# THE IMPACT OF CONCUSSION HISTORY ON THE NEAR REFLEX AND PUPILLARY LIGHT REFLEX IN VIRTUAL REALITY

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# ABSTRACT

**Background:** Mild traumatic brain injury (mTBI) is a major public health concern, with over 1 million concussions estimated to occur each year in the United States alone. TBI impacts a diverse array of brain regions, producing a wide range of cognitive, physical, and psychological symptoms. Notably, there is currently no agreed-upon gold-standard concussion assessment, as the type, severity, and duration of impairments can vary widely between patients. To establish a valid and reliable biomarker for concussions, oculomotor behavior has gained increased attention as a promising avenue for detecting post-injury deficits caused by mTBI. Importantly, numerous oculomotor functions, including convergence, accommodation, and pupillary response, are commonly found to be impacted by mTBI. Deficits in oculomotor responses produce significant impairments to a patient's quality of life, such as difficulties reading, challenges driving, motion sickness, and sensitivity to light. These deficits also suggest that the near reflex triad, the joint response consisting of convergence, accommodation, and pupillary constriction associated with near vision, as well as the pupillary light reflex (PLR), hold promise as sensitive biomarkers. However, there has yet to be a comprehensive assessment of these reflexes in patients with a history of concussions.

**Aims:** Our primary aim was to determine whether differences in the near response and the PLR could be detected between healthy young adults (HCG) and patients with a history of concussions (CHX). It was hypothesized that the CHX group's responses would be reduced, slowed, or delayed, across ocular metrics. Further, we aimed to establish the ability of our novel virtual reality (VR) assessments to elicit stimulus-specific changes in oculomotor behavior by determining whether responses were altered by manipulations in visual stimuli across test conditions. We hypothesized that differences would be found across PLR, vergence, and accommodation tests as a function of manipulations in the VR scene similar to the stimulus-dependent changes in oculomotor response found across commonly used clinical tests.

**Methods:** Forty-five young adults (29 females, 16 males; Age M = 26.18, SD 4.18 years [18-35 years]) were recruited to either a Concussion History Group, which included participants with a history of one or more diagnosed concussions (CHX; n = 24), or a Healthy Control Group (HCG; n = 21). Participants performed a novel suite of oculomotor assessments in a SensoMotoric Instruments (SMI) HTC Vive with built-in video oculography (VOG) eye-tracking, which recorded eye movements and pupillometry at 89.6 Hz while simultaneously presenting an immersive VR environment. Tests were divided into three primary categories, (1) PLR tests (VR_PLR) – participants fixated on a target while the intensity (dim vs bright) and duration (pulse vs step) of a light stimulus was manipulated in the visual scene, (2) Vergence tests (VR_VA) – participants performed four tasks which primarily assessed convergence (VR_NPC), divergence (Divergence Fusion), Vergence Tracking, and Vergence Holding, by manipulating the movement of a virtual target in depth during binocular viewing, (3) Accommodation tests (VR_AT) – participants viewed two targets (near vs far, 35 cm apart) with a blur filter randomly applied to either stimulus and were tasked with judging which had greater clarity across progressively more difficult trials in binocular and monocular assessments. Standard clinical oculomotor data outside of a VR environment was also collected using an accommodative slide ruler to establish near-point of convergence (NPC) scores and a Snellen chart to assess visual acuity.

**Statistical Analysis:** For the PLR tests, a mixed repeated measures ANOVA of four conditions (2 intensity x 2 duration) and two participant groups (CHX vs HCG) was used to analyze eleven standard pupillary response variables (minimum diameter, maximum diameter, baseline diameter, final diameter, amplitude, peak and average constriction velocity, peak and average dilation velocity, and latency). For the vergence tests, independent samples t-tests were used for the VR_NPC and Divergence Fusion tests to compare interpupillary distance (IPD) metrics between CHX and HCG (minimum IPD, maximum IPD, IPD amplitude, correlations between IPD and target position, subjective breakpoint, and target fusion position). Repeated measures ANOVAs were used for the Convergence Tracking and Holding tasks to further assess whether IPD metrics were altered through changes across target cycles or steps. For the accommodation tasks, repeated measures ANOVAs were also used to assess differences in response metrics (reaction time, accuracy, target distance, target blur) between groups and across binocular and monocular conditions. Additional repeated measures ANOVAs were conducted by subdividing the HCG based on the number of concussions to determine whether there was an additive effect of multiple concussions, single, or no concussions. Follow-up pairwise comparisons with Bonferroni adjusted alpha levels to account for multiple comparisons were conducted if significant main effects were found across experiments. Regression analyses were conducted using time since injury as a factor to determine whether recovery time impacted PLR and near response metrics.

**Results:** No differences were seen between the CHX and HCG groups across clinical visual and oculomotor tests. Limited significant differences in oculomotor metrics were found between the CHX and HCG groups, but they were inconsistent across tests.

Similarly, follow-up analysis was unable to detect consistent differences based on time since injury or the number of concussions. Across assessments, significant differences within testing conditions were consistently found for VR_PLR, VR_VA, and VR_AT.

**Conclusion:** Our results demonstrated that VR technology is capable of eliciting and measuring the near reflex triad through manipulations in the virtual environment producing changes in VOG metrics that are stimulus-dependent. Our assessments produced expected response patterns that were similar to clinical tests (e.g., a brighter light produced greater pupil constriction). Limited significant differences as a result of a history of concussions conflict with past research but are likely attributed to notable heterogeneity in our patient population’s time since injury. Notably, the auditory pupil response, divergence fusion, and monocular accommodation tests appeared to be the most sensitive to potential deficits as a result of chronic mTBI. Our findings suggest that VR technology is capable of clinical oculomotor testing, but more research is needed to understand the utility of these tests among mTBI patients.

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# CHAPTER 1

# INTRODUCTION AND REVIEW OF THE LITERATURE

## Traumatic Brain Injury as a Public Health Risk

Traumatic brain injury (TBI) can be defined as “an alteration in brain function, or other evidence of brain pathology, caused by an external force” (Menon et al., 2010). TBIs are a major public health concern, with estimates suggesting over 1 million concussions occur each year in the United States alone (Laker, 2011; Langlois et al., 2006). Importantly, TBIs also represent a major economic burden, with the total direct and indirect costs estimated at $60 billion per year in the United States and individual cases costing patients tens to hundreds of thousands of dollars throughout their lifetime (Humphreys et al., 2013; Langlois J A et al., 2004). Most injuries are classified as concussions, which are generally categorized as mild traumatic brain injuries (mTBI) (Laker, 2011; Langlois et al., 2006; Menon et al., 2010). These can be caused by a wide range of incidents and activities, ranging from falls, motor vehicle accidents, assaults, and sports injuries (Harmon et al., 2019; Laker, 2011; Langlois et al., 2006; Menon et al., 2010). Further, evidence suggests that numerous populations are at-risk for concussions, with young children, older adolescents, and the elderly at increased risk and are more often hospitalized due to their injuries (Harmon et al., 2019; Langlois et al., 2006). Concussions can produce a diverse array of signs and symptoms ranging from cognitive (e.g., disorientation, difficulty concentrating, memory loss), physical (e.g., dizziness, blurred vision, headache, postural instability), emotional (anxiety, depression, irritability), changes in sleep habits, amnesia, and even loss of consciousness (Laker, 2011; Langlois et al., 2006; Menon et al., 2010; Scorza & Cole, 2019). Clinical selfreports suggest that in as many as 80% to 90% of patients, symptom resolution occurs within approximately 7 days (Harmon et al., 2019; Langlois et al., 2006). However, more recent attention has been given to the long-term deficits associated with concussions, which may not be detected through traditional symptom evaluation (Berkner et al., 2017; Maddocks & Saling, 1996; Slobounov et al., 2008; Wright et al., 2018). This has prompted a novel line of research exploring the chronic deficits that may not necessarily be resolved within the traditional symptom recovery timeline.

## Concussions Present Long-term Risks to Patients

Emerging evidence suggests that concussions are not just acute injuries but may be associated with extensive chronic deficits. Patients with a previous concussion are 2 to 5.8 times more likely to experience a subsequent injury and are at the greatest risk for a mTBI compared to those who have not been previously diagnosed (Harmon et al., 2019). This may be due to long-term neurophysiological changes, which may follow a different time course of recovery compared to self-reported symptoms (Scorza & Cole, 2019; Silverberg & Iverson, 2011). Emerging evidence also suggests that patients who are diagnosed with a concussion may experience post-concussion syndrome (PCS), in which symptoms persist after the typical acute recovery phase (Heitger et al., 2009; Ryan & Warden, 2003; Silverberg & Iverson, 2011). Importantly, there is significant variability in the prevalence of PCS, as well as the length and type of persistent symptoms. Conflicting reports suggest that there is significant heterogeneity in mTBI cases, with studies suggesting as many as 20-84% of patients experiencing PCS that can last from a few weeks to decades (Heitger et al., 2009; Ryan & Warden, 2003; Silverberg & Iverson, 2011). There is a notable overlap between common concussion symptoms and PCS, which often include physical (headaches, dizziness, and light sensitivity), cognitive (memory, attention, and executive deficits), and emotional (depression, anxiety, and irritability) factors (Heitger et al., 2009; Ryan & Warden, 2003; Silverberg & Iverson, 2011).

mTBI has also been linked to chronic physiological changes, including neurodegenerative disorders such as chronic-traumatic encephalopathy (CTE) (Gardner et al., 2014; Manley et al., 2017; Maroon et al., 2015). Importantly, CTE is commonly found in sports where players are at-risk for repeated impacts and multiple mTBI diagnoses, such as in boxing and football (Gardner et al., 2014; Manley et al., 2017; Maroon et al., 2015). While a confirmed diagnosis of CTE is not currently possible in living patients, those with verified cases often had a recorded history of clinical signs ranging from cognitive deficits, motor dysfunction, and psychological disorders (Gardner et al., 2014; Maroon et al., 2015; Ryan & Warden, 2003). Autopsies frequently reveal notable changes in cerebral mass, the presence of certain biomarkers such as abnormal TDP-43, tau protein, and amyloid accumulation, and notable brain lesions (Gardner et al., 2014; Manley et al., 2017; Maroon et al., 2015). CTE and other neurodegenerative disorders are not necessarily linked to a single acute event but may develop through repeated injuries over time (Gardner et al., 2014; Manley et al., 2017; Maroon et al., 2015). However, it is currently unclear why certain patients experience these and other long-term changes associated with mTBI, while others with similar concussion history may appear clinically normally. This uncertainty highlights the importance of early detection and an increased understanding of long-term deficits that may otherwise go undetected to help improve health professionals’ ability to protect patients at risk for repeated injuries, limit future neurological disorders, and optimize patient outcomes.

## Concussions Linked to Chronic Deficits Despite Normal Clinical Recovery

Research has recently uncovered underlying deficits associated with concussions that are not detected through traditional clinical means. Slobounov et al., (2008) studied the effects of a sports concussion on postural control before and 30 days after injury. Notably, patients were asymptomatic through traditional neuropsychological assessments and were cleared to return to their respective sports at the time of a follow-up assessment (Slobounov et al., 2008).  Participants’ postural control was found to be normal as measured through traditional center of pressure (COP) measures, including sway area, velocity, and stability index. However, using a virtual time-to-contact (VTC) measure, which reflects the time it takes a virtual trajectory of COP to reach a stability boundary, notable changes in postural control were uncovered (Slobounov et al., 2008). Notably, VTC absolute values, range, and mode, were found to be altered in the most challenging dynamic balance task, but not during static tasks (Slobounov et al., 2008). In this condition, participants were tasked with swaying forward and backward to their limits of stability, which produced significantly higher VTC values in the forward and backward directions, as well as increased VTC variability (Slobounov et al., 2008). These findings suggest that a participant’s ability to sway within a safe margin of stability, a decreased stability boundary, and/or altered postural control strategy may be characteristic of the post-concussion period, especially during challenging balance tasks (Slobounov et al., 2008). Importantly, these deficits would have otherwise gone undetected had it not been for a novel method for assessing postural control which suggests that the current battery of balance assessments may not be sufficient to detect all the chronic changes that occur following a mTBI.

Wright et al., (2018) studied military personnel who had previously reported one or more mTBIs greater than one-year post-injury and who were free from self-reported symptoms. Notably, postural deficits were uncovered using a novel VR balance task using a Wii Fit Balance Board, with significantly greater postural sway found in patients with more than one concussion in the most challenging test condition (Wright et al., 2018). In this condition, conflicting somatosensory and visual information, in the form of a foam support surface and rotating scene, respectively, produced significantly greater COP sway area, highlighting potential ongoing deficits integrating visual, vestibular, and somatosensory input during quiet stance (Wright et al., 2018). Acoustic startle response, which involves a diverse network of cortical and subcortical structures, was also found to be suppressed in mTBI patients despite the normal neurocognitive performance, providing further evidence for long-term neuroanatomical and/or physiological changes that may not be detected through traditional assessments (Wright et al., 2018).

In addition to the extensive findings across clinical tests, evidence for long-term behavioral changes following a concussion is supported by neuroimaging studies, which suggest neuroanatomical and physiological changes can persist long after the initial injury. Significant differences in electroencephalogram (EEG) activity have been noted in patients with a history of concussions who continue to experience residual deficits following injury (Cao et al., 2008; de Beaumont et al., 2007; Munia et al., 2016). Munia et al., (2016) investigated 6 adolescent football players, 4 of whom had been previously diagnosed with a concussion 8 months before initial data collection. The concussed group experienced worse neurocognitive performance as measured by the ImPACT assessment in both processing speed and impulse control sections (Munia et al., 2016). Importantly, these deficits were accompanied by significant differences in EEG power, such as an increased delta frequency band in frontal brain regions, and decreased beta and gamma frequency bands in the frontal, central, and parietal regions (Munia et al., 2016). While these differences in EEG activity dissipated over time, they were still found during a more demanding vigilance task at the final testing session 1 year later (Munia et al., 2016). Across tests, deficits were most pronounced in participants who had experienced more than one concussion, highlighting the potential cumulative effect of multiple injuries on delayed recovery and cognitive function (Munia et al., 2016). Importantly increased delta frequency is associated with increased mental effort during cognitive tasks, while decreased gamma frequency is associated with neurological disorders that include cognitive deficits such as Alzheimer's and depression, suggesting chronic EEG changes in concussion could be associated with decreased executive function, similar to other diseases (Casula et al., 2022; Harmony 2013; Harmony et al., 1996; Munia et al., 2016).

Research also suggests that chronic neurological changes can persist in patients who display normal symptomology and who otherwise appear to have recovered from one or more mTBIs. Notably, changes in specific EEG event-related potentials (ERPs) such as P300 have been found in chronic mTBI patients (de Beaumont et al., 2007; Dupuis et al., 2000; Pratap-Chiand & Sinniah, 1988). P300 is associated with memory, allocation of attentional resources, and may reflect CNS activity during tasks that require the generation of mental representations (de Beaumont et al., 2007). In one study, participants were divided into single-, multiple-, and no-concussion groups, and tested an average of 3 years post-injury in a visual-oddball task (de Beaumont et al., 2007). Despite being asymptomatic, significant between-group differences in P300 amplitude were noted when participants were presented with a rare visual stimulus (de Beaumont et al., 2007). P300 amplitude was found to be suppressed in the multiple-concussion group compared to the no-concussion group and trended toward significance compared to the single-concussion group (de Beaumont et al., 2007). Therefore, despite equivalent performance between groups during symptom screening and neuropsychological evaluations, chronic deficits in ERPs associated with cognitive performance suggest underlying deficits in executive function as a result of mTBI that would otherwise go undetected through clinical testing.

In addition to alterations in synapsing, there are also changes in neuronal structure. EEG studies are supported by other neuroimaging methods such as structural (e.g., magnetic resonance imaging) and functional (positron emission tomography) techniques, which show significant CNS alterations following mTBI (Davis et al., 2009; McCrea et al., 2017; Tremblay et al., 2013). Structural imaging has identified potential abnormalities including lesions, changes in white and grey matter, enlargement of the lateral ventricles, and cerebral hemorrhaging (Davis et al., 2009; McCrea et al., 2017; Tremblay et al., 2013). Functional imaging studies have also identified diverse changes following a mTBI, including alterations in brain metabolism, blood oxygenation-level dependent (BOLD) activity during neuropsychological and motor tasks, and changes in levels of compounds such as N-acetyl aspartate (NAA), creatine, and choline (Davis et al., 2009; McCrea et al., 2017; Tremblay et al., 2013). In a review by David et al. (2009), these changes were noted in patients months and even years after injury. Taken together, neuroimaging studies suggest long-term structural and functional neurological changes can accompany mTBI and may explain why chronic symptoms may persist outside of the traditional recovery period.

Despite these promising findings, the clinical potential of many neuroimaging techniques has not been extensively evaluated (McCrea et al., 2017). Further, these methods are often cost-prohibitive, lack portability, and are unable to be used outside of medical facilities, limiting their application for managing patient rehabilitation. Therefore, there is a growing need for tests that can infer the integrity of neurological systems and can address these limitations, while remaining both valid and reliable.

## Effect of Concussions on Visual and Oculomotor Systems

To address some of the limitations of neuroimaging assessments, researchers have investigated other means of assessing mTBI symptoms to infer changes in neuroanatomy and physiology. The oculomotor and visual systems have been identified as promising resources for detecting long-term deficits following a history of mTBI. Research has found significant changes in a variety of biomarkers in mTBI patients including saccades, smooth pursuit, convergence, pupillary light response, and the accommodative reflex (Cheever et al., 2018; Duprey et al., 2017; Galetta et al., 2011; Heitger et al., 2009; Kawata et al., 2016; Podolak et al., 2019; Wright et al., 2017). Notably, oculomotor deficits have been detected in mTBI patients in the subacute, acute, and chronic recovery phases following a concussion. (Cheever et al., 2018; J. McDevitt et al., 2016; Kawata et al., 2016; Wright et al., 2017).  With such a diverse array of impacted oculomotor functions, numerous clinical tests have been developed in an attempt to provide a valid and reliable means of detecting deficits post-injury.

Among the wide range of tests available, the near point of convergence test (NPC) has proven particularly sensitive to post-concussion deficits across the recovery timeline. Kawata et al., (2016) found that the patient’s NPC distance increased immediately following, and 24 hours after, a soccer heading session. Notably, these findings suggest even mild impacts below the threshold of a concussion diagnosis, termed subconcussions, may disrupt the oculomotor system, pointing to its sensitivity as a biomarker that can infer neurological changes (Kawata et al., 2016).

Another study by Cheever et al. (2018) found significant differences in NPC subjective break point (the distance at which participants report diplopia) at baseline across healthy, acute concussion participants. and those with prolonged recovery periods. Significant differences between healthy and prolonged recovery groups at a 2-week follow-up were also found (Cheever et al., 2018). Symptom provocation (i.e., the sum of rating headache, dizziness, and nausea on a scale of 0-6) following a battery of oculomotor assessments was also found to be significantly greater in the prolonged recovery group compared to the healthy controls (Cheever et al., 2018). Cheever et al. (2018) discriminated between healthy and concussed groups with 90% accuracy by combining symptom provocation and NPC scores, however, the distinction between acute and prolonged recovery groups was not made.

The utility of oculomotor testing is further supported by McDevitt et al. (2016) who found that a concussion assessment protocol consisting of the NPC, optokinetic stimulation (OKS), and gaze stability assessments can produce a highly sensitive model (accuracy 94.4%, AUC = 0.951) for discriminating between healthy and concussed individuals in the subacute phase. Similarly, Wright et al., (2017) found that NPC subjective break point was significantly worse in participants who had received a concussion within the past 6 months (M = 7.51 ± 1.34) compared to healthy controls (M = 3.42 ± 0.26). Importantly, NPC scores were significantly correlated with health status, but other oculomotor assessments such as the King-Devick (a number naming test which assesses saccadic eye movements; KD) and dynamic visual acuity test were not (Wright et al., 2017).

Taken together, these findings suggest that the oculomotor system provides a promising avenue for assessing the impact of mTBI, as clinical assessments appear to be an effective means of discriminating between healthy participants and those who have been diagnosed with a concussion. Assessments of the NPC response appear particularly sensitive to deficits following a concussion, highlighting their potential importance in clinical concussion testing.

## Current Oculomotor Assessments are Insufficient as Clinical Diagnostic Tools

Despite the promise that oculomotor testing holds, there is currently no gold-standard assessment for mTBI. Ongoing research has found conflicting results regarding which measures are the most sensitive to deficits following mTBI and what subpopulation these tests are most applicable for. For instance, while saccades are generally believed to be impacted by concussions, a review by Ventura et al (2016) found that both the type (memory-guided, anti, self-paced, etc.) and the dependent measure assessed (latency, number of errors, or accuracy) may impact whether results differ significantly between groups. Additionally, despite research often finding significant differences between healthy participants and those with one or more concussions, reliability among commonly used concussion assessments may not meet the acceptable levels for widespread clinical use and may vary across studies (Broglio et al., 2018). This may be due to the diverse and complex nature of the brain circuitry involved in regulating eye movements, coupled with the diffuse changes across the central nervous system caused by a mTBI. Notably, oculomotor dysfunction can present in the form of changes to smooth pursuit, saccades, vergence, accommodation, visual acuity, and other deficits (Broglio et al., 2018; Comeau & Pfeifer, 2019; Ventura et al., 2016). The heterogenous nature of these changes is further complicated by a diverse array of devices and techniques for measuring eye movements, ranging from video-oculography, reaction time tasks, computer-based neurocognitive assessments, and other methods. Therefore, research is needed to address not only which oculomotor variables are most sensitive to the post-injury state of the CNS following a concussion but should also explore the most reliable methods for measuring these deficits.

## The Near Reflex Triad

The near reflex triad is an underexplored function of the oculomotor system which could prove sensitive to concussions due to its use in tests such as the NPC. It consists of three linked oculomotor responses to an approaching target in a person's visual field. Namely, the pupils constrict to increase the depth of field and focus, the eyes rotate medially to prevent binocular disparity (convergence), and the lens changes shape to increase power and keep an object in focus (accommodation) (Mays & Gamlin, 1995; Myers & Stark, 1990). The relationship between these actions is reciprocal; a voluntary shift in attention from a target that is far away to one that is near will stimulate convergence to maintain binocular vision, which then stimulates accommodation to maintain focus on the target (Mays & Gamlin, 1995; Myers & Stark, 1990) . The relationship is reciprocal - as voluntarily focusing on a near object will drive the convergence of the eyes toward that target (Mays & Gamlin, 1995; Myers & Stark, 1990). Importantly, this reflex is necessary for a wide range of activities ranging from reading, driving, computer work, sports participation, and numerous other tasks, making it an essential component of everyday life. Elements of the NPC are commonly assessed clinically outside of a VR environment using tests such as the NPC and visual push-up tests and using devices such as an accommodative slide ruler and auto-refractor across patient populations (Campbell & Westheimer, 1960; Kawata et al., 2016).

The neural pathways involved in controlling the near reflex include a diverse network of sensory and motor structures, yet they are not fully understood. The accommodation reflex's afferent circuit begins at the retina, which acts as the sensor and detects retinal blur through the cones (Campbell & Westheimer, 1960; Green et al., 2010; Mays & Gamlin, 1995; Myers & Stark, 1990). From there, the signal travels through the optic nerve, optic chiasm, optic track, lateral geniculate body, and optic radiation to the primary visual cortex, where contrast-related neurons form a blur signal via cortical responses (Campbell & Westheimer, 1960; Green et al., 2010; Mays & Gamlin, 1995; Myers & Stark, 1990). The supranuclear signal then travels from the striate cortex to the Edinger-Westphal nucleus (EW), where the motor signal for an accommodation response is generated (Green et al., 2010; Mays & Gamlin, 1995; Myers & Stark, 1990). The motor command travels from the EW to the ciliary muscle via the oculomotor nerve, ciliary ganglion, and finally, short ciliary nerve (Campbell & Westheimer, 1960; Green et al., 2010; Mays & Gamlin, 1995; Myers & Stark, 1990). This signal causes the ciliary muscle to contract, which causes the lens to change shape to improve visual clarity and focus on a nearby target (Campbell & Westheimer, 1960; Green et al., 2010; Mays & Gamlin, 1995; Myers & Stark, 1990). Conversely, far responses are not well understood but are believed to involve a release of excitatory control via the abducens nucleus, perhaps through the posterior interposed nucleus in the cerebellum (Campbell & Westheimer, 1960; Green et al., 2010; Mays & Gamlin, 1995; Myers & Stark, 1990). This allows the lens to adjust to the target distance via a flat or rounded lens shape for near and far vision respectively (Campbell & Westheimer, 1960; Green et al., 2010; Mays & Gamlin, 1995; Myers & Stark, 1990).

