td><td>***Linear Mixed effects model***
Random effect for subject
Fixed effects:
• Sequence length as a grouping variable
• Task performance
• Heart rate variability</td></tr>
<tr><td colspan="3">**Aim 2:** To examine the effect of prior concussion injury, and task performance accuracy and heart rate variability response dynamics on pupillary response dynamics, across levels of task difficulty within a digit-span working memory task in healthy collegiate club sport athletes.</td></tr>
<tr><td>**DV**</td><td>**IV**</td><td>**Analysis Plan**</td></tr>
<tr><td>Pupillary Response: Change in pupil size from baseline to retention response periods</td><td>• Digit sequence length (5 levels; 3, 5, 7, 9, 11)
• Concussion history group (yes versus no)
Included Co-variates:
Task Performance
Heart Rate Variability
Considered Co-variates:
• Sex
• Prior contact/collision sport participation</td><td>***Linear Mixed effects model***
Random effect for subject
2 Fixed effects:
• Sequence length as a grouping variable
• Concussion history (yes/no) as a grouping variable
• Task performance
• Heart rate variability</td></tr>
</table>

CHAPTER 4: RESULTS

4.1 Descriptive results

Sixty-two Athletes from 18 club sports, participated in this study between December 2019 and March 2020. Participants' average age was 20.48 ± 1.86 years, and 34 (57.63%) were male. Twenty-four (40.6%) participants self-reported a concussion history, and subgroups representing total lifetime concussion injuries and injury chronicity (i.e., time since most recent injury in years), are further summarized in **Table 4.1**. Participants' self-reported, average lifetime contact/collision sport participation was 7.2 ± 5.1 years, and 61.6 ± 57.9 total hours during high school—including all pre-, in-, and post-season practices and games. Self-perceived performance measures were completed by 58 participants (see **Table 4.2)**, whereby most participants felt they performed 'moderately well' on the task (n=26, 44%).

Following data processing, 39 (3.3%) pupillary response trials were excluded due to signal loss greater than 20% within response intervals of interest. One participant was excluded due to excessive signal loss within response intervals, that resulted in less than 10 total valid trials—and two participants were excluded following accuracy response inspection for non-credible performance. A total of 59 participants had valid measures for pupillary response, task performance and heart rate variability and were included in all analyses.

Average baseline-corrected pupillary responses are temporally summarized by sequence length for a single participant in **Figure 4.1** to illustrate typical response dynamics. Grand average pupillary response means by sequence length followed expected response dynamics, consistent with previous observations by Klingner et al during a similar task.[111] Participant's pupils gradually dilated as the digits were encoded with each presentation and reached a peak within the 3 seconds following final digit presentation—during the retention period—while digits were being reordered.

Grand average pupillary response, task performance and HRV measures are summarized across sequence-lengths in **Table 4.3.** Consistent with prior work, mean baseline-corrected pupillary responses during the retention period systematically increased with longer sequence lengths (cognitive load) within resource availability (working memory capacity of 7 ± 2 digits) then declined with overload (11-digits).[34,35,111] Mean accuracy scores exhibited a steady decline from 98% to 27% as sequence-lengths increased from 3- to 11-digits. Mean RMSSD measures exhibited little fluctuation across sequence-lengths, within previously established normal ranges (i.e., between 27-72 ms).[112] **Figures 4.2.** and **4.3.** overlay grand average pupillary response and heart rate variability measures (respectively) with task performance to further illustrate response dynamics in these clinical and physiological metrics.

Bivariate correlations (**Table 4.4**) exhibited significant relationships between longer sequence-lengths and both larger pupillary responses ($p<0.001$) and lower accuracy scores ($p<0.001$). Higher accuracy scores were also weakly related to higher heart rate variability ($p=0.04$). Age, and both variables representing prior contact collision sport participation (i.e., average total years playing and average total hours participating in high school) demonstrated negligible relationships with pupillary response ($p>0.05$).

4.2 Aim 1 Results

Participants' average pupillary response, task performance and heart rate variability measures—summarized across sequence-lengths— are depicted in **Figures 4.4-4.6**. Results from the mixed effects model are summarized in **Table 4.5** (Type III model results) and **Table 4.6**

(Simple effects). There was a significant main effect of sequence-length on mean pupillary response ($F_{4,232}$=3.69, *p*=0.006), whereby longer sequence-lengths elicited greater average dilation responses. Accuracy and RMSSD demonstrated non-significant effects in the model ($F_{1,1076}$=0.00, *p*=0.974) and ($F_{1,1076}$=1.58, *p*=0.208), respectively. Model-derived pupillary response means are plotted for each participant as a function of sequence-length in **Figure 4.7**.

4.3 Aim 2 Results

In table **4.7.** pupillary response, task performance and heart rate variability grand means and 95% CIs are summarized as a function of sequence length for concussion history groups (yes versus no). **Figures 4.8.** and **4.9.** overlay grand average pupillary response and heart rate variability measures (respectively) with task performance to further illustrate response dynamics in these clinical and physiological metrics for each group. While not statistically significant, mean pupillary responses in the concussion history group were smaller at the lower 3- and 5-digit sequence lengths, and larger across sequence lengths between 7- and 11-digits—compared to those without a concussion history. In contrast, task performance and heart rate variability responses follow very similar response dynamics across sequence lengths (cognitive load) for both groups.

For descriptive purposes only, pupillary response dynamics for the concussion history group were further summarized across sequence lengths, based on total lifetime concussion injuries and injury chronicity (i.e., time since most recent injury in years) **(Table 4.8)**. Participants reporting only 1 prior concussion represented 54% of the concussion history group (n=13), 25% reported 2 (n=6), and 21% reported 3+ (n=5). Concussion chronicity ranging between 6months to 2 years prior to testing, was reported by 25% of those in the concussion history group (n=6), 25% reported chronicity between 2 to 3 years (n=5), and 54% reported 3 or more years (n=13). Pupillary response dynamics within these subgroups are illustrated in **Figures 4.10** and **4.11**. Upon visual inspection, concussion injury dose and chronicity effects appear negligible at lower sequence lengths within resource availability, though diverge with higher loads that approach and exceed working memory capacity (7 ± 2 digits). Participants' individually averaged pupillary response dynamics are also plotted as a function of sequence length and paneled by total lifetime concussion number and chronicity sub-groups in **Figures 4.12** and **4.13.**

For the primary analysis, participants' average pupillary response, task performance and heart rate variability are plotted as a function of sequence length and grouped by concussion history in **Figures 4.14—4.16** respectively. The significant main effect of sequence-length on greater average pupillary response outcomes was similar to the previous model ($F_{4,232}$=3.67, *p*=0.006). Accuracy, RMSSD, and concussion history demonstrated non-significant effects in the model ($F_{1,1076}$=0.00, *p*=0.972), ($F_{1,1076}$=1.62, *p*=0.204), and ($F_{1,57}$=0.04, *p*=0.833) respectively **(Table 4.8).** Aim 2 mixed effects model simple effects are also outlined in **Table 4.9.** Both aim 1 and aim 2 models demonstrated significant random intercepts, though non-significant random responses across sequence lengths. With respect to random effects in both models, significant residual error and moderate variability were present, along with non-significant random load effects—which supports general load dependent pupillary response dynamics during the overloaded digit span task across participants. Model-derived pupillary response means are plotted by concussion history group for each participant as a function of sequence-length in **Figure 4.17**.

Table 4.1. Participant demographic information

Demographics	
Age	20.48 ± 1.86
Sex	**Males** = 34 (58%) **Females** = 25 (42%)
Concussion History	
Group	**No** = 35 (60%) **Yes** = 24 (40%)
Lifetime Concussions	**1** = 13 (54%) **2** = 6 (25%), **3+** = 5 (21%)
*Concussion Chronicity	**6 months to 2 years** = 6 (25%) **2 to 3 years** = 5 (21%) **3+ years** =13 (54%)

*Time since most recent injury

Table 4.2. Self-reported performance

Self-Performance	Frequency	Percent
Not Well at All	11	18.6
Slightly Well	20	33.9
Moderately Well	25	42.4
Very Well	1	1.7
Extremely Well	0	0

A total of 58 participants completed a question following task completion that asked how well they felt they performed on the digit span task with the following response options.

Table 4.3. Average pupillary response, task performance, and heart rate variability summarized across sequence-length and 95% confidence intervals

		N	Mean	95% CI	
Average Pupillary Response (baseline corrected response in mm)	**Sequence-Length**				
	3-digits	233	0.11	0.08	0.15
	5-digits	233	0.14	0.10	0.18
	7-digits	227	0.22	0.18	0.26
	9-digits	223	0.25	0.21	0.30
	11-digits	225	0.23	0.18	0.28
Average Task Performance (accuracy—percent correct)	**Sequence-Length**				
	3-digits	236	98%	97%	100%
	5-digits	236	80%	77%	84%
	7-digits	236	52%	48%	56%
	9-digits	236	34%	31%	36%
	11-digits	236	27%	25%	29%
Average Heart Rate Variability (RMSSD in ms)	**Sequence-Length**				
	3-digits	236	40.7	37.2	44.2
	5-digits	236	41.7	38.0	45.5
	7-digits	236	39.3	35.6	43.0
	9-digits	236	40.9	37.4	44.3
	11-digits	236	39.6	36.2	43.0

Average responses for each variable are summarized across all valid trials for all 59 participants.

Table 4.4. Bivariate correlation matrix among numeric variables of interest

	Age	[a]Average Total Years	[a]Average Total Hours	Pupillary Response	Task Performance	Heart Rate Variability	Sequence Length
Age	1						
[a]Average Total Years	0.04	1					
[b]Average Total Hours	**-0.11**	**0.64**	1				
Pupillary Response (baseline corrected response in mm)	0.06	-0.03	0.01	1			
Task Performance (accuracy--percent correct)	-0.01	-0.02	-0.03	**-0.11**	1		
Heart Rate Variability (RMSSD in ms)	-0.04	**-0.14**	**-0.09**	-0.04	**0.06**	1	
Sequence Length	0	0	0	**0.15**	**-0.77**	-0.02	1

Correlations include total number of observations for all 59 participants. Bolded numbers represent significant relationships where *p*=0.05. RMSSD = root mean square of successive differences (in milliseconds).

[a] Total Years = Lifetime (years) participating in contact/collision sport

[b] Total Hours = total hours participating in contact/collision sport in high school—including practice and game hours in pre- in- and post-season.

Table 4.5. Aim 1 mixed effects model for the effect of cognitive load, task performance, and heart rate variability on pupillary response (type III results)

Variable	F Value	*p*
Sequence-Length	3.69	0.006
Task Performance (accuracy--percent correct)	0.00	0.974
Heart Rate Variability (RMSSD in ms)	1.58	0.208

--RMSSD (root mean square of successive differences) in milliseconds.

Table 4.6. Aim 1 mixed effects model for the effect of cognitive load, task performance, and heart rate variability on pupillary response (simple effects)

	Estimate	DF	t Value	95% CI		*p*
Sequence Length						
5-digits	0.03	232	.98	-0.03	0.09	0.326
7-digits	0.10	232	3.03	0.04	0.17	0.003
9-digits	0.14	232	3.50	0.06	0.21	0.001
11-digits	0.12	232	2.58	0.03	0.19	0.010
3-digits	0	.	.	.	.	.
Task Performance (accuracy--percent correct)	-0.00	1076	-0.06	-0.08	0.08	0.974
Heart Rate Variability (RMSSD in ms)	-0.00	1076	-0.94	-0.00	0.00	0.208

--RMSSD (root mean square of successive differences) in milliseconds.

Table 4.7. Concussion history group differences in average study measures across sequence lengths and 95% confidence intervals

		Concussion History Groups					
		No (n=35)			**Yes (n=24)**		
		Mean	**95% CI**		**Mean**	**95% CI**	
Average Pupillary Response (baseline corrected response in mm)	**Sequence-Length**						
	3-digits	0.14	0.09	0.18	0.08	0.03	0.13
	5-digits	0.17	0.11	0.22	0.10	0.04	0.17
	7-digits	0.20	0.15	0.25	0.26	0.19	0.32
	9-digits	0.24	0.18	0.31	0.27	0.21	0.34
	11-digits	0.22	0.15	0.29	0.24	0.17	0.31
Average Task Performance (accuracy--percent correct)	**Sequence-Length**						
	3-digits	98%	100%	96%	99%	100%	98%
	5-digits	80%	85%	76%	79%	85%	74%
	7-digits	53%	58%	48%	51%	57%	44%
	9-digits	32%	35%	29%	36%	40%	32%
	11-digits	26%	28%	23%	28%	31%	25%
Average Heart Rate Variability (RMSSD in ms)	**Sequence-Length**						
	3-digits	43.4	48.4	38.4	36.8	41.0	32.7
	5-digits	44.0	49.4	38.6	38.7	43.1	34.3
	7-digits	42.5	48.0	36.9	34.9	38.8	31.1
	9-digits	43.5	48.6	38.5	37.3	41.3	33.4
	11-digits	42.5	47.5	37.5	35.9	39.7	32.2

Average responses for each variable are summarized above by concussion history group, for all 59 participants. RMSSD (root mean square of successive differences) in milliseconds.

Table 4.8. Pupillary response means by sequence length for concussion history subgroups

Concussion History Group Means

Total Lifetime Concussions

	1		**2**		**3+**	
	n= 13		**n=6**		**n=5**	
	Mean	95% CI	Mean	95% CI	Mean	95% CI
Sequence-Length						
3-digits	0.03	-0.03 0.09	0.13	0.01 0.26	0.15	0.07 0.23
5-digits	0.10	0.00 0.20	0.13	0.02 0.24	0.09	-0.06 0.23
7-digits	0.22	0.13 0.31	0.36	0.17 0.55	0.24	0.18 0.31
9-digits	0.22	0.13 0.31	0.42	0.27 0.58	0.23	0.15 0.31
11-digits	0.18	0.09 0.28	0.41	0.24 0.58	0.18	0.09 0.28

Concussion Chronicity

	6 Months to 2 Years		**2 to 3 Years**		**3+ Years**	
	n=6		**n=5**		**n=13**	
	Mean	95% CI	Mean	95% CI	Mean	95% CI
Sequence-Length						
3-digits	0.07	-0.01 0.15	0.05	-0.06 0.16	0.10	0.03 0.17
5-digits	0.05	-0.08 0.17	0.08	-0.10 0.26	0.14	0.05 0.23
7-digits	0.21	0.09 0.34	0.32	0.19 0.45	0.25	0.15 0.35
9-digits	0.15	0.06 0.23	0.28	0.14 0.42	0.32	0.23 0.42
11-digits	0.12	0.04 0.21	0.12	0.00 0.24	0.35	0.24 0.46

Table 4.9 Aim 2 mixed effects model for the effect of concussion history, cognitive load, task performance, and heart rate variability on pupillary response (type III results)

Variable	F Value	*p*
Sequence-Length	3.67	0.006
Task Performance (accuracy--percent correct)	0.00	0.96
Heart Rate Variability (RMSSD in ms)	0.97	0.32
Concussion History	0.45	0.50

--RMSSD (root mean square of successive differences) in milliseconds.

Table 4.10. Aim 2 mixed effects model for the effect of concussion history, cognitive load, task performance, and heart rate variability on pupillary response (simple effects)

	Estimate	DF	t Value	95% CI		*p*
Sequence lengths						
5-digits	0.03	232	0.98	-0.02	0.82	0.326
7-digits	0.10	232	3.02	0.04	0.17	0.003
9-digits	0.14	232	3.50	0.06	0.21	<0.001
11-digits	0.11	232	2.58	0.03	0.18	0.010
3-digits	0	.	.	.	.	.
Task Performance (accuracy--percent correct)	-0.001	1076	-0.03	-0.09	0.08	0.973
Heart Rate Variability (RMSSD in ms)	-0.00	1076	-1.27	-0.00	0.00	0.204
Concussion History (Yes v No)	-0.01	57	-0.21	-0.1	0.08	0.838

--RMSSD (root mean square of successive differences) in milliseconds.

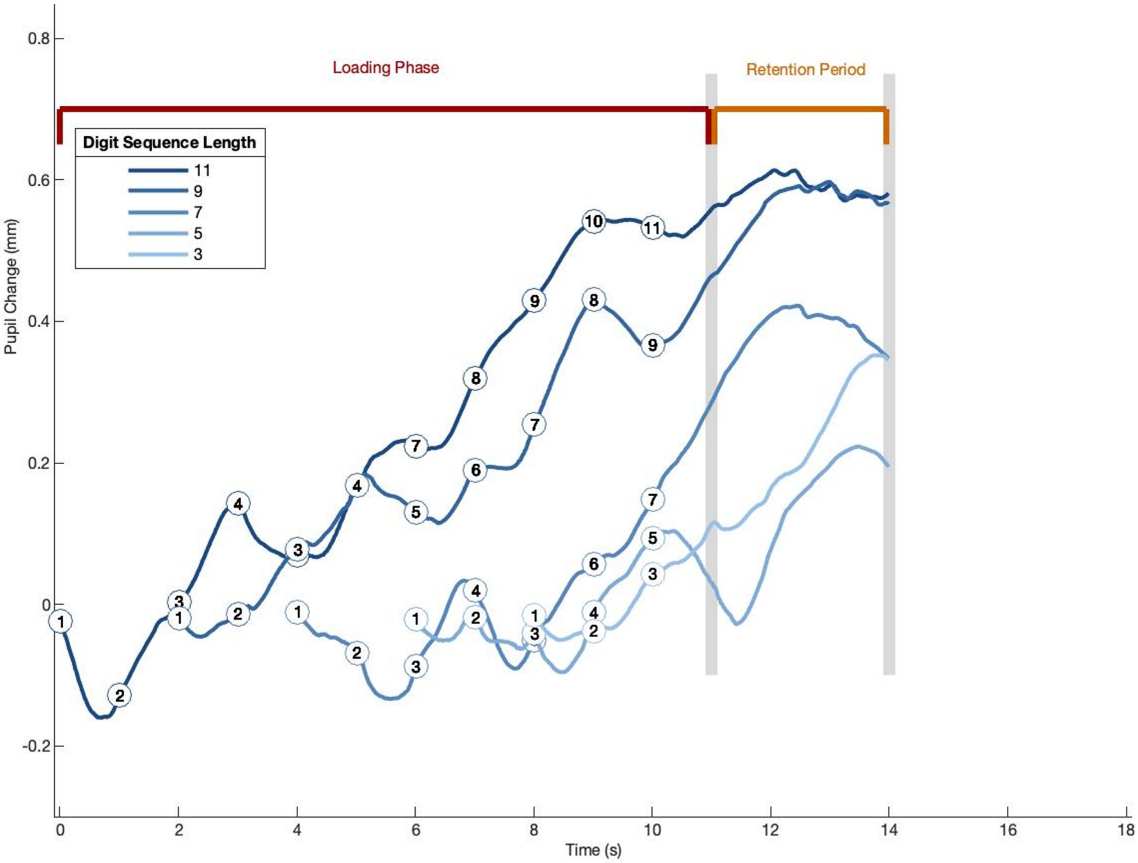


Figure 4.1. Prototypical time trace for pupillary response dynamics by sequence-length. Similar to time trace representation described by Klingner et al.—numbered circles on each line represent digit presentation. Each curve is horizontally shifted to align at final digit presentation, with the 11-digit sequence starting furthest to the left. Baseline-corrected pupillary response outcome response region of interest is within the last 2 seconds of the retention period (grey bars).