The vergence response shares a similar afferent pathway to the accommodation loop, however, it is primarily driven by a disparity signal, reflecting the difference in retinal images due to the eyes being offset by 5-6 centimeters. (Cumming & Judge, 1984; Mays & Gamlin, 1995; Myers & Stark, 1990; Rucker et al., 2019; Searle & Rowe, 2016). While the pathways for vergence are less understood, the mesencephalic reticular formation (MRF) has been strongly implicated as responsible for the generation of vergence motor commands (Cumming & Judge, 1984; Mays & Gamlin, 1995; Myers & Stark, 1990; Rucker et al., 2019; Searle & Rowe, 2016). Additionally, distinct tonic and burst cells have been identified which are believed to regulate vergence angle and velocity respectively and are primarily active during convergence (Cumming & Judge, 1984; Mays & Gamlin, 1995; Myers & Stark, 1990; Rucker et al., 2019; Searle & Rowe, 2016).

Separate fast and slow vergence centers have also been identified, which allow for different types of vergence responses for target acquisition and target tracking (Rucker et al., 2019). Fast vergence responses are believed to involve the rostral superior colliculus, which provides input to the MRF and projects to the oculomotor nucleus that houses fast singly innervated motoneurons (Rucker et al., 2019). This region may also project to the supra-oculomotor area, which houses near-response cells that provide input to the EW. Conversely, the C group of the oculomotor complex contains multiply innervated extraocular motoneurons that project to the oculomotor nucleus and may be responsible for slow eye movements (Rucker et al., 2019). From there motor commands are sent primarily via the abducens nerve to the medial and lateral rectus muscles which control horizontal eye movement and signal rotation of the eyes (Cumming & Judge, 1984; Rucker et al., 2019; Searle & Rowe, 2016).

In addition to the primary pathways for vergence and accommodation, crosslinks between both pathways suggest that disparity and blur signals also provide input to the other oculomotor reflex (Cumming & Judge, 1984; Mays & Gamlin, 1995; Myers & Stark, 1990). Convergence-accommodation (CA) crosslinks from vergence sensory processors provide input to the accommodation motor controller in the absence of a blur signal (e.g., viewing a stimulus through a pinhole) (Cumming & Judge, 1984; Mays & Gamlin, 1995; Myers & Stark, 1990). Conversely, in the absence of a disparity signal during monocular viewing, accommodation-convergence (AC) crosslinks provide input to the vergence motor controller from the accommodation sensory processors (Cumming & Judge, 1984; Mays & Gamlin, 1995; Myers & Stark, 1990). Despite the need for additional research, these studies suggest there are several key areas of the vergence and accommodation pathways that have been established as important for proper oculomotor responses to visual stimuli. Evidence for key neuroanatomical structures involved in the near reflex triad also provides the foundation for understanding what areas of the brain may be damaged following a mTBI.

## The Pupillary Reflex

The pupillary light reflex (PLR) is an oculomotor response to changes in luminance levels through the regulation of pupil size to control the amount of light reaching the retina (Belliveau et al., 2021; Heller et al., 1990; Wang et al., 2016). After the introduction of a light stimulus, the typical PLR is categorized by rapid pupillary constriction to a minimum diameter, a short duration peak dilation period, and a longer duration period of slower dilation over several seconds to minutes until a baseline diameter is achieved (Ciuffreda et al., 2017; Podolak et al., 2019; Thiagarajan & Ciuffreda, 2015; Truong & Ciuffreda, 2016; Urosevich & Capo-Aponte, 2013). The amplitude of the PLR response increases with increased stimulus intensity, duration, and adaptation to a dark environment (Ciuffreda et al., 2017). Notably, pupillary response is affected by age, sleep, fatigue, attention, and emotional state, among other factors (Ciuffreda et al., 2017; Spector, 1990). In healthy individuals, pupillary diameter has been found to range between 2 to 5 mm in adults under clinical testing conditions but may expand to 8 mm in dark environments (Ciuffreda et al., 2017; Spector, 1990). The PLR has commonly been measured through the swinging light test, or more objectively through pupillometers which capture pupillary radius in response to controlled light stimuli (Belliveau et al., 2021; Binda & Gamlin, 2017; Ciuffreda et al., 2017; Thiagarajan & Ciuffreda, 2015).

Pupillary constriction is regulated through the parasympathetic nervous system in response to an increasing light stimulus (Belliveau et al., 2021; Heller et al., 1990; Wang et al., 2016). As light enters the eye, photosensitive retinal ganglion cells are activated, then transmit a signal related to stimulus intensity through the optic nerve, optic chiasm, and optic tract (Belliveau et al., 2021; Heller et al., 1990; Wang et al., 2016). This signal then travels to the midbrain and pretectal nuclei, where luminance neurons are housed in the olivary nucleus and are likely responsible for regulating the PLR response (Gamlin et al., 1995). Luminance neurons synapse with the EW via interneurons, which contain preganglionic parasympathetic fibers and generate an excitatory signal to the ciliary ganglion via the 3rd oculomotor nerve (Belliveau et al., 2021; Heller et al., 1990; Wang et al., 2016). Finally, a signal is sent via the short ciliary nerve to acetylcholine receptors in the iris sphincter muscles, which induces contraction of the pupil (Belliveau et al., 2021; Heller et al., 1990; Wang et al., 2016).

The pupillary constriction that accompanies the near reflex and that occurs during the PLR are believed to share many common neural pathways. However, the exact nature of their relationship is not fully understood. One possible source of differences between paths may be the location of pretectal connections, with PLR predominantly linked to the posterolateral/medial pretectum and the near reflex linked to the posteromedial/anterior pretectum (Binda & Gamlin, 2017; Thompson, 1984; Trejo & Cicerone, 1984). Additionally, the pathway for visual pupillary response is believed to involve the lateral geniculate nucleus, where optic tract fibers terminate for processing images (H. Yoo & Mihaila, 2021). While some fibers from the visual pathway do make their way to the pretectal nucleus, it is the primary destination for optic tract fibers involved in the PLR

(H. Yoo & Mihaila, 2021). Therefore, while differences exist in the pathways that regulate the PLR and near reflex, there is also evidence suggesting that they share key structures that may be responsible for regulating the motor signal for both pupillary responses.

## Near Reflex and Pupillary Light Reflex Deficits are Commonly Found in Concussed Patients

Deficits in one or more aspects of the near response have been found in patients with a history of concussions. Convergence insufficiency (CI), or abnormal convergence response categorized by the eyes drifting outward at nearer distances and a receded NPC (the point at which the eyes can maintain binocular vision while focusing on a near object is farther away than normal), has been found at significantly greater rates in participants following a head injury (47-64%) compared to healthy controls (Ventura et al., 2016). Accommodative disorders are also commonly found in concussion patients, with some estimates suggesting 51% of patients may have some detectable impairment even several months after injury (Master et al., 2016). Pupillary response deficits have been studied in mTBI patients through the pupillary light reflex. Particularly, measures such as constriction velocity, dilation velocity, maximum pupil diameter, and constriction amplitude among others were found to be slower, smaller, and delayed in patients with mTBI (Capó-Aponte et al., 2013, Thiagarajan & Ciuffreda, 2015; Ciuffreda et al., 2016).  However, more research is needed to determine how these measures vary across light conditions in the concussed population. Finally, the NPC is commonly used to assess the near response in mTBI patients. While its sensitivity has been demonstrated across multiple studies, by relying on patient's self-reported diplopia it lacks objective measures of ocular response such as interpupillary distance and pupil diameter (McDevitt et al., 2016; Wright et al., 2017, Cheever et al., 2018). These findings support the immense promise of leveraging the near reflex and PLR for a comprehensive assessment of oculomotor deficits following a concussion, as well as the research gaps that such a study could fill.

## Using Virtual Reality to Assess the Visual and Oculomotor Systems

Virtual reality (VR) technology has received growing attention as a potential diagnostic and rehabilitative tool in a variety of patient populations, including those with postural control disorders, mTBI, brain lesions, and oculomotor deficits (Bell et al., 2020; Munsamy & Paruk, 2021; Park et al., 2007; Piron et al., 2001; Schultheis & Rizzo, 2001; Vincent et al., 2021; Wright et al., 2017). The potential advantages of using VR are extensive, ranging from improved portability, cost-effectiveness, enhanced patient engagement, more individualized treatments, and possibly even better patient outcomes (Bell et al., 2020; Munsamy & Paruk, 2021; Park et al., 2007; Piron et al., 2001; Schultheis & Rizzo, 2001; Vincent et al., 2021; Wright et al., 2017). While VR has been used to study the oculomotor and visual systems, research focused on designing and testing the oculomotor assessments in concussion patients is limited (Santos et al., 2020). Therefore, establishing the ability of VR-based assessments to detect oculomotor deficits due to mTBI could represent a promising avenue for improving patient outcomes.

## Innovation and Significance

A comprehensive investigation of the near reflex triad in participants with a history of concussions has yet to receive adequate attention from researchers, yet it may hold immense promise for understanding mTBI-related changes in the brain. This may contribute to the development of more valid and reliable clinical assessments of the oculomotor system, as well as increase our overall understanding of the persistent deficits associated with concussions. For instance, a recently published case study by Rucker et al. (2019) investigated the chronic impairments a patient experienced years after a mTBI. The patient suffered from several difficulties, including an unstable pupillary response to close targets in light, an inability to maintain convergence after initiating eye movements toward a near target, and a reduced accommodative response to near targets. These clinical deficits were accompanied by lesions to the pretectum, right pulvinar in the caudal thalamus, and rostral superior colliculus found via functional magnetic resonance imaging (fMRI), which are regions of the brain well-established as key for visual processing, orientation, and oculomotor control (Rucker et al., 2019). Shockingly, these deficits remained 11 years after the patient's initial injury and severely restricted her ability to read, use technology, and return to work. This case represents the debilitating nature of deficits of the near reflex triad, which are frequently noted in mTBI populations even after initial recovery from injury.

As such, a comprehensive test of the near reflex triad has the potential to offer valuable insight into the diagnosis, management, and treatment of oculomotor symptoms following concussions. The use of VOG and VR technology allows for an objective assessment of the oculomotor system, as both pupillary response and vergence can be directly measured. The utility of VR allows for custom-designed scenes that can stimulate vergence, accommodation, and pupillary response across a variety of manipulations, allowing for a comprehensive assessment of the near response and pupillary light reflex.

## Summary

Patients of all ages can experience a concussion throughout their lifetime. With millions of concussions occurring each year, and the threat of post-concussion syndrome and chronic deficits producing long-term dysfunction, there is a clear need for more objective tests, as well as measurable biomarkers, that can assess the effects of a history of mTBIs. As the neurodegenerative effects of a mTBI have been linked to not just single impact, but repeated injuries, valid and reliable clinical assessments that can be easily used are needed to maximize patients' health and safety. Oculomotor tests have gained popularity due to the numerous deficits commonly associated with mTBI, however, there is currently no gold-standard assessment. While elements of the near reflex triad have been studied in research settings and utilized to develop limited clinical assessments, a comprehensive, objective examination of this reflex has not been well-studied in the mTBI population. Evidence pointing to deficits in convergence, pupillary response, and visual accommodation provides a strong framework for studying the near reflex triad. The development of a test that is portable, cost-effective, valid, and reliable, holds immense importance in advancing mTBI diagnosis and treatment.

## Pilot Study

Pilot data was collected on 4 participants to assess how manipulations in VR stimuli and subconcussions impacted oculomotor responses. We aimed to investigate whether manipulations to target distance would produce vergence responses and whether these responses would be significantly larger compared to responses to changes in target size in VR. We hypothesized that target size changes would produce relatively small vergence responses compared to target distance changes based on prior work showing that binocular disparity, and not size, accounts for a majority of the vergence response (Regan et al., 1986). Our second aim was to investigate whether subclinical impacts following a bout of soccer heading would produce alterations in vergence. This would provide evidence for the ability of the VR VOG system to detect potential disruption of the near-response system following lower-intensity impacts. We hypothesized that vergence would be impacted following a bout of soccer heading compared to baseline, similar to the findings of Kawata et al. (2016) who found that soccer heading produced changes in the NPC subjective break point.

In this pilot study participants were presented with a virtual target presented using a SensoMotoric Industries HTC Vive that either changed in distance or size throughout a 20-second trial and were asked to continuously track the target as it moved or changed size. Participants were tested at baseline and immediately following a bout of 10 soccer headers using a model incorporated by Kawata et al. (2016).

A repeated measures ANOVA found that interpupillary distance (IPD) differed between the target distance change and size change conditions. For minimum IPD (reflecting convergence; the point where the pupils are closest together) a significant main effect of target type was found (F (1, 3) = 17.10, p = 0.026, partial $\eta^2 = 0.851$), indicating that more convergence was seen while tracking the target that changed distance (5.76, SEM = 0.24) compared to when it changed size (M = 6.00, SEM = 0.19) Additionally, for IPD Difference (maximum IPD vs minimum IPD; reflecting the overall divergence and convergence response) a significant main effect of target type was found (F (1, 3) = 10.80, p = 0.046, $\eta^2 = 0.783$), indicating that the total vergence response was significantly larger when the target changed distance (M = 0.23, SEM = 0.05), while virtually no change between convergence and divergence occurred when the target changed size  (M = 0.02, SEM = 0.01). These findings supported our hypothesis, suggesting changes in target distance in VR produce vergence responses, while changes in size do not to a significant degree.

Notably, evidence supporting the impact of soccer heading on vergence response was also found. An interaction between session and target type was found for IPD Difference (F (1, 3) = 27.05, p = 0.014, $\eta^2 = 0.900$). A follow-up post-hoc analysis for target type revealed that at baseline, the IPD Difference was significantly larger in response to the target changing distance (M = 0.27, SEM = 0.044) compared to size (M = 0.04, SEM = 0.03). Following the soccer heading there was no significant difference in IPD Difference between the conditions where the target changed distance (M = 0.19, SEM = 0.07) or size (M = 0.01, SEM = 0.003). This suggests that soccer heading altered vergence responses in a manner that decreased overall convergence and divergence in response to target movement. This would, in-turn, produce an IPD that was relatively unchanged between near and far targets, as seen in this data. This supported our hypothesis, as even low-intensity impacts altered vergence responses as measured in our VR-based protocol.

## Specific Aims

**Primary Aim 1.** To assess how the PLR is impacted in participants with a history of concussions by comparing response amplitude, diameter, velocity, and latency metrics to the Healthy Control Group.

**Primary Hypothesis 1.** Pupillary responses will be reduced, slowed, and delayed in the Concussion History Group compared to the Healthy Control Group.

**Secondary Aim 1.** To assess the ability of a VR HMD to elicit and measure the PLR by comparing pupillary responses between conditions that manipulate the duration and intensity of a light stimulus.

**Secondary Hypothesis 1.** Pupil metrics will differ based on stimulus length and intensity between conditions. We will report descriptive statistics for each group across the 4 pupillary light tests.

**Tertiary Aim 1.** To investigate how the number of lifetime concussions impacts the PLR by comparing the Healthy Control Group, those with one diagnosed concussion, and those with 2 or more diagnosed concussions.

**Tertiary Hypothesis 1.** Participants with two or more concussions will have reduced, receded, slowed, and delayed pupillary responses compared to those with one concussion and the Healthy Control Group.

**Primary Aim 2.** To assess how vergence response is impacted in participants with a history of concussions by comparing near points of convergence, divergence, vergence holding, and vergence tracking to the Healthy Control Group.

**Primary Hypothesis 2.** Vergence metrics will be receded, more variable, and less accurate in the Concussion History Group.

**Secondary Aim 2.** To assess the ability of a VR HMD to elicit and measure vergence by comparing differences in interpupillary distance based on manipulations in target position.

**Secondary Hypothesis 2.** Interpupillary distance will differ significantly based on the target position in the VR space across tests. We will report descriptive statistics for each group across the 4 vergence tests.

**Tertiary Aim 2.** To assess the relationship between vergence in the physical world and VR by comparing subjective break points in the NPC and VR_NPC.

**Tertiary Hypothesis 2.** Subjective break point will be strongly correlated between both tests, indicating similar vergence responses in both settings. However, overall values may differ.

**Primary Aim 3.** To assess how accommodation is impacted in participants with a history of concussions by comparing reaction time, accuracy of responses, and level of accommodative and vergence demand in a novel VR accommodation test to the Healthy Control Group.

**Primary Hypothesis 3.** The Concussion History Group will have slower, less accurate responses, and worse performance compared to the Healthy Control Group.

**Secondary Aim 3.** To assess how reaction time and accuracy of responses are impacted across test phases which manipulate cues for accommodation and vergence in a novel VR accommodation test.

**Secondary Hypothesis 3.** Outcome measures will differ across the three test phases in the VR accommodation test because of changes in accommodation and vergence demand.

**Tertiary Aim 3.** To assess the differences in reaction time, accuracy of responses, and level of accommodative and vergence demand during monocular versus binocular test conditions.

**Tertiary Hypothesis 3.** Reaction time, accuracy, and level of accommodative and vergence demand will significantly differ between the binocular and monocular conditions in the Healthy Control Group and Concussion History Group.

# CHAPTER 2

# EFFECT OF A HISTORY OF CONCUSSIONS ON THE PUPILLARY LIGHT REFLEX IN A NOVEL VIRTUAL REALITY ENVIRONMENT

## Introduction

Impairments of the pupillary light reflex (PLR) are commonly found in patients following a concussion and may persist across the subacute, acute, and chronic recovery periods (Ciuffreda et al., 2017; Podolak et al., 2019; Thiagarajan & Ciuffreda, 2015; Truong & Ciuffreda, 2016; Urosevich & Capo-Aponte, 2013). The PLR is often assessed in clinical settings following a concussion through the swinging light test, or more objectively through pupillometers which directly measure pupil diameter in response to controlled light stimuli (Belliveau et al., 2021; Binda & Gamlin, 2017; Ciuffreda et al., 2017; Thiagarajan & Ciuffreda, 2015). Dynamic PLR deficits are typically categorized by changes in the magnitude or diameter, velocity, and latency of response, and have been found across numerous studies investigating pupillary response in mild traumatic brain injury (mTBI) patients across the recovery timeline (Ciuffreda et al., 2017). Notably, mTBI patients have been found to have slower (peak and average constriction and dilation velocity), delayed (constriction latency and dilation recovery), and reduced (maximum and minimum diameter, constriction amplitude) responses following the presentation of a light stimulus as measured through objective pupillometry (Ciuffreda et al., 2017; Podolak et al., 2019; Thiagarajan & Ciuffreda, 2015; Truong & Ciuffreda, 2016; Urosevich & Capo-Aponte, 2013). Importantly, deficits appear to be consensual across both eyes in a majority of testing conditions, as asymmetric PLR responses are not typically found in concussion patients (Ciuffreda et al., 2017).

The presence of PLR deficits may depend on stimulus parameters and testing conditions utilized during testing, as the intensity, duration, and color of light may produce differential effects on the pupillary response (Ciuffreda et al., 2017; Podolak et al., 2019; Thiagarajan & Ciuffreda, 2015; Truong & Ciuffreda, 2016; Urosevich & Capo-Aponte, 2013). Recent studies using dynamic pupillometry have incorporated both dim (~4 lux) and bright (~251 lux) conditions, as well as pulse (~100 ms) and step (~1000 ms) stimulus durations, which produced stereotypical response profiles reflecting changes in intensity and duration of stimuli (Ciuffreda et al., 2017; Podolak et al., 2019; Thiagarajan & Ciuffreda, 2015; Truong & Ciuffreda, 2016; Urosevich & Capo-Aponte, 2013). While conditions with greater intensity and longer duration have been found to produce larger responses (e.g., greater response amplitude), response saturation may also occur and hide subtle differences in the PLR between healthy participants and those with mTBI (Ciuffreda et al., 2017; Podolak et al., 2019; Thiagarajan & Ciuffreda, 2015; Truong & Ciuffreda, 2016; Urosevich & Capo-Aponte, 2013).

An important consideration that is often overlooked when assessing the pupillary response to a light stimulus in patient populations is determining whether confounding injuries to other structures or neural pathways could mask or present PLR deficits. Notably, changes in pupillary response can also be caused by factors such as changes in executive function, aging, and psychological disorders (Tapper et al., 2021; Zekveld et al., 2018a). Further, due to the diverse nature of TBIs, they can elicit a variety of abnormal pupillary responses which may not necessarily reflect direct changes to the afferent and efferent arms of the PLR pathway, but instead more global central or peripheral nervous system damage (Adoni & McNett, 2007; Tapper et al., 2021).

Specifically, mTBIs have been found to produce symptoms that are also associated with changes in pupil response, such as fatigue, impaired working memory, and difficulty concentrating (Harmon et al., 2019; Laker, 2011; Maddocks & Saling, 1996; Tapper et al., 2021). Therefore, it may be important to understand the pupillary response to non-visual or light stimuli to isolate the presence of more global pupillary response deficits from those related to the PLR. An auditory stimulus may act as a promising reference point, as extensive research suggests the presence of sound, especially loud sounds, can elicit a pupil dilation response (Cajal, 2011; Wang et al., 2016; Zekveld et al., 2018).

The color of the light stimulus has also been found to produce differences in pupillary parameters, as retinal photoreceptors and retinal ganglion cells preferentially respond to different wave lengths of light (Ciuffreda et al., 2017). For instance, white light is thought to target primarily the rod and cones, with the intrinsic photosensitivity retinal ganglion (ipRGC) cells having little influence over pupillary responses (Ciuffreda et al., 2017). Conversely, a blue light stimulus primarily activates the melanopsin-containing ipRGCs, with little involvement of the rods and cones. Despite these differences, there has been little evidence to suggest that different colored light stimuli offer inherent advantages over white light for eliciting more robust findings or diagnostic capabilities (Ciuffreda et al., 2017). With artificial light emitted from devices such as head-mounted displays (HMDs) and other displays being common sources of stimuli across activities of daily living, using a full-spectrum light may have value for recreating the conditions typically experienced by a mTBI patient regularly (Kim et al., 2018).

Despite consistent findings which suggest that the PLR is affected by concussions, more research is needed to fully address three important questions: 1) How are PLR deficits categorized across the recovery timeline in the subacute, acute, and chronic phases, 2) What aspects of the PLR are most sensitive to concussion-related deficits, and 3) How is the PLR impacted by stimuli which vary in intensity and duration? Furthermore, the ability of a virtual reality (VR) head-mounted display (HMD) to stimulate and assess the PLR has yet to be extensively explored. Therefore, the current study focuses on the impact of a history of concussions on the PLR within a VR environment using varied stimulus conditions to extend the literature comprehensively and innovatively. We hypothesized that participants with a history of concussions would have delayed, slowed, and reduced pupillary responses compared to healthy control participants. We also predicted that pupillary response would depend on the intensity and duration of the light stimulus, as seen through increased response rate, velocity, and amplitude. Finally, we aimed to establish normative values for the PLR assessment in VR, with responses matching the characteristic pupil response pattern seen in the literature.

## Methods

### Participants

Forty-five young adults gave informed consent to participate in this study approved by Temple University's Institutional Review Board (IRB). Participants were recruited through flyers posted on Temple University's main campus, as well as emails sent throughout the College of Public Health. For their time, participants were compensated with a $25 Amazon gift card. The study was conducted within the Motion-Action-Perception Laboratory (MAP Lab), at Temple University in Philadelphia, PA.

### ***Eligibility***

Participants in the Concussion History Group were defined as having one or more diagnosed concussions in their lifetime, while the Healthy Control Group had to have never been diagnosed or suspected of having experienced a concussion. Inclusion criteria were as follows: 1) Age between 18-35, 2) Normal or corrected to normal vision (binocular visual acuity of 20/40 or better), 3) No history of oculomotor or vision disorders unrelated to a concussion (e.g., strabismus), 4) No history of neurological disorders unrelated to a concussion (e.g., Parkinson’s disease), 5) Participants must avoid alcohol and drug use 24 hours before testing. Participants completed a medical history form to determine concussion status, the presence of symptoms, and potential neurological and visual disorders before testing. A short visual assessment using a Tumbling E chart was conducted to verify visual acuity fell within normal ranges for the right and left eyes, as well as binocularly.

## Procedures

### ***Instrumentation***

Virtual Reality Pupillary Light Reflex Tests (VR_PLR): The VR_PLR was presented through a wired HTC Vive HMD (HTC Corporation, Taoyuan City, Taiwan) that was fitted with a SensoMotoric Instruments (SMI) video-oculography (VOG) system (SensoMotoric Instruments, Teltow, Germany). The HMD uses a 110° diagonal field of view and 89.6 Hz refresh rate to stream the VR environment through the Unity Real-Time Development Platform (Unity Technologies, San Francisco, US). The HTC Vive also utilizes on-ear headphones to stream audio from the host computer. VOG data (pupil diameter) was recorded at 89.6 Hz.

### ***Experimental Protocol***

#### Clinical Visual Assessment

Participants' visual acuity was measured using a standard tumbling E chart at a distance of 2 meters. The right and left eyes were assessed separately by having participants cover one eye and verbally indicate the direction the letter E was facing. Participants started from the top of the chart and read down line by line until errors were made and they were unable to meaningfully read or guess the direction of the E (Camparini et al., 2001). The procedure was then repeated for both eyes to establish binocular visual acuity.

Near visual acuity was measured using an accommodative slide rule. Participants held the card holder at a fixed distance of 40 cm and read down the reduced Snellen chart line by line until errors were made or they were unable to meaningfully continue (Holladay, 1997).

#### Auditory Pupil Response

Before the VR_PLR assessment, participants were seated in a chair and fitted with the HTC Vive HMD. Participants were then seated at a comfortable height with their chin placed on a headrest to control for head position. A standard five-point calibration and validation procedure was conducted through SMI's internal software, which ensured that the gaze angle was within 1° tolerance.

Participants were placed in a dark VR environment and instructed to focus on a small red cross (5 cm diameter) centered in the scene. After 5 seconds, a 440 Hz auditory tone was played through the HTC Vive's headphones lasting 500 ms, followed by an additional 5 seconds of recording time. Three trials were performed separated by 30 seconds of rest.

### VR_PLR

Before testing, participants sat with the headset in a dark environment for 1 minute to control for light adaptation between participants. During testing, participants were presented with a black VR environment with a small red cross (5 cm diameter) centered in the scene for 2 seconds. Then, a white light stimulus was presented which varied in intensity and duration across four experimental conditions: 1) Dim Pulse - a 4 lux 167 ms light 2) Dim Step - a 4 lux 1000 ms light, 3) Bright Pulse - a 250 lux, 167 ms light, 4) Bright Step - 250 lux 1000 ms light. Following the light stimulus, the VR scene returned to a black environment and data was captured for an additional 6 seconds (Figure 1). The presence of “light” vs. “dark” was simulated by changing the surrounding color from black to white, which produced a measurable increase in illuminance through the display of the HMD as measured objectively through a lux meter (Urceri Handheld Digital Light Meter).

Participants performed three trials per condition with 30 seconds of rest between conditions to allow for the pupil to return to baseline based on prior research on the PLR (Y. J. Yoo et al., 2017). The dim conditions were presented first, followed by the bright conditions, and the order of pulse vs. step trials was counterbalanced between participants. In each condition, participants were instructed to maintain their fixation on the center of the target and to minimize blinking during testing.

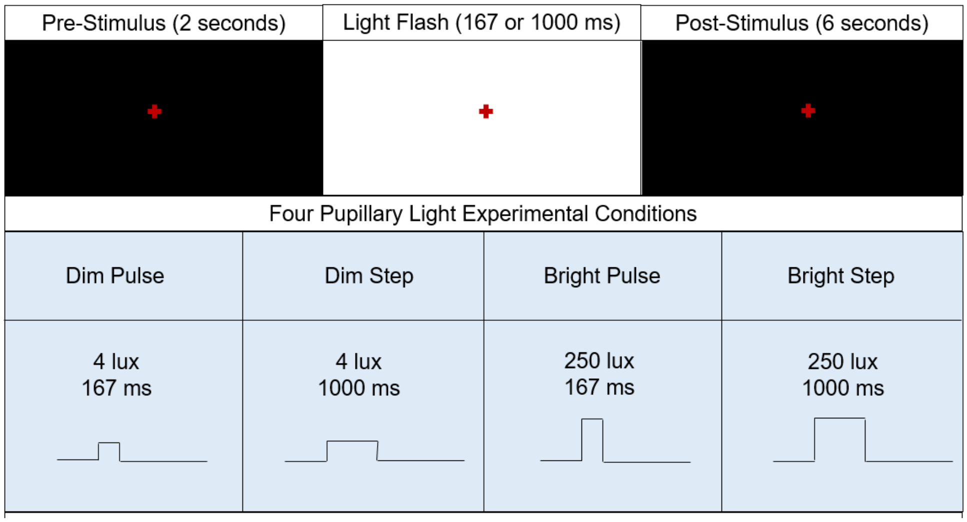


Figure 1. Diagram of VR_PLR stimulus and experimental conditions.

### *Outcome Measures*

To create a comprehensive assessment of the PLR, as well as determine the ability of a VR system to measure pupillary response like a traditional dynamic pupillometry device, we selected a range of dependent variables to match those used across PLR studies in the mTBI population (Thiagarajan & Ciuffreda, 2015). Dependent variables were as follows: 1) Baseline Diameter (mean of the pre-stimulus pupil diameter), 2) Maximum Diameter (maximum pre-stimulus diameter), 3) Minimum Diameter (minimum post-stimulus diameter), 4) Constriction Amplitude (maximum - minimum diameter), 5) Final Diameter (last value in the post-stimulus period), 6) Constriction Latency (time of peak constriction acceleration – time of stimulus onset), 7) Peak Constriction Velocity (minimum value for velocity [change in position/change in time] from the time of stimulus onset to the time of minimum pupil diameter; negative values reflected constriction in our system), 8) Peak Dilation Velocity (maximum value for velocity from the time of maximum constriction to the end of the trial), 9) Average

Constriction Velocity (mean of the same period for Peak Constriction Velocity), 10) Average Dilation Velocity (mean of the same period for Peak Dilation Velocity) (Thiagarajan & Ciuffreda, 2015). A diagram demonstrating a stereotypical response with outcome variables overlayed can be seen in Figure 2.

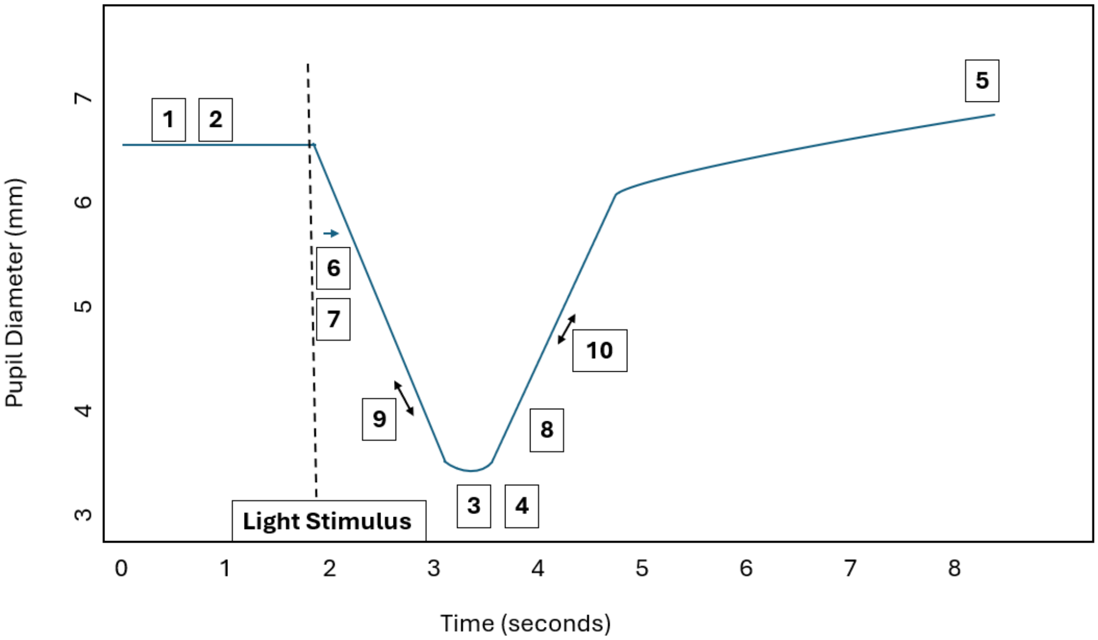


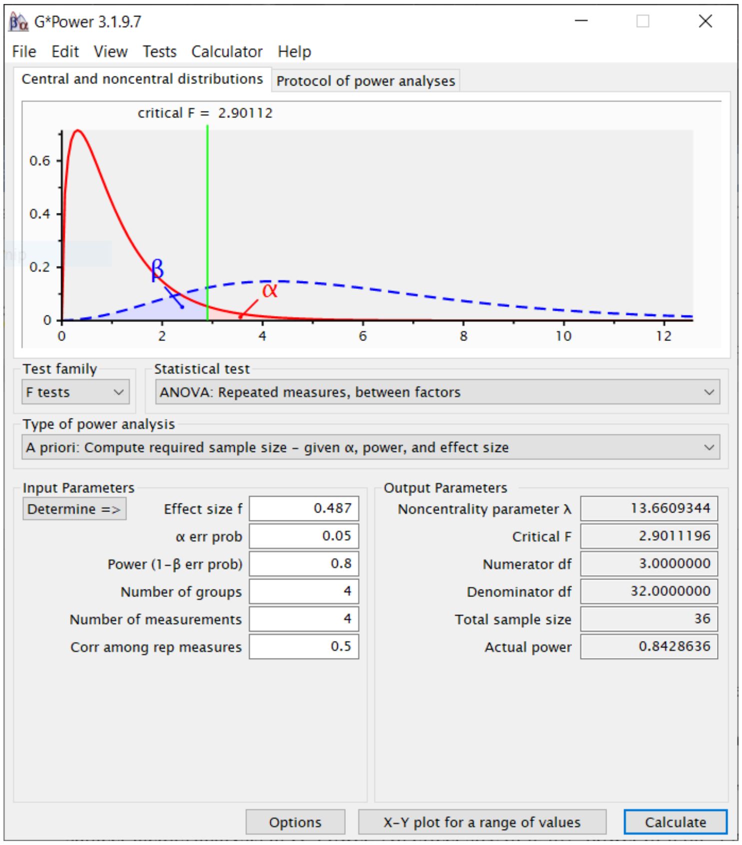
Figure 2. Diagram illustrating the VR_PLR outcome measure during a stereotypical response.

For the clinical visual assessment, standard outcome measures were used. Visual acuity was recorded as Snellen Score (e.g., 20/20) and then converted to LogMAR for statistical comparison (Holladay, 1997).

For the Auditory Pupil Response Test variables were selected based on prior research and were similar to those used for the VR_PLR, as both were aimed at capturing the pupillary response to an external stimulus (Ciuffreda et al., 2017; Zekveld et al., 2018a). However, because the auditory pupil response elicits dilation, where the PLR elicits constriction followed by dilation during recovery, the constriction metrics were not included for analysis in this test (Zekveld et al., 2018a). Outcome measures were as follows: 1) Baseline Diameter, 2) Maximum Diameter, 3) Minimum Diameter, 4) Response Amplitude, 5) Final Diameter, 6) Peak Dilation Velocity, 7) Average Dilation Velocity.

## Power Analysis

Our sample size was powered based on our Primary Aim 1. Effect sizes were calculated using prior research which investigated the PLR in participants with TBI compared to healthy control participants, as the methods and sample closely matched our proposed study (Thiagarajan & Ciuffreda, 2015; Urosevich & Capo-Aponte, 2013). Across studies, numerous aspects of the PLR are found to be significantly impacted by mTBI, with large effect sizes across variables. The absolute values of effect sizes from these studies were ordered, and a median value was chosen for conversion for a repeated measures ANOVA between subject factors analysis in G*Power. An effect size of 0.487, power of 0.80, 4 groups (concussion history vs control; two counterbalanced test orders within each), 4 measurements (bright step, bright pulse, dim step, dim pulse), and an alpha level of 0.05 were used for this analysis. A sample size of 36 was determined to achieve sufficient power (Figure 3). This aligns with the sample size used by Thiagarajan & Ciuffreda (2015) (n = 32) and Urosevich & Capo-Aponte (2013) (n = 40), suggesting our study will be sufficiently powered to achieve numerous significant findings. This sample also aligns with prior studies of the NPC with mTBI populations, which have used 40 participants and found significant findings – suggesting our calculation provides adequate power for our other tests (Santo et al., 2020). An additional 4 participants will be added to account for data loss, giving us a targeted sample size of 40 (20 healthy control; 20 concussion history).



Test family: F tests

Statistical test: ANOVA: Repeated measures, between factors

Type of power analysis: A priori: Compute required sample size – given α, power, and effect size

Input Parameters

Effect size f 0.487
α err prob 0.05
Power (1–β err prob) 0.8
Number of groups 4
Number of measurements 4
Corr among rep measures 0.5

Output Parameters

Noncentrality parameter λ 13.6609344
Critical F 2.9011196
Numerator df 3.0000000
Denominator df 32.0000000
Total sample size 36
Actual power 0.8428636

Figure 3. Sample size determination (from G*Power).

## Statistical Analysis

Eye data was processed in MATLAB (MathWorks, Portola Valley, CA) and followed a standardized pipeline for managing pupillary data. This process first required the removal of invalid data or "NaN" values, which indicate blinks or data loss (Kret & Sjak-Shie, 2019). Then, raw data was filtered for artifacts such as temporally isolated samples, which may indicate repeated blinks or an occluded pupil (Kret & Sjak-Shie, 2019). Finally, valid data was processed using a zero-phase low-pass filter before analysis (Kret & Sjak-Shie, 2019).

For the pupillary light reflex tests, a mixed model repeated measures ANOVA was used to address Primary Aim 1 and Secondary Aim 1. This allowed us to detect the presence of between-group differences (concussion status), within-group differences (condition), and interaction effects. An alpha level of 0.05 was set for the main effects. If significant main effects were found, follow-up post-hoc analysis was used with Bonferroni-adjusted alpha levels to account for multiple comparisons. The analyses were conducted for each pupillary DV listed above. (Thiagarajan & Ciuffreda, 2015; Truong & Ciuffreda, 2016).

To address Tertiary Aim 1, our statistical approach was repeated with the Concussion History Group subdivided into a Single Concussion Group (1 diagnosed concussion) and a Multiple Concussion Group (2 or more diagnosed concussions). For the concussion history subgroup analysis, within-subject effects were only reported if not previously found, as the focus of this analysis was primarily to determine whether there was a unique effect of having multiple concussions on the PLR compared to either one or no concussions. Otherwise, only significant between-group differences and subgroup x condition interaction effects were reported.

A regression analysis was also conducted to determine whether there was a relationship between time since injury and pupillary response. A multiple regression was completed for each dependent variable using the four VR_PLR conditions. For the Auditory Pupil Response Test, a simple linear regression was used as there was only one testing condition. Healthy control participants' time since injury was entered as their age at the time of testing to incorporate their dataset into the regression analysis, as they had not been diagnosed with a concussion.

## Results

### Demographics

Forty-five participants (24 females; 21 males; 26.17 ± 4.18 years [18-35 years]) completed the study. Independent samples t-tests did not show any significant differences in age between the Concussion History and Healthy Control Groups. A chi-squared test did not find significant differences in gender between both groups. The number of concussions reported by the Concussion History Group ranged from 1 to 4 (1.75 ± 0.94 concussions) and the time since their most recent injury ranged from 0.75 to 20 years (6.56 ± 4.33 years) (Table 1). Self-reported medical history did not reveal ongoing symptoms across our Concussion History Group as a result of mTBI.

Table 1. Demographic and clinical characteristics of participants – VR_PLR. (n = 45)

| **Variable** | **Healthy Control (n = 21)** | **Concussion History (n = 24)** | **p** |
|---|---|---|---|
| Age (yrs) | 25.90 ± 3.56 | 26.42 ± 4.67 | 0.685 |
| Male (%) | 5 (24%) | 11 (46%) | 0.124 |
| Female (%) | 16 (76%) | 13 (54%) | |
| Number of Concussions | 0 | 1.75 ± 0.94 | |
| Time Since Injury (yrs) | 25.90 ± 3.56 | 6.56 ± 4.33 | **< 0.001**** |

Age, number of concussions, and time since injury (mean ± standard deviation); sex (frequency). ** Indicates p < 0.001.

Table 2. Demographic characteristics by number of concussions – VR_PLR. (n = 45)

| **Variable** | **Healthy Control (n = 21)** | **Single Concussion (n = 12)** | **Multiple Concussion (n = 12)** | **p** |
|---|---|---|---|---|
| Age (yrs) | 25.90 ± 3.56 | 25.67 ± 4.18 | 27.16 ± 5.18 | 0.631 |
| Male (%) | 5 (24%) | 3 (25%) | 8 (66.6%) | 0.085 |
| Female (%) | 16 (76%) | 9 (75%) | 4 (33.3%) | |
| Number of Concussions | 0 | 1 | 2.50 ± 0.80 | |
| Time Since Injury (yrs) | 25.90 ± 3.56 | 7.75 ± 4.43 | 5.38 ± 4.06 | **< 0.001**** |

Age, number of concussions, and time since injury (mean ± standard deviation); sex (frequency). ** Indicates p < 0.001

## Clinical Visual Assessment

Independent samples t-tests did not show any significant differences in visual acuity or near visual acuity between the concussion history and Healthy Control Groups (Table 3). For the concussion history subgroup analysis, a one-way ANOVA did not show any significant differences between the Healthy Control, Single Concussion, or Multiple Concussion Groups for visual acuity or near visual acuity (Table 4).

Table 3. Clinical visual assessment results – VR_PLR.

| **Variable** | **Healthy Control (n = 21)** | **Concussion History (n = 24)** | **p** |
|---|---|---|---|
| Right Visual Acuity (LogMar) | 0.14 ± 0.16 | 0.15 ± 0.12 | 0.690 |
| Left Visual Acuity (LogMar) | 0.10 ± 0.10 | 0.13 ± 0.11 | 0.512 |
| Binocular Visual Acuity (LogMar) | 0.05 ± 0.10 | 0.07 ± 0.09 | 0.625 |
| Binocular Near Visual Acuity (LogMar) | 0.11 ± 0.10 | 0.10 ± 0.10 | 0.388 |

Reported as mean ± standard deviation.

Table 4. Clinical visual assessment results by number of concussions – VR_PLR.

| Variable | Healthy Control (n = 21) | Single Concussion (n = 12) | Multiple Concussion (n = 12) | p |
|---|---|---|---|---|
| Right Visual Acuity (LogMar) | 0.14 ± 0.16 | 0.15 ± 0.13 | 0.16 ± 0.12 | 0.911 |
| Left Visual Acuity (LogMar) | 0.10 ± 0.10 | 0.11 ± 0.11 | 0.14 ± 0.11 | 0.580 |
| Binocular Visual Acuity (LogMar) | 0.05 ± 0.10 | 0.07 ± 0.09 | 0.07 ± 0.10 | 0.889 |
| Binocular Near Visual Acuity (LogMar) | 0.11 ± 0.10 | 0.13 ± 0.10 | 0.14± 0.11 | 0.672 |

Reported as mean ± standard deviation.

## Auditory Pupil Response Test

### ***Response Amplitude***

A one-tailed independent samples t-test revealed that response amplitude was significantly larger in the Healthy Control Group (M = 0.47, SD = 0.28) compared to the Concussion History Group (M = 0.33, SD = 0.21); t (43) = 1.89, p = 0.033.

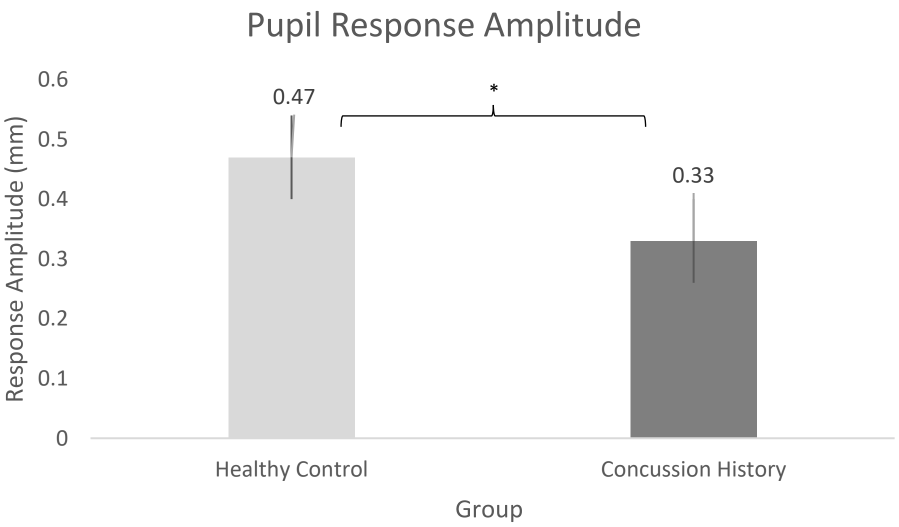


Figure 4. Auditory Pupil Response Test Response Amplitude differences between groups. * Indicates $p < 0.05$.

### *Average Dilation Velocity*

A one-tailed independent samples t-test revealed that average dilation velocity was significantly faster in the Healthy Control Group (M = 0.09, SD = 0.09) compared to the Concussion History Group (M = 0.04, SD = 0.06); t (43) = 2.45, p = 0.009. No additional significant between-group differences were found across the Auditory Pupil Response Test.

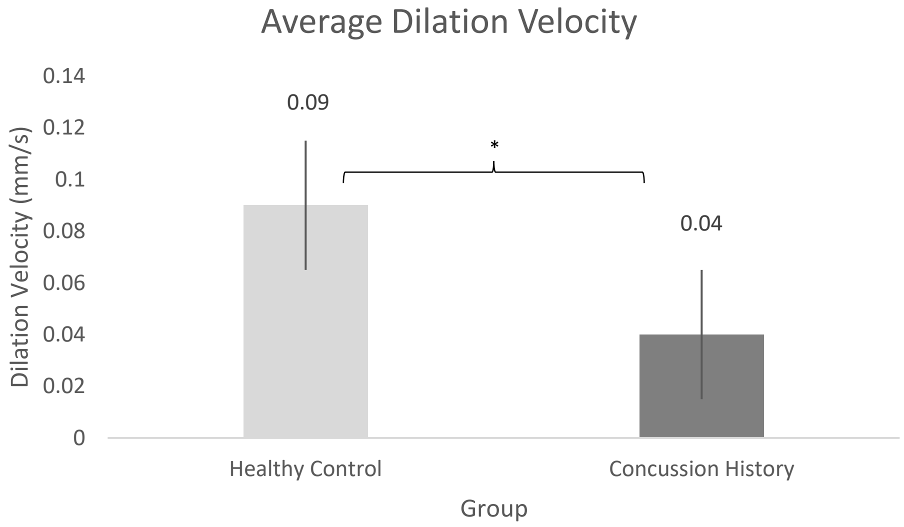


Figure 5. Auditory Pupil Response Test Average Dilation Velocity differences between groups. * Indicates $p < 0.05$.

## Concussion History Subgroup Analysis

### *Average Dilation Velocity*

A one-way ANOVA revealed that there was a trend towards significance for average dilation velocity between groups (F (2, 44) = 3.06, p = 0.058).

### *Peak Dilation Velocity*

A one-way ANOVA revealed that there was a trend towards significance for peak dilation velocity between groups (F (2, 44) = 3.03, p = 0.059). No additional significant between-group differences were found across the Auditory Pupil Response Test for the concussion history subgroup analysis.

Table 5. Auditory Pupil Response Test Results Between Groups.

| **Variable** | **Healthy Control** | **Concussion History** |
|---|---|---|
| Baseline Pupil Diameter (mm) | 6.90 ± 0.88 | 7.20 ± 1.06 |
| Minimum Pupil Diameter (mm) | 6.75 ± 0.93 | 7.10 ± 1.10 |
| Maximum Pupil Diameter (mm) | 7.22 ± 0.78 | 7.42 ± 1.02 |
| Pupil Constriction Amplitude (mm) | 0.47 ± 0.28 | 0.33 ± 0.21 |
| Final Pupil Diameter (mm) | 6.75 ± 0.93 | 7.10 ± 1.10 |
| Peak Constriction Velocity (mm/s) | 0.72 ± 0.28 | 0.61 ± 0.28 |
| Average Constriction Velocity (mm/s) | 0.09 ± 0.09 | 0.04 ± 0.06 |

Reported as mean ± standard deviation.

## Concussion History Time-Since-Injury Analysis

### *Average Dilation Velocity*

A simple linear regression found that time since injury significantly explained the variance in average dilation velocity across participants (F (1, 43) = 5.22, p = 0.027, $R^2$ = 0.108). The regression coefficient (B = 0.002) indicated that an increase in 1 year since a concussion increased average dilation velocity, on average, by 0.002 (mm/sec).