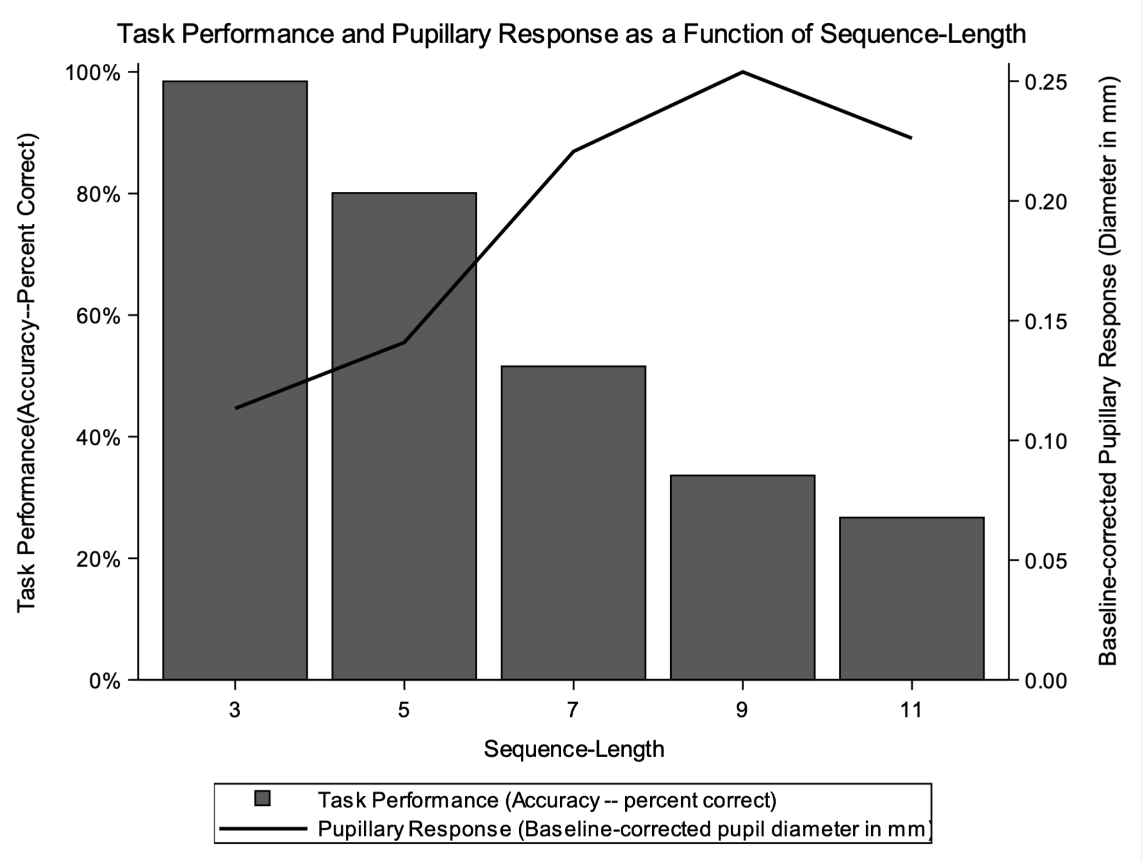


Figure 4.2. Grand means: pupillary response and task performance summarized across sequence-lengths

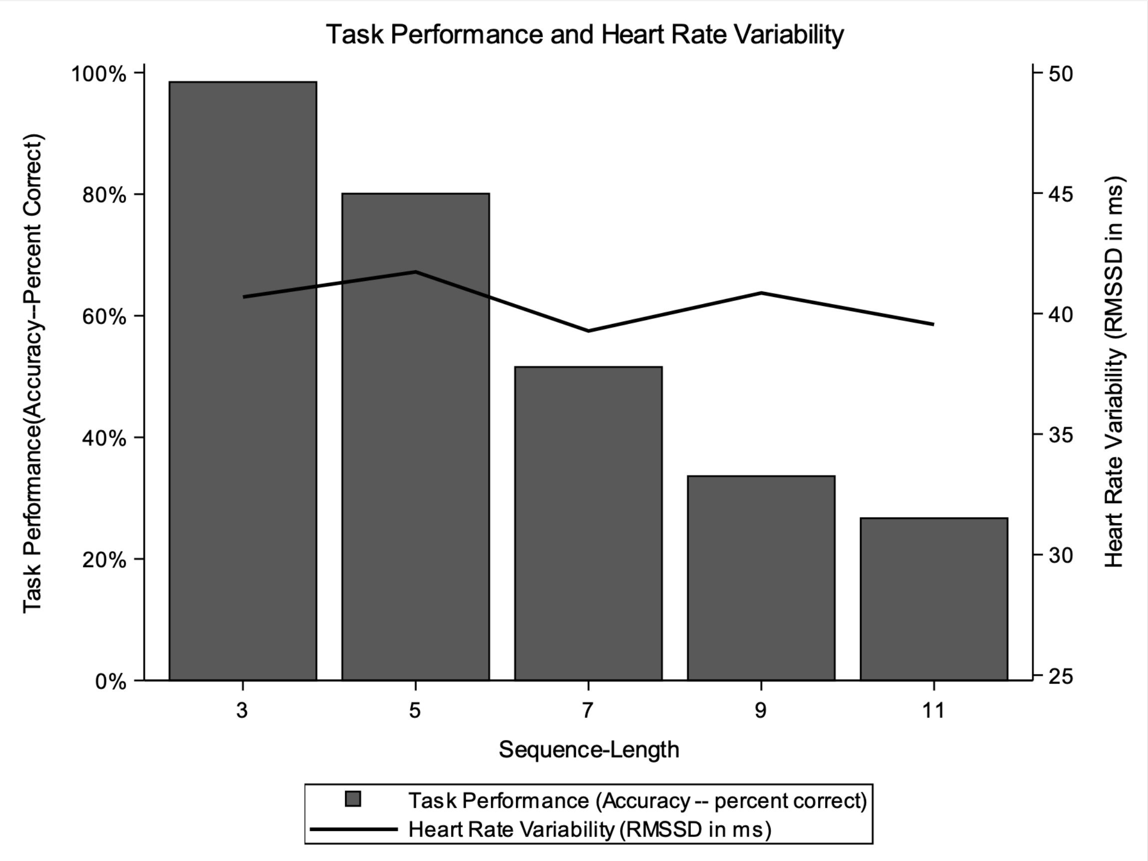


Figure 4.3. Grand means: task performance and heart rate variability summarized across sequence-lengths

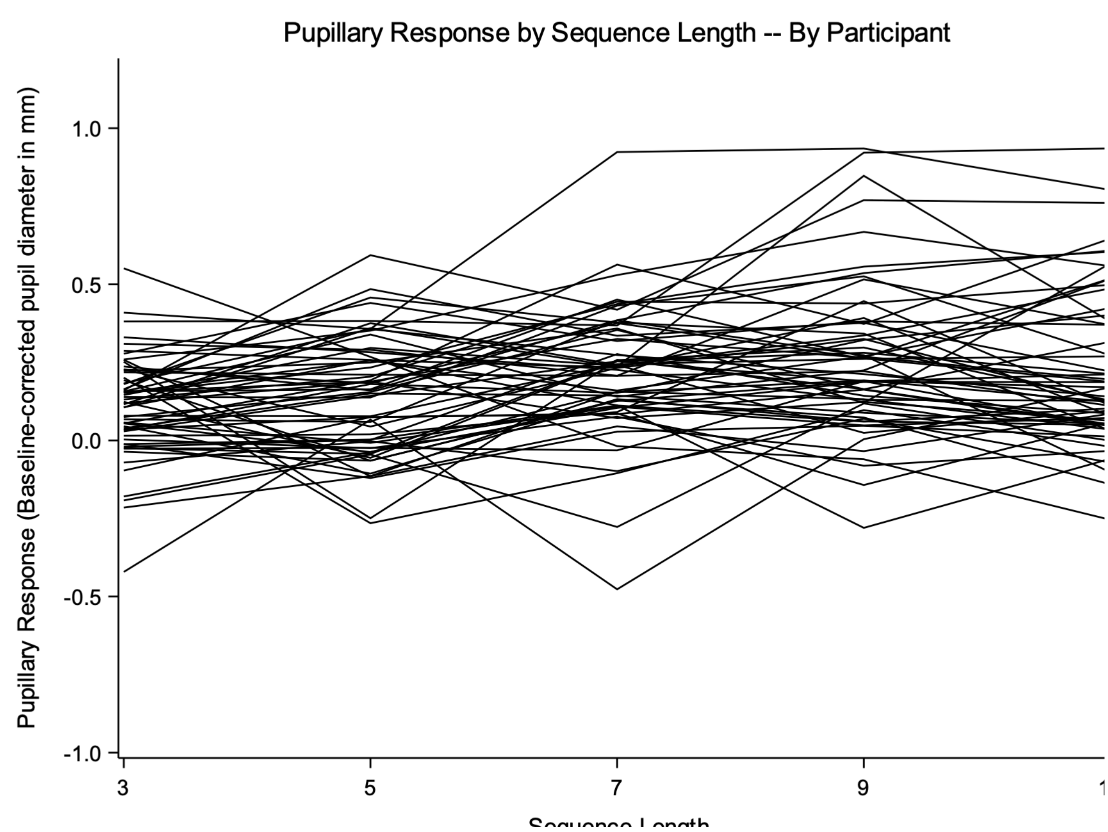


Figure 4.4. Participants' pupillary responses by sequence-length

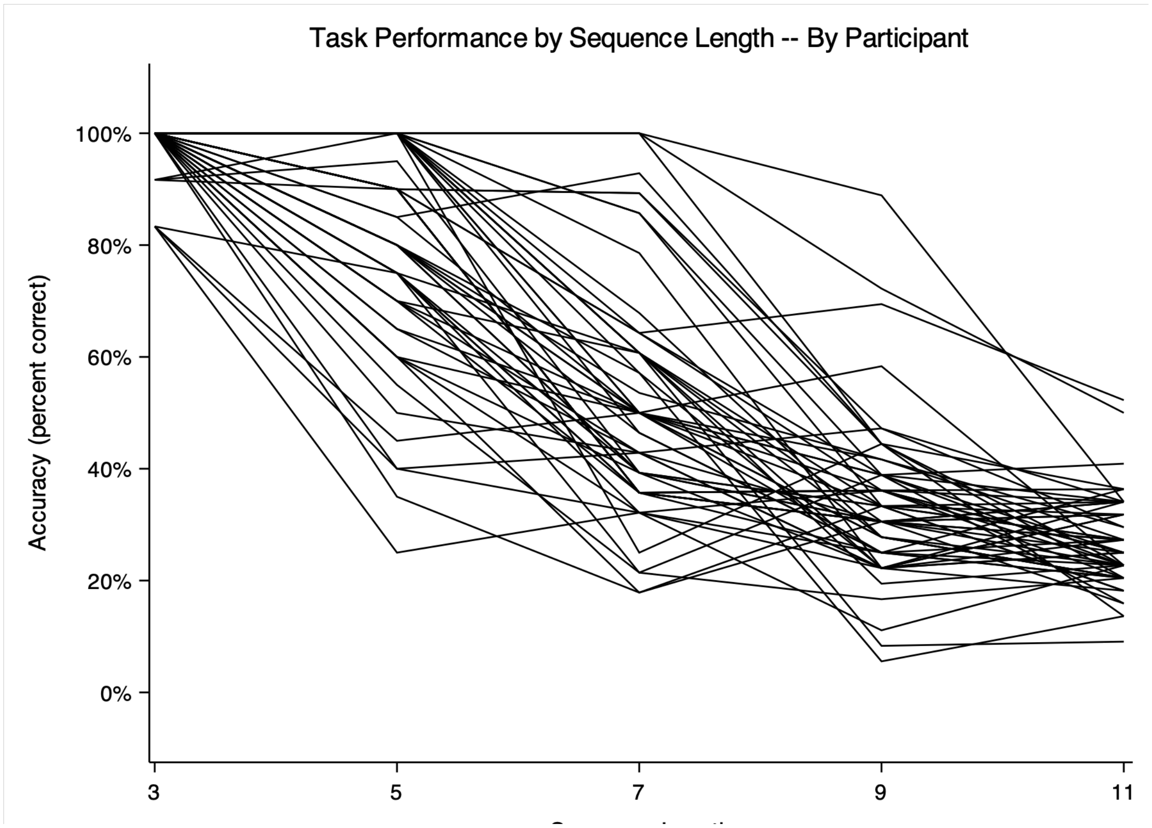


Figure 4.5. Participants' task performance by sequence-length

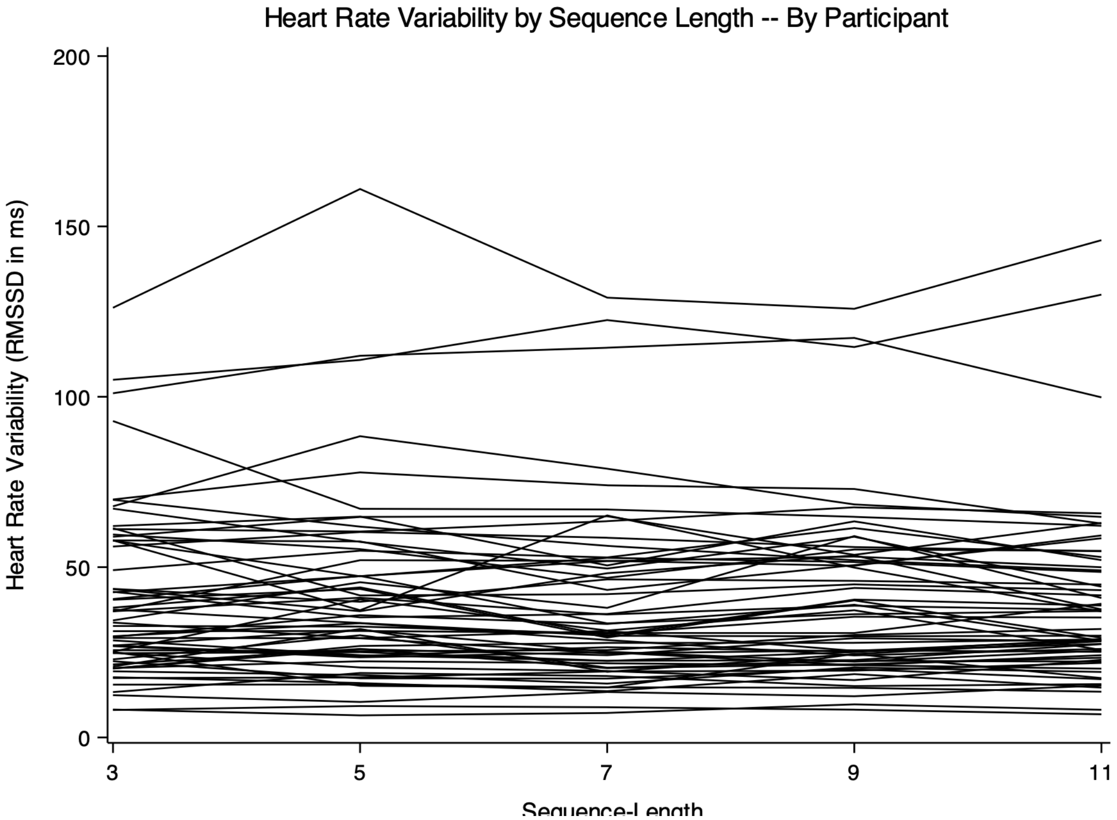


Figure 4.6. Participants' heart rate variability by sequence-length

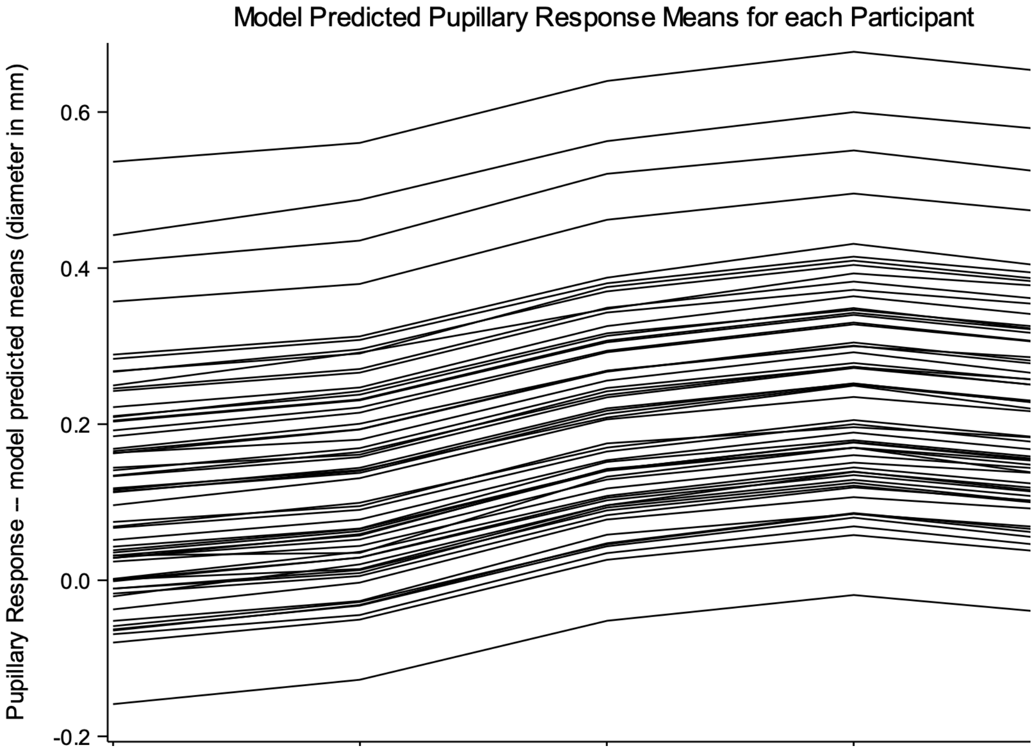


Figure 4.7. Aim 1 model predicted pupillary response means by sequence-length

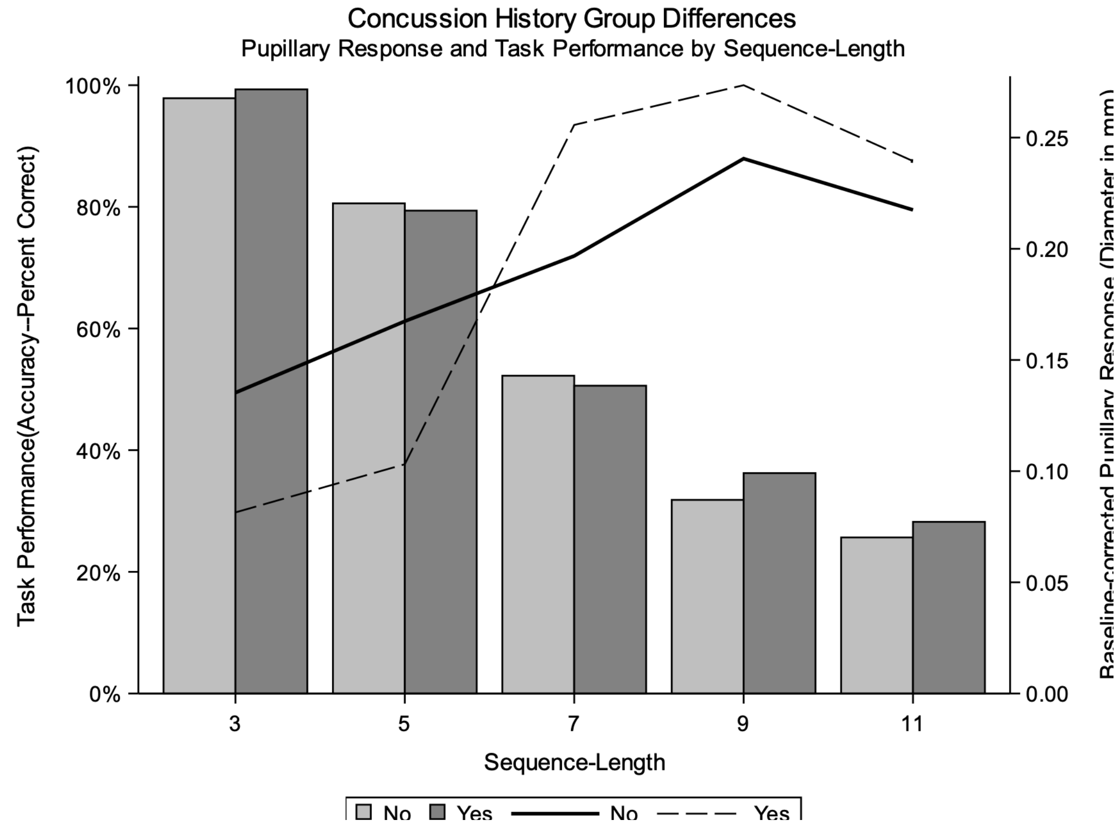


Figure 4.8. Group differences in pupillary response and task performance by sequence-length