No additional significant regression equations were found for the Auditory Pupil Response Test.

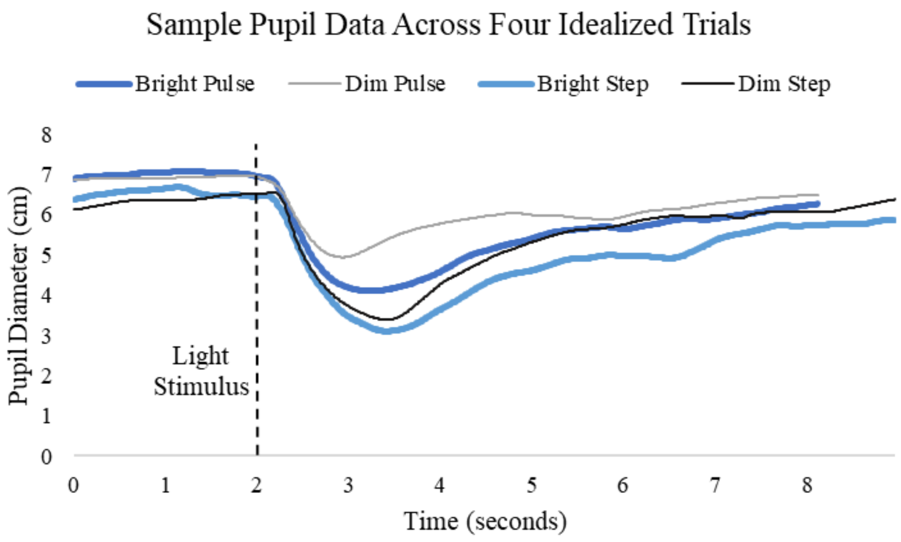


Figure 6. Graph showing representative pupillary response patterns across four test conditions in the VR_PLR assessment.

### *Baseline Pupil Diameter*

Mauchly's Test of Sphericity revealed that the assumption of sphericity had been violated ($\chi^2(5) = 37.85$, $p < 0.001$). A repeated measures ANOVA with a Greenhouse-Geisser adjustment showed a significant main effect for condition (F (2.06, 88.63) = 9.84, $p < 0.001$). A follow-up post-hoc analysis with Bonferroni adjusted alpha levels revealed that the baseline pupil diameter was significantly smaller in the Bright Step condition (M = 6.29, SEM = 0.15) compared to the Bright Pulse condition (M = 6.54, SEM = 0.15, p = 0.001). Additionally, baseline pupil diameter was significantly smaller in the Dim Step condition (M = 6.19, SEM = 0.15) compared to the Bright Pulse ($p < 0.001$) and Dim Pulse (M = 6.47, SEM = 0.15, $p < 0.001$) conditions. No significant between-group differences or concussion status and condition differences were found.

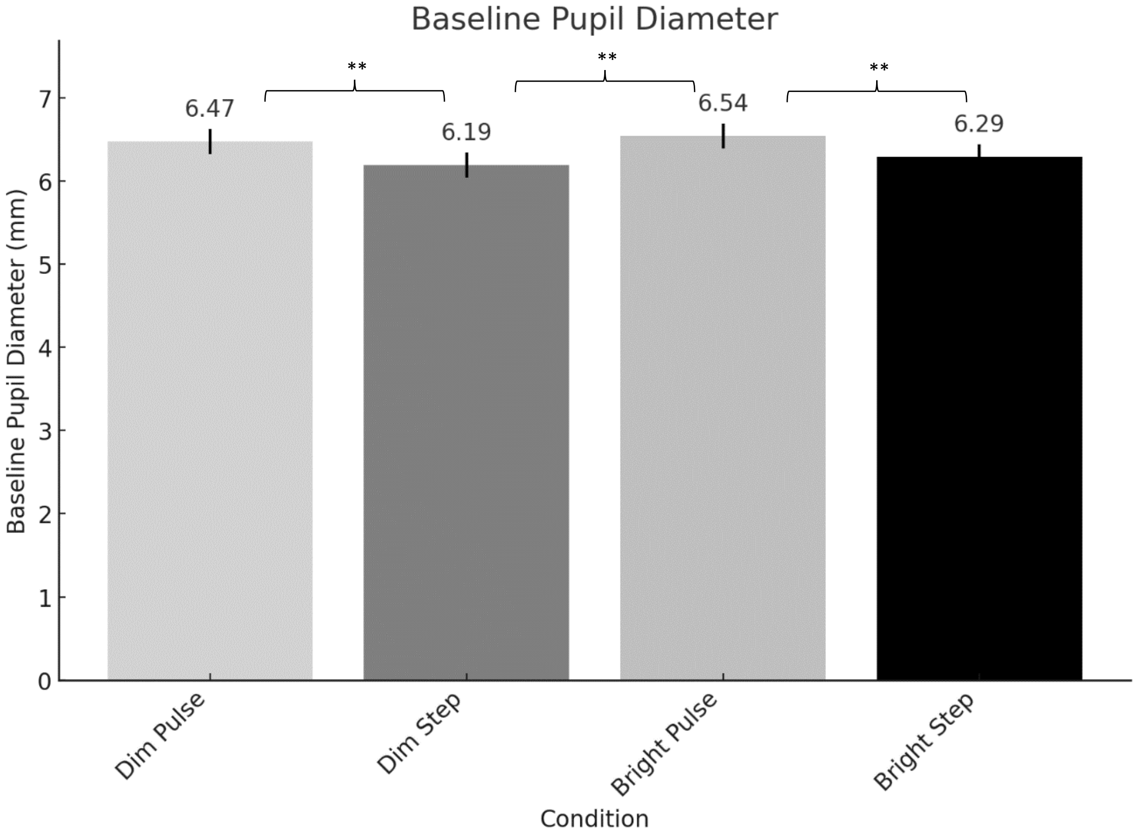


Figure 7. VR_PLR Baseline Pupil Diameter averages for each condition across participants. ** Indicates $p < 0.001$.

### *Minimum Pupil Diameter*

Mauchly's Test of Sphericity revealed that the assumption of sphericity had been violated ($\chi^2(5) = 48.57$, $p < 0.001$). A repeated measures ANOVA with a Greenhouse-Geisser adjustment showed a significant main effect for condition (F (1.99, 85.66) = 230.67, $p < 0.001$). A follow-up posthoc analysis with Bonferroni adjusted alpha levels revealed that the Bright Step condition produced a significantly smaller minimum pupil diameter (M = 3.11, SEM = 0.07) compared to Dim Step (M = 3.45, SEM = 0.08, $p < 0.001$), Bright Pulse (M = 4.2, SEM = 0.11, $p < 0.001$), and Dim Pulse (M = 4.56, SEM =

0.12), $p < 0.001$). Additionally, the Dim Step condition produced a significantly smaller minimum pupil diameter compared to the Bright Pulse ($p < 0.001$) and Dim Pulse ($p < 0.001$). Finally, the Bright Pulse condition produced a significantly smaller minimum pupil diameter compared to the Dim Pulse condition ($p < 0.001$). No significant between-group differences or concussion status and condition differences were found.

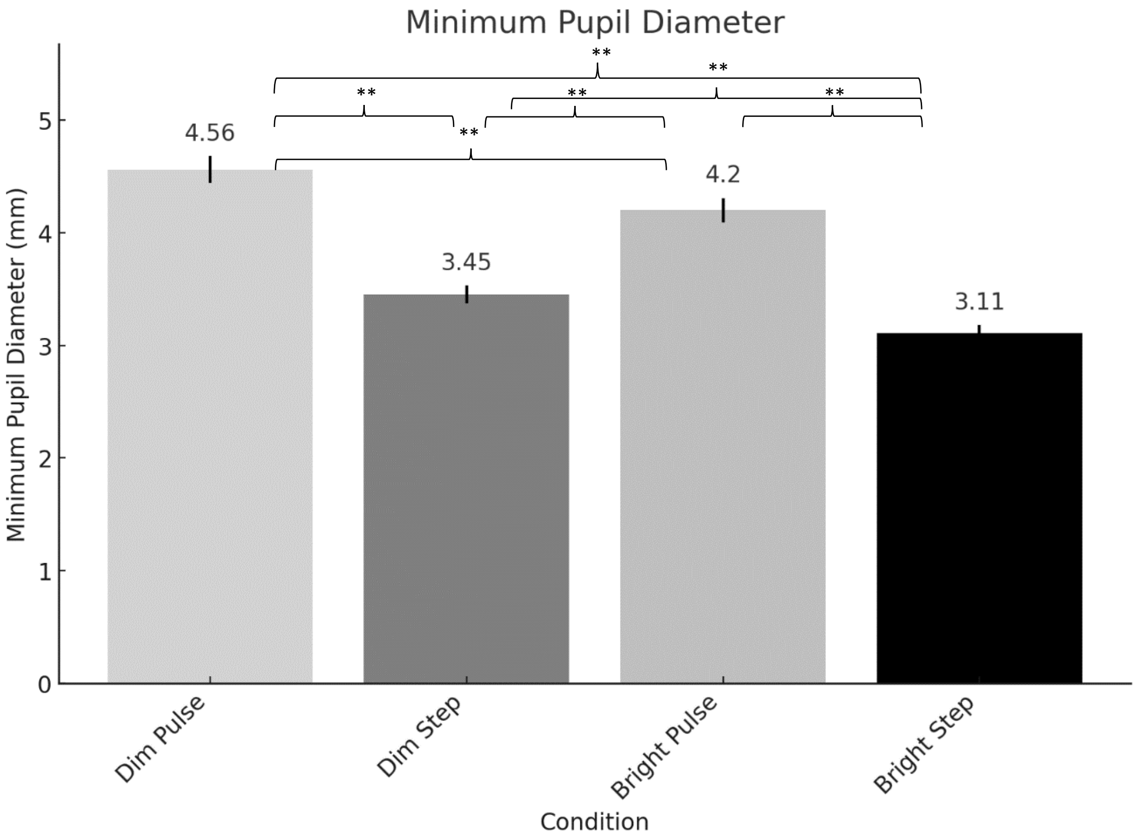


Figure 8. VR_PLR Minimum Pupil Diameter averages for each condition across participants. ** Indicates $p < 0.001$.

### ***Maximum Pupil Diameter***

Mauchly’s Test of Sphericity revealed that the assumption of sphericity had been violated ($\chi^2(5) = 39.79$, $p < 0.001$). A repeated measures ANOVA with a Greenhouse-

Geisser adjustment showed a significant main effect for condition (F (1.99, 85.67) = 10.09, $p < 0.001$). A follow-up post-hoc analysis with Bonferroni adjusted alpha levels revealed that the Bright Step condition produced significantly smaller maximum pupil diameter (M = 6.47, SEM = 0.14) compared to the Bright Pulse condition (M = 6.71, SEM = 0.14, $p < 0.001$). Additionally, the Dim Step condition produced a significantly smaller maximum pupil diameter (M = 6.39, SEM = 0.14) compared to the Bright Pulse ($p < 0.001$) and Dim Pulse conditions (M = 6.63, SEM = 0.15, $p < 0.001$). No significant between-group differences or concussion status and condition differences were found.

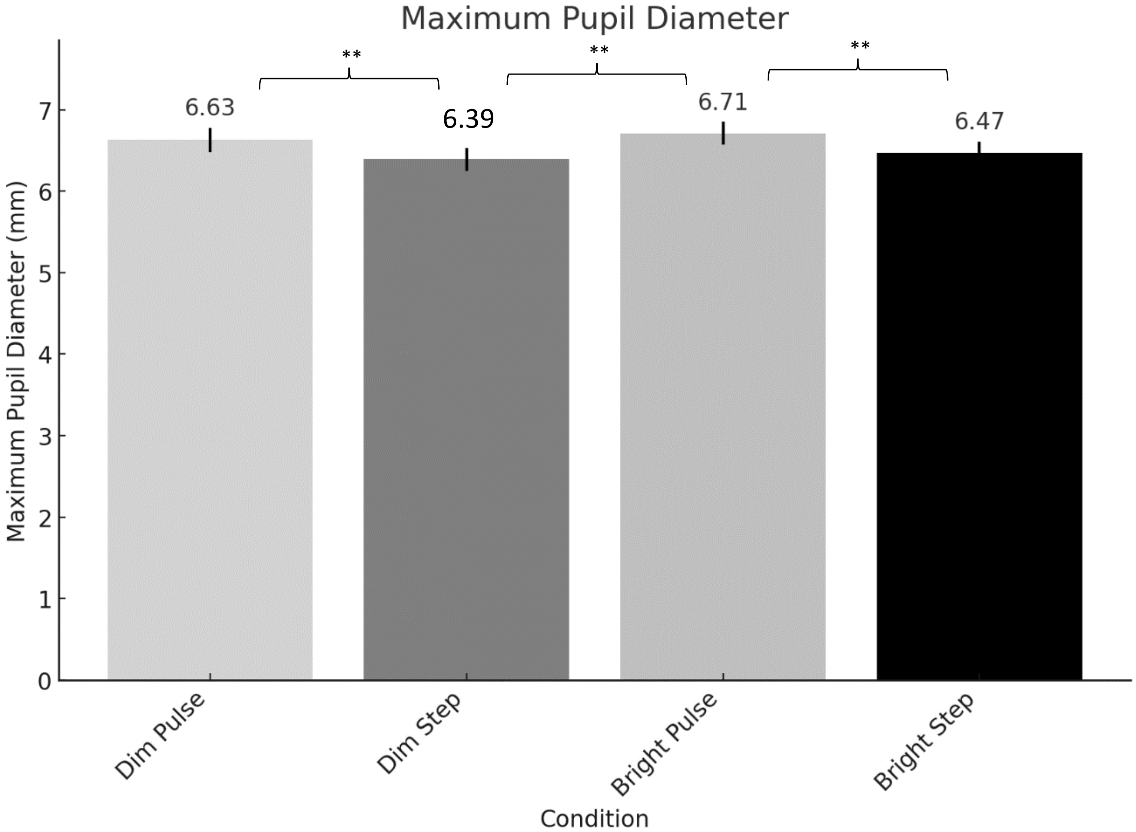


Figure 9. VR_PLR Maximum Pupil Diameter averages for each condition across participants. ** Indicates $p < 0.001$.

### ***Pupil Constriction Amplitude***

Mauchly’s Test of Sphericity revealed that the assumption of sphericity had been violated ($\chi^2(5) = 32.13$, $p < 0.001$). A repeated measures ANOVA with a Greenhouse-Geisser adjustment showed a significant main effect for condition (F (2.08, 89.22) = 159.71, $p < 0.001$). A follow-up post-hoc analysis with Bonferroni adjusted alpha levels revealed that the Bright Step condition produced a significantly larger pupil amplitude (M = 3.18, SEM = 0.09) compared to Dim Step (M = 2.75, SEM = 0.09, $p < 0.001$), Bright Pulse (M = 2.34, SEM = 0.06, $p < 0.001$), and Dim Pulse (M = 1.91, SEM = 0.07, $p < 0.001$). Additionally, the Dim Step condition produced significantly larger pupil amplitude compared to the Bright Pulse ($p < 0.001$) and Dim Pulse ($p < 0.001$). Finally, the Bright Pulse condition produced a significantly larger pupil amplitude compared to the Dim Pulse condition ($p < 0.001$). No significant between-group differences or concussion status and condition differences were found.

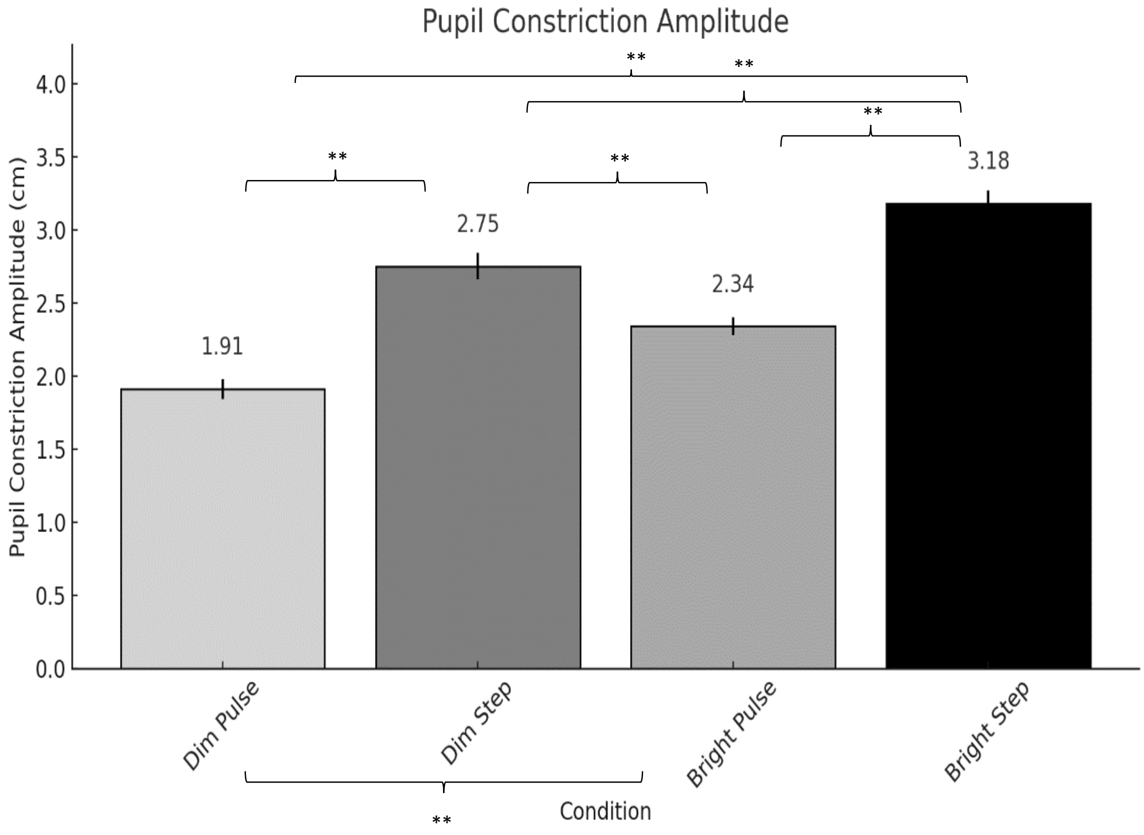


Figure 10. VR_PLR Pupil Constriction Amplitude averages for each condition across participants. ** Indicates $p < 0.001$.

### *Final Pupil Diameter*

Mauchly's Test of Sphericity revealed that the assumption of sphericity had been violated ($\chi^2(5) = 19.42$, $p = 0.002$). A repeated measures ANOVA with a Greenhouse-Geisser adjustment showed a significant main effect for condition (F (2.29, 98.49) = 42.59, $p < 0.001$). A follow-up post-hoc analysis with Bonferroni adjusted alpha levels revealed that the Bright Step condition produced a significantly smaller final pupil diameter (M = 5.55, SEM = 0.12) compared to the Dim Step (M = 5.86, SEM = 0.14, $p < 0.001$), Bright Pulse (M = 6.17, SEM = 0.14, $p < 0.001$), and Dim Pulse (M = 6.36, SEM = 0.15), $p < 0.001$) conditions. Additionally, the Dim Step condition produced a significantly smaller final pupil diameter compared to the Bright Pulse ($p < 0.001$) and Dim Pulse ($p < 0.001$). Finally, the Bright Pulse condition produced a significantly smaller final pupil diameter compared to the Dim Pulse condition ($p = 0.005$). No significant between-group differences or concussion status and condition differences were found.

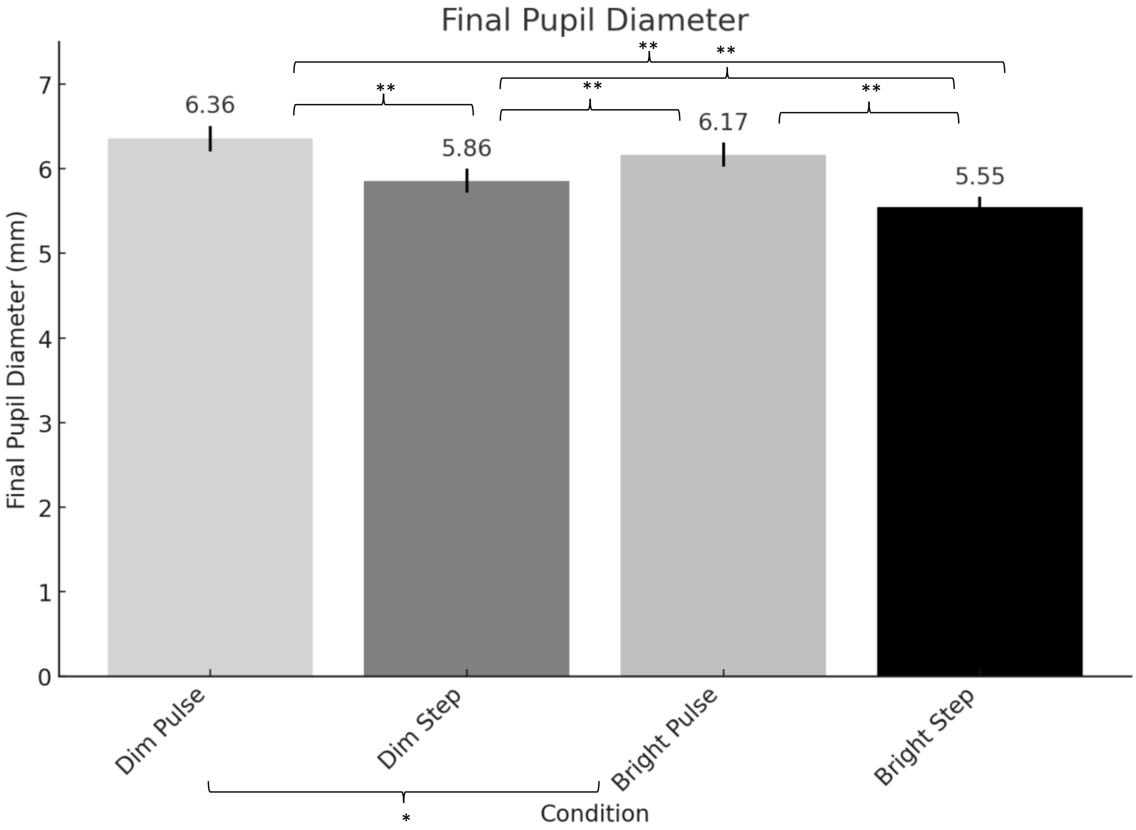


Figure 11. VR_PLR Final Pupil Diameter averages for each condition across participants. * Indicates $p < 0.05$, ** indicates $p < 0.001$.

### *Final Pupil Diameter with Baseline Pupil Diameter as Covariate*

Repeated measures ANOVAs using baseline pupil diameter for each condition as covariates found that there was a significant effect of baseline pupil diameter on final pupil diameter across conditions for Dim Pulse (F (3, 117) = 3.77, p = 0.013), Bright Pulse (F (3, 117) = 4.57, p = 0.005), Dim Step (F (3, 117) = 4.70, p = 0.004), Bright Step (F (3, 117) = 9.04, p < 0.001). When controlling for baseline pupil diameter, the differences in final pupil diameter between conditions were no longer significant.

### ***Peak Constriction Velocity***

Mauchly's Test of Sphericity revealed that the assumption of sphericity had been violated ($\chi^2(5) = 32.74$, $p < 0.001$). A repeated measures ANOVA with a Greenhouse-Geisser adjustment showed a significant main effect for condition (F (1.96, 84.31) = 7.88, p = 0.001). A follow-up post-hoc analysis with Bonferroni adjusted alpha levels revealed that the Bright Step condition produced significantly larger peak constriction velocity (M = 6.39, SEM = 0.21) compared to the Bright Pulse (M = 5.71, SEM = 0.16, p = 0.001) and Dim Pulse (M = 5.66, SEM = 0.15, p = 0.005) conditions. Finally, the Dim Step condition (M = 6.26, SEM = 0.18) produced significantly larger peak constriction velocity compared to the Bright Pulse (p = 0.001) and Dim Pulse (p < 0.001) conditions. No significant between-group differences or concussion status and condition differences were found.

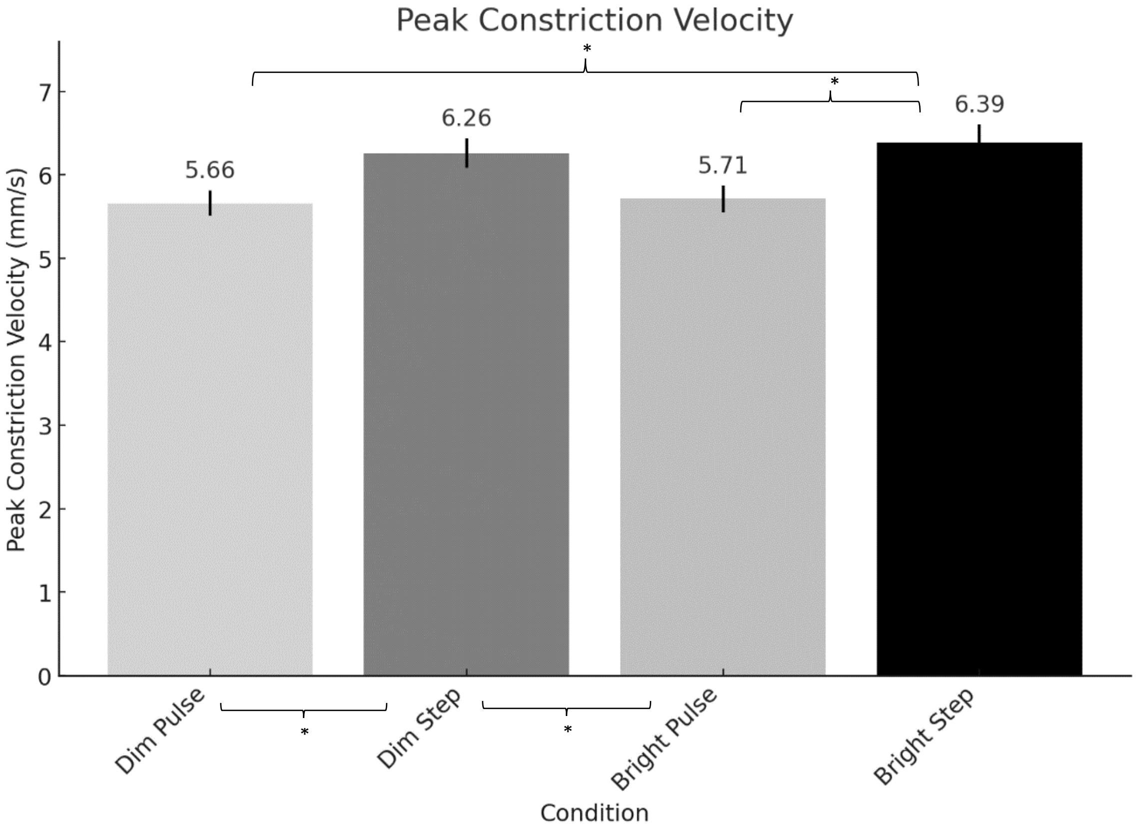


Figure 12. VR_PLR Peak Constriction Velocity averages for each condition across participants. * Indicates $p < 0.05$, ** indicates $p < 0.001$.

### *Average Constriction Velocity*

Mauchly's Test of Sphericity revealed that the assumption of sphericity had been violated ($\chi^2(5) = 19.09$, $p = 0.002$). A repeated measures ANOVA with a Greenhouse-Geisser adjustment showed a significant main effect for condition (F (2.45, 105.51) = 7.47, $p < 0.001$). A follow-up post-hoc analysis with Bonferroni adjusted alpha levels revealed that the Bright Step condition produced a significantly larger average constriction velocity (M = 2.28, SEM = 0.05) compared to the Dim Step condition (M = 2.02, SEM = 0.05, $p < 0.001$). Finally, the Dim Step condition produced a significantly smaller average constriction velocity compared to the Bright Pulse (M = 2.19, SEM =

0.06, p = 0.004) and Dim Pulse (M = 2.2, SEM = 0.07, p < 0.001) conditions. No significant between-group differences or concussion status and condition differences were found.

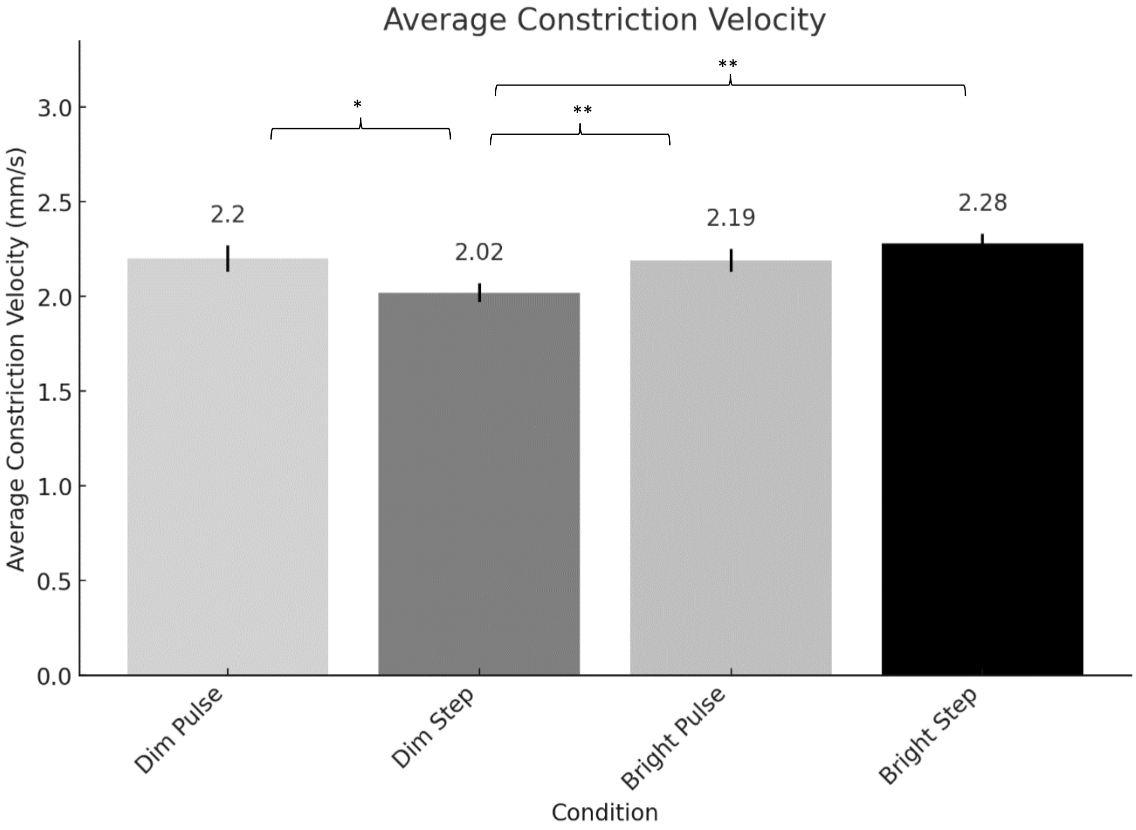


Figure 13. VR_PLR Average Constriction Velocity averages for each condition across participants. * Indicates p < 0.05, ** indicates p < 0.001.

### *Peak Dilation Velocity*

A repeated measures ANOVA showed a significant main effect for condition (F (3, 129) = 23.55, p < 0.001). A follow-up post-hoc analysis with Bonferroni adjusted alpha levels revealed that the Bright Step condition produced significant peak dilation velocity (M = 2.11, SEM = 0.12) compared to the Bright Pulse condition (M = 1.62, SEM

= 0.06, p < 0.001). Additionally, the Dim Step condition (M = 2.45, SEM 0.11) produced significantly larger peak dilation velocity compared to the Bright Step (p = 0.21), Bright Pulse (p < 0.001), and Dim Pulse (M = 2.08, SEM = 0.09, p = 0.001) condition. Finally, the Dim Pulse condition produced a significantly larger peak dilation velocity compared to the Bright Pulse condition (p < 0.001). No significant between-group differences or concussion status and condition differences were found.

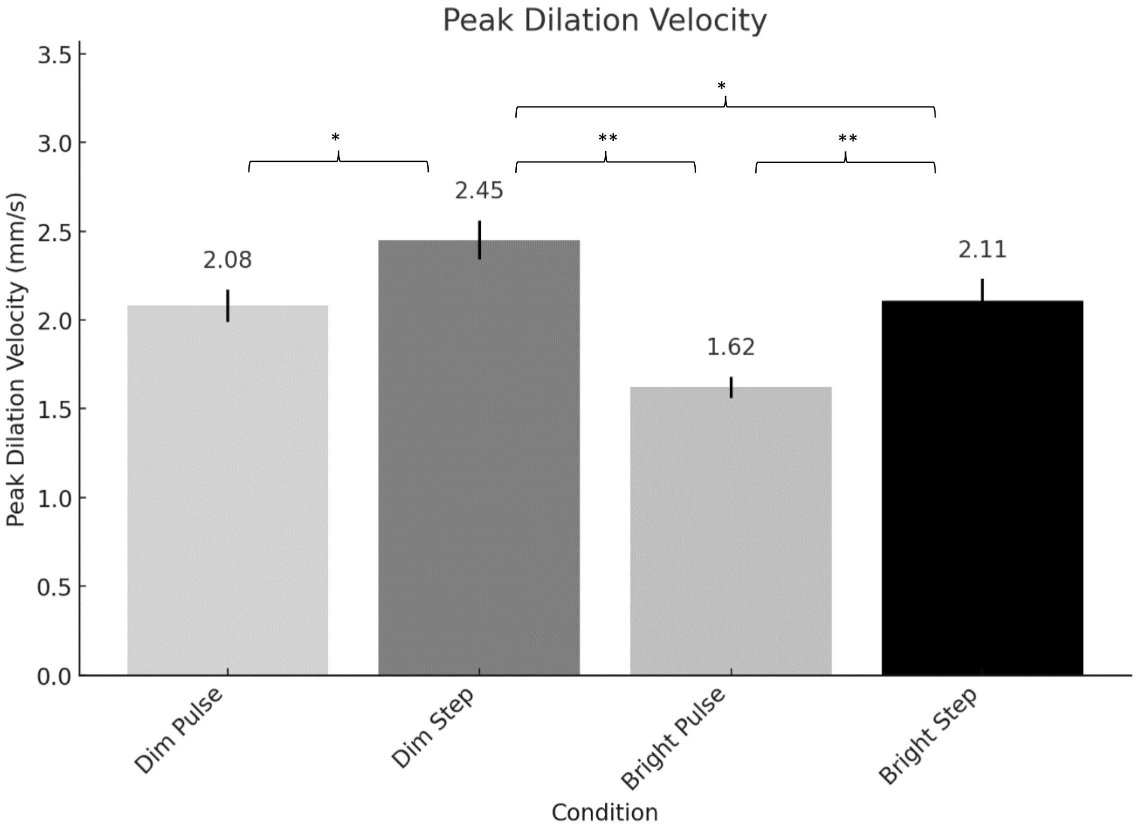


Figure 14. VR_PLR Peak Dilation Velocity averages for each condition across participants. * Indicates p < 0.05, ** indicates p < 0.001.

### *Average Dilation Velocity*

Mauchly's Test of Sphericity revealed that the assumption of sphericity had been violated ($\chi^2(5) = 14.35$, $p = 0.014$). A repeated measures ANOVA with a Greenhouse-Geisser adjustment showed a significant main effect for condition ($F (2.54, 109.01) = 39.67$, $p < 0.001$). A follow-up posthoc analysis with Bonferroni adjusted alpha levels revealed that the Bright Step condition produced a significantly larger average dilation velocity ($M = 0.44$, $SEM = 0.01$) compared to the Bright Pulse ($M = 0.39$, $SEM = 0.01$, $p < 0.001$) and Dim Pulse ($M = 0.34$, $SEM = 0.01$, $p < 0.001$) conditions. Additionally, the Dim Step condition ($M = 0.43$, $SEM = 0.01$) produced significantly larger average dilation velocity compared to the Bright Pulse ($p < 0.001$) and Dim Pulse ($p < 0.001$) conditions. Finally, the Bright Pulse condition produced a significantly larger average dilation velocity compared to the Dim Pulse condition ($p < 0.001$). No significant between-group differences or concussion status and condition differences were found.

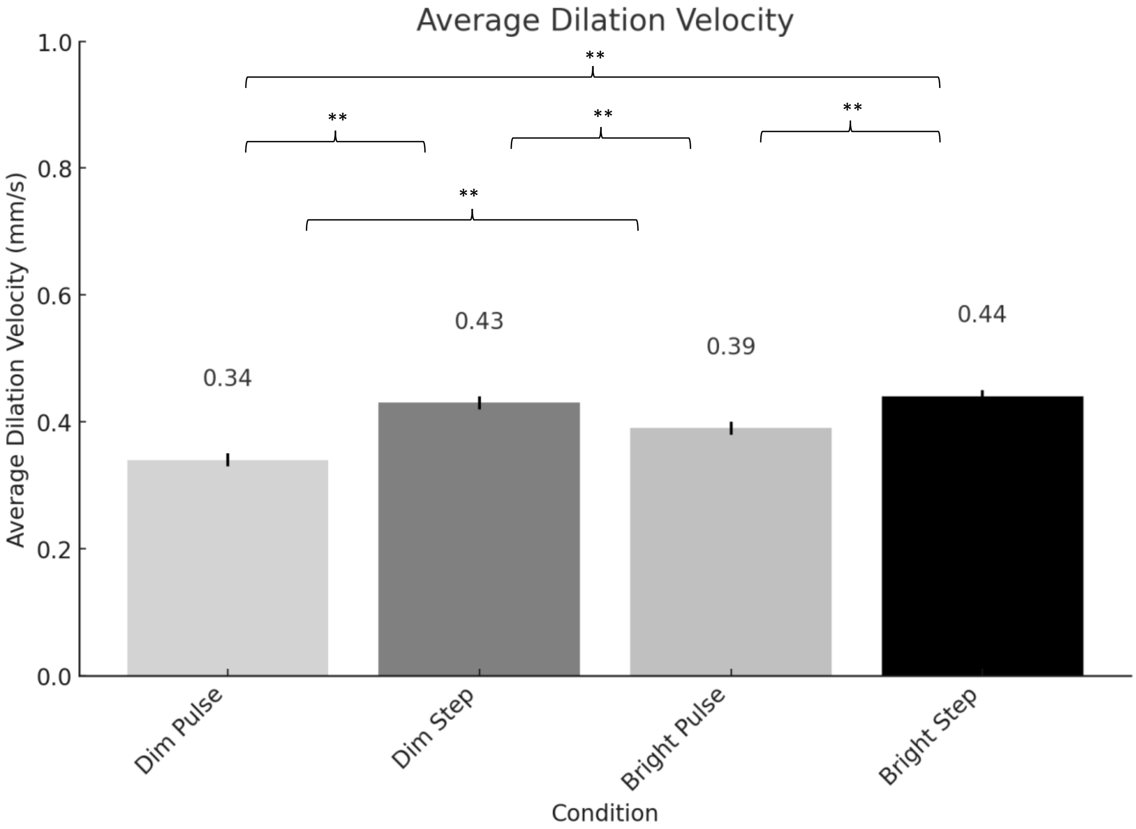


Figure 15. VR_PLR Average Dilation Velocity averages for each condition across participants. ** Indicates p < 0.001.

### ***Constriction Latency***

No significant within-group differences, between-group differences, or interaction effects were found for constriction latency.

Table 6. VR_PLR variables - results across conditions.

| | Condition | | | |
|---|---|---|---|---|
| **Variable** | **Dim Pulse** | **Dim Step** | **Bright Pulse** | **Bright Step** |
| **Baseline Pupil Diameter (mm)** | 6.47 ± 0.15 | 6.19 ± 0.15 | 6.54 ± 0.15 | 6.29 ± 0.15 |
| **Minimum Pupil Diameter (mm)** | 4.56 ± 0.12 | 3.45 ± 0.08 | 4.2 ± 0.11 | 3.11 ± 0.07 |
| **Maximum Pupil Diameter (mm)** | 6.63 ± 0.15 | 6.39 ± 0.14 | 6.71 ± 0.14 | 6.47 ± 0.14 |
| **Pupil Constriction Amplitude (mm)** | 1.91 ± 0.07 | 2.75 ± 0.09 | 2.34 ± 0.06 | 3.18 ± 0.09 |
| **Final Pupil Diameter (mm)** | 6.36 ± 0.15 | 5.86 ± 0.14 | 6.17 ± 0.14 | 5.55 ± 0.12 |
| **Peak Constriction Velocity (mm/s)** | 5.66 ± 0.15 | 6.26 ± 0.18 | 5.71 ± 0.16 | 6.39 ± 0.21 |
| **Average Constriction Velocity (mm/s)** | 2.20 ± 0.07 | 2.02 ± 0.05 | 2.19 ± 0.06 | 2.28 ± 0.05 |
| **Peak Dilation Velocity (mm/s)** | 2.08 ± 0.09 | 2.45 ± 0.11 | 1.62 ± 0.06 | 2.11 ± 0.12 |
| **Average Dilation Velocity (mm/s)** | 0.34 ± 0.01 | 0.43 ± 0.01 | 0.39 ± 0.01 | 0.44 ± 0.01 |
| **Constriction Latency (ms)** | 261.81 ± 6.54 | 260.04 ± 6.27 | 243.77 ± 7.83 | 250.23 ± 12.61 |

Reported as mean ± standard error.

Table 7. VR_PLR variables - results across conditions by participant status.

| | Healthy Control | | | | Concussion History | | | |
|---|---|---|---|---|---|---|---|---|
| **Variable** | **Dim Pulse** | **Dim Step** | **Bright Pulse** | **Bright Step** | **Dim Pulse** | **Dim Step** | **Bright Pulse** | **Bright Step** |
| **Baseline Pupil Diameter (mm)** | 6.32 ± 0.97 | 6.04 ± 0.89 | 6.33 ± 1.08 | 6.40 ± 0.92 | 6.61 ± 1.03 | 6.33 ± 1.04 | 6.74 ± 0.95 | 6.29 ± 0.15 |
| **Minimum Pupil Diameter (mm)** | 4.49 ± 0.78 | 3.41 ± 0.55 | 4.08 ± 0.81 | 3.10 ± 0.48 | 4.63 ± 0.77 | 3.49 ± 0.53 | 4.31 ± 0.66 | 3.13 ± 0.41 |
| **Maximum Pupil Diameter (mm)** | 6.53 ± 0.94 | 6.28 ± 0.88 | 6.54 ± 0.99 | 6.37 ± 0.99 | 6.74 ± 1.01 | 6.49 ± 1.01 | 6.88 ± 0.90 | 6.57 ± 0.90 |
| **Pupil Constriction Amplitude (mm)** | 1.89 ± 0.46 | 2.73 ± 0.57 | 2.31 ± 0.38 | 3.16 ± 0.54 | 1.93 ± 0.46 | 2.76 ± 0.58 | 2.38 ± 0.41 | 3.20 ± 0.65 |
| **Final Pupil Diameter (mm)** | 6.25 ± 1.01 | 5.75 ± 0.87 | 6.05 ± 0.96 | 5.44 ± 0.85 | 6.48 ± 1.00 | 5.98 ± 0.96 | 6.29 ± 0.93 | 5.67 ± 0.78 |
| **Peak Constriction Velocity (mm/s)** | 5.56 ± 1.07 | 6.25 ± 1.15 | 5.72 ± 1.00 | 6.61 ± 1.20 | 5.76 ± 0.95 | 6.27 ± 1.21 | 5.69 ± 1.10 | 6.16 ± 1.51 |
| **Average Constriction Velocity (mm/s)** | 2.13 ± 0.48 | 1.98 ± 0.38 | 2.19 ± 0.41 | 2.25 ± 0.35 | 2.27 ± 0.44 | 2.05 ± 0.34 | 2.18 ± 0.39 | 2.31 ± 0.36 |
| **Peak Dilation Velocity (mm/s)** | 2.05 ± 0.60 | 2.43 ± 0.92 | 1.65 ± 0.35 | 2.14 ± 0.68 | 2.10 ± 0.57 | 2.48 ± 0.50 | 1.58 ± 0.46 | 2.08 ± 0.90 |
| **Average Dilation Velocity (mm/s)** | 0.34 ± 0.08 | 0.42 ± 0.08 | 0.39 ± 0.05 | 0.43 ± 0.08 | 0.34 ± 0.07 | 0.44 ± 0.10 | 0.39 ± 0.08 | 0.45 ± 0.09 |
| **Constriction Latency (ms)** | 276.93 ± 31.59 | 279.53 ± 42.35 | 255.68 ± 38.51 | 272.23 ± 97.82 | 266.70 ± 52.07 | 260.56 ± 41.58 | 251.86 ± 62.05 | 248.23 ± 70.71 |

Reported as mean ± standard deviation.

## Concussion History Subgroup Analysis

No additional significant within-group differences, between-group differences, or interaction effects were found across the VR_PLR assessment for the concussion history subgroup analysis.

### Time-Since-Injury Analysis

The multiple regression analysis revealed that time since injury did not predict pupillary response across dependent variables or VR_PLR conditions.

## Discussion and Conclusion

This study used a novel application of VR HMD technology to elicit and measure the PLR to determine whether pupillary responses differed between participants with a history of concussions and healthy individuals. The primary objective was to assess potential long-term deficits to the pupillary system that may follow one or more mTBI(s). However, we did not find evidence that pupillary response differed between the concussion history or heathy control groups. Importantly, our study showed the VR HMD with built-in VOG was able to elicit and detect the presence of the PLR in response to manipulations of a light stimulus.

We found strong criterion validity that a VR-based was able to elicit and detect the presence of the PLR in response to manipulations of a light stimulus. Notably, light stimuli with greater intensity through increased luminance and/or duration consistently yielded pupillary responses with greater magnitude, length, and velocity compared to conditions with less intense or lower duration stimuli. Using a VR HMD to elicit and measure the PLR produced results that followed stereotypical patterns like those seen via objective pupillometry, which has traditionally been used to assess the dynamic pupillary response in the mTBI population (Figure 6) (Ciuffreda et al., 2017; Podolak et al., 2019; Urosevich & Capo-Aponte, 2013). Notably, in the Bright Step condition we saw significantly more pupillary constriction compared to all other conditions following exposure to a bright, long-duration stimulus, as measured through minimum pupil diameter and pupillary amplitude. In this condition, greater peak and average constriction velocity were seen, indicating that the more intense stimulus not only impacted the magnitude of response but also the rate at which pupillary constriction reached its minimum point. Across metrics, we also found evidence that step conditions produced greater pupillary responses compared to pulse conditions, even when comparing the bright pulse and dim step conditions. This suggests that the duration of light stimuli may have a greater effect on pupillary constriction compared to its brightness alone, supporting prior research (Alpern et al., 1963).

Our VR-based assessment also found that pupillary recovery (dilation) following a light stimulus, matched the stereotypical response profile seen in prior research (Ciuffreda et al., 2017; Podolak et al., 2019; Urosevich & Capo-Aponte, 2013). Notably, peak and average dilation velocities were the largest in the Bright Step condition, suggesting that a more intense stimulus elicited a faster return toward baseline pupil diameter. However, our findings also suggest that temporal summation may have occurred between trials, indicating that the pupil size may not have sufficient time to fully return to baseline in our study design. The Bright Step condition produced the smallest final pupil diameter at the end of the 6-second recovery period and values across conditions were smaller than baseline values, suggesting that 30 seconds may have not been enough time to allow the pupil to dilate to pre-stimulus values. To investigate this further, a follow-up ANCOVA analysis revealed that baseline pupil diameter significantly impacted final pupil diameter across conditions. Further, the differences in final pupil diameter between conditions were no longer significant when using baseline pupil diameter as a covariate, suggesting that the pupil was likely not fully recovered between trials. The exact time used between PLR tests varies greatly in the literature, ranging between 30 seconds to up to 10 or more minutes (Ciuffreda et al., 2017; Podolak et al., 2019; Urosevich & Capo-Aponte, 2013; Y. J. Yoo et al., 2017). Our study opted for 30 seconds due to the extensive oculomotor testing that participants completed, as well as prior research suggesting it may be sufficient for PLR assessments (Y. J. Yoo et al., 2017). While the order of pulse versus step conditions was counterbalanced and bright conditions were performed last to try and limit the intense stimuli's influence on subsequent trials, future studies may opt for longer rest periods to ensure that complete recovery occurs between measurements.