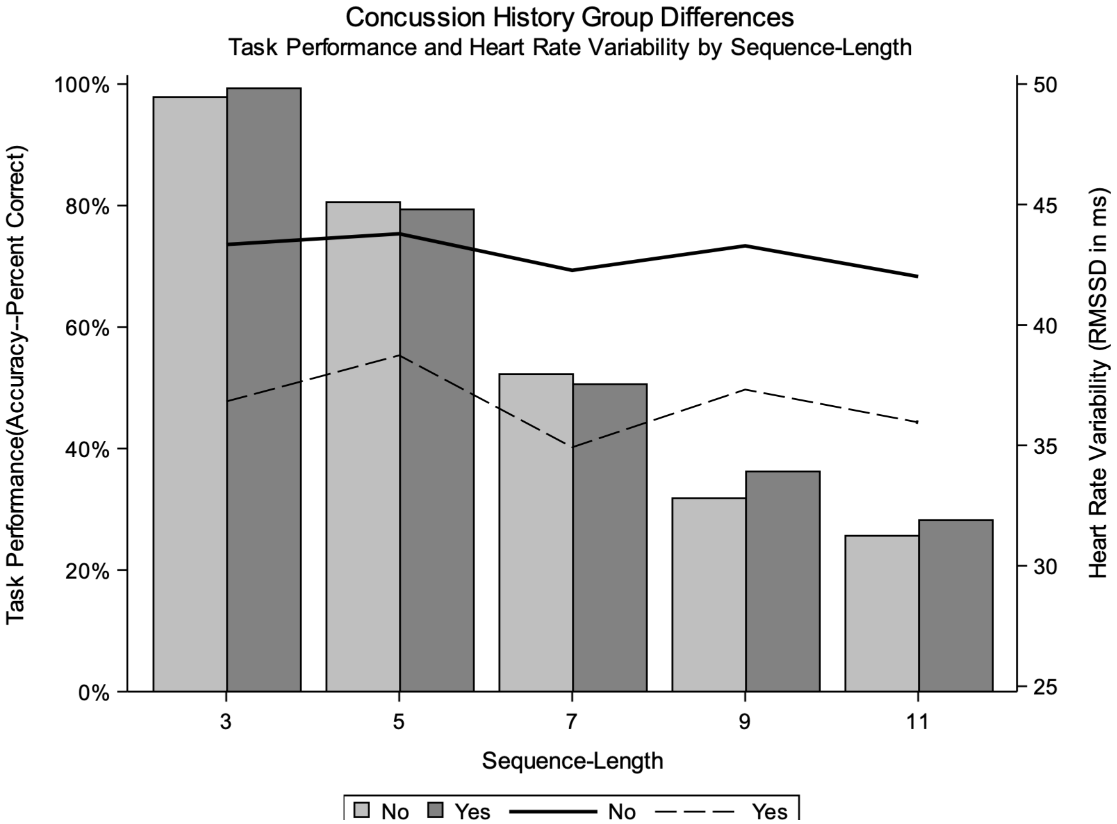


Figure 4.9. Group differences in heart rate variability and task performance by sequence-length

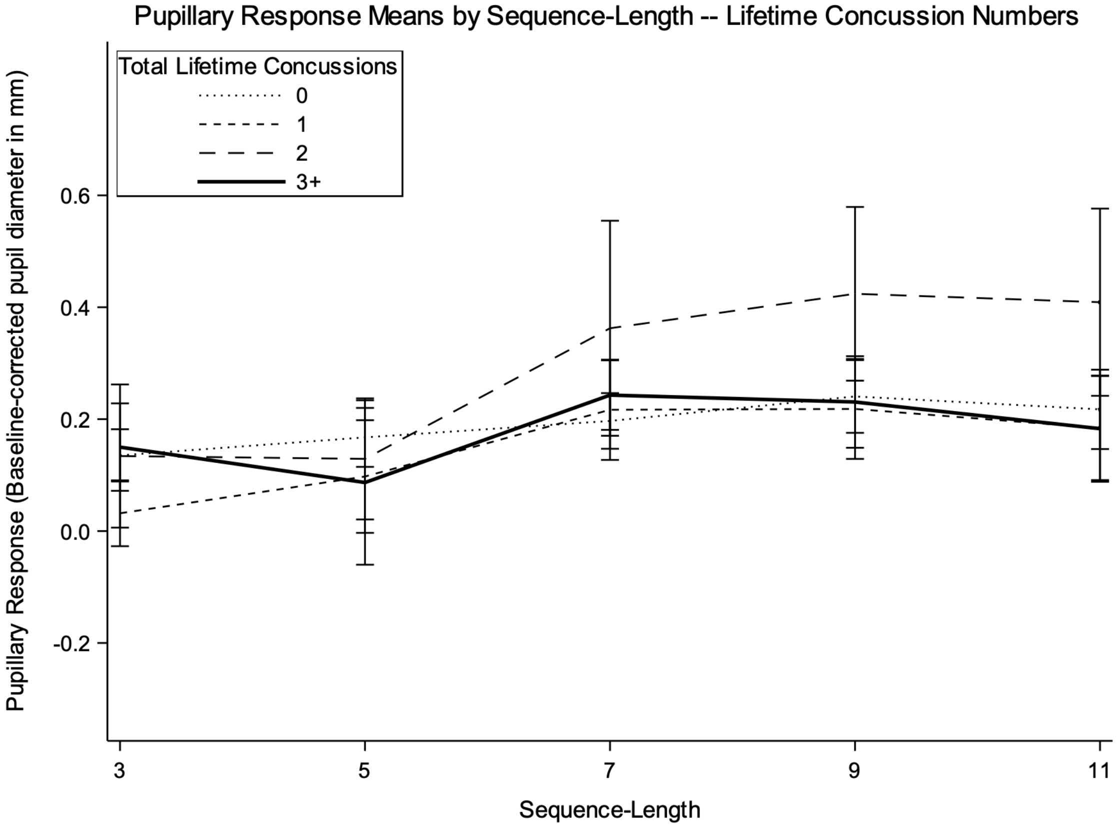


Figure 4.10 Average pupillary response by sequence-length–split by total lifetime concussion subgroups (0, 1, 2, and 3+). Error bars represent 95% Confidence Intervals.

Total participants per subgroup: **0** (n=35), **1** (n=13), **2** (n=6), and **3+** (n=5).

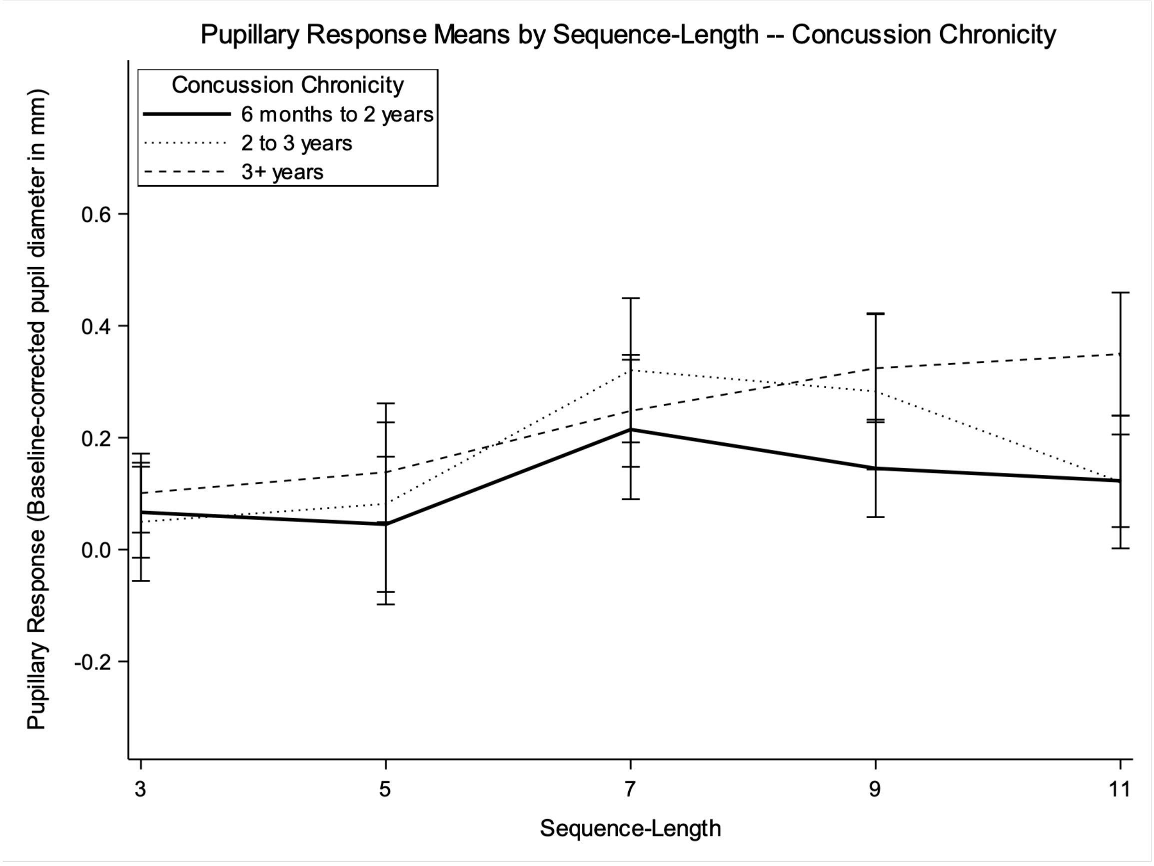


Figure 4.11 Average pupillary response by sequence-length–split by concussion chronicity subgroups.

Total participants per subgroup: (**6months to 2 years** (n=6)**; 2 to 3 years** (n=5)**; 3+ years** (n=13).

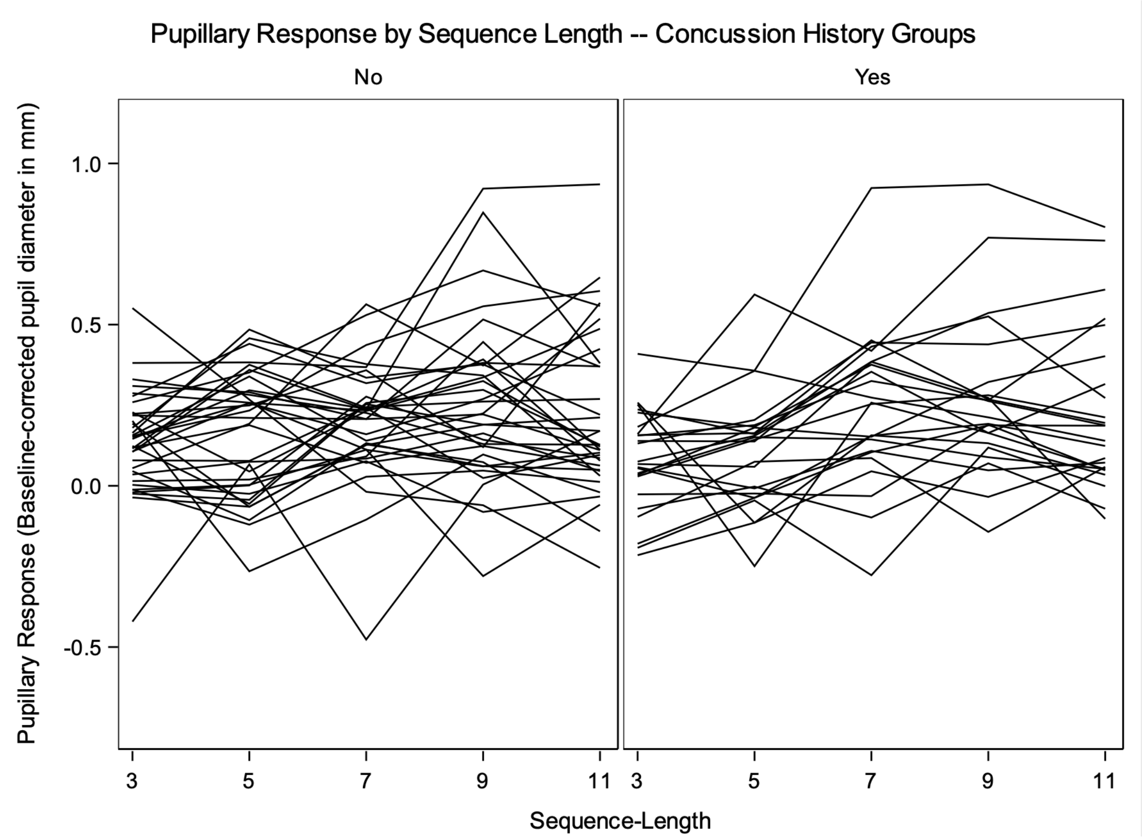


Figure 4.12 Pupillary response by sequence-length for each participant– split by concussion history groups

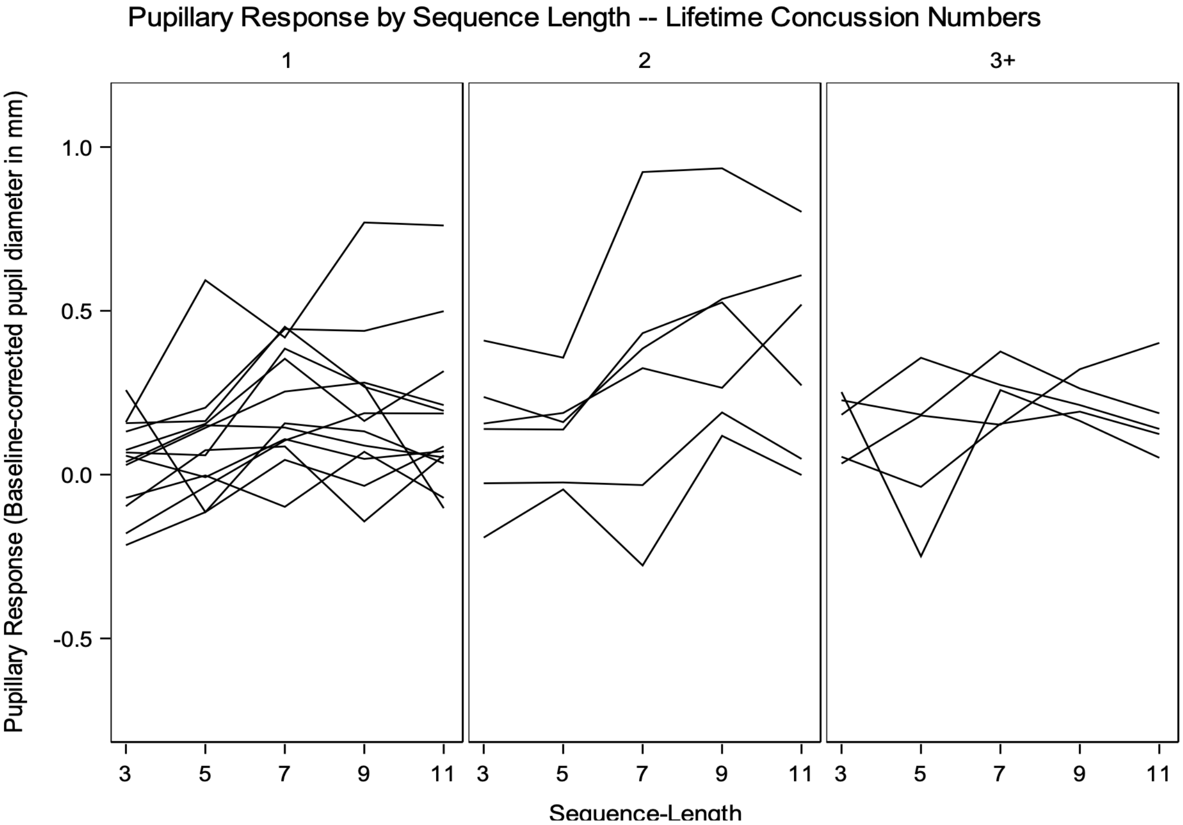


Figure 4.13. Pupillary response by sequence-length for each participant–split by concussion history subgroups for lifetime concussions

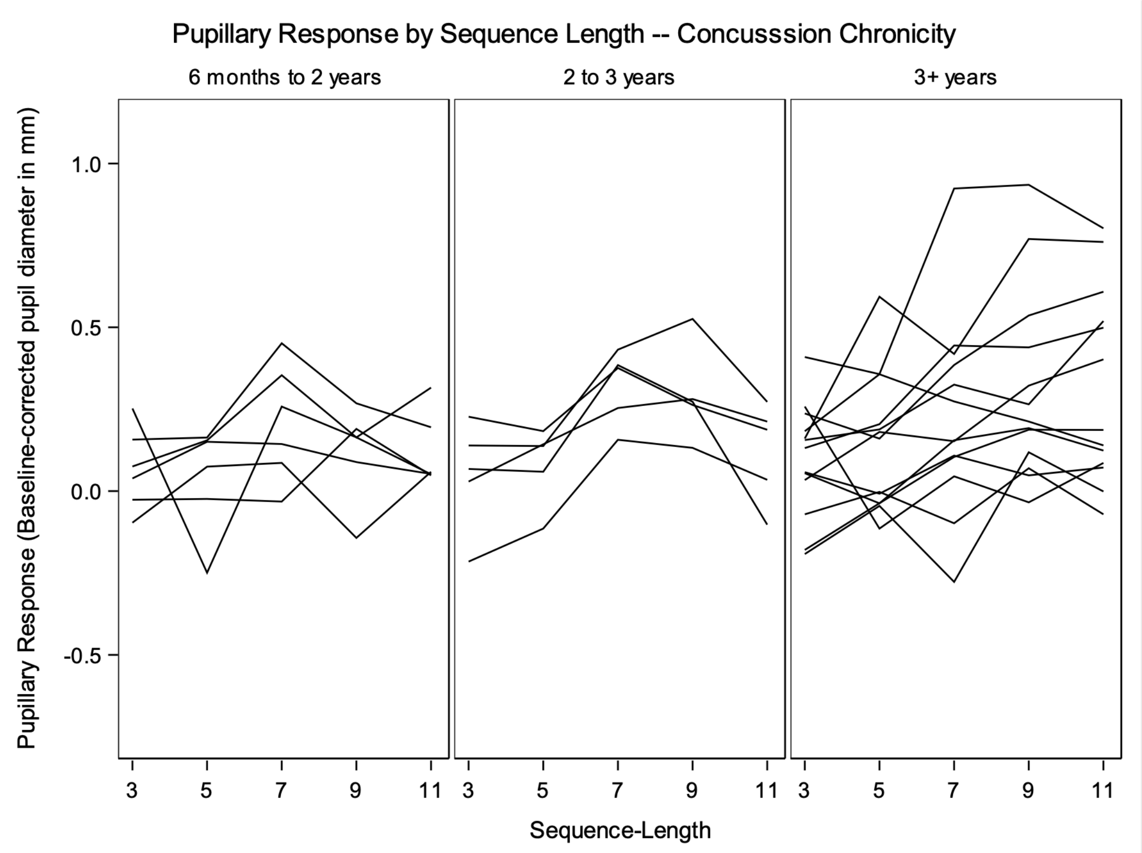


Figure 4.14. Pupillary response by sequence-length for each participant–split by concussion history subgroups for concussion chronicity