Despite strong evidence that the VR_PLR elicits and measures the PLR, we did not find evidence that pupillary response differed between the Concussion History or Heathy Control Groups. This finding is surprising given the abundance of research that suggests that changes to the PLR are commonly found in mTBI patients across the recovery timeline (Ciuffreda et al., 2017; Podolak et al., 2019; Thiagarajan & Ciuffreda, 2015; Truong & Ciuffreda, 2016; Urosevich & Capo-Aponte, 2013). Given the heterogeneity in our patient population, with time since injury ranging between 0.75 to 20 years, a significant limitation of our study is the large variability in the injury profiles of participants, which likely contributed to the lack of significant PLR differences between groups. For one, many studies focusing on the chronic effects of concussions have limited time since an injury to several years (Cheever et al., 2018; J. McDevitt et al., 2016; Master et al., 2016; Podolak et al., 2019; Tapper et al., 2021). Currently, the long-term visual deficits associated with mTBI are not well-understood, due in-part to the numerous types of deficits that may or may not present across patients (e.g., impacting optic neuropathy, motor pathways, the eye itself, etc.), as well as the complex interaction between physiological factors, genetics, and injury circumstances impacting symptom intensity, duration, and recovery (Armstrong, 2018; Sen, 2017). Therefore, when studying participants who experienced injuries as far as 20 years prior, there is a strong likelihood that any potential oculomotor deficits could have recovered. Secondly, while our study used asymptomatic participants as has been done in prior studies (e.g., Thiagarajan & Ciuffreda (2015), other research has focused on recruiting participants specifically reporting pupil-related deficits following a mTBI, increasing the homogeneity of injury characteristics and the likelihood of detecting clinically significant findings (Ciuffreda et al., 2017; Podolak et al., 2019; Thiagarajan & Ciuffreda, 2015; Truong & Ciuffreda, 2016; Urosevich & Capo-Aponte, 2013). Doing so would have allowed us to better understand the sensitivity of our novel VR_PLR assessment, but we were limited in our ability to specifically recruit such a patient population. Therefore, future research should investigate whether VR_PLR differences can be seen in a more controlled sample of mTBI participants, possibly in the subacute or acute stages of recovery, to attempt to recreate traditional PLR studies using objective pupillometry.

Finally, this study found some evidence that a history of concussions can produce changes in auditory pupil response in the form of dilation velocity differences. Average dilation velocity was found to be significantly slower in the Concussion History Group compared to the Healthy Control Group, with other metrics such as peak dilation velocity and amplitude trending towards significance. While the finding of slowed pupillary response aligns with PLR research, research examining pupillary response to a simple auditory tone has yet to be conducted in the mTBI population. While there is a possibility that our finding simply occurred by chance, our finding is strengthened by the regression analysis, which showed that average dilation velocity was positively related to the time since injury in the auditory task.

The lack of significant findings in PLR and the presence of differences in auditory pupil response between groups suggests that a history of concussions may produce longer-term changes involved in the neural pathways responsible for regulating the pupil's response to sound. While not yet fully understood, the auditory pupil response is hypothesized to involve inhibitory signals from the locus coeruleus (LC) to the EW, which leads to the relaxation of the constrictor muscles of the pupil, producing a stereotypical dilation response (Eckstein et al., 2017; Zekveld et al., 2018b). Additionally, the release of norepinephrine from the LC is believed to stimulate the sympathetic nervous system, leading to the activation of the dilator pupillae muscles (Eckstein et al., 2017; Zekveld et al., 2018b).

The LC has been implicated as one of the structures consistently altered in participants diagnosed with CTE, even in more mild cases such as Stage 1 diagnoses, implicating its sensitivity as a biomarker to mTBI (Mavroudis et al., 2022). The LC is also involved in tasks requiring cognitive resources allocation and attention, which aligns with research demonstrating chronic concussion participants may present cognitive deficits outside of the normal recovery period (Gallo et al., 2020; McInnes et al., 2017; Munia et al., 2016; Zekveld et al., 2018b). Therefore, an altered auditory pupil response may suggest that a history of concussion produces chronic alternations in LC and sympathetic activity, whereas the parasympathetic activity and structures such as photosensitive retinal ganglion cells involved in the PLR may not have been impacted or could be more likely to recover over time. This suggests that the auditory pupil response may hold promise as a biomarker present in chronic mTBI participants that reflects deficits related to attentional resources and other executive processes. Future research should delineate how both are impacted by mTBI across the recovery timeline to better understand whether concussions can differentially impact either pupillary reflex or whether there is a relationship between them.

# CHAPTER 3

# EFFECT OF CONCUSSION HISTORY ON THE VERGENCE RESPONSE IN A NOVEL VIRTUAL REALITY ENVIRONMENT

## Introduction

Vergence deficits are frequently reported in concussion patients across the acute, subacute, and chronic recovery periods (Duprey et al., 2017; Kawata et al., 2016; Pearce et al., 2015; Rucker et al., 2019). The most common vergence impairment among mild traumatic brain injury (mTBI) patients is convergence insufficiency (CI), which may affect as much as 49% of those with visual dysfunction associated with a concussion (Gallaway et al., 2017). CI is characterized by 1) greater outward eye-drift (exophoria) during near distance fixation, 2) a receded near point of convergence (NPC), and 3) a decreased ability of the eyes to overcome retinal image disparity and create a single image when fixating on a nearby target (positive fusional convergence), which limits the ability of participants to maintain binocular vision at close distances. (Cooper & Jamal, 2012; Duprey et al., 2017; Gallaway et al., 2017; Pearce et al., 2015). Importantly, while CI is commonly the focus of vergence-related deficits in mTBI patients, other aspects of the vergence system have received growing attention including convergence excess, vergence endurance, and divergence impairments (Master et al., 2016; Rucker et al., 2019; Yaramothu et al., 2019). Notably, these deficits can impact a wide range of activities of daily living including reading, driving, computer use, and other near work, supporting the importance of the vergence system in maintaining quality of life in those diagnosed with a mTBI (Master et al., 2016; Rucker et al., 2019; Yaramothu et al., 2019).

Vergence is typically assessed in clinical settings using the NPC test, which measures the closest point that a patient can maintain convergence on a moving target without double vision (diplopia) occurring (Kawata et al., 2016; Pearce et al., 2015; Scheiman et al., 2003). To quantify NPC, an accommodative slide rule is typically used, which allows the test administrator to determine the subjective breakpoint or distance in which the patient indicates that diplopia has occurred, and a deviation of the patient's eye laterally (exophoria) is observed (Kawata et al., 2016; Pearce et al., 2015; Scheiman et al., 2003). While the traditional NPC test has widespread use across settings, several concerns have been noted, including the inability to measure other aspects of vergence impairments common in mTBI patients, fatigue effects across trials, and the reliance on participants' subjective experience of diplopia, not to mention administrator expertise and issues of inter- and intra-rater reliability (Pearce et al., 2015; Rucker et al., 2019; Yaramothu et al., 2019). These potential shortcomings suggest a new approach to assessing concussion-related deficits in the vergence system may be warranted. This is further exacerbated by recent research which calls into question the traditional assumption that a receded NPC is diagnostic of CI, which may contribute to misdiagnoses of vergence-related deficits in patients (Raghuram et al., 2019). Importantly, impaired NPC performance can be indicative of a diverse array of vergence deficits that require more comprehensive assessments than the NPC test alone to accurately diagnose (Raghuram et al., 2019).

Recent attention has been given to virtual reality (VR) technology as an avenue for developing comprehensive diagnostic and treatment options that are cost-effective and highly portable. For instance, a novel VR vergence endurance test uncovered numerous significant differences in vergence performance between concussed and healthy individuals, including convergence and divergence peak velocity, divergence response amplitude, and a binocular accuracy index (Yaramothu et al., 2019). Notably, the binocular accuracy index and divergence peak velocity metrics were able to discriminate between groups with a high degree of accuracy, supporting the potential of VR technology to objectively assess the integrity of the vergence system (Yaramothu et al., 2019). However, the effects of VR on vergence are far from being fully understood, with more research needed to establish the effect of manipulations in a VR environment on convergence and divergence.

## Methods

### Participants

Forty-five young adults gave informed consent to participate in this study approved by Temple University's Institutional Review Board (IRB). Participants were recruited through flyers posted on Temple University's main campus, as well as emails sent throughout the College of Public Health. For their time, participants were compensated with a $25 Amazon gift card. The study was conducted within the Motion-Action-Perception Laboratory (MAP Lab), at Temple University, Philadelphia, PA.

#### ***Eligibility***

Participants in the Concussion History Group were defined as having one or more diagnosed concussions in their lifetime, while the Healthy Control Group had to have never been diagnosed or suspected of having experienced a concussion. Inclusion criteria were as follows: 1) Age between 18-35, 2) Normal or corrected to normal vision (binocular visual acuity of 20/40 or better), 3) No history of oculomotor or vision disorders unrelated to a concussion (e.g., strabismus), 4) No history of neurological disorders unrelated to a concussion (e.g., Parkinson's disease), 5) Participants must avoid alcohol and drug use 24 hours before testing. Participants completed a medical history form to determine concussion status, the presence of symptoms, and potential neurological and visual disorders before testing. A short visual assessment using a Tumbling E chart was conducted to verify visual acuity fell within normal ranges for the right and left eyes, as well as binocularly.

## Procedures

### ***Instrumentation***

Virtual Reality Vergence Assessment (VR_VA): The VR_VA was presented through a wired HTC Vive HMD (HTC Corporation, Taoyuan City, Taiwan) that was fitted with a SensoMotoric Instruments (SMI) video-oculography (VOG) system (SensoMotoric Instruments, Teltow, Germany). The HMD uses a 110° diagonal field of view and 89.6 Hz refresh rate to stream the VR environment through the Unity Real-Time Development Platform (Unity Technologies, San Francisco, US). The HTC Vive also utilizes on-ear headphones to stream audio from the host computer. VOG data (interpupillary distance, IPD) was recorded at 89.6 Hz. In some conditions, participants used a Bluetooth button press device to respond based on test instructions.

### ***Experimental Protocol***

#### Clinical Visual Assessment

Participants' visual acuity was measured using a standard tumbling E chart at a distance of 2 meters. The right and left eyes were assessed separately by having participants cover one eye and verbally indicate the direction the letter E was facing. Participants started from the top of the chart and read down line by line until errors were made and they were unable to meaningfully read or guess the direction of the E

(Camparini et al., 2001). The procedure was then repeated for both eyes to establish binocular visual acuity.

Near point of convergence (NPC) was then measured using the standard accommodative slide rule (Bernell Corporation, Mishawaka, IN). Participants positioned the ruler below their nose and held the slide card holder at 30 cm while focusing on a central letter on the reduced Snellen chart. The participant then moved the slide card holder toward them at approximately 1 cm/s while maintaining focus on the central letter until the letter appeared to double in their vision. The experimenter recorded the distance at which diplopia was reported. This procedure was repeated 3 times.

Near visual acuity was measured using the same accommodative slide rule. Participants held the card holder at a fixed distance of 40 cm and read down the reduced Snellen chart line by line until errors were made or they were unable to meaningfully continue (Holladay, 1997).

### VR_VA

Participants were then seated at a comfortable height with their chin placed on a headrest to control for head position. After being fitted with the HTC Vive, a standard five-point calibration and validation procedure was conducted through SMI’s internal software, which ensured that the gaze angle was within 1° tolerance.

Before testing, participants sat with the headset in a dark environment for 1 minute to control for light adaptation between participants. During testing, participants were presented with a black VR environment with a small (20 cm) circular target with a tan outer ring and red center. In each condition, participants were instructed to maintain their fixation on the center of the target and to minimize blinking during testing.

Vergence was assessed across four subtests, which targeted difference aspects of the vergence response: 1) VR_NPC – the virtual target was positioned at a distance of 40 cm, and moved towards participant' visual center at a speed of 1 cm/s while they used a Bluetooth button press device to indicate when the target doubled in their vision 2) Divergence Fusion – the target was positioned at a distance of 3 cm, moved away from the participant' visual center at a speed of 1 cm/s while they used a Bluetooth button press device to indicate when the target fused in their vision 3) Vergence Tracking – participants tracked a target which started at a distance of 40 cm and moved at 5 cm/s to a distance of 3 cm before reversing, with the trial lasting 20 seconds and the target completing 4 full cycles of back and forth movement 4) Vergence Holding – participants tracked a target which started at a distance of 40 cm and made 5 cm jumps every 1 second toward and then away from the participants' visual center for a total of 15 positions (Figure 16). Participants performed three trials per condition with 30 seconds of rest between conditions. A standard order of VR_NPC, Divergence Fusion, Vergence Tracking, and Vergence Holding was used across participants.

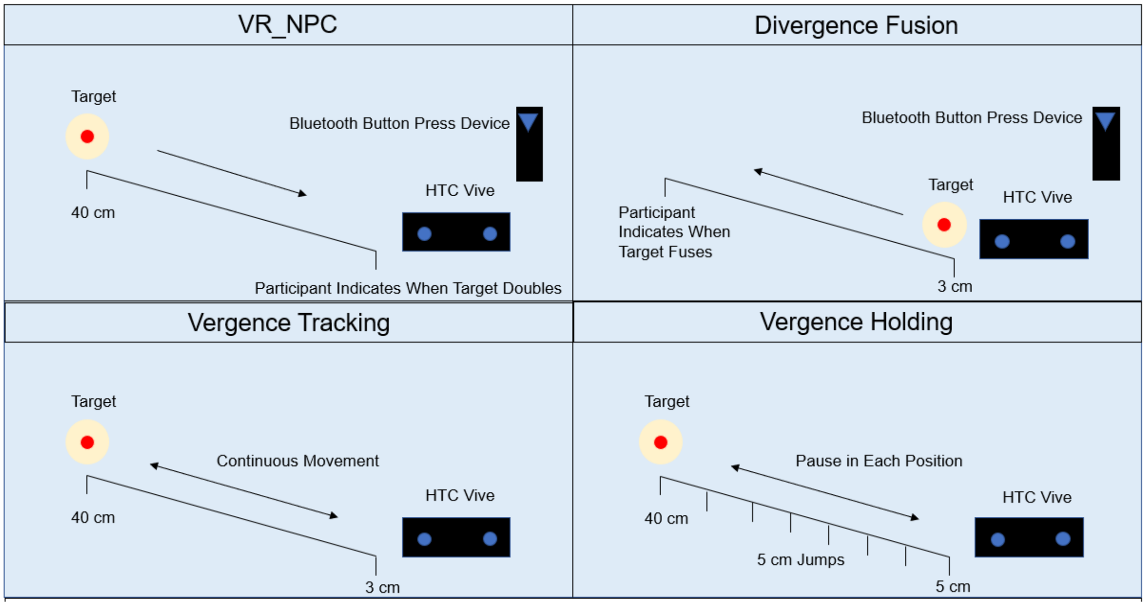


Figure 16. Diagram showing the VR_VA test conditions and methodology.

### *Outcome Measures*

To create a comprehensive assessment of vergence, as well as determine the ability of a VR system to elicit and measure vergence response like clinical and eye-tracker-based assessments, we selected a range of dependent variables to match those used across vergence studies in the mTBI population (Duprey et al., 2017; Kawata et al., 2016; Rucker et al., 2019; Santo et al., 2020). Dependent variables for the VR_NPC were as follows: 1) Minimum IPD (smallest IPD value), 2) Maximum IPD (largest IPD value) 3) IPD Amplitude (difference between maximum and minimum IPD), 4) Target Distance at Minimum IPD (objective position of the VR target at the minimum IPD), 5) Subjective Break Point (target position where participants indicated the target doubled), 6) Z Score (from a time series correlation between the target position and IPD, correlation coefficients were converted to z scores for comparison using Fisher's Z transformations (Silver & Dunlap, 1987). Dependent variables for the Divergence Fusion test were as follows: 1) Baseline IPD (IPD at the start of the test), 2) Fusion IPD (IPD where participants indicated the target fused), 3) IPD Difference (Fusion IPD – Baseline IPD), 4) Max Target Distance (Target distance at Fusion IPD), and 5) Z Score. Dependent variables for the Vergence Tracking and Vergence Holding tests were as follows: 1) Minimum IPD, 2) Maximum IPD, 3) IPD Difference, 4) IPD Standard Deviation, 5) Z Score.

For the clinical visual assessment, standard outcome measures were used. Visual acuity was recorded as Snellen Score (e.g., 20/20) and then converted to LogMAR for statistical comparison (Holladay, 1997). For the NPC assessment, a subjective breakpoint (the distance at which participants indicated that diplopia was experienced) was recorded (Duprey et al., 2017; Kawata et al., 2016; Santo et al., 2020).

## Power Analysis

Our sample size was powered based on our Primary Aim 1 using a pupillary light reflex test (PLR), as this is a well-established methodology for objectively assessing the oculomotor system following a concussion (Chapter 2, Figure 3) (Thiagarajan & Ciuffreda, 2015; Urosevich & Capo-Aponte, 2013). While convergence is commonly assessed in concussion patients, the limitations of eliciting and measuring vergence in VR are not fully understood (Hoffman et al., 2008; Kramida, 2016; Paulus et al., 2017; Zabels et al., 2019). As investigating these limitations was one of the major aims of this project, we were more confident that the PLR would provide a reliable sample size estimation, given that the light stimulus produced by the HMD closely matched the characteristics (i.e., luminance) of pupilometers used in prior studies (Santo et al., 2020). Finally, this sample aligns with prior studies of the NPC with mTBI populations, which have used 40 participants and found significant findings – suggesting our calculation provides adequate power for our other tests (Santo et al., 2020).

## Statistical Analysis

Eye data was processed in MATLAB (MathWorks, Portola Valley, CA) and followed a standardized pipeline. This process first required the removal of invalid data or "NaN" values, which indicate blinks or data loss (Kret & Sjak-Shie, 2019). Then, raw data was filtered for artifacts such as temporally isolated samples, which may indicate repeated blinks or an occluded pupil (Kret & Sjak-Shie, 2019). Finally, valid data was processed using a zero-phase low-pass filter before analysis (Kret & Sjak-Shie, 2019).

To address our Primary and Secondary Aim 2, for the VR_NPC and Divergence Fusion tests, a series of independent samples t-tests were used to determine whether there is a difference in vergence metrics between groups. Pearson's correlations were also reported between interpupillary distance and target position across both groups to establish the efficacy of the VR-based vergence assessment to both elicit a vergence response and verify the ability of participants to effectively track a virtual target moving in depth in a VR environment. Z scores were averaged across participants and then converted back to correlation coefficients using Inverse Fisher transformations (Silver & Dunlap, 1987).

For the Vergence Tracking and Vergence Holding tests, mixed model repeated measures ANOVAs were used to detect the presence of between-group differences (concussion status), within-group differences (target cycle or target position respectively), and interaction effects. An alpha level of 0.05 was set for determining significant main effects. If significant main effects were found in Vergence Tracking variables, a follow-up post-hoc analysis was used with Bonferroni adjusted alpha levels to account for multiple comparisons. If significant main effects were found for the Vergence Holding variables, follow-up planned contrast comparisons were conducted using the first target position (40 cm) as a reference, as this required the least convergence demand and would not be subject to fatigue effects at the start of the trial. For the Vergence Holding task, an independent samples t-test was also used for the z score describing the correlation between eye and target position to compare the strength of the relationship between the healthy control and Concussion History Groups.

To address our Tertiary Aim 2, a series of statistical tests were conducted to determine whether vergence responses in VR mimicked a natural environment for the VR_NPC test. A mixed model repeated measures ANOVA was used to determine whether there was a difference between minimum and maximum IPD, and whether this relationship was impacted by concussion status, to determine whether a significant degree of convergence occurred in VR. A Pearson's correlation was conducted between interpupillary distance and target position to establish the relationship between vergence response and a VR target moving in depth. Additionally, a Pearson's correlation was conducted to determine whether there was a significant relationship between subjective break point values achieved from the traditional NPC and VR_NPC tests.

To address Tertiary Aim 1, our statistical approach was repeated with the Concussion History Group subdivided into subgroups: a Single Concussion Group (1 diagnosed concussion) and a Multiple Concussion Group (2 or more diagnosed concussions). For the concussion history subgroup analysis, within-subject effects were only reported if not previously found, as the focus of this analysis was primarily to determine whether there was a unique effect of having multiple concussions on the vergence response compared to either one or no concussions. Otherwise, only significant between-group differences and subgroup x condition interaction effects were reported.

A regression analysis was also conducted to determine whether there was a relationship between time since injury and vergence response. A simple linear regression was completed for each of the dependent variables across the VR_NPC, Divergence Fusion, and Vergence Holding tests. For the Vergence Tracking test, a multiple regression was used which treated each cycle of target movement as a separate independent variable. This was done to determine whether the integrity of vergence was maintained throughout the entire trial, or if fatigue may produce different vergence behavior at the end of a trial compared to the beginning. Healthy control participants' time since injury was entered as their age at the time of testing to incorporate their dataset into the regression analysis, as they had not been diagnosed with a concussion.

## Results

### Demographics

Forty-five participants (24 females; 21 males; 26.17 ± 4.18 years [18-35 years]) completed the study. Independent samples t-tests did not show any significant differences in age or gender between the Concussion History and Healthy Control Groups. The number of concussions reported by the Concussion History Group ranged from 1 to 4 (1.75 ± 0.94 concussions) and the time since their most recent injury ranged from 0.75 to 20 years (6.6 ± 4.3 years) (Table 8). Self-reported medical history did not reveal ongoing symptoms across our Concussion History Group as a result of mTBI.

Table 8. Demographic and clinical characteristics of participants. (n = 45)

| **Female (%)** | **Healthy Control (n = 21)** | **Concussion History (n = 24)** | **p** |
|---|---|---|---|
| Age (yrs) | 25.90 ± 3.56 | 26.42 ± 4.67 | 0.685 |
| Male (%) | 5 (24%) | 11 (46%) | 0.124 |
| Female (%) | 16 (76%) | 13 (54%) | |
| Number of Concussions | 0 | 1.75 ± 0.94 | |
| Time Since Injury (yrs) | 25.90 ± 3.56 | 6.56 ± 4.33 | **< 0.001**** |

Age, number of concussions, and time since injury (mean ± standard deviation); sex (frequency); ** indicates p < 0.001

Table 9. Demographic and clinical characteristics of participants by number of concussions. (n = 45)

| **Variable** | **Healthy Control (n = 21)** | **Single Concussion (n = 12)** | **Multiple Concussion (n = 12)** | **p** |
|---|---|---|---|---|
| Age (yrs) | 25.90 ± 3.56 | 25.67 ± 4.18 | 27.16 ± 5.18 | 0.631 |
| Male (%) | 5 (24%) | 3 (25%) | 8 (66.6%) | 0.085 |
| Female (%) | 16 (76%) | 9 (75%) | 4 (33.3%) | |
| Number of Concussions | 0 | 1 | 2.50 ± 0.80 | |
| Time Since Injury (yrs) | 25.90 ± 3.56 | 7.75 ± 4.43 | 5.38 ± 4.06 | **< 0.001**** |

Age, number of concussions, and time since injury (mean ± standard deviation); sex (frequency). ** Indicates p < 0.001.

## Clinical Visual Assessment

Independent samples t-tests did not show any significant differences in visual acuity, near visual acuity, or mean subjective break point between the Concussion History and Healthy Control Groups (Table 10).

Table 10. Clinical visual assessment results – VR_VA.

| Variable | Healthy Control (n = 21) | Concussion History (n = 24) | p |
|---|---|---|---|
| Right Visual Acuity (LogMar) | 0.14 ± 0.16 | 0.15 ± 0.12 | 0.690 |
| Left Visual Acuity (LogMar) | 0.10 ± 0.10 | 0.13 ± 0.11 | 0.512 |
| Binocular Visual Acuity (LogMar) | 0.05 ± 0.10 | 0.07 ± 0.09 | 0.625 |
| Binocular Near Visual Acuity (LogMar) | 0.11 ± 0.10 | 0.10 ± 0.10 | 0.388 |
| Mean NPC Subjective Break Point (cm) | 4.37 ± 2.21 | 5.47 ± 2.66 | 0.153 |

Reported as mean ± standard deviation.

A one-way ANOVA found a significant main effect for the mean subjective break point in the NPC test (F (2,42) = 4.34, p = 0.019) (Table 11). A follow-up posthoc analysis with Bonferroni adjusted alpha levels revealed that the mean subjective break point was significantly greater in the Single Concussion Group compared to the healthy control (p = 0.031) and multiple concussion (p = 0.048) groups (Figure 17).

Table 11. Clinical visual assessment results by number of concussions – VR_VA.

| Variable | Healthy Control (n = 21) | Single Concussion (n = 12) | Multiple Concussion (n = 12) | p |
|---|---|---|---|---|
| Right Visual Acuity (LogMar) | 0.14 ± 0.16 | 0.15 ± 0.13 | 0.16 ± 0.12 | 0.911 |
| Left Visual Acuity (LogMar) | 0.10 ± 0.10 | 0.11 ± 0.11 | 0.14 ± 0.11 | 0.580 |
| Binocular Visual Acuity (LogMar) | 0.05 ± 0.10 | 0.07 ± 0.09 | 0.07 ± 0.10 | 0.889 |
| Binocular Near Visual Acuity (LogMar) | 0.11 ± 0.10 | 0.13 ± 0.10 | 0.14± 0.11 | 0.672 |
| Mean NPC Subjective Break Point (cm) | 4.37 ± 2.21 | 6.68 ± 2.74 | 4.25 ± 2.24 | **0.019*** |

Reported as mean ± standard deviation.

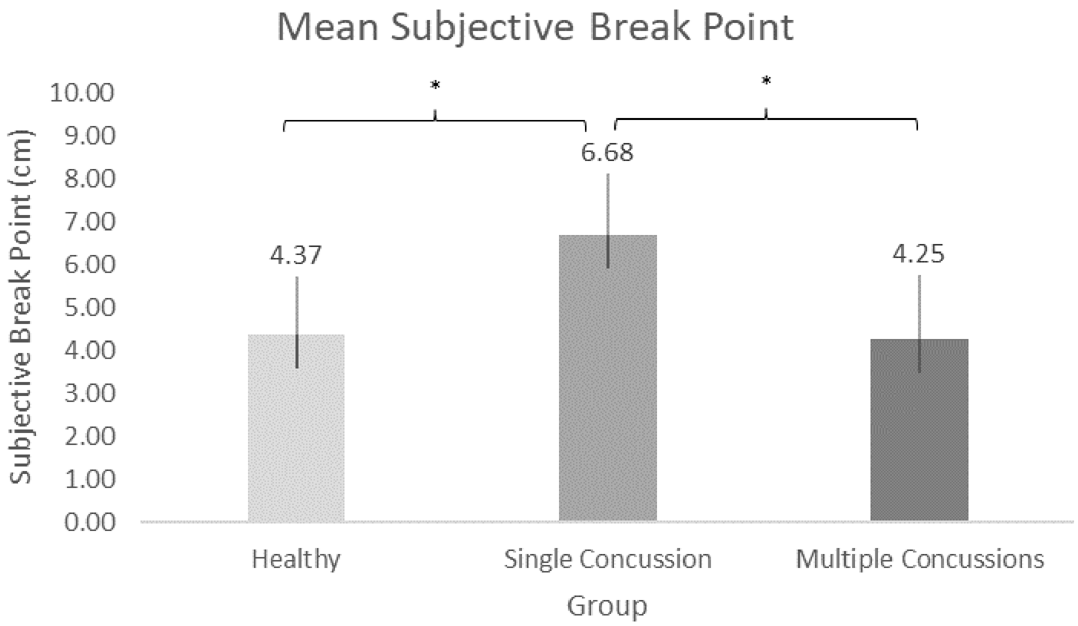


Figure 17. Mean Subjective Breakpoint by concussion history subgroup in the physical NPC test. * Indicates $p < 0.05$.

### VR_NPC

No significant between-group differences were found across the VR_NPC variables.

### VR_NPC Concussion History Subgroup Analysis

No significant within-group differences, between-group differences, or interaction effects were found across the VR_NPC variables for the concussion history subgroup analysis.

### VR_NPC Concussion History Time-Since-Injury Analysis

The simple regression analysis revealed that time since injury did not predict vergence response across VR_NPC variables.

### Correlation between Interpupillary Distance and Target Position VR_NPC

A Pearson's correlation revealed a significant, strong, positive relationship between interpupillary distance and target position in the VR_NPC test (r = 0.84, p = 0.004).

### Difference between Min and Max IPD VR_NPC

A repeated measures ANOVA showed a significant main effect for IPD difference (F (1,43) = 91.49, p < 0.001), with Min IPD (M = 59.15, SD = 0.74) found to be significantly smaller than Max IPD (M = 62.85, SD = 0.66). No significant between-group differences or interaction effects were found.

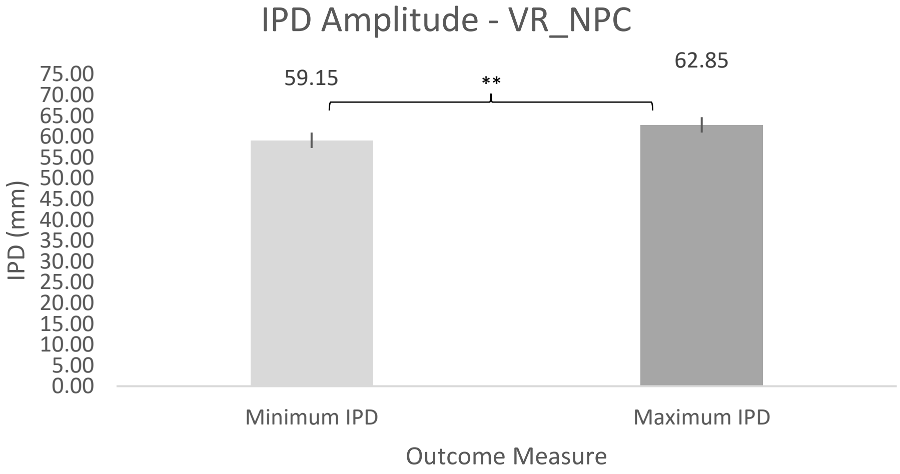


Figure 18. IPD Amplitude (Maximum IPD – Minimum IPD) in the VR_NPC test.
** indicates p < 0.001.

### Comparison NPC and VR_NPC Subjective Break Points

A repeated measures ANOVA showed a significant main effect for subjective break point (F (1,43) = 74.80, p < 0.001), with VR_NPC subjective break point (M = 15.00, SD = 1.22) found to be significantly larger than NPC subjective break point (M =

4.92, SD = 0.38). No other significant between-group differences or interaction effects were found.

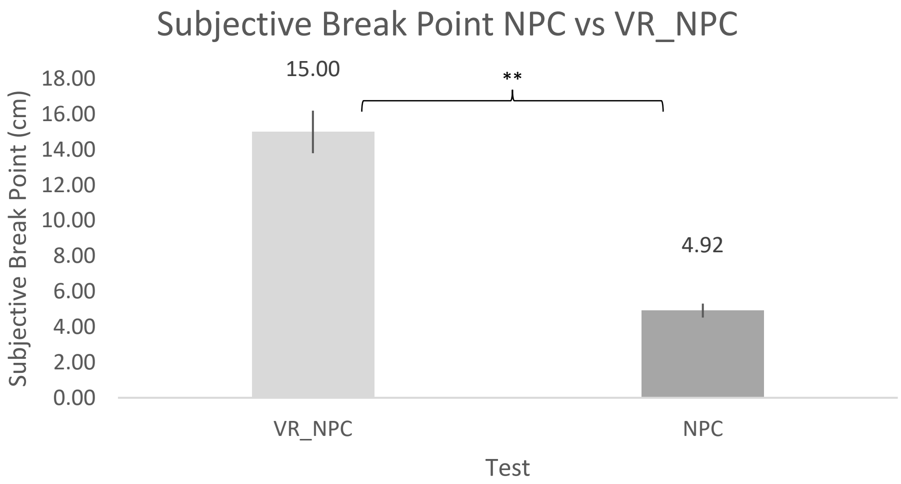


Figure 19. Subjective Break Point in the VR_NPC vs physical NPC test.
** Indicates p < 0.001.

### Correlation between NPC and VR_NPC Subjective Break Points

Pearson’s correlation revealed that the relationship between the NPC and VR_NPC subjective break points trended toward significance (r = 0.28, p = .063).

Table 12. VR_NPC variables – averages by participant status.

| Group | Subjective Break Point (cm) | Target Distance at Min IPD (cm) | Min IPD (mm) | Max IPD (mm) | IPD Difference (mm) | Z-Score |
|---|---|---|---|---|---|---|
| Healthy Control | 15.27 ± 9.17 | 19.15 ± 11.70 | 58.98 ± 5.07 | 62.78 ± 4.28 | 3.80 ± 2.78 | 1.13 ± 0.80 |
| Concussion History | 14.73 ± 7.03 | 18.40 ± 8.79 | 59.31 ± 4.89 | 62.92 ± 4.53 | 3.61 ± 2.42 | 1.29 ± 0.66 |

Reported as mean ± standard deviation.

## Divergence Fusion

### *Z-Score*

An independent samples t-test found a significant between-group difference for the z-score which described the correlation between IPD and target position (t (43) = 2.49, p = 0.017). Z-score was significantly larger and negative (reflecting a stronger negative correlation) in the Concussion History Group (M = -0.75, SD = 0.61) compared to the Healthy Control Group (M = -0.22, SD = 0.81). No other significant differences were found across the Divergence Fusion variables.

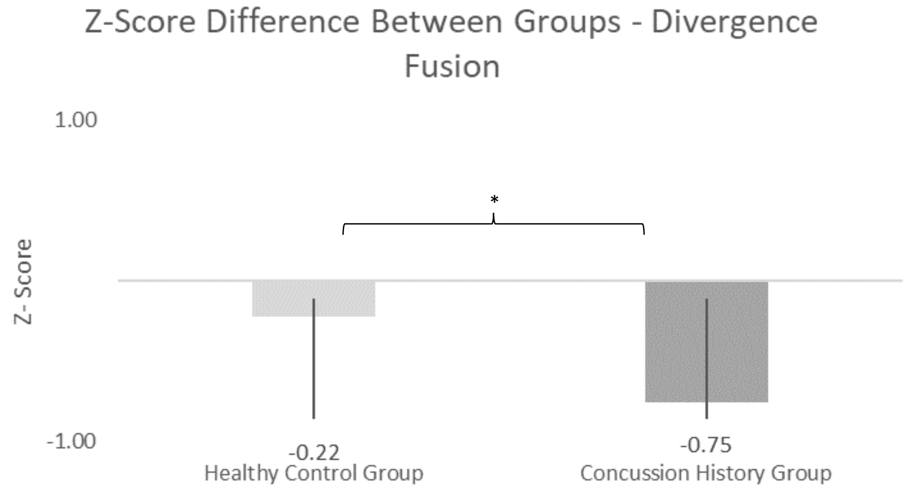


Figure 20. Z-Scores difference between groups in the Divergence Fusion test. * Indicates $p < 0.05$.

## Divergence Fusion Concussion History Subgroup Analysis

### *Z-Score*

A one-way ANOVA revealed a trend toward significance for the Z score describing the relationship between the vergence angle and target position between the

Healthy Control Group, Single Concussion Group, and Multiple Concussion Group (t (2,44) = 3.04, p = 0.059).

No other significant within-group differences, between-group differences, or interaction effects were found across other Divergence Fusion variables for the concussion history subgroup analysis.

### Divergence Fusion Time-Since-Injury Analysis

A simple linear regression found that time-since-injury significantly explained the variance in the Z score describing the relationship vergence angle and target position across participants (F (1, 43) = 5.98, p < 0.019, $R^2 = 0.12$). The regression coefficient (B = 0.025) indicated that an increase in 1 year since a concussion increased the strength of the Z score by 0.025 (reflecting a stronger positive correlation). No other significant regression equations were found for the Divergence Fusion test.

### Correlation between Interpupillary Distance and Target Position Divergence Fusion

Pearson's correlation revealed a significant, moderate, negative relationship between interpupillary distance and target position in the Divergence Fusion test (r = -0.47, p = 0.003).

Table 13. Divergence Fusion variables – averages by participant status.

| Group | Fusion IPD (mm) | Max Target Distance (cm) | Min IPD (mm) | IPD Difference (mm) | Z-Score |
|---|---|---|---|---|---|
| Healthy Control | 61.74 ± 4.27 | 26.70 ± 5.08 | 62.53 ± 4.15 | 0.79 ± 2.02 | -0.22 ± 0.81 |
| Concussion History | 61.68 ± 4.54 | 26.26 ± 4.84 | 63.08 ± 4.35 | 1.41 ± 1.06 | -0.75 ± 0.61 |

Reported as mean ± standard deviation.

## Vergence Tracking

### ***Minimum IPD***

Mauchly's Test of Sphericity revealed that the assumption of sphericity had been violated ($\chi^2(5) = 42.85$, $p < 0.001$). A repeated measures ANOVA with a Greenhouse-Geisser adjustment showed a significant main effect for the four target cycles (F (2.00, 86.15) = 6.44, p = 0.002). A follow-up posthoc analysis with Bonferroni adjusted alpha levels revealed that minimum IPD was significantly smaller in cycle 1 (M = 59.22, SEM = 0.68) compared to cycle 2 (M = 59.69, SEM = 0.67, p = 0.006), cycle 3 (M = 59.66, SEM = 0.65, p = 0.027), and cycle 4 (M = 59.80, SEM = 0.66), p = 0.005). No significant between-group differences or interaction effects were found.

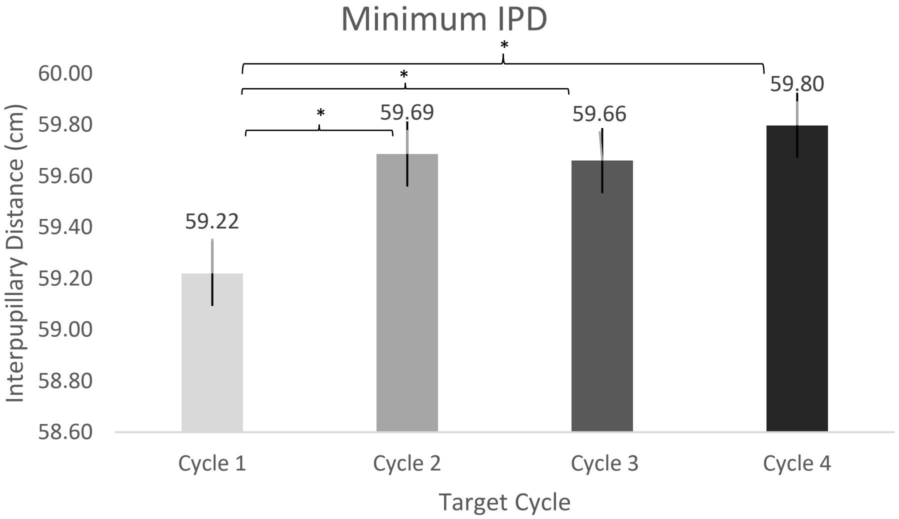


Figure 21. <u>Minimum IPD in the Vergence Tracking Test across target cycles.</u> One target cycle occurs when the target moves from 40 cm to 3 cm and back. * Indicates $p < 0.05$.

### *Maximum IPD*

Mauchly's Test of Sphericity revealed that the assumption of sphericity had been violated ($\chi^2(5) = 182.94$, $p < 0.001$). A repeated measures ANOVA with a Greenhouse-Geisser adjustment showed a significant main effect for the target cycle (F (1.30, 55.74) = 25.18, $p < 0.001$). A follow-up posthoc analysis with Bonferroni adjusted alpha levels revealed that maximum IPD was significantly larger in cycle 1 (M = 63.24, SEM = 0.69) compared to cycle 2 (M = 62.24, SEM = 0.65, $p < 0.001$), cycle 3 (M = 62.22, SEM = 0.64, $p < 0.001$), and cycle 4 (M = 62.22, SEM = 0.62, $p < 0.001$). No significant between-group differences or interaction effects were found.

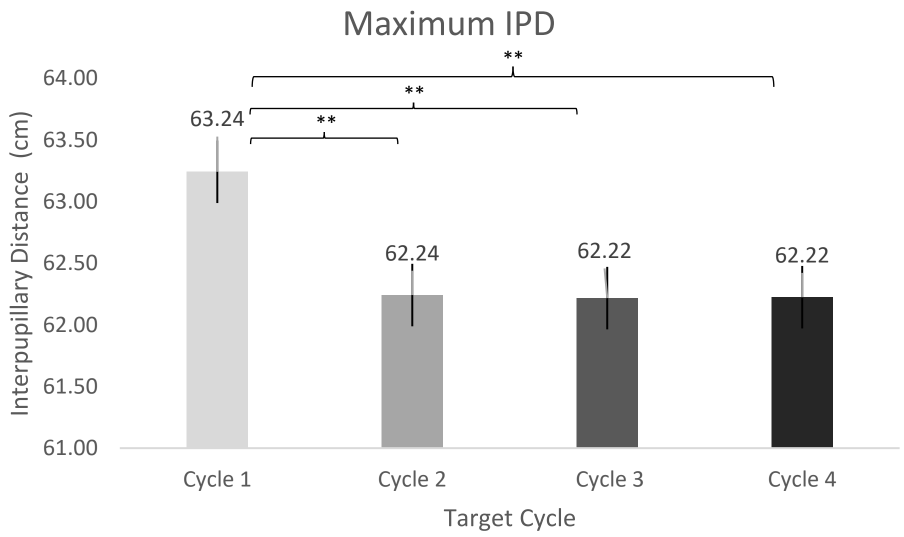


Figure 22. Maximum IPD in the Vergence Tracking Test across target cycles. One target cycle occurs when the target moves from 40 cm to 3 cm and back. ** Indicates $p < 0.001$.

### *IPD Difference*

Mauchly's Test of Sphericity revealed that the assumption of sphericity had been violated ($\chi^2(5) = 51.57$, $p < 0.001$). A repeated measures ANOVA with a Greenhouse-

Geisser adjustment showed a significant main effect for the target cycle (F (1.74, 74.81) = 48.82, $p < 0.001$). A follow-up posthoc analysis with Bonferroni adjusted alpha levels revealed that the IPD difference was significantly larger in cycle 1 (M = 4.02, SEM = 0.42) compared to cycle 2 (M = 2.56, SEM = 0.31, $p < 0.001$), cycle 3 (M = 2.56, SEM = 0.33, $p < 0.001$), and cycle 4 (M = 2.43, SEM = 0.29, $p < 0.001$). No significant between-group differences or interaction effects were found.

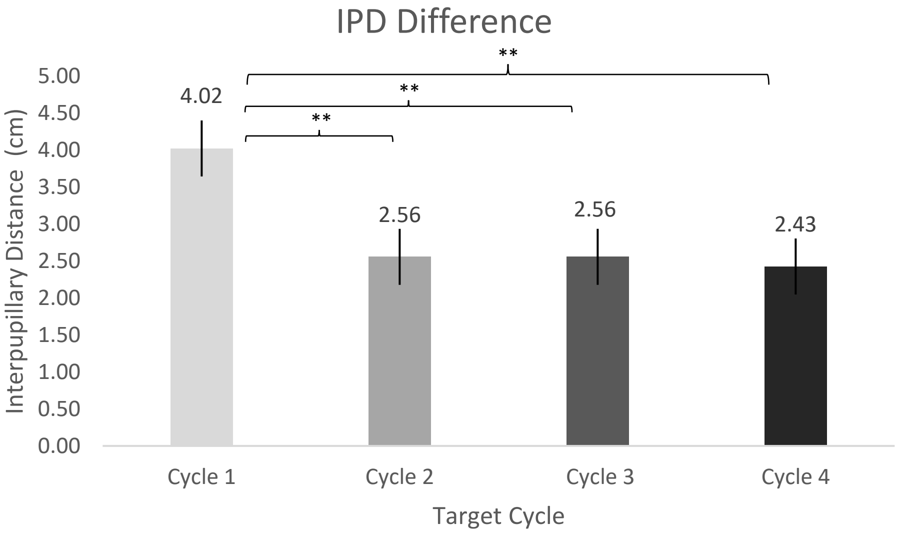


Figure 23. IPD Difference (Maximum IPD – Minimum IPD) in the Vergence Tracking Test across target cycle. One target cycle occurs when the target moves from 40 cm to 3 cm and back. ** Indicates $p < 0.001$.

### *IPD Standard Deviation*

Mauchly's Test of Sphericity revealed that the assumption of sphericity had been violated ($\chi^2(5) = 27.15$, $p < 0.001$). A repeated measures ANOVA with a Greenhouse-Geisser adjustment showed a significant main effect for the target cycle (F (2.13, 91.49) = 24.27, $p < 0.001$). A follow-up posthoc analysis with Bonferroni adjusted alpha levels revealed that IPD standard deviation was significantly larger in cycle 1 (M = 1.12, SEM = 0.12) compared to cycle 2 (M = 0.87, SEM = 0.11, p < 0.001), cycle 3 (M = 0.85, SEM = 0.11, p < 0.001), and cycle 4 (M = 0.79, SEM = 0.1, p < 0.001). No significant between-group differences or interaction effects were found.

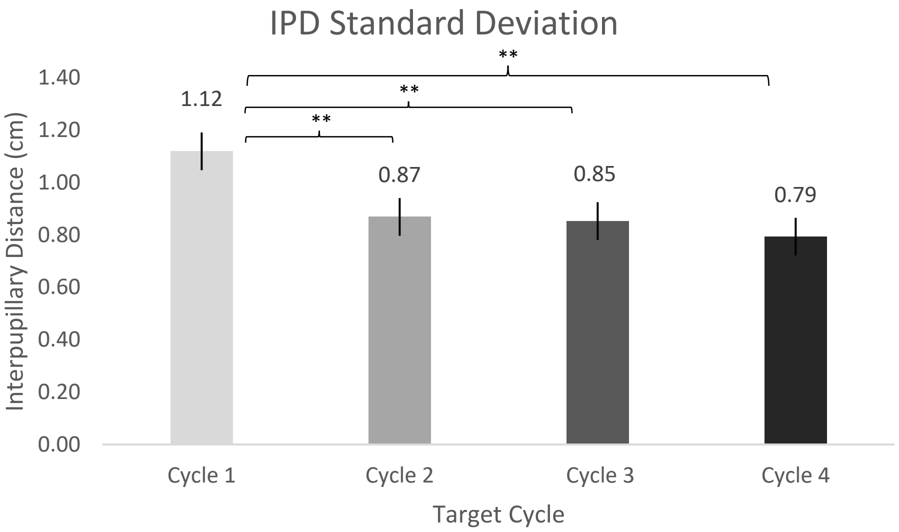


Figure 24. <u>IPD Standard Deviation in the Vergence Tracking Test across target cycles.</u> One target cycle occurs when the target moves from 40 cm to 3 cm and back. ** Indicates p < 0.001.

### ***Z-Score***

No significant within-group, between-group, or interaction effects were found for Z-score.

## Vergence Tracking Concussion History Subgroup Analysis

### ***Z-Score***

A repeated measures ANOVA revealed a significant interaction effect for the z-score which described the correlation between IPD and target position between the target cycle and concussion history subgroup (F (6, 126) = 2.19, p = 0.048). A follow-up post-hoc analysis with Bonferroni adjusted alpha levels did not reveal any significant differences between concussion history subgroups across target cycles.

No other significant within-group differences, between-group differences, or interaction effects were found across Vergence Tracking variables for the concussion history subgroup analysis.

## Vergence Tracking Time-Since-Injury Analysis

The simple regression analysis revealed that time since injury did not predict vergence response across Vergence Tracking variables.

Table 14. Vergence Tracking variables – averages by participant status across target cycles.

| | Healthy Control | | | | Concussion History | | | |
|---|---|---|---|---|---|---|---|---|
| **Variable** | **Cycle 1** | **Cycle 2** | **Cycle 3** | **Cycle 4** | **Cycle 1** | **Cycle 2** | **Cycle 3** | **Cycle 4** |
| **Minimum IPD (cm)** | 58.93 ± 4.23 | 59.6 ± 4.02 | 59.43 ± 3.84 | 59.46 ± 3.94 | 59.51 ± 4.81 | 59.78 ± 4.86 | 59.89 ± 4.77 | 60.13 ± 4.74 |
| **Maximum IPD (cm)** | 63.28 ± 5.00 | 62.2 ± 4.49 | 62.11 ± 4.30 | 62.01 ± 3.94 | 63.2 ± 4.30 | 62.29 ± 4.24 | 62.32 ± 4.29 | 62.43 ± 4.32 |
| **IPD Difference (cm)** | 4.35 ± 3.39 | 2.60 ± 2.37 | 2.68 ± 2.75 | 2.55 ± 2.28 | 3.69 ± 2.20 | 2.51 ± 1.82 | 2.43 ± 1.64 | 2.30 ± 1.63 |
| **IPD Standard Deviation (cm)** | 1.21 ± 1.02 | 0.89 ± 0.84 | 0.90 ± 0.93 | 0.83 ± 0.78 | 1.03 ± 0.62 | 0.85 ± 0.64 | 0.81 ± 0.57 | 0.75 ± 0.56 |
| **Z-Score** | 1.16 ± 0.82 | 1.10 ± 0.91 | 1.19 ± 0.86 | 1.13 ± 0.93 | 1.36 ± 0.83 | 1.27 ± 0.92 | 1.15 ± 0.92 | 1.26 ± 0.81 |

Reported as mean ± standard deviation.