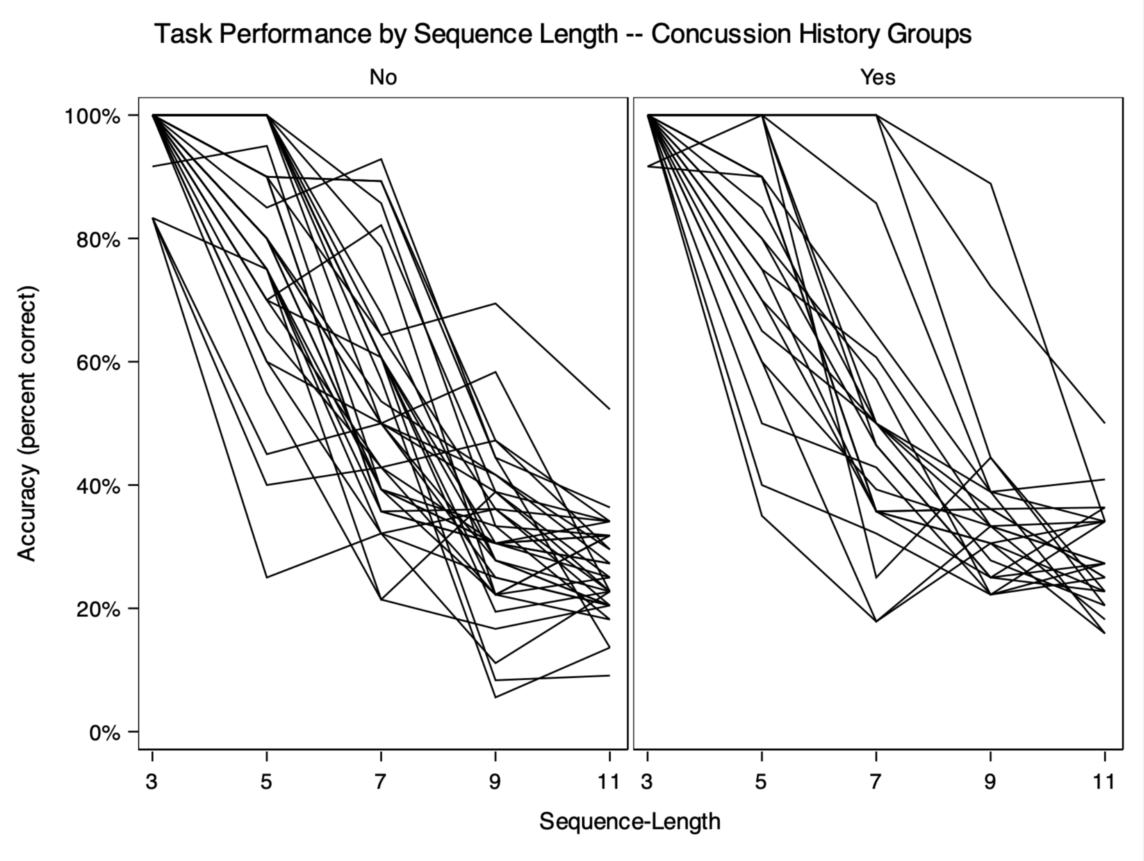


Figure 4.15. Task performance by sequence-length for each participant–split by concussion history groups

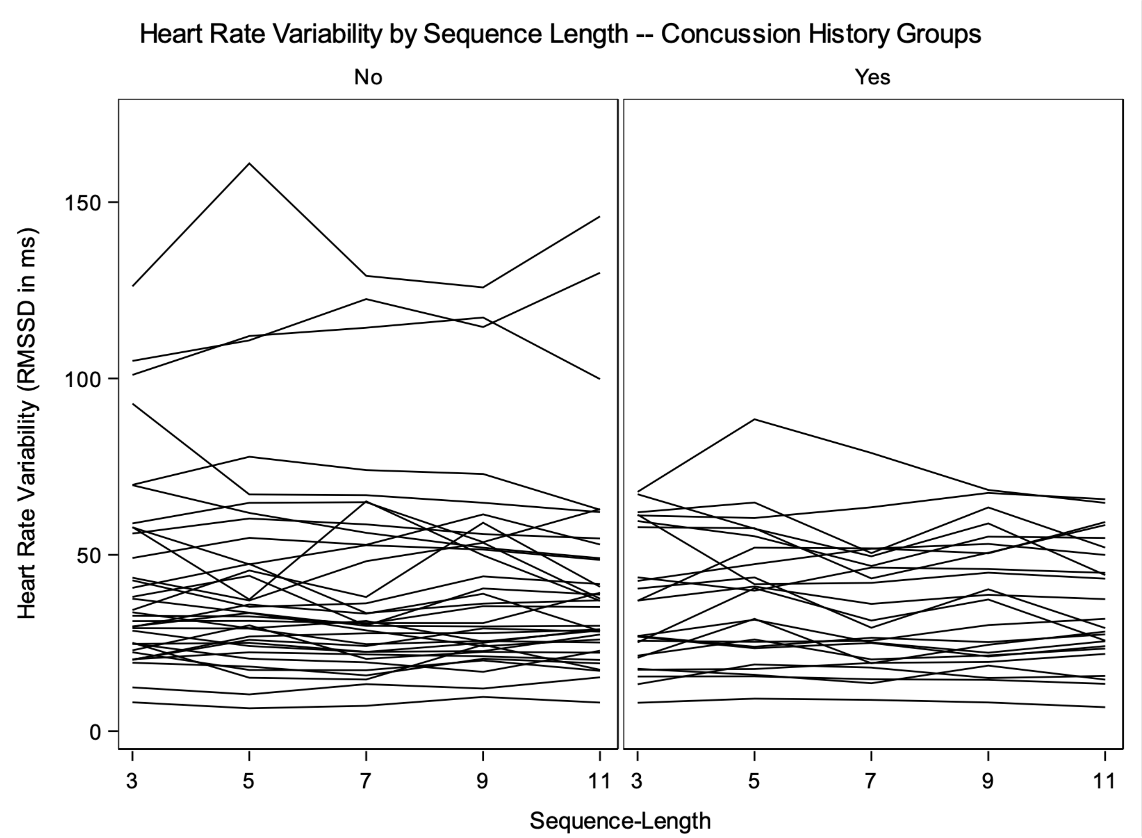


Figure 4.16 Heart rate variability by sequence-length for each participant–split by concussion history groups

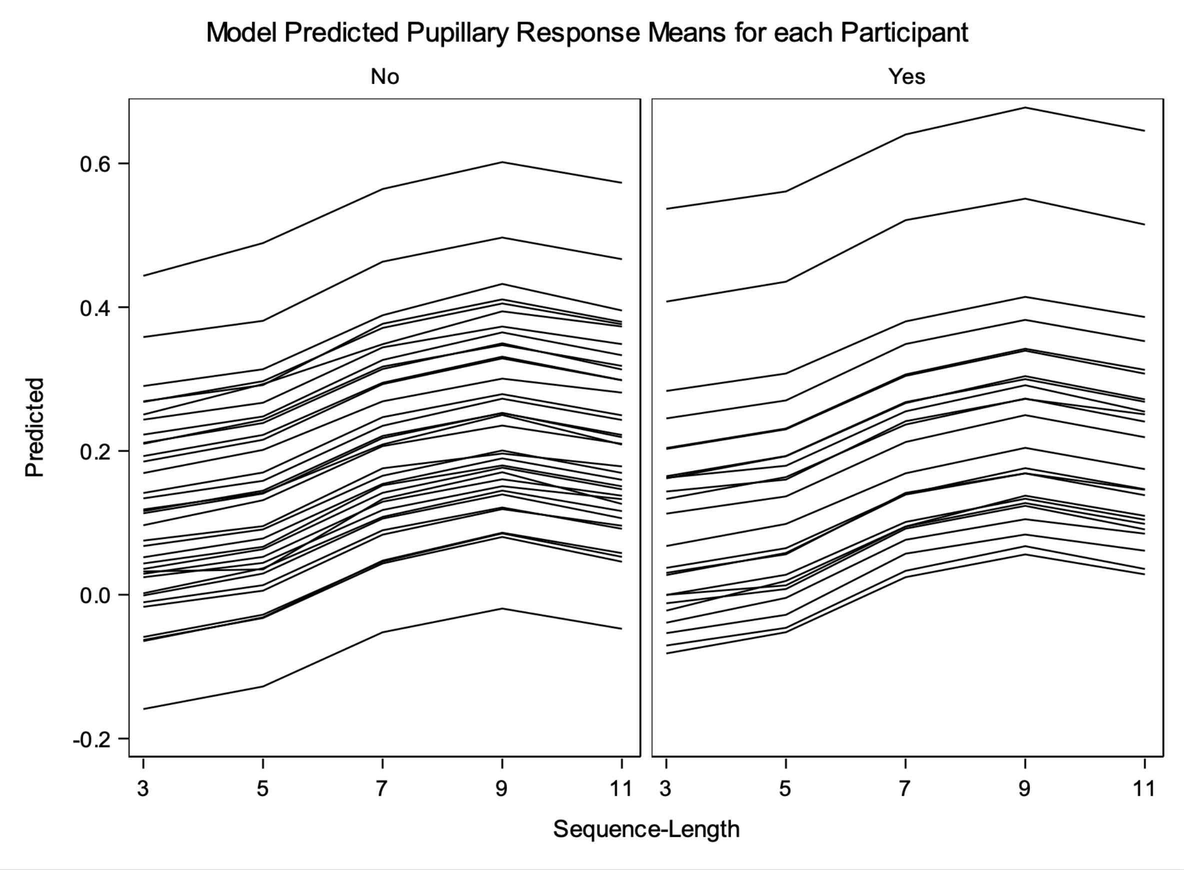


Figure 4.17 Aim 2 model predicted pupillary response means by sequence-length—split by concussion history groups

CHAPTER 5: DISCUSSION

We examined relationships between task-performance, heart rate variability, and cognitive load associated with a backwards digit-span task, and pupillary response measures—followed by concussion history effects, in collegiate club sports athletes. Virtual reality eye tracking technology was used to record pupillary responses, as a more ecologically valid mechanism for cognitive pupillometry, compared to desktop-based trackers and neuroimaging techniques such as fMRI. Consistent with previous literature, using more invasive and costly instrumentation, our results demonstrated load dependent pupillary response dynamics. Specifically, participants demonstrated larger pupillary responses with greater cognitive loads (i.e., longer sequence lengths), within working memory capacity. As such, our findings support advancement for future cognitive pupillometry investigations to expand into more applied settings—which may have important clinical implications for concussion assessment future and management.

5.1 General Findings Informing Cognitive Efficiency

5.1.1 Overall descriptives

Combined examination of clinical task performance and physiological metrics for resource allocation relative to imposed cognitive demands, are essential to assess neurocognitive processing effectiveness, and efficiency. Clinical task performance (accuracy) in our study demonstrated a steady decline from 98% to 27% in response to increasing cognitive loads from 3- to 11-digits. Our accuracy response dynamics are consistent with previous literature and support appropriate task design, whereby cognitive load was adequately modulated beyond working memory capacity and resource availability.[28,34,35] Our pupillary response outcomes were similar to previous cognitive pupillometry studies whereby greater cognitive load elicited larger dilation responses, when the load was within resource availability (working memory capacity of 7 ± 2 digits), then declined with overload (11-digits).[34,35,111] Task performance measures alone (e.g., accuracy), provide little insight relative to participants' neural resource utilization to meet task demands and achieve a specific level of accuracy or performance.[29,31] Task performance measures from neuropsychological tests may be indirect measures of the applied cognitive demands—though they are not continuous. Our pupillary response measure on the other hand, illustrated the ability to assess the cognitive load and associated neural resource allocation in-real time and provided a continuous recording of data over time. [29] Therefore, the neurophysiological measure in our study may capture cognitive change which may appear before manifestation of cognitive symptoms and decreased task performance.[29,34] Future work should examine such outcomes in clinical populations as well as extend this work from a human performance perspective.

5.1.2 Heart Rate Variability

Average heart rate variability measures did not descriptively demonstrate a load dependent response and exhibited negligible relationships with pupillary responses. Our findings indicate that on average participants' HRV was within normal range (i.e., RMSSD between 27-72ms), with little to no change in mean RMSSD across sequence lengths.[54,112] Previous studies have examined changes in short-term HRV measures to examine relationships with cognitive load and task performance.[113–115] However, differences in trial durations, study samples, and the explored cognitive domains likely explain the disagreement with the findings of the present study.[45,54]

Other studies such as that by Hess and Ennis have examined other working memory tasks such as mental arithmetic, that were shorter in nature to assess 3 measures for cardiac physiological resource allocation (i.e., Heart rate—HRV, and systolic and diastolic blood pressure)—whereby systolic blood pressure demonstrated greater sensitivity to age related differences.[29] Moreover, the short measurement duration of our heart rate variability outcome may not have been long enough to capture vagally mediated changes in HRV, by the autonomic nervous system due to acute mental stress during our task.[54] Additionally, not all measures were of the same duration in this study, given the prioritization of self-paced recall periods in our task.

Evidence suggesting cardiac measures' ability to reflect changes in cognitive load, also highlight their sensitivity to experience levels and training, the type of task observed and the time of day—consequently these measures require high levels of task demand in order to be reflected in HRV.[113] Much of the HRV literature however, has examined cardiac activity measurements over longer durations i.e., more "chronic" cognitive or physical loads (>5min), and suggest that more extreme cognitive demands are needed to elicit more prominent responses during shorter measurement durations—similar to trial durations in our study. A few studies have shown elevated HR and subsequently lower HRV during logical and dynamic reasoning tasks demanding high levels of verbal working memory and high visual attention, though HRV measurements were recorded over longer trial durations ≥30s.[45,54] One particular study by Rivecourt et al examined HRV measures during a flight simulation task and measures were recorded over longer durations.[54] This task likely elicited a greater cardiac and autonomic stress response relative to our digit-span task due to the task nature, which might explain the lack of significance in the present study. These findings combined with those from the present study, may also suggest that pupillary responses are more sensitive to real time changes in neural resource utilization and therefore a better measure for clinical monitoring in with respect to cognitive testing than this specific HRV outcome.

5.2 Discussion for Specific Aim 1

Aim 1 examined the relationships between measures of task performance, heart rate variability, and pupillary response across digit-span levels of task difficulty in healthy collegiate club sports athletes. Our mixed effects model demonstrated a significant effect of load on pupillary response –consistent with previous literature.[28,34,35] However, neither task performance or heart rate variability demonstrated significant effects on pupillary response. While descriptively we saw the expected load dependent relationship between task performance and digit-sequence-length, the negligible effect of task performance on pupillary response in the model suggests that resource utilization is not influenced by overall task performance.

Performance effects on pupillary response have been reported during other cognitive control and/or working memory tasks such as the Stroop or n-back tasks, whereby some physiological responses increase after a poor performance trial indicating compensatory neural recruitment for sustained performance.[25,32] These tasks typically maintain a static level of cognitive load for several trials, (e.g., Stroop task will first assess correct identification on both color and word, before the assessing the Stroop effect when asking participants to respond when these things are congruent versus incongruent). [32] The task in the present study requires different aspects of working memory processing given the load is constantly changing.[32] The aspects of cognitive control for our task instead include encoding, and manipulation of information which may make it more difficult—especially on the longer trials—for individuals ascertain their trial-by-trial-performance accuracy. Self-performance and subsequent compensatory recruitment for sustained performance may be more prevalent in tasks that require updating and inhibition such as is Stroop or n-back tasks.[32]

Heart rate variability also demonstrated negligible effects in the model. To our knowledge this is the first study to examine the relationship between pupillary responses and heart rate variability as physiological markers for resource allocation to meet task demands during a digit span task. Previous studies have examined the effects of cognitive load on each of these measures independently as separate dependent variables.[41,113] The negligible effect of heart rate variability on pupillary response may be explained by poor sensitivity of our chosen HRV measure to detect acute changes in cognitive load, as described above, or may support the independence in these two physiological measures to capture aspects of physiological resource mobilization to meet cognitive task demands.[113] Future studies should consider splitting these two physiological metrics to inform cognitive efficiency given they likely reflect different aspects of ANS activity. Moreover, additional studies are needed to determine the utility of various short-term HRV measures to capture acute cognitive load. The lack of convergence between pupillary response and this particular cardiovascular measure for resource mobilization may be further explained by the differing sensitivity of these measures to various factors, as outlined in **Table 5.1**. In light of our findings, and those of previous literature, it is important to note the sensitivity in both pupillary response and HRV measures relative to their ANS foundations to various confounding factors. However, our findings suggest pupillary responses may be a more sensitive measure—especially to acute, short-term changes in cognitive load—given our ability to mitigate potential confounds through vigorous environmental control in VR.

5.3 Discussion for Specific Aim 2

Aim 2 investigated the potential added effect of a prior concussion injury on the relationships examined in aim 1. Model results for cognitive load, task performance and HRV were similar to those observed in Aim 1 and were relatively unchanged with the addition of concussion history in the model. Concussion history groups did not demonstrate statistically significant differences in pupillary response dynamics across levels of task difficulty. Our study results differ from previous cognitive pupillometry studies reporting group differences in pupillary response outcomes, within clinical populations with cognitive deficits. Additionally, our model findings differ from those concerning the pupillary light reflex as well as other eye tracking methodologies that identified differences in those with and without a concussion history.[90,91] Discrepancies between our findings and previous work in clinical populations may be explained by differences in task design and the selected pupillary response outcome.

A recent study by Hershaw et al. examined several pupillary response metrics (e.g., cue- and response-locked means, peaks, peak latencies) during an n-back cued attention task, to describe variability and reliability across concussion history groups, with respect to cognitive load sensitivity. Results from the Hershaw's study showed greater pupillary responses outcomes at higher cognitive loads in the concussion history group—whereby, dilation response latencies demonstrated the greatest sensitivity to group differences.[101] Few cognitive pupillometry studies have examined dilation/constriction latency outcomes, more often used when evaluating the pupillary light reflex. However, Hershaw's results warrant future consideration and examination of latency outcomes, especially in clinical populations.

Granholm and colleagues reported similar findings when examining pupillary responses during a digit span task in those with amnestic single domain-mild cognitive impairment associated with Alzheimer's, compared to healthy controls. [38] Participants in the Granholm study exhibited similar task-performance measures though greater pupil responses at lower cognitive loads (i.e., shorted digit-sequences) were exhibited in those with mild cognitive impairment, compared to cognitively normal participants.[38] Suggesting compensatory neurophysiological resource utilization and decreased cognitive efficiency when cognitive load is lower in this unique population with mild cognitive impairment.[116] Response visual examination suggests a potential trend developing and group by load interaction which warrants future examination, as differences may be isolated to a certain cognitive load level.

Preliminarily, we examined pupillary response dynamics as a function of cognitive load, within concussion history subgroupings to probe potential dose and concussion chronicity responses. Visual examination shows a potential dose response, whereby those with more than on prior concussion may be utilizing greater neural resources when cognitive loads exceed capacity. Plot inspection for average pupillary responses by cognitive load for concussion subgroups representing time since most recent injury also warrants further examination, as they depict potential response differences with respect to overall resource utilization and response change points for working memory capacity. Specifically, those reporting shorter chronicity exhibit more distinct change points at capacity, that might suggest overall limited resource availability. Given these preliminary reviews, future studies should further elucidate potential dose and concussion chronicity responses through within group analyses.

5.4 Summary of Findings Related to Original Hypotheses

Our hypotheses that greater cognitive load (e.g., sequence length) would elicit larger pupillary responses (dilation) were supported by our findings. Null findings were exhibited in our hypothesized effects of our other independent variables, including task performance, heart rate variability, and concussion history, on pupillary responses. It is important to recognize the utility in the results of our study despite these negligible effects, especially as it pertains to the robustness and potential sensitivity and specificity pupillary responses may offer when examining cognitive efficiency in future studies using a more parsimonious model.

5.5 Limitations

Our study was not without limitations. Shifting cognitive pupillometry assessments into a VR headset helped to mitigate potential non-cognitive environmental influence on pupillary response outcomes. However, other potential confounds for pupillary response metrics and heart rate variability relative to ANS function may have played a role in measurement variability. [28,29] For example, we did not examine effects of daily medication and other co-morbidities (e.g., anxiety, depression, etc.) on these measures, or standardized the testing time of day. [28,29,101]

The pupillary response outcome measure used in this study, while common in cognitive pupillometry studies examining working memory, may not have been sensitive to concussion group differences. Future studies should consider using alternative pupillary response outcomes given recent Hershaw et al. results demonstrating greater sensitivity in latency metrics to concussion group differences compared to baseline-corrected responses.[101] Consensus around metric selection will eventually lend towards normative values, and improved generalizability of study findings. Participant heterogeneity and differences in task design within previous literature further complicate generalizability of our findings. Despite these limitations, we sought to control many confounders by enrolling a sample within a tight age range, similar athletic demands, and in a virtual reality environment.

5.6 Future Research

There is a great paucity of cognitive pupillometry studies in applied clinical settings comparing healthy and clinical groups (e.g., post-concussion) which greatly compromises the generalizability of our findings. Our findings do however, support advancement for cognitive pupillometry investigations to expand into applied settings in the future—which may have important clinical implications for concussion assessment and management.

Longitudinal examination of within and between subjects' comparative analyses among pupillary response and other relevant clinical performance-based metrics. This will provide initial information to establish normative values around intra-individual changes and inter-individual variation in cognitive processing. Studies aimed to discriminate among clinical populations should consider more discriminatory inclusion criteria, clinically meaningful assessment timepoints and within group analyses to control for potential injury comorbidities and/or confounding variables which may include injury frequency and chronicity.

5.6 Conclusions

Our study was the first to examine an assessment for cognitive efficiency that accounts for both physiological and performance-based response dynamics, using VR and eye tracking technology, which may have implications for clinical practice and future cognitive interventions. Our study findings support pupillary responses' sensitivity to cognitive load beyond performance-based measures alone, in an athletic population. These findings provide additive evidence for the need to include ecologically valid and cost-efficient neurophysiological metrics when assessing cognitive performance and efficiency. Employing VR and eye tracking technologies to examine these responses may be useful for human performance and concussion management paradigms.

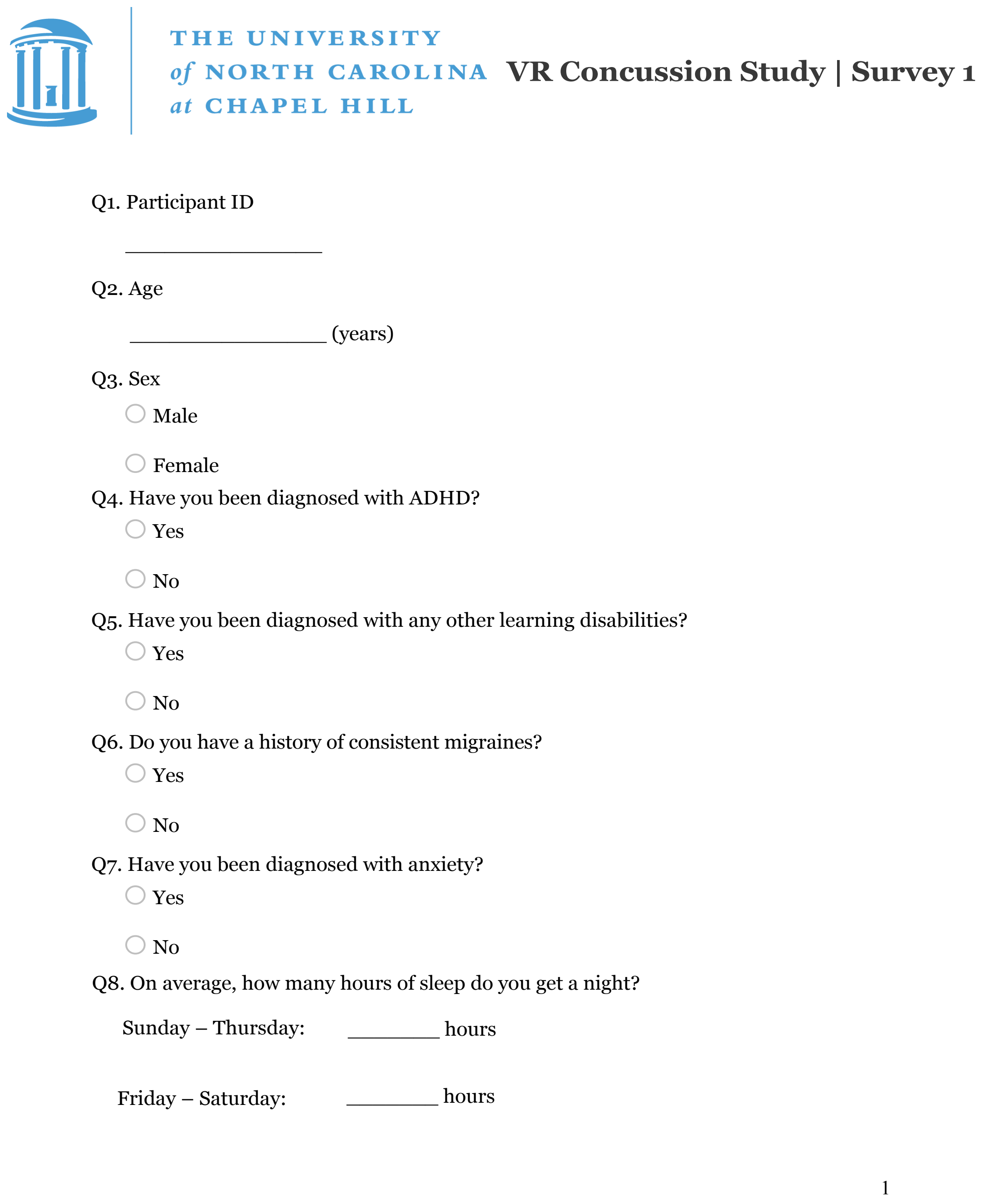
Table 5.1 Factors Know to Influence Heart Rate Variability versus Pupillary Response Measures

Heart Rate Variability	Pupillary Response
Respiration versus paced breathing	** Environmental luminance
Health/training status	** Testing distance (distance between camera & eyes)
Testing position (supine, seated, etc.)	** Accommodation response
Movement during testing	** Environmental distractions and saccadic eye movement
Recency of physical activity	Medication/Alcohol/Drug use
Medication/Alcohol/Drug use	Emotional state
Emotional state	

--Due to the modulation of each of these measures by the autonomic nervous system, each are uniquely sensitive to latent variables that could influence outcomes. Different factors identified by previous literature to influence heart rate variability and pupillary response measures are outlined above.
** Indicates the factors we are able to potentially mitigate with pupillary response testing in a VR environment.

APPENDIX A. TESTING SESSION MATERIALS

Testing Session Materials -- Questionnaire



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VR Concussion Study | Survey 1

Q1. Participant ID

____

Q2. Age

________________ (years)

Q3. Sex

○ Male

○ Female

Q4. Have you been diagnosed with ADHD?

○ Yes

○ No

Q5. Have you been diagnosed with any other learning disabilities?

○ Yes

○ No

Q6. Do you have a history of consistent migraines?

○ Yes

○ No

Q7. Have you been diagnosed with anxiety?

○ Yes

○ No

Q8. On average, how many hours of sleep do you get a night?

Sunday – Thursday: ________ hours

Friday – Saturday: ________ hours

1

Q9. **Concussion**

A **concussion** is a change in brain function following a force to the head, which may be accompanied by temporary loss of consciousness and is identified in awake individuals with measures of neurological and cognitive dysfunction.

Common concussion symptoms include:

headache
irritability
nausea
poor balance
fatigue
drowsiness
feeling slowed down
sensitivity to light/noise
dizziness
feeling in a fog
loss of balance
memory loss
loss of energy
blurred vision
difficulty focusing
difficulty concentrating

Q10 Have you ever had a concussion?

○ Yes

○ No **If 'No' Go to Page 5**

Q11 How many concussions have you had in your lifetime? (CIRCLE one)

1 **2** **3** **4** **5+**

Q12 How long ago was your most recent concussion? (Days)

________ Days

Q13 How long ago was your most recent concussion? (Months)

________ Months

Q14 How long ago was your most recent concussion? (Years)

________ Years

Please answer the following questions regarding your **most recent concussion** (if applicable)**:**

Q15. Date of prior concussion: (mm/dd/yyyy)

___ __/__ __/__ __ __ __

Q16. How long did it take for you to recover?

○ Within 2 Weeks

○ Within a month

○ > one month

○ > one year

Q17. Did you lose consciousness upon injury?

○ Yes

○ No

Q18. Describe below how you sustained your concussion:

Please answer the following questions regarding your **second (most recent) concussion** (if applicable)**:**

Q19. Date of prior concussion: (mm/dd/yyyy)

___ __/__ __/__ __ __ __

Q20. How long did it take for you to recover?

○ Within 2 Weeks

○ Within a month

○ > one month

○ > one year

Q21. Did you lose consciousness upon injury?

○ Yes

○ No

Q22. Describe below how you sustained your concussion :

Sport History

Below is a list of contact/collision sports:

Football
Baseball
Soccer
Pole Vault
Diving
Softball
Lacrosse
Field Hockey
Field Hockey
Rugby
Equestrian
Water Polo
Wrestling
Basketball
Ice Hockey
Cheerleading
Gymnastics

Q23. How many of these sports listed above have you played competitively (club, school, etc.)? Please CIRCLE one:

0 **1** **2** **3+**

If you feel as though you have participated in a collision sport that is **NOT** listed, please let the research assistant know.

**You will now answer questions regarding the sports you have played competitively. Please use one sheet per sport--reporting on up to 3 sports max.

If you have **not played any of the sports listed above competitively, **<u>please go to page 12.</u>**

Sport 1) Please circle which sport you are referring to when completing this page:

Football
Baseball
Soccer
Pole Vault
Diving
Softball
Lacrosse
Field Hockey
Field Hockey
Rugby
Equestrian
Water Polo
Wrestling
Basketball
Ice Hockey
Cheerleading
Gymnastics

Q24. What position did you play?

____

Q25. How old were you when you participated in your FIRST competitive season?
________________ (years)

Q26. How many years did you spend playing competitively?
________________ (years)

Q27. Please provide your average number of **PRACTICE** contact hours *per week* in high school. If not applicable, please leave the field blank.

	Freshman	Sophomore	Junior	Senior	Other
Pre-Season Contact Hours					
Regular Season Contact Hours					
Post-Season Contact Hours					

Q28. Please provide your average number of **GAME** contact hours *per week* in high school. If not applicable, please leave the field blank.

	Freshman	Sophomore	Junior	Senior	Other
Pre-Season Contact Hours					
Regular Season Contact Hours					
Post-Season Contact Hours					

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VR Concussion Study | Survey 2

Demographics

Participant ID

Self-Assessment on Digit-Span Task

Please use the following scales to summarize the **clarity of the task instructions** AND **your compliance with the task instructions**.

	How **clear** were the following instructions?					How often were you **compliant** with the following instructions?				
	Extremely Clear	Somewhat clear	Neither clear nor unclear	Somewhat unclear	Extremely unclear	Always	Most of the time	About half of the time	Sometimes	Never
Look straight ahead	○	○	○	○	○	○	○	○	○	○
Try to avoid looking up while remembering	○	○	○	○	○	○	○	○	○	○
Try to avoid closing your eyes while remembering	○	○	○	○	○	○	○	○	○	○
Press the handle grips when you have finished reporting all of the digits you remember	○	○	○	○	○	○	○	○	○	○
Pull both handle triggers when you are ready to advance to the next trial	○	○	○	○	○	○	○	○	○	○

How well do you think you did on the digit-span task overall?

○ Extremely well
○ Very well
○ Moderately well
○ Slightly well
○ Not well at all

You saw digit-sequences between 3 and 11 digits long. What is the **maximum number of digits** you think you were able to accurately report backwards?

__________Digits

What strategy/strategies did you use to help you remember the digits that were presented? (Please SELECT ALL that apply)

○ Rehearsal--reciting them over and over

○ Writing them down in your mind

○ Chunking the numbers together

○ Other:____

Did you experience any discomfort in the virtual environment during the testing session? (CIRCLE one)

No Yes

If 'Yes' --Please describe: ____

____

____

Please provide any additional information you feel is important with respect to your performance on the task.

____

____

____

PHQ-15 During the **past 4 weeks**, how much have you been **bothered** by any of the following problems?

	Not Bothered At All	Bothered A Little	Bothered A Lot
Stomach pain	○	○	○
Back pain	○	○	○
Pain in your arms, legs, or joints (knees, hips, etc..)	○	○	○
Headaches	○	○	○
Chest pain	○	○	○
Dizziness	○	○	○
Faint spells	○	○	○
Feeling your heart pound or race	○	○	○
Shortness of breath	○	○	○
Pain or problems during sexual intercourse	○	○	○
Constipation, loose bowels, or diarrhea	○	○	○
Nausea, gas, or indigestion	○	○	○
Feeling tired or having low energy	○	○	○
Trouble sleeping	○	○	○

GAD7-1 Over the **last 2 weeks**, how often have you been bothered by the following problems?

	Not at all	Several days	Over half the days	Nearly every day
Feeling nervous, anxious, or on edge	○	○	○	○
Not being able to stop or control worrying	○	○	○	○
Worrying too much about different things	○	○	○	○
Trouble relaxing	○	○	○	○
Being so restless that it's hard to sit still	○	○	○	○
Becoming easily annoyed or irritable	○	○	○	○
Feeling afraid as if something awful might happen	○	○	○	○

GAD7-2 If you checked off any problems, how difficult have these made it for you to do your work, take care of things at home, or get along with other people?

○ Not difficult at all ○ Somewhat Difficult ○ Very Difficult ○ Extremely Difficult

PSS-4 The following questions ask you about your thoughts and feelings over **the last month**. In each case, indicate how often you felt or thought a certain way. Although some questions are similar, there are differences between them and you should treat each one as a separate question.

	Never	Almost never	Sometimes	Fairly Often	Very Often
How often do you feel that you are unable to control the important things?		○	○	○	○
How often do you feel confident about your abilities to handle your problems?	○	○	○	○	○
How often do you feel things were going your way?	○	○	○	○	○
How often do you feel that difficulties were piling up so high that you could not overcome them?	○	○	○	○	○

ER89 Please read the below statements about yourself and indicate how well it applies to you. Indicate how true the following characteristics are as they apply to you generally:

	Does not apply at at all	Applies slightly	Applies somewhat	Applies very strongly
I am generous with my friends	○	○	○	○
I quickly get over and recovery from being startled	○	○	○	○
I enjoy dealing with new and unusual situations	○	○	○	○
I usually succeed in making favorable impressions on people	○	○	○	○
I enjoy trying new foods I have never tasted before	○	○	○	○
I am regarded as a very energetic person	○	○	○	○
I like to take different paths to familiar places	○	○	○	○
I am more curious than most people	○	○	○	○
Most of the people I meet are likeable	○	○	○	○

APPENDIX B. REFINEMENT PROJECT RESULTS

Preliminary Analyses:

Pupillary response evoked by a digit-span working memory task

Our pilot data were summarized and referenced in order to determine various task refinement parameters. We examined the qualitative shape of our pupillary response time traces for each digit-sequence length—to ensure consistency with previous literature. Within each digit-sequence length, we identified appropriate response curves across the task, whereby pupil size demonstrated incremental increases as digits were presented in sequence, until reaching a point of capacity at which point, they would begin to constrict. In cases of longer sequence-lengths, this point would occur prior to the retention phase—resulting in smaller 'change scores' during retention.

A)

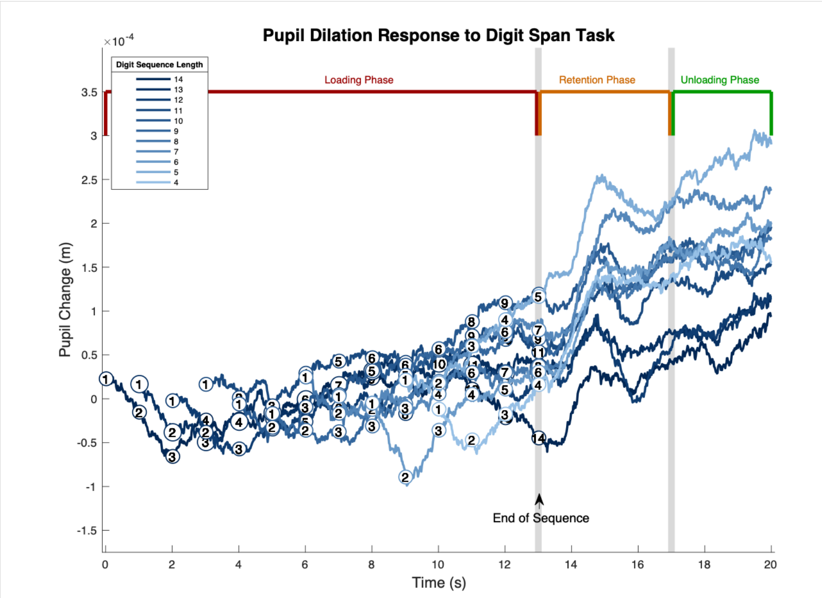

Figure 1. Pupillary Response. A) Average change in pupil diameter (mm) during a digit-span working memory task. The numbered circles represent the time each digit was presented, and time traces are shifted horizontally to align at 'final digit presentation' for each level of difficulty (sequence-length). Therefore, the longest sequence (14-digits) is depicted the furthest to the left.

Task performance—Digit-span Working Memory Task

Task refinement was largely informed by our task performance data. Our first refinement being to adjust our scoring method of this response to allow for greater granularity and precision of measure. Our initial ‘all or nothing’ scoring approach was been previously described and recognized as standard practice when evaluating working memory digit-span tasks. However, consistent with recent literature, our initial summary of these data revealed blunted response variability, especially at higher sequence-lengths **(Table 1).** Therefore, we adapted our scoring approach to allow for partial credit assignment for each correctly identified digit by serial position. Greater variability in task performance data where partial credit scoring is applied (**Table 2**) compared to that of traditional ‘all or nothing’ scoring (**Table 1**). Greater variability allows for more robust analyses and ability to detect individual differences—also more appropriate considering the fluidity of working memory rather than a fixed capacity by reporting task performance dynamics within and beyond capacity for perfect performance.

All or Nothing Scoring

Table 1. Overall behavioral response by sequence length for all trials (proportion perfectly recalled trials)

Sequence Length	Proportion of perfectly recalled sequences	Percent
4-digits	205/240	85%
5-digits	170/240	71%
6-digits	95/240	40%
7-digits	42/240	18%
8-digits	26/240	11%
9-digits	15/240	6%
10-digits	6/240	3%
11-digits	2/240	1%
12-digits	1/240	1%
13-digits	0/240	0
14-digits	0/240	0

Partial Credit Scoring

Table 2. Overall behavioral response by sequence length for all trials (avg. correctly recalled digits)

Sequence Length	Mean	SD
4-digits	.91	0.23
5-digits	.85	0.25
6-digits	.70	0.31
7-digits	.55	0.29
8-digits	.48	0.26
9-digits	.41	0.24
10-digits	.34	0.21
11-digits	.29	0.17
12-digits	.26	0.15
13-digits	.24	0.13
14-digits	.23	0.12

Following this adaptation, we were able to then use these data to understand the maximum level of task difficulty we would include in the refined task, in order to sufficiently overload working memory. Participant task performances were plotted across sequence-lengths for each group in our pilot study to again examine qualitative shape. Each plot demonstrates deteriorating task performances as sequence-length increases, as expected. However, beyond the 11-digit sequence-length responses appear to plateau. Therefore, we determined that including sequences beyond this length may not provide any additional information in either group.

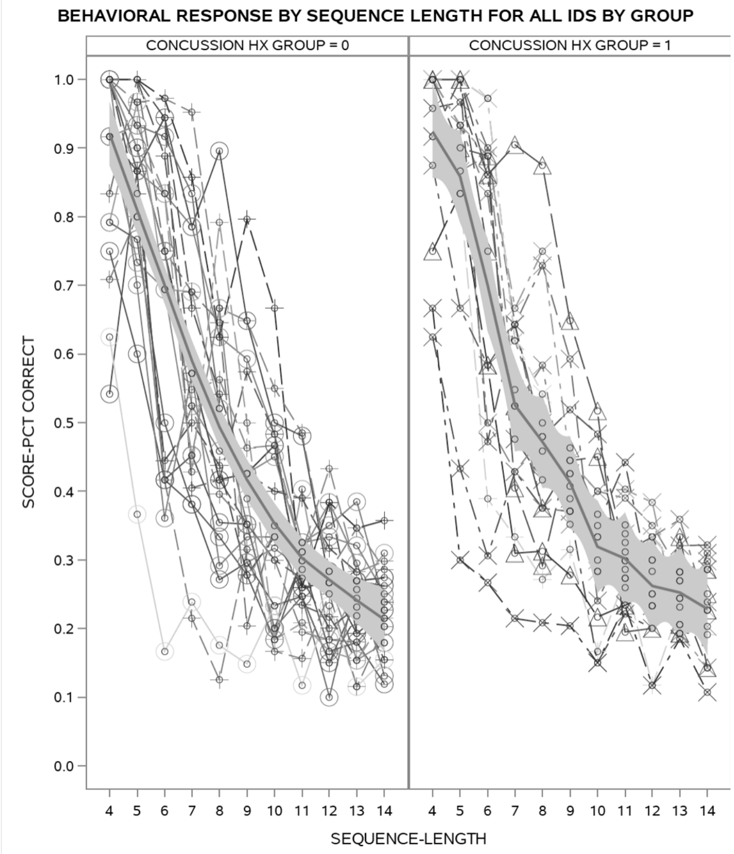

Figure 2. Individual behavioral responses across sequence-lengths in those with and without a concussion history.

Sex Differences in Pupillary Response

Our preliminary analyses showed significant sex differences in pupillary response as our primary outcome of interest mean differences outlined in **Table 3.** And depicted in **Figure 3**. Whereby females demonstrated overall smaller pupil size changes across sequence-lengths—especially at lengths at and above 11. Therefore, we plan to continue to investigate this effect in the proposed study as a potential model covariate.

Table 3. Sex differences in pupillary responses by sequence-length

Sequence-Length	**Male**		**Female**	
	Mean	std	Mean	std
4-digits	0.17	0.35	0.09	0.36
5-digits	0.32	0.44	0.13	0.39
6-digits	0.19	0.38	0.12	0.40
7-digits	0.22	0.45	0.18	0.40
8-digits	0.22	0.55	0.04	0.50
9-digits	0.23	0.48	0.09	0.38
10-digits	0.20	0.48	0.13	0.44
11-digits	0.15	0.48	0.01	0.43
12-digits	0.12	0.37	0.01	0.46
13-digits	0.14	0.52	-0.01	0.46
14-digits	0.13	0.48	-0.07	0.47

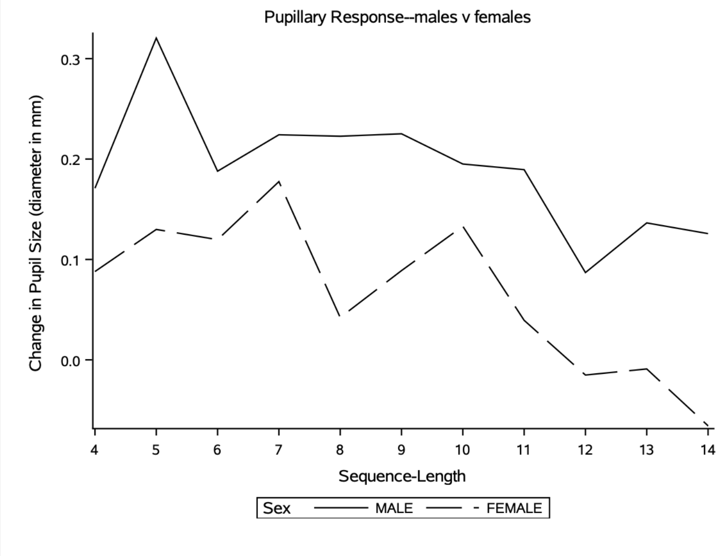

Figure 3. Sex differences in average pupillary response measures by sequence length

APPENDIX C. DISSERTATION MANUSCRIPT – MEDICINE & SCIENCE IN SPORT & EXERCISE

Physiological correlates of working memory and cognitive efficiency: implications for concussion management in college aged student-athletes

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Running title: Cognitive Load and Concussion

Purpose: To examine effects of cognitive load, task performance, heart rate variability (HRV), and concussion history on pupillary responses in healthy collegiate club sports athletes using virtual reality (VR) and eye track and technology.

Methods: Participants (n=59) self-reported concussion history (yes vs. no) and completed a backwards digit-span task in a single testing session. A virtual reality headset with 90Hz infrared eye tracking displayed the task and recorded pupil size diameter (mm), and participants were a chest strap heart rate monitor. Cognitive load was represented as the levels of difficulty with in a backwards overloaded digit span task i.e., digit sequence-length). Heart rate variability and pupil size were continuously recorded throughout the duration of the cognitive task. Pupillary responses were calculated as the baseline corrected mean size during the retention period of each digit-span trial. Task performance was calculated as accuracy (proportion of correctly recalled digits by serial position) and HRV as the root mean square of successive differences in R-R intervals (RMSSD). A linear mixed effects model examined accuracy, HRV and concussion history effects on PR across sequence lengths (*a priori* $\alpha=0.05$).

Results: 59 participants were included [age=20.48 ± 1.86years; males=58%; 24 (40%) with concussion history]. There was a significant effect of cognitive load ($F_{4,232}=3.67$, $p=0.006$) on pupillary response. Specifically, higher loads exhibited larger mean pupillary responses (i.e., greater neural resource utilization). Task performance, heart rate variability, and concussion history demonstrated non-significant effects in the model ($F_{1,1076}=0.00$, $p=0.972$), ($F_{1,1076}=1.62$, $p=0.204$), and ($F_{1,57}=0.04$, $p=0.833$) respectively.

Conclusion: Our study findings support pupillary responses’ sensitivity to cognitive load beyond performance-based measures alone, in an athletic population. Our assessment for cognitive efficiency accounts for both physiological and performance-based response dynamics, using VR

and eye tracking technology, which may have implications for clinical practice and future cognitive interventions.

Key Words: cognitive load, mTBI, cognitive efficiency, cognitive pupillometry

INTRODUCTION

Recent literature favors symptom limited physical and cognitive activity following concussion over strict rest to support injury recovery.[117,118] While moderate levels of cognitive activity following concussion are recommended, clinicians are in need of evidence-based guidance to ensure appropriate post-injury management of cognitive load, within return to learn and activity paradigms. Specifically, cognitive load refers to the inherent demands of a cognitive task, often represented clinically with respect to levels of task difficulty (e.g., sequence-lengths of a digit-span task). [24,29,31] Importantly, cognitive load can also represent the extent to which cognitive resources are allocated to meet task demands and is typically informed using various physiological metrics. As such, when combined with task performance outcome measures (i.e., score on digit-span task) these two aspects of cognitive load, may inform the broader construct of cognitive efficiency, (e.g., the relationship between neural resource utilization and task performance-based outcomes).[24,29,31] Clinical examination of cognitive efficiency is currently limited due to the complexities associated with physiological assessment and monitoring in this setting (e.g., costly equipment, processing time, difficulty interpreting results, etc.).

Cognitive pupillometry has been extensively examined and responses validated as indirect neurophysiological markers for global neural resource utilization in healthy and clinical populations with mild cognitive impairment. [28,29] Simultaneous pupil size recording, with other spatial and temporal brain activity measures such as electroencephalography (EEG) and functional magnetic resonance imaging (fMRI) demonstrate significant temporal associations with pupillary responses, relative to varying cognitive demands.[29–33]

Pupillary response dynamics across a single digit-span trial include incremental dilation as each digit is encoded, reaching maximum dilation during the retention period following final digit presentation (while manipulating and reordering information), when individuals have been fully loaded for a given sequence-length.[28,32,35] Greater pupillary responses during the retention window are exhibited with increasing cognitive load levels within resource availability/capacity .[28,34] Baseline corrected average pupil size during retention therefore reflects the neural resource utilization for each trial as the physiological aspect of cognitive load.[30,35] Greater pupillary dilation responses, and degrading task performance[25,27] are typically demonstrated under conditions of high cognitive load. However, as cognitive load exceeds neural resource availability—or individuals reach working memory capacity, pupillary responses exhibit a plateau followed by constriction. [28,34,35] Few studies have examined pupillary responses and associated task performance measures under overloaded conditions (e.g., digit sequence-lengths > 9-digits) when neural resources are limited. This may be an important area to investigate as we consider such measures for cognitive load and efficiency in human performance and clinical management paradigms.[31,41]

Pupillary response dynamics may be useful to inform cognitive efficiency in isolation—though recent psychophysiological investigations suggests monitoring multiple metrics when examining associations between physiological and performance outcomes associated with complex cognitive constructs and processes.[29,34,41,42] Juxtaposed with concussion literature, recent systematic reviews regarding the physiological response to injury concludes that a single 'perfect metric' that accounts for the complexities associated with concussion is highly unlikely—rather, a combination of measures may be more appropriate.[8,18] Moreover, physiological metrics regulated by the ANS require careful consideration given the many latent variables that may contribute to variability in response dynamics (e.g., stress, emotional response, etc.).[41,43–45] Heart rate variability (HRV) may be a meaningful supplement to pupillary response monitoring during an overloaded digit-span task to inform cognitive efficiency, as a secondary marker for resource allocation.[44,46,47] Significant effects of acute cognitive load on HRV measures have been reported in healthy populations. [18,50,51] However, few studies have examined relationships between HRV and pupil measures in response to cognitive demands.[18,50,51] Simultaneous monitoring of pupillary and HRV response dynamics may be more sensitive to cognitive load in healthy and clinical populations when task demands exceed resource availability and compensatory mechanisms are pursued to maintain performance.

Examining cognitive efficiency as the dynamic interplay between physiological indicators of individuals' cognitive effort—alongside the cognitive task demands (task difficulty), and performance-based outcomes—may expand our understanding of post-concussion cognitive impairment and clinical management paradigms [24,25]. However, considerations for ecological validity are equally, if not more important than measurement sensitivity when investigating physiological markers for potential clinical applications. Previous literature clearly acknowledges the importance of experimental control when investigating physiological metrics such as pupillary responses and HRV, given the various potential confounds.[32] Recent advancements in virtual reality (VR) head mounted displays with embedded infrared eye tracking technology provide a controlled, portable, and cost-effective solution to this problem and improved ecological validity for pupillary response parameter assessment to inform cognitive efficiency.

Therefore, the purpose of this study was to examine relationships among physiological (pupillary response and heart rate variability) and performance-based (task accuracy) measures, in collegiate club sports athletes, during an overload backwards digit-span task, in the context of cognitive efficiency. We also investigated potential concussion history effects on these relationships as a preliminary examination of the sensitivity of these assessments collected using VR and eye tracking technology, to group differences. We anticipated significant associations to exist across participants between task performance accuracy, heart rate variability, and pupillary response dynamics with respect to the levels of task difficulty within an overloaded backwards digit-span task. Whereby individuals would demonstrate decreasing heart rate variability and task performance accuracy across increasing digit sequence-lengths—while pupillary responses would increase to a point, then plateau or decrease beyond resource availability.

METHODS

We completed a quasi-experimental cross-sectional study with club sports athletes at a single university during the spring or 2020. Participants reported for a single testing session that lasted approximately 1 hour. This study was approved by the institution's Office of Human Research Ethics board.

Participants

All athletes included in the present provided written informed consent prior to participation. Specific inclusion criteria for study participation required all individuals to be between the ages of 18 and 30 and a rostered UNC club sport athlete. Individuals were excluded if they did not meet the above inclusion requirements and/or if they were unable to complete vision testing for whatever reason, had permanent vision loss in one or both eyes, had any visual surgery in the last year that would inhibit testing completion, were currently being treated to address balance or vision problems, and/or had strabismus or amblyopia. To support appropriate group designation using participants' self-reported concussion history, we employed methods similar to those reported by the NCAA–DOD CARE Consortium[102]—whereby all participants reviewed an injury definition and common signs and symptoms prior to reporting. The concussion definition and associated signs and symptoms were informed by evidence-based guidelines and the latest international consensus statement on concussion in sport.[9]

Following provision of the definition the concussion history group included those who reported having sustained at least one concussion—via any mechanism (i.e., sports-related or not) and those who reported concussion recency time frames during or since high school, but not in the past 6 months.

Digit-Span Working Memory Task

Task Design

The backward digit-span task was developed for the proposed study in order to examine task performance and physiological response dynamics associated with working memory processes. Traditional task design and administration parameters were adapted to coincide with recent applications in healthy and clinical samples within psychophysiological and concussion-based research domains.[35,37,38,92] The task design was randomized blocked, containing 4 consecutive testing blocks of 5 randomized digit sequence-lengths (i.e., 3, 5, 7, 9, and 11)—20 total trials. Sequence-length presentation order within the first testing block was determined using a random number generator followed by a Latin Square to counter-balance sequence-length presentation order for the remaining 3 blocks. The individual digits presented within each digit-sequence were randomly generated—consistent with previous literature that excludes within-trial immediate duplicates and consecutive integers.[35–37]

Task Presentation and VR Integration

The custom developed digit-span task was designed using Unity 3D® engine software, to be presented within the HTC VIVE™ VR head-mounted display integrated with Tobii Pro VR infrared eye tracking technology (Tobii Technology, Inc.). Pupil size (diameter in mm) in both eyes was continuously recorded at 90Hz during the task. Tobii Pro VR Solution software and infrared illuminators (dark pupil tracking) worked together to record time-locked pupillary response and specific digit-span task event markers for each trial (i.e., baseline, loading phase, retention period, and recall).

Each trial begins with a 5-second pre-stimulus baseline period. Digit-sequences are then presented at the rate of one digit per second, followed by a 3-second retention pause. Participants are then prompted to verbally recall as many digits as they can remember in the exact reverse order. Recall was self-paced, and all trials were participant initiated. **Figure 1** presentation parameters for a single trial of a 4-digit sequence. The VR design parameters applied in the present study were consistent with previous pupillary response literature that highlight the importance of strict environmental control and equiluminant conditions for sufficient measurement precision and accuracy.[101]

All stimuli were custom designed to ensure equiluminance (size= 200pixels, color= R:46 G:46 B:46 A: 255). A fixation 'X' was displayed for each trial baseline and retention periods (display time = 5 and 3 seconds respectively) to ensure consistency in environmental luminance during baseline corrected response intervals of interest. Digits were 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 promoted to provide their response. Participants completed all trials until reaching an 'end of trial' slide, without reinforcement or feedback from study personnel.[40,102] In order to further minimize the influence of non-intended effects associated with saccades and blinks on pupil size, the task was centrally presented within participants' field of view and participants' vision within the headset was fixated to imitate a 2D display, and avoid any potential accommodative effects.[40,106]

Task Performance

Participants' verbal recall for each trail was recorded in real-time by study personnel and compared with digits presented, to determine accuracy for each trial. Each trial was scored separately and credit was assigned as a percent correct, for each digit recalled in the correct serial position, i.e., total digits correct divided by digit-sequence length.

Heart Rate Variability

Heart rate and inter-beat interval data (RR interval) were continuously sampled during the digit-span task at 1000Hz using a Polar H10 chest strap. Raw unfiltered RR data were then processed using Kubios HRV software, version 3.3 software (Biosignal Analysis and Medical Group, Kupio, Finland). Trial event markers exported from Unity were used to time-lock RR interval data for each participant. Average HRV via the root mean square of successive differences (RMSSD), as previously reported by Mandrick et al[41] was determined for each trial. Specifically, the signal was derived into RR intervals to across each trial (from baseline to baseline). Kubios HRV software 2.2 (University of Eastern Finland, http:// kubios.uef.fi) was then used to preprocess RR data for each participant and calculate mean RMSSD for each trial.[64]

Pupillary Response

Pupillary response data were sampled at 90 Hz and exported variables included left and right absolute pupil size (mm). Timestamps for key components within the digit-span task were recorded by Unity including 1) trial number (1-20), 2) sequence-length (3, 5, 7, 9, and 11), 3) digit presentation(s), 4) baseline and retention X displays, 5) response box display, and 6) recall completion and trial advancement, to ensure appropriate time-locking across pupillary and HRV variables of interest.

Raw data were directly exported into a spreadsheet and imported into Matlab (MATLAB and Statistic Toolbox Release 2017b, The MathWorks, Inc., Natick, MA, USA). A custom Matlab program adapted from the suggested procedures for processing pupil size data by Kret and colleagues in 2018[56] was then used to complete all pupil size data processing and reduction. The adapted processing program employs 6 procedural levels as follows: 1) raw data are prepared for filtering by removing identified blinks; 2) additional artifacts are 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); 3) the remaining valid samples are then smoothed using linear interpolation; 5) pupil response regions of interest are segmented (i.e., the last 40 samples of each baseline period and the first 200 samples of each retention period) and individually averaged; and 6) segment averages for both tonic and phasic responses are baseline corrected to represent pupil size change within response regions of interest for each trial (i.e., average pupil size during retention – average baseline pupil).[28,36,37] Finally, processed data were exported from Matlab and SAS 9.4 (Cary, NC) was used for all descriptive statistics and inferential analyses.

General Testing Procedures

Participants completed a single testing session, that lasted approximately one hour. A demographic and health history questionnaire was completed first—including questions regarding participant’s sport and medical history. Participants then fitted themselves with the heart rate monitor and were then seated and fitted with the VR headset. A five-point calibration sequence was completed before each testing session for all participants.[40,101,106] Additionally, parameters within our task design (e.g., standard and equiluminant baseline/accommodation periods before each trial) allow for pseudo-calibration via trial by trial standard baseline subtraction procedures and reliable/valid assessment of our pupillary response outcome. [40,101,106]

Participants were then familiarized with the task and allowed at least 4 practice trials (all 5-digits in length) in order to familiarize them with task presentation and sequencing—and to ensure understanding of task demands and required responses.[25,37,77,110] Participants were not informed of the digit sequence-length for each trial, though they were told that the task includes sequences between 3- and 11-digits long. Emphasis was placed on participants do their best to recall as many digits as they can possibly remember (in exact reverse order), for each trial. Recall periods were self-paced, and all trials were participant initiated using HTC VIVE handheld controller triggers. Participants were given compliance feedback (e.g., appropriate response timing, trial initiation accuracy, etc.) during practice, and encouraged to ask any questions they may have about the testing procedures prior to beginning the experimental trials. No feedback was given during experimental trials.

Statistical Analysis

Continuous variables are summarized using means and 95% confidence intervals, and categorical variables are summarized with frequencies and associated percentages**.** Bivariate correlations were used to examine the strength of association between continuous variables.

We then used a linear mixed effects model to examine how HRV and task performance together may influence pupillary response changes across sequence lengths of a digit-span task—in the context of cognitive efficiency. To account for latent heterogeneity between subjects and their responses to each trial, the model will include two random effects—one ‘by-subject’ effect of participant, and sequence-length as a grouping variable. Behavior and HRV were added as fixed effects and tonic pupillary response will serve as the outcome variable as an index of neural resource utilization. Finally, we employed a second linear mixed effects model with an additional random effect of concussion history as a grouping variable. Correlation and distributive properties of pupillary response outcomes by sequence length were examined for curvilinear relationships and the potential need for model adjustment to account for quadratic mean structure in both models.

RESULTS

Sixty-two athletes from 18 club sports participated in this study between December 2019 and March 2020. Men's Rugby athletes made up 18.33% of our study sample (n=11), followed by Softball (n=10), Hockey (n=8), and Men's Soccer (n=7). Four participants were participated in Rock Climbing (6.67%), and Cheer, Swimming and Diving, and Women's Soccer each represented 5% (n=3) of our sample. The remaining 9 participants each represented 1.67% of our sample (n=1) from the following sports: Baseball, Golf, Gymnastics, Jiu Jitsu, Jump Rope, Marathon Running, Racquetball, Women's Lacrosse, and Cross Country. Participants' average age was 20.48 ± 1.86 years, and 34 (57.63%) were male. Twenty-four (41%) participants self-reported a concussion history, average lifetime contact/collision sport participation was 7.2 ± 5.1 years, and 61.6 ± 57.9 total hours during high school—including all pre-, in-, and post-season practices and games. Task perception measures were completed by 58 participants, whereby the greatest proportion of participants felt they performed 'moderately well' on the task (n=26, 44%).

Following data processing, 39 (3.3%) pupillary response trials were excluded due to signal loss greater than 20% within response intervals of interest. One participant was excluded due to excessive signal loss within response intervals, that resulted in less than 10 total valid trials—and two participants were excluded following accuracy response inspection for non-credible performance. A total of 59 participants had valid measures for pupillary response, task performance and heart rate variability and were included in all analyses.

General Descriptives

Grand average pupillary response means by sequence length followed expected response dynamics, consistent with previously reported observations by Klingner et al. who examined pupil size changes during a visually presented digit-span task.[111] Participant's pupils gradually dilated as the digits were encoded with each presentation and reached a peak within the 3 seconds following final digit presentation—during the retention period—while digits were being reordered.

Grand means and 95% confidence intervals for pupillary response, task performance and HRV, are summarized across sequence-lengths in **Table 1**. Consistent with prior work, mean baseline-corrected pupillary responses during the retention period exhibited systematic increases with longer sequence lengths (cognitive load) within resource availability (working memory capacity of 7 ± 2 digits), then declined with overload (11-digits).[34,35,111] Mean accuracy scores exhibited a steady decline from 98% to 27% as sequence-lengths increased from 3- to 11-digits. Mean RMSSD measures exhibited little fluctuation across sequence-lengths, within previously established normal ranges (i.e., between 27-72 ms).

Bivariate correlations demonstrated significant relationships between longer sequence-lengths and both larger pupillary responses ($r= 0.13$; $p=<0.001$) and lower accuracy scores ($r=-.77$; $p=<0.001$). Higher accuracy scores were also weakly related to higher heart rate variability ($r=0.06$; $p=0.04$). Age, and both variables representing prior contact collision sport participation (i.e., average total years playing and average total hours participating in high school) demonstrated negligible relationships with pupillary response ($p>0.05$).

Original Model

There was a significant main effect of sequence-length on mean pupillary response ($F_{4,232}$=3.69, *p*=0.006), whereby longer sequence-lengths elicited greater average dilation responses. Accuracy and RMSSD demonstrated non-significant effects in the model ($F_{1,1076}$=0.00, *p*=0.974) and ($F_{1,1076}$=1.58, *p*=0.208), respectively.

Concussion History Model

The significant main effect of sequence-length on greater baseline corrected pupillary response outcomes was retained in the second model ($F_{4,232}$=3.67, *p*=0.006). Concussion history group means and 95% CIs for pupillary response, task performance and heart rate variability are summarized as a function of sequence length for concussion history groups (yes versus no) in **Table 2.** Accuracy, RMSSD, and concussion history demonstrated non-significant effects in the model ($F_{1,1076}$=0.00, *p*=0.972), ($F_{1,1076}$=1.62, *p*=0.204), and ($F_{1,57}$=0.04, *p*=0.833) respectively. Model results are summarized in **Table 3.** While not statistically significant, mean pupillary responses in the concussion history group were smaller at the lower 3- and 5-digit sequence lengths, and larger across sequence lengths between 7- and 11-digits—compared to those without a concussion history. In contrast, task performance and heart rate variability responses follow very similar response dynamics across sequence lengths (cognitive load) for both groups. Participants' averaged responses for pupillary response, task performance and heart rate variability are depicted by concussion history group in **Figures 1-3.**

DISCUSSION

We examined relationships between task-performance, heart rate variability, and cognitive load associated with a backwards digit-span task, and pupillary response measures—followed by concussion history effects, in collegiate club sports athletes. Virtual reality eye tracking technology was used to record pupillary responses, as a more ecologically valid mechanism for cognitive pupillometry, compared to desktop-based trackers and neuroimaging techniques such as fMRI. Consistent with previous literature, using more invasive and costly instrumentation, our results demonstrated load dependent pupillary response dynamics. Specifically, participants demonstrated larger pupillary responses with greater cognitive loads (i.e., longer sequence lengths), within working memory capacity. As such, our findings support advancement for future cognitive pupillometry investigations to expand into more applied settings—which may have important clinical implications for concussion assessment future and management.

General Findings Informing Cognitive Efficiency

Combined examination of clinical task performance and physiological metrics for resource allocation relative to imposed cognitive demands, are essential to assess neurocognitive processing effectiveness, and efficiency. Clinical task performance (accuracy) in our study demonstrated a steady decline from 98% to 27% in response to increasing cognitive loads from 3- to 11-digits. Our accuracy response dynamics are consistent with previous literature and support appropriate task design, whereby cognitive load was adequately modulated beyond working memory capacity and resource availability.[28,34,35] Our pupillary response outcomes were similar to previous cognitive pupillometry studies whereby greater cognitive load elicited larger dilation responses, when the load was within resource availability (working memory capacity of 7 ± 2 digits), then declined with overload (11-digits).[34,35,111] Task performance measures alone (e.g., accuracy), provide little insight relative to participants' neural resource utilization to meet task demands and achieve a specific level of accuracy or performance.[29,31] Task performance measures from neuropsychological tests may be indirect measures of the applied cognitive demands—though they are not continuous. Our pupillary response measure on the other hand, illustrated the ability to assess the cognitive load and associated neural resource allocation in-real time and provided a continuous recording of data over time. [29] Therefore, the neurophysiological measure in our study may capture cognitive change which may appear before manifestation of cognitive symptoms and decreased task performance.[29,34] Future work should examine such outcomes in clinical populations as well as extend this work from a human performance perspective.

Heart Rate Variability

Average heart rate variability measures did not descriptively demonstrate a load dependent response and exhibited negligible relationships with pupillary responses. Our findings indicate that on average participants' HRV was within normal range (i.e., RMSSD between 27-72ms), with little to no change in mean RMSSD across sequence lengths.[54,112] Previous studies have examined changes in short-term HRV measures to examine relationships with cognitive load and task performance.[113–115] However, differences in trial durations, study samples, and the explored cognitive domains likely explain the disagreement with the findings of the present study.[45,54]

Other studies such as that by Hess and Ennis have examined other working memory tasks such as mental arithmetic, that were shorter in nature to assess 3 measures for cardiac physiological resource allocation (i.e., Heart rate—HRV, and systolic and diastolic blood pressure)—whereby systolic blood pressure demonstrated greater sensitivity to age related differences.[29] Moreover, the short measurement duration of our heart rate variability outcome may not have been long enough to capture vagally mediated changes in HRV, by the autonomic nervous system due to acute mental stress during our task. Additionally, not all measures were of the same duration in this study, given the prioritization of self-paced recall periods in our task.

Evidence suggesting cardiac measures' ability to reflect changes in cognitive load, also highlight their sensitivity to experience levels and training, the type of task observed and the time of day—consequently these measures require high levels of task demand in order to be reflected in HRV.[113] Much of the HRV literature however, has examined cardiac activity measurements over longer durations i.e., more "chronic" cognitive or physical loads (>5min), and suggest that more extreme cognitive demands are needed to elicit more prominent responses during shorter measurement durations—similar to trial durations in our study. A few studies have shown elevated HR and subsequently lower HRV during logical and dynamic reasoning tasks demanding high levels of verbal working memory and high visual attention, though HRV measurements were recorded over longer trial durations ≥30s.[45,54] One particular study by Rivecourt et al examined HRV measures during a flight simulation task and measures were recorded over longer durations.[54] This task likely elicited a greater cardiac and autonomic stress response relative to our digit-span task due to the task nature, which might explain the lack of significance in the present study. These findings combined with those from the present study, may also suggest that pupillary responses are more sensitive to real time changes in neural resource utilization and therefore a better measure for clinical monitoring in with respect to cognitive testing than this specific HRV outcome

Our initial mixed effects model examined the relationships between measures of task performance, heart rate variability, and pupillary response across digit-span levels of task difficulty in healthy collegiate club sports athletes. Our mixed effects model demonstrated a significant effect of load on pupillary response –consistent with previous literature.[28,34,35]

However, neither task performance or heart rate variability demonstrated significant effects on pupillary response. While descriptively we saw the expected load dependent relationship between task performance and digit-sequence-length, the negligible effect of task performance on pupillary response in the model suggests that resource utilization is not influenced by overall task performance.

Performance effects on pupillary response have been reported during other cognitive control and/or working memory tasks such as the Stroop or n-back tasks, whereby some physiological responses increase after a poor performance trial indicating compensatory neural recruitment for sustained performance.[25,32] These tasks typically maintain a static level of cognitive load for several trials, (e.g., Stroop task will first assess correct identification on both color and word, before the assessing the Stroop effect when asking participants to respond when these things are congruent versus incongruent). [32] The task in the present study requires different aspects of working memory processing given the load is constantly changing.[32] The aspects of cognitive control for our task instead include encoding, and manipulation of information which may make it more difficult—especially on the longer trials—for individuals ascertain their trial-by-trial-performance accuracy. Self-performance and subsequent compensatory recruitment for sustained performance may be more prevalent in tasks that require updating and inhibition such as is Stroop or n-back tasks.[32]

Heart rate variability also demonstrated negligible effects in the model. To our knowledge this is the first study to examine the relationship between pupillary responses and heart rate variability as physiological markers for resource allocation to meet task demands during a digit span task. Previous studies have examined the effects of cognitive load on each of these measures independently as separate dependent variables.[41,113] The negligible effect of heart rate variability on pupillary response may be explained by poor sensitivity of our chosen HRV measure to detect acute changes in cognitive load, as described above, or may support the independence in these two physiological measures to capture aspects of physiological resource mobilization to meet cognitive task demands.[113] Future studies should consider splitting these two physiological metrics to inform cognitive efficiency given they likely reflect different aspects of ANS activity. Moreover, additional studies are needed to determine the utility of various short-term HRV measures to capture acute cognitive load. The lack of convergence between pupillary response and this particular cardiovascular measure for resource mobilization may be further explained by the differing sensitivity of these measures to various factors. In light of our findings, and those of previous literature, it is important to note the sensitivity in both pupillary response and HRV measures relative to their ANS foundations to various confounding factors. However, our findings suggest pupillary responses may be a more sensitive measure—especially to acute, short-term changes in cognitive load—given our ability to mitigate potential confounds through vigorous environmental control in VR.

Our follow-up mixed effects model investigated the potential added effect of a prior concussion injury. Concussion history groups did not demonstrate statistically significant differences in pupillary response dynamics across levels of task difficulty, however upon visual examination alone, we see a potential trend developing and a group by load interaction which may warrant future examination of such interactions in a larger sample. Our findings concerning load, task performance and HRV were similar to those observed in the initial model and were relatively unchanged after including concussion history. These results differ from previous cognitive pupillometry studies reporting group differences in pupillary response outcomes, within clinical populations with cognitive deficits. Additionally, our model findings differ from those concerning the pupillary light reflex as well as other eye tracking methodologies that identified differences in those with and without a concussion history.[90,91] Discrepancies between our findings and previous work in clinical populations may be explained by differences in task design and the selected pupillary response outcome.

A recent study by Hershaw et al. examined several pupillary response metrics (e.g., cue- and response-locked means, peaks, peak latencies) during an n-back cued attention task, to describe variability and reliability across concussion history groups, with respect to cognitive load sensitivity. Results from the Hershaw's study showed greater pupillary responses outcomes at higher cognitive loads in the concussion history group—whereby, dilation response latencies demonstrated the greatest sensitivity to group differences.[101] Few cognitive pupillometry studies have examined dilation/constriction latency outcomes, more often used when evaluating the pupillary light reflex. However, Hershaw's results warrant future consideration and examination of latency outcomes, especially in clinical populations.

Granholm and colleagues reported similar findings when examining pupillary responses during a digit span task in those with amnestic single domain-mild cognitive impairment associated with Alzheimer's, compared to healthy controls. [38] Participants in the Granholm study exhibited similar task-performance measures though greater pupil responses at lower cognitive loads (i.e., shorted digit-sequences) were exhibited in those with mild cognitive impairment, compared to cognitively normal participants.[38] Suggesting compensatory neurophysiological resource utilization and decreased cognitive efficiency when cognitive load is lower in this unique population with mild cognitive impairment.[116] Response visual examination suggests a potential trend developing and group by load interaction which warrants future examination, as differences may be isolated to a certain cognitive load level.

Overall, our hypotheses that greater cognitive load (e.g., sequence length) would elicit larger pupillary responses (dilation) were supported by our findings. Null findings were exhibited in our hypothesized effects of our other independent variables, including task performance, heart rate variability, and concussion history, on pupillary responses. It is important to recognize the utility in the results of our study despite these negligible effects, especially as it pertains to the robustness and potential sensitivity and specificity pupillary responses may offer when examining cognitive efficiency in future studies, using a more parsimonious model.

Limitations

Our study was not without limitations. Shifting cognitive pupillometry assessments into a VR headset helped to mitigate potential non-cognitive environmental influence on pupillary response outcomes. However, other potential confounds for pupillary response metrics and heart rate variability relative to ANS function may have played a role in measurement variability. [28,29] For example, we did not examine effects of daily medication and other co-morbidities (e.g., anxiety, depression, etc.) on these measures, or standardized the testing time of day. [28,29,101]

The pupillary response outcome measure used in this study, while common in cognitive pupillometry studies examining working memory, may not have been sensitive to concussion group differences. Future studies should consider using alternative pupillary response outcomes given recent Hershaw et al. results demonstrating greater sensitivity in latency metrics to concussion group differences compared to baseline-corrected responses.[101] Consensus around metric selection will eventually lend towards normative values, and improved generalizability of study findings. Participant heterogeneity and differences in task design within previous literature further complicate generalizability of our findings. Despite these limitations, we sought to control many confounders by enrolling a sample within a tight age range, similar athletic demands, and in a virtual reality environment.

Future Research

There is a great paucity of cognitive pupillometry studies in applied clinical settings comparing healthy and clinical groups (e.g., post-concussion) which greatly compromises the generalizability of our findings. Our findings do however, support advancement for cognitive pupillometry investigations to expand into applied settings in the future—which may have important clinical implications for concussion assessment and management.

Longitudinal examination of within and between subjects' comparative analyses among pupillary response and other relevant clinical performance-based metrics. This will provide initial information to establish normative values around intra-individual changes and inter-individual variation in cognitive processing. Studies aimed to discriminate among clinical populations should consider more discriminatory inclusion criteria, clinically meaningful assessment timepoints and within group analyses to control for potential injury comorbidities and/or confounding variables which may include injury frequency and chronicity.

Conclusions

Our study was the first to examine an assessment for cognitive efficiency that accounts for both physiological and performance-based response dynamics, using VR and eye tracking technology, which may have implications for clinical practice and future cognitive interventions. Our study findings support pupillary responses' sensitivity to cognitive load beyond performance-based measures alone, in an athletic population. These findings provide additive evidence for the need to include ecologically valid and cost-efficient neurophysiological metrics when assessing cognitive performance and efficiency. Employing VR and eye tracking technologies to examine these responses may be useful for human performance and concussion management paradigms.

Acknowledgments

The authors would like to acknowledge the academic faculty, research staff, and trainees in the Matthew Gfeller Center at UNC-Chapel Hill for their assistance with various aspects of this project. Specifically, we would like to thank Emily Barron and Kou Yang for their significant efforts in making this project successful. This study was funded in part by the Sarah Steel Danhoff Undergraduate Research Award at the University of North Carolina at Chapel Hill

Table 1. Average pupillary response, task performance, and heart rate variability summarized across sequence-length and 95% confidence intervals

		N	Mean	95% CI	
	Sequence-Length				
Average Pupillary Response (baseline corrected response in mm)	3-digits	233	0.11	0.08	0.15
	5-digits	233	0.14	0.10	0.18
	7-digits	227	0.22	0.18	0.26
	9-digits	223	0.25	0.21	0.30
	11-digits	225	0.23	0.18	0.28
	Sequence-Length				
Average Task Performance (accuracy—percent correct)	3-digits	236	98%	97%	100%
	5-digits	236	80%	77%	84%
	7-digits	236	52%	48%	56%
	9-digits	236	34%	31%	36%
	11-digits	236	27%	25%	29%
	Sequence-Length				
Average Heart Rate Variability (RMSSD in ms)	3-digits	236	40.7	37.2	44.2
	5-digits	236	41.7	38.0	45.5
	7-digits	236	39.3	35.6	43.0
	9-digits	236	40.9	37.4	44.3
	11-digits	236	39.6	36.2	43.0

Average responses for each variable are summarized across all valid trials for all 59 participants.

Table 2. Concussion history group average study measures across sequence lengths and 95% confidence intervals

		Concussion History Groups					
		No (n=35)			**Yes (n=24)**		
		Mean	**95% CI**		**Mean**	**95% CI**	
Average Pupillary Response	**Sequence-Length**						
	3-digits	0.14	0.09	0.18	0.08	0.03	0.13
	5-digits	0.17	0.11	0.22	0.10	0.04	0.17
	7-digits	0.20	0.15	0.25	0.26	0.19	0.32
	9-digits	0.24	0.18	0.31	0.27	0.21	0.34
	11-digits	0.22	0.15	0.29	0.24	0.17	0.31
Average Task Performance	**Sequence-Length**						
	3-digits	98%	100%	96%	99%	100%	98%
	5-digits	80%	85%	76%	79%	85%	74%
	7-digits	53%	58%	48%	51%	57%	44%
	9-digits	32%	35%	29%	36%	40%	32%
	11-digits	26%	28%	23%	28%	31%	25%
Average HRV	**Sequence-Length**						
	3-digits	43.4	48.4	38.4	36.8	41.0	32.7
	5-digits	44.0	49.4	38.6	38.7	43.1	34.3
	7-digits	42.5	48.0	36.9	34.9	38.8	31.1
	9-digits	43.5	48.6	38.5	37.3	41.3	33.4
	11-digits	42.5	47.5	37.5	35.9	39.7	32.2

Average responses for each variable are summarized above by concussion history group, for all 59 participants.

Table 3. Mixed effects model results for the effect of concussion history, cognitive load, task performance, and heart rate variability on pupillary response

	Estimate	DF	t Value	95% CI		*p*
Sequence lengths						
5-digits	0.03	232	0.98	-0.02	0.82	0.326
7-digits	0.10	232	3.02	0.04	0.17	0.003
9-digits	0.14	232	3.50	0.06	0.21	<0.001
11-digits	0.11	232	2.58	0.03	0.18	0.010
3-digits	0	.	.	.	.	.
Task Performance (accuracy--percent correct)	-0.001	1076	-0.03	-0.09	0.08	0.973
Heart Rate Variability (RMSSD in ms)	-0.00	1076	-1.27	-0.00	0.00	0.204
Concussion History- Yes v No	-0.01	57	-0.21	-0.1	0.08	0.838

--RMSSD (root mean square of successive differences) in milliseconds.

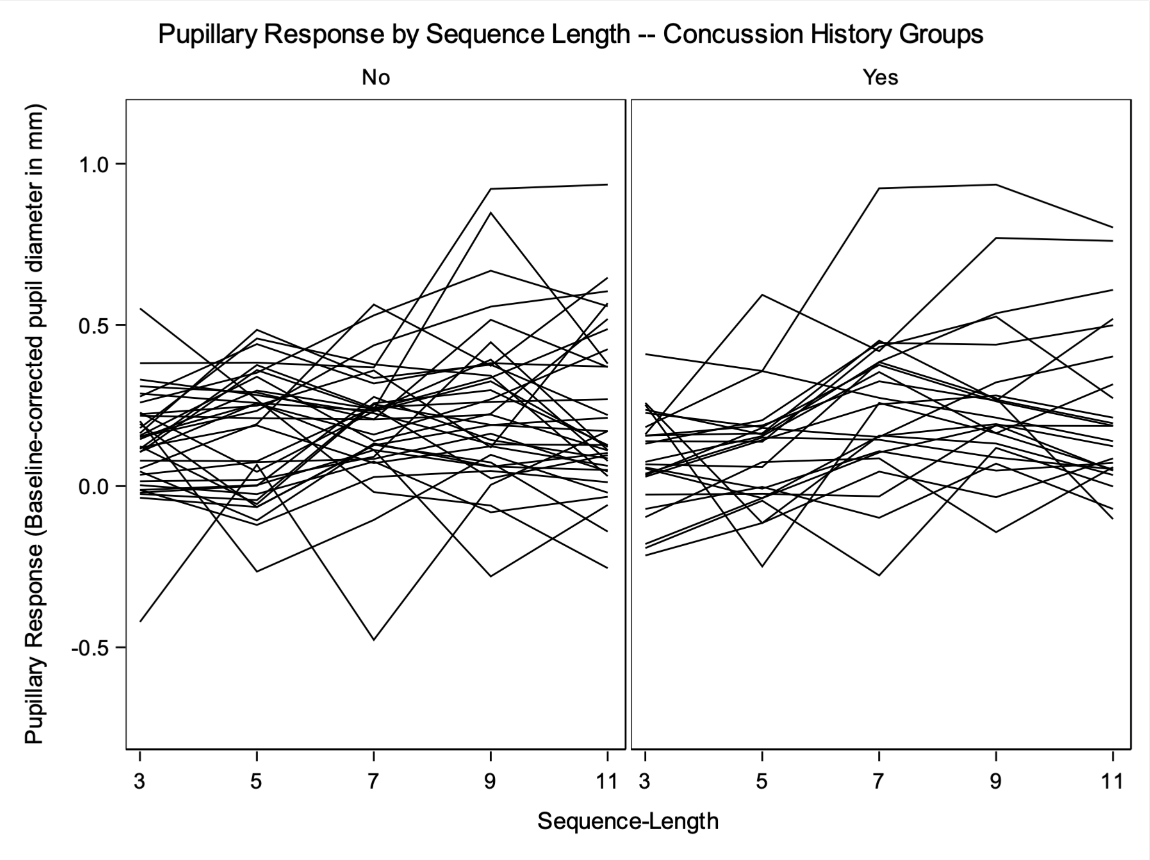


Figure 1. Pupillary response by sequence-length for each participant – split by concussion history groups

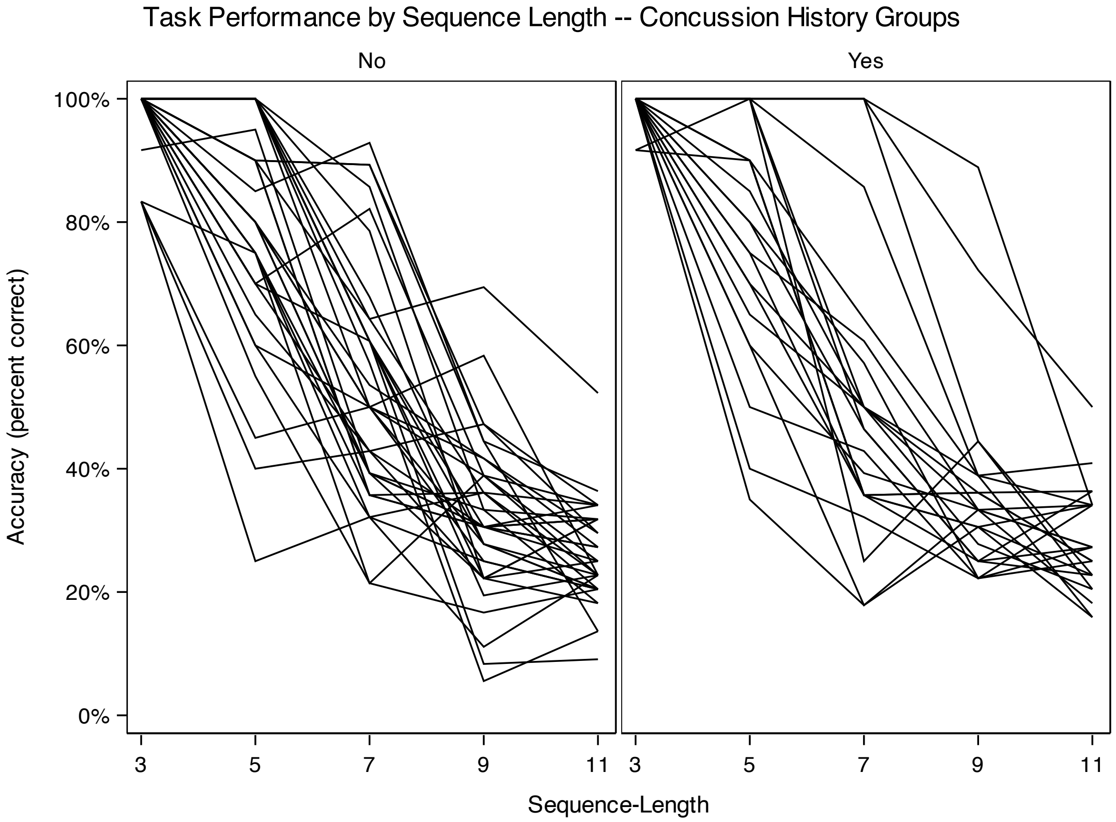


Figure 2. Task performance by sequence-length for each participant – split by concussion history groups

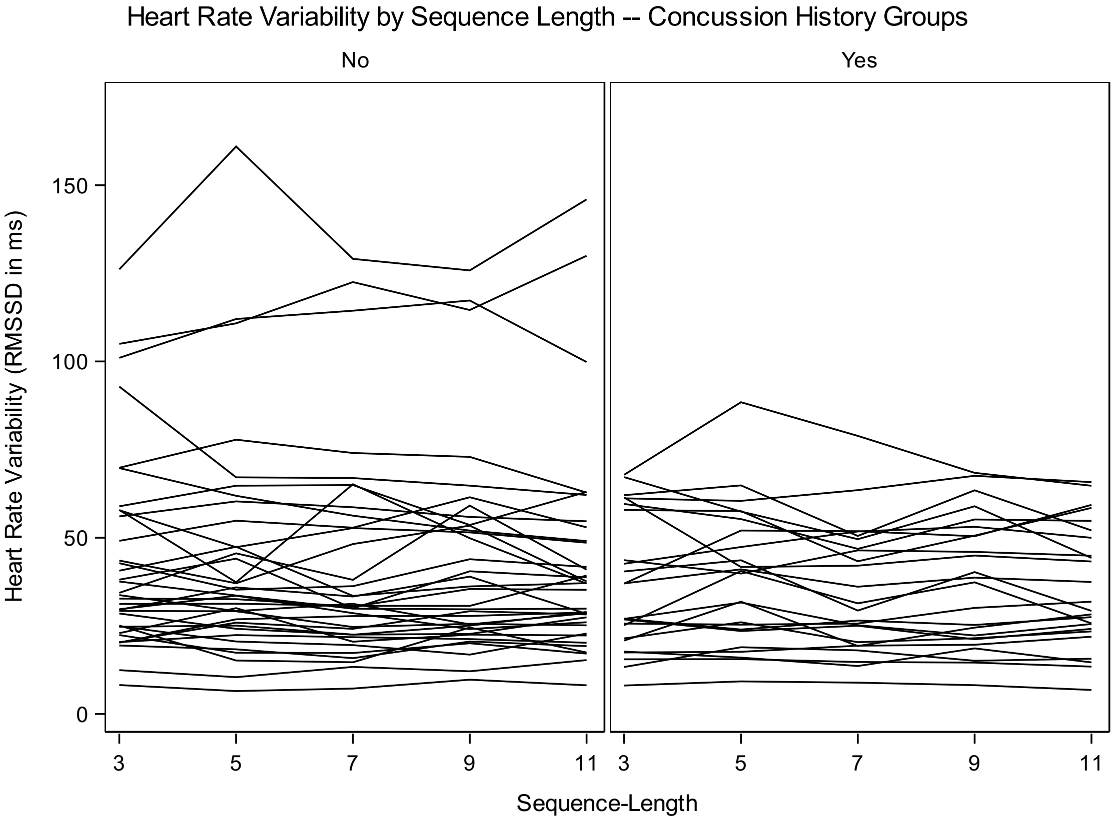
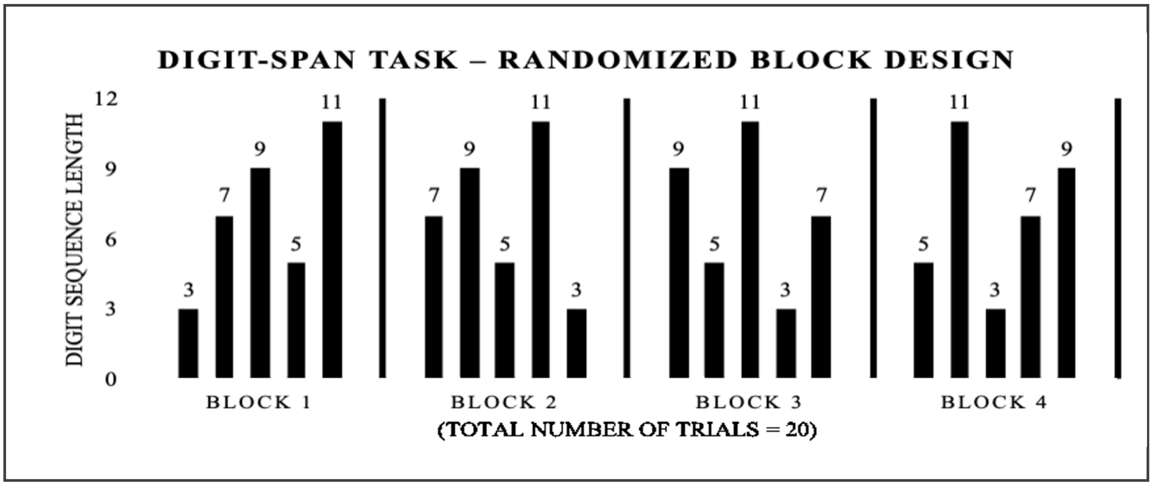
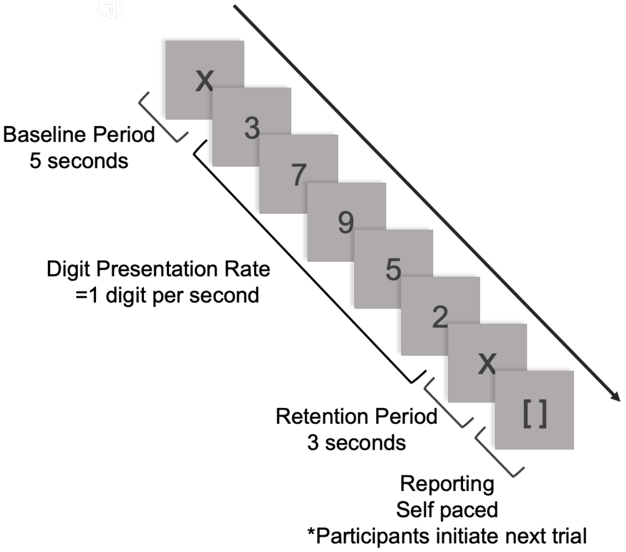


Figure 3. Heart rate variability by sequence-length for each participant – split by concussion history groups

SUPPLEMENTARY MATERIAL



Digit-span randomized blocked design: Each block randomly presents a single trial at each level of difficulty. A random number generator was used to determine testing order for the first block and a Latin square was used to counterbalance order for each subsequent block.



Sample Trial: Sample five-digit-sequence presentation illustrating the block design for a single trial within the digit-span task. Digits were displayed at a rate of one digit per second. Shaded areas represent response regions of interest within baseline and retention period, used to calculate pupil size change for each trial.

APPENDIX D. EXECUTIVE SUMMARY

Background:

Clinicians and researchers need objective measures to better characterize behavioral and physiological response dynamics associated with cognitive inefficiency following concussion. While cognitive efficiency can be described relative to various cognitive processes, working memory is most often examined with respect to relationships between task demands and available cognitive resources. Working memory is also one of the most common cognitive impairments following concussion—where associated post-injury clinical measures (digit-span task) demonstrate high diagnostic sensitivity.[11,12] Task Performance and physiological response dynamics associated with cognitive efficiency are difficult to characterize following concussion due to rapidly deteriorating signal detection and poor ecological validity of current clinical assessments and advanced neuroimaging modalities.[8,18] Dynamic cognitive efficiency characterization via physiological and task performance metrics may better inform concussion recovery response dynamics—which may hold important implications for improved concussion clinical assessment and management regarding readiness to return to athletic and or military activity. The study aims were to first examine relationships among behavioral and physiological metrics of heart rate variability and pupillary response during a working memory digit-span task to inform cognitive efficiency, and 2) determine the effects of concussion injury on these response dynamics.

Methods Overview:

a. ***Design****:* Cross sectional

b. ***Participants***: UNC Club Sports Athletes

c. ***Instrumentation & Study Measures****:*

Digit-Span Task –Task Design

- 20 Trials (4 blocks / 5 trials). Sequence Lengths -- 5 Levels (3, 5, 7, 9, 11)
- Backwards Recall

Task Presentation, VR and Eye Tracking Integration

- HTC VIVE Virtual reality – Head mounted display
- Infrared Eye tracking--Tobii Technologies

Task Performance/Accuracy

- Participant responses recorded
- Credit assigned for accurate digit recall, by serial position
- Represented as % correct for each trial [Higher = Better]

Hear Rate Variability

- Inter-beat intervals (RR intervals) recorded during task
- HRV represented as the RMSSD -- calculated for each trial [Higher = Better]
- Pupillary Response represented as the baseline corrected pupil diameter in mm, during the retention period– for each trial. [Larger diameter = Greater neural resource utilization]

d. ***General Testing Procedures***

Participants presented for a single testing session where they filled out a demographics and sport participation questionnaire, were then fitted with a chest strap heart rate monitor and VR headset, then completed the digit-span task.

e. Statistical Approach

We first examined relationships among pupillary response, task performance, and heart rate variability measures across levels of cognitive load (i.e., digit-sequence lengths). Then we employed two separate linear mixed effects models to first examine the effects of cognitive load, task performance, and heart rate variability on pupillary responses during our overloaded backwards digit span task—then the added effect of a prior concussion history.

Summary Results and Discussion Points:

There was a significant effect of cognitive load on our pupillary response outcomes. Specifically, higher cognitive load elicited greater pupillary dilation responses indicating greater neural resource utilization. Measures of task performance, heart rate variability, and concussion history group effects were non-significant.

Overall, our study was the first to examine an assessment for cognitive efficiency that accounts for both physiological and performance-based response dynamics, using VR and eye tracking technology, which may have implications for clinical practice and future cognitive interventions. Our study findings support pupillary responses' sensitivity to cognitive load beyond performance-based measures alone, in an athletic population. These findings provide additive evidence for the need to include ecologically valid and cost-efficient neurophysiological metrics when assessing cognitive performance and efficiency. Employing VR and eye tracking technologies to examine these responses may be useful for human performance and concussion management paradigms.

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