## Vergence Holding

### ***Minimum IPD***

Mauchly's Test of Sphericity revealed that the assumption of sphericity had been violated ($\chi^2(104) = 1535.76$, $p < 0.001$). A repeated measures ANOVA with a Greenhouse-Geisser adjustment showed a significant main effect for condition ($F(1.55, 66.44) = 45.60$, $p < 0.001$). Follow-up within-subjects contrasts showed that minimum IPD was significantly different across target distances as the trial progressed (e.g., 35 cm, 30 cm, etc.) compared to IPD at the initial target distance. No significant between-group differences or concussion status x cycle differences were found.

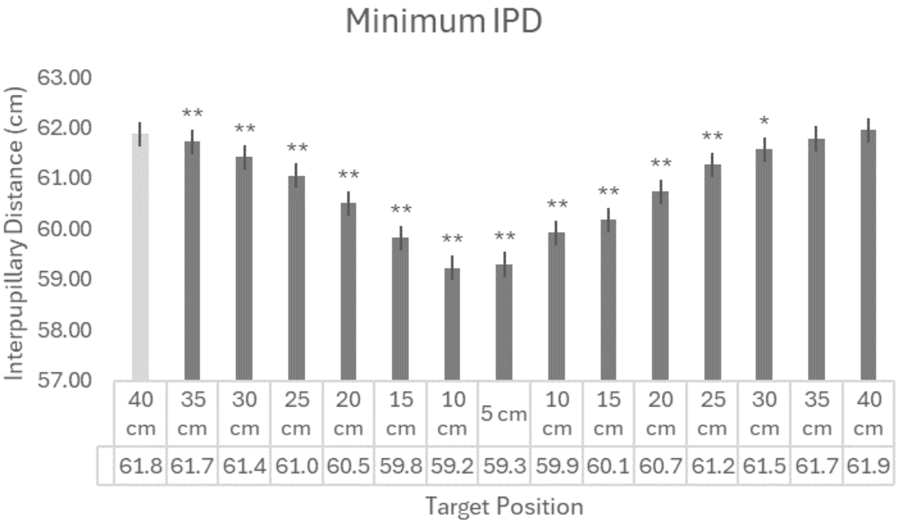


Figure 25. Minimum IPD in the Vergence Holding Test across target positions. Contrast testing done versus the initial target distance (40 cm; light grey bar). * Indicates $p < 0.05$, ** indicates $p < 0.001$.

### *Maximum IPD*

Mauchly's Test of Sphericity revealed that the assumption of sphericity had been violated ($\chi^2(104) = 1170.72$, $p < 0.001$). A repeated measures ANOVA with a Greenhouse-Geisser adjustment showed a significant main effect for condition (F (2.04, 87.62) = 42.80, $p < 0.001$). Follow-up within-subjects contrasts showed that maximum IPD was significantly different across target distances as the trial progressed (e.g., 35 cm, 30 cm, etc.) compared to IPD at the initial target distance (40 cm). No significant between-group differences or concussion status x cycle differences were found.

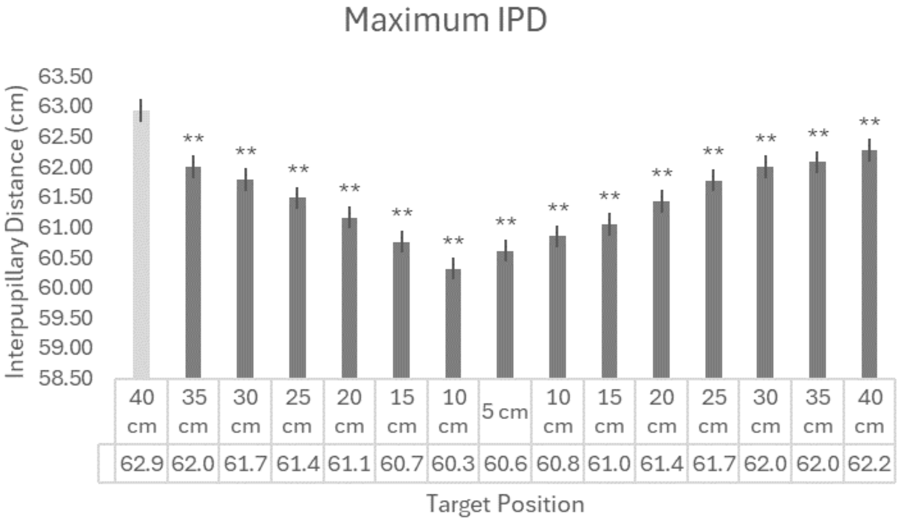


Figure 26. Minimum IPD in the Vergence Holding Test across target positions. Contrast testing done versus the initial target distance (40 cm; light grey bar). ** Indicates $p < 0.001$.

### *IPD Difference*

Mauchly's Test of Sphericity revealed that the assumption of sphericity had been violated ($\chi^2(104) = 765.61$, $p < 0.001$). A repeated measures ANOVA with a Greenhouse-Geisser adjustment showed a significant main effect for condition ($F (3.22, 138.23) = 26.55$, $p < 0.001$). Follow-up within-subjects contrasts showed that IPD Difference was significantly different across numerous target distances compared to the initial target distance. No significant between-group differences or concussion status x cycle differences were found.

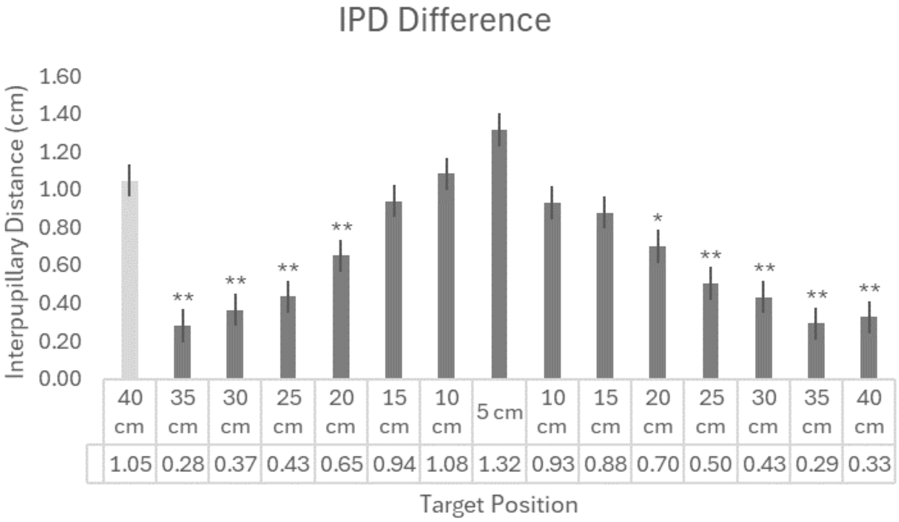


Figure 27. IPD Difference in the Vergence Holding Test across target positions. Contrast testing done versus the initial target distance (40 cm; light grey bar). * Indicates $p < 0.05$, ** indicates $p < 0.001$.

### *IPD Standard Deviation*

Mauchly's Test of Sphericity revealed that the assumption of sphericity had been violated ($\chi^2(104) = 767.56$, $p < 0.001$). A repeated measures ANOVA with a Greenhouse-Geisser adjustment showed a significant main effect for condition ($F(3.26, 140.11) = 25.14$, $p < 0.001$). Follow-up within-subjects contrasts showed that IPD Standard Deviation was significantly different across numerous target distances compared to the initial target distance. No significant between-group differences or concussion status x cycle differences were found.

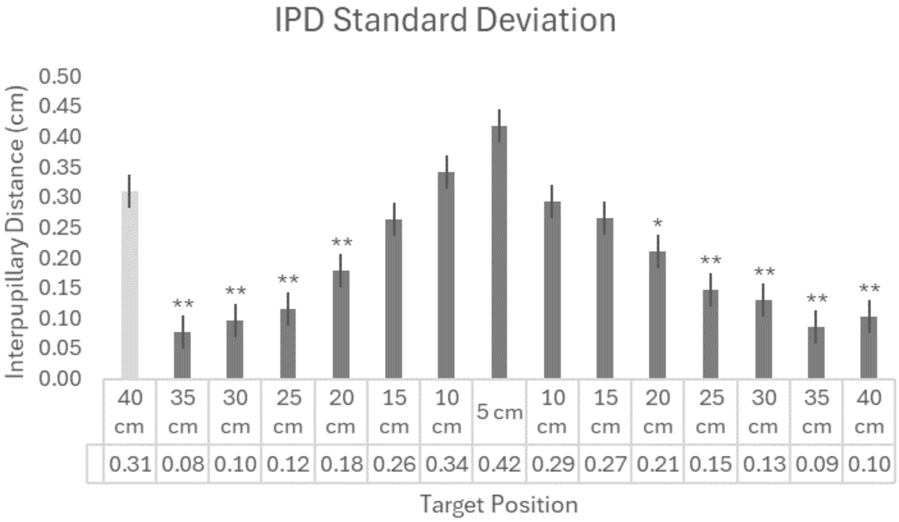


Figure 28. IPD Standard Deviation in the Vergence Holding Test across target positions. Contrast testing done versus the initial target distance (40 cm; light grey bar). * Indicates $p < 0.05$, ** indicates $p < 0.001$.

### *Z-Score*

No significant within-group, between-group, or interaction effects were found for Z-score.

## Vergence Holding Concussion History Subgroup Analysis

No additional significant within-group differences, between-group differences, or interaction effects were found across the Vergence Holding variables for the concussion history subgroup analysis.

## Vergence Holding Time-Since-Injury Analysis

The simple regression analysis revealed that time since injury did not predict vergence response across Vergence Holding variables.

## Discussion and Conclusion

This study investigated the ability of an HMD to elicit and measure convergence and divergence responses in a VR environment. We also investigated whether convergence and divergence responses differed between participants with a history of concussions and healthy individuals to assess potential long-term deficits to the vergence system associated with mTBI. Our findings demonstrated extensive evidence for stereotypical convergence and divergence responses to manipulations in virtual stimuli, which mimicked the expected response patterns.

In the VR_NPC assessment, interpupillary distance was found to be strongly correlated with the target position, suggesting that as the virtual target moved closer in the visual field a greater convergence response was produced. This stereotypical response pattern serves as the basis for clinical vergence testing, including the NPC, suggesting that our VR version produces oculomotor responses similar to those found in physical stimuli (Kawata et al., 2016; Pearce et al., 2015; Scheiman et al., 2003). This finding is further supported by significant differences in minimum and maximum IPD, which demonstrate a clear convergence response to a virtual target (Table 23).

However, it should be noted that significant differences in subjective break point values were found between the NPC and VR NPC. This suggests that, while the response patterns appear to be similar, the actual magnitude of convergence occurring in VR and the physical world appear to differ. Though the correlation between the NPC and VR_NPC subjective break point values trended toward significance, our study suggests that additional research is needed to fully understand what cues influence vergence response in VR when designing virtual environments. In our VR_NPC test, a black scene without depth cues was used to minimize confounding variables present in the testing environment. Therefore, it is unsurprising that differences in the magnitude of response between the virtual and physical tests were found, given the strong evidence for inaccurate depth perception and motor responses to virtual targets in VR, especially when visual cues are missing (Armbrüster et al., 2008; el Jamiy & Marsh, 2019; Gerig et al., 2018). Unlike the physical world, even rich VR scenes lack all the binocular and monocular depth cues that allow for accurate judgments of distance (Hartle & Wilcox, 2022; Hoffman et al., 2008; Kramida, 2016; Paulus et al., 2017; Zabels et al., 2019). Additionally, VR headsets produce a vergence-accommodation conflict due to the physical distance of the lens the eye accommodates versus the perceived distance of the target the eyes converge to (Hartle & Wilcox, 2022; Hoffman et al., 2008; Kramida, 2016; Paulus et al., 2017; Zabels et al., 2019). Therefore, while our findings suggest some vergence does occur, it may not have been to the same degree as that which occurs in the physical world, aligning with past research (Hartle & Wilcox, 2022; Hoffman et al., 2008; Kramida, 2016; Paulus et al., 2017; Zabels et al., 2019).

In the divergence fusion, a moderate, negative correlation between the target position and IPD was found, suggesting vergence occurred but that it may not have followed an expected pattern of initial convergence to acquire the target, followed by divergence as the target moved away. This negative correlation implies as the target moved further away participants attempted to converge to try to fuse the target successfully. Notably, a major limitation of our test was the starting and ending position of the VR target. We used the closest possible target position where the entire target was still visible in the VR space and opted for an ending distance that mimicked the length of the NPC test (40 cm). We found that many participants were unable to fuse the target within the parameters of the test and reported doing so to be challenging, which aligns with research showing the lack of depth cues in a VR environment makes judgments of distance unreliable and alters the vergence-accommodation relationship (Hartle & Wilcox, 2022; Hoffman et al., 2008; Kramida, 2016; Paulus et al., 2017; Zabels et al., 2019). Therefore, future research should manipulate the start and end positions to determine the proper parameters to allow for a more robust assessment and account for the increased difficulty VR may present for fusing a target as it changes distance.

Our last two tests, the vergence tracking and holding assessments, investigated aspects of vergence that are less well understood in the literature but have been given recent attention in mTBI patients (Rucker et al., 2019; Yaramothu et al., 2019). In the convergence tracking test, we found that vergence response was impacted by tracking a VR target that moved in depth in a cyclical pattern, with the strongest response found in the first cycle compared to others. In the Vergence Holding assessment, we found numerous differences in IPD metrics based on target position, providing further support for the capabilities of a VR HMD to elicit and measure vergence response. Interestingly, vergence response appeared to be degraded in the closest target positions, with significant differences between the minimum and maximum IPD disappearing and greater variability in responses being found. This finding supports the notion of “endurance” being a measurable aspect of the vergence system in VR environments, as it appears participants had difficulty maintaining a convergence response on a target position closer to the visual field over multiple cycles in a single trial (Yaramothu et al., 2019). The lack of rich depth cues may have contributed to the difficulty participants had fusing the target once it is perceived as being two separate images (Hartle & Wilcox, 2022; Hoffman et al., 2008; Kramida, 2016; Paulus et al., 2017; Zabels et al., 2019).

Across all the VR assessments, we found little evidence for vergence differences between the Concussion History Group and healthy controls, even when considering the number of concussions and time since injury. This is surprising, given that vergence deficits are well established as one of the most common impairments of the visual system in concussion patients across subacute, acute, and chronic periods (Duprey et al., 2017; Kawata et al., 2016; Pearce et al., 2015; Rucker et al., 2019). Given the heterogeneity in our patient population, with time since injury ranging between 0.75 to 20 years, it is very likely that large variability in the injury profiles of participants was a limitation in our study and could have contributed to the lack of significant vergence differences between groups. Additionally, because we did not specifically screen for participants with a history or oculomotor or vision disorders, there is a chance that any deficits may have recovered by the time testing occurred or may have never been present at all. While vergence deficits are common in mTBI patients across the recovery timeline, many studies have focused on recruiting participants with known concussion-related oculomotor disorders and/or more acute recovery periods where clinically relevant findings are more likely to be found (Duprey et al., 2017; Kawata et al., 2016; Pearce et al., 2015; Rucker et al., 2019). While studies have found deficits in chronic mTBI patients who were not experiencing visual symptoms (e.g., Wicek et al., 2021), participants still experienced other symptoms which would imply more severe and/or persistent deficits. Because our participants were asymptomatic and time-since-injury varied greatly between participants, this presented a significant limitation that should be addressed in future studies to determine whether a VR-based vergence assessment is sensitive to mTBI-related deficits in a more strictly defined chronic mTBI population.

While there were a small number of significant between-group differences across our vergence assessments and analyses, the inconsistency of results makes it difficult to attribute a history of concussions or time since injury as causes. As previously discussed, our Divergence Fusion test found that the correlation between target position and IPD was stronger, but negative, in the Concussion History Group. This suggests that the vergence angle of the eyes and the distance of the virtual target were more closely related in participants with a history of concussion compared to healthy control participants. This contradicts extensive research that suggests vergence response is degraded following a mTBI and one would expect that participants with a history of concussions would have greater difficulty regulating vergence as a target changes depth (Cooper & Jamal, 2012; Duprey et al., 2017; Gallaway et al., 2017; Pearce et al., 2015). Conversely, the time since injury regression analysis found that the strength of the relationship between IPD and target distance during the Divergence Fusion Test increased over time, suggesting participants who had greater recovery time were able to diverge their eyes more effectively as the target moved away in virtual space. This finding supports the literature which shows, unsurprisingly, symptoms tend to be more severe during the subacute and acute periods following an mTBI, while injuries that occurred months or years prior would allow for greater recovery. Therefore, the lack of consistency across an already limited number of differences would suggest the impact of a history of concussions on vergence response in our VR assessment is inconclusive.

Taken together, we demonstrated that while vergence tests in physical and virtual environments produce responses that differ in magnitude, IPD follows similar patterns to changes in target distance in both tests. We also found that VR-based tests can measure a variety of aspects of the vergence system, including convergence, divergence, vergence tracking, and vergence holding, supporting the potential of VR as a tool for eliciting and measuring oculomotor responses. However, our study was limited by the significant heterogeneity in our patient characteristics, with the time since injury differing across participants by as much as a decade. Therefore, future research should focus on testing acute and subacute mTBI participants to determine whether vergence deficits can be detected in VR among a more tightly controlled patient population.

# CHAPTER 4

# EFFECT OF A HISTORY OF CONCUSSIONS ON THE ACCOMMODATION RESPONSE IN VIRTUAL REALITY

## Introduction

Impairments of the accommodation response are commonly found in patients following a concussion and may persist across the subacute, acute, and chronic recovery periods (Fraser & Mobbs, 2021; Green et al., 2010; Master et al., 2016; Raghuram et al., 2021) Accommodative insufficiency, or difficulty focusing at nearby targets due to impairments in the focal lens' ability appropriately change its shape, is perhaps the most studied accommodative dysfunction associated with a mild traumatic brain injury (mTBI) (Fraser & Mobbs, 2021; Green et al., 2010; Master et al., 2016; Raghuram et al., 2021). As many as 51-65% of patients may experience accommodative insufficiency, with a significant portion of diagnoses persisting into the chronic recovery period despite improvements in other aspects of the visual system (Green et al., 2010). Accommodation spasm (pseudomyopia) when switching from near to far targets and accommodation infacility (a slowed accommodative response to changes in target distance) can also present following a mTBI and may persist for years following an injury (Green et al., 2010; Raghuram et al., 2021). These deficits contribute to numerous difficulties with activity daily, limiting patients' ability to read, drive, use a computer, and perform a variety of other tasks (Fraser & Mobbs, 2021; Green et al., 2010; Master et al., 2016). Altogether, one or more accommodative deficits may be present in as many as 84% of concussion patients across the subacute and chronic recovery periods, highlighting the importance of being able to identify deficits following a mTBI accurately, efficiently, and without significant financial burden (Raghuram et al., 2021).

There are currently major challenges measuring accommodation in clinical populations, especially for lower-income communities, those who have difficulty accessing medical facilities, and the elderly (Rubio et al., 2019). Objective accommodation testing is frequently done using an autorefractor, which assesses the refraction of the eye when changing focus from a far to a near target to determine accommodative amplitude (Aldaba et al., 2015; Win-Hall et al., 2007). While autorefractors are quick and reliable, these devices are prohibitively expensive, typically costing several thousand dollars, and rarely found outside of medical or research facilities (Goss & Grosvenor, 1996). A much more economical and portable solution is the clinical assessment of accommodative response. Techniques such as the push-up and pull-away tests are commonly used, which require participants to track a moving stimulus at set distances and report when the target blurs or is detected (Koslowe et al., 2010). While these tests can be effective for assessing accommodation in certain contexts, their validity and reliability are limited due to relying on subjective reporting from patients. For instance, compared to objective methods, subjective methods may over-estimate accommodative amplitude (Wold et al., 2003). Therefore, there are ongoing efforts to develop new methods for measuring accommodation that are affordable and portable, while maintaining validity and reliability (Rubio et al., 2019).

To develop new assessment tools, research focused on utilizing virtual reality technology (VR) to measure the accommodative response has gained recent attention (Koulieris et al., 2017). An important consideration when using VR is the vergence-accommodation conflict, in which the visual system has difficulty adapting to the conflicting stimuli of the stereoscopic image presented on a screen relatively close to the eyes, while the object appears to be distant in the virtual space (Hoffman et al., 2008; Kramida, 2016; Paulus et al., 2017; Zabels et al., 2019). Despite this conflict, there is evidence that the accommodation response still occurs in VR (Kramida, 2016). Importantly, while the accommodation response is impacted and reduced in VR environments, the stimulus-response pattern appears to match that of real-world environments (Koulieris et al., 2017).

The preservation of an accurate, albeit reduced, accommodation response suggests that there is potential to measure the integrity of this system in clinical populations using VR (Koulieris et al., 2017). Currently, this represents a massive gap in the literature, with most of the research focusing on the negative effects of the convergence-accommodation conflict in virtual environments (Hoffman et al., 2008; Kramida, 2016; Paulus et al., 2017). Therefore, understanding how traumatic brain injury impacts the accommodation response in a VR head-mounted display (HMD) presents a unique opportunity to assess the oculomotor deficits associated with concussions. To our knowledge, this has yet to be investigated and could offer a new avenue for developing affordable and portable assessments of the visual and oculomotor systems.

## Methods

### Participants

Forty-five young adults gave informed consent to participate in this study approved by Temple University's Institutional Review Board (IRB). Participants were recruited through flyers posted on Temple University's main campus, as well as emails sent throughout the College of Public Health. For their time, participants were compensated with a $25 Amazon gift card. The study was conducted within the Motion-Action-Perception Laboratory (MAP Lab), at Temple University, Philadelphia, PA.

### ***Eligibility***

Participants in the Concussion History Group were defined as having one or more diagnosed concussions in their lifetime, while the Healthy Control Group had to have never been diagnosed or suspected of having experienced a concussion. Inclusion criteria were as follows: 1) Age between 18-35, 2) Normal or corrected to normal vision (binocular visual acuity of 20/40 or better), 3) No history of oculomotor or vision disorders unrelated to a concussion (e.g., strabismus), 4) No history of neurological disorders unrelated to a concussion (e.g., Parkinson’s disease), 5) Participants must avoid alcohol and drug use 24 hours before testing. Participants completed a medical history form to determine concussion status, the presence of symptoms, and potential neurological and visual disorders before testing. A short visual assessment using a Tumbling E chart was conducted to verify visual acuity fell within normal ranges for the right and left eyes, as well as binocularly.

## Procedures

### ***Instrumentation***

Virtual Reality Accommodation Tests (VR_AT): The VR_AT was presented through a wired HTC Vive HMD (HTC Corporation, Taoyuan City, Taiwan) that was fitted with a SensoMotoric Instruments (SMI) video-oculography (VOG) system (SensoMotoric Instruments, Teltow, Germany). The HMD uses a 110° diagonal field of view and 89.6 Hz refresh rate to stream the VR environment through the Unity Real-Time Development Platform (Unity Technologies, San Francisco, US).

### ***Experimental Protocol***

#### Clinical Visual Assessment

Participant's visual acuity was measured using a standard tumbling E chart at a distance of 2 meters. The right and left eyes were assessed separately by having participants cover one eye and verbally indicate the direction the letter E was facing. Participants started from the top of the chart and read down line by line until errors were made and they were unable to meaningfully read or guess the direction of the E (Camparini et al., 2001). The procedure was then repeated for both eyes to establish binocular visual acuity.

Near visual acuity was measured using the same accommodative slide rule. Participants held the card holder at a fixed distance of 40 cm and read down the reduced Snellen chart line by line until errors were made or they were unable to meaningfully continue (Holladay, 1997).

#### VR_AT

The scene, target, and manipulation were chosen based on prior research which showed reduced, but present, accommodation in VR (Koulieris et al., 2017). Importantly, using a cue-rich environment has been shown to significantly improve depth constancy in a near-perfect manner compared to a cue-poor environment (Vienne et al., 2020). Further, blur has been well-established as the primary driver of the accommodative response, with binocular disparity acting as a secondary driver through the cross-coupling of the vergence and accommodation systems (Cumming & Judge, 1984; Judge & Cumming, 1986). Therefore, blur and binocular disparity (via target distance) were selected as the cues that were manipulated in this task.

Participants were seated at a comfortable height with their chin placed on a headrest to control for head position. After being fitted with the HTC Vive, a standard five-point calibration and validation procedure was conducted through SMI's internal software, which ensured that the gaze angle was within 1° tolerance. Participants view a black 20 cm Maltese cross presented at 40 cm for 1 second followed by a second Maltese cross at 75 cm presented for 1 second in a complex forest scene in multiple bocks of 5 trials (Figure 29).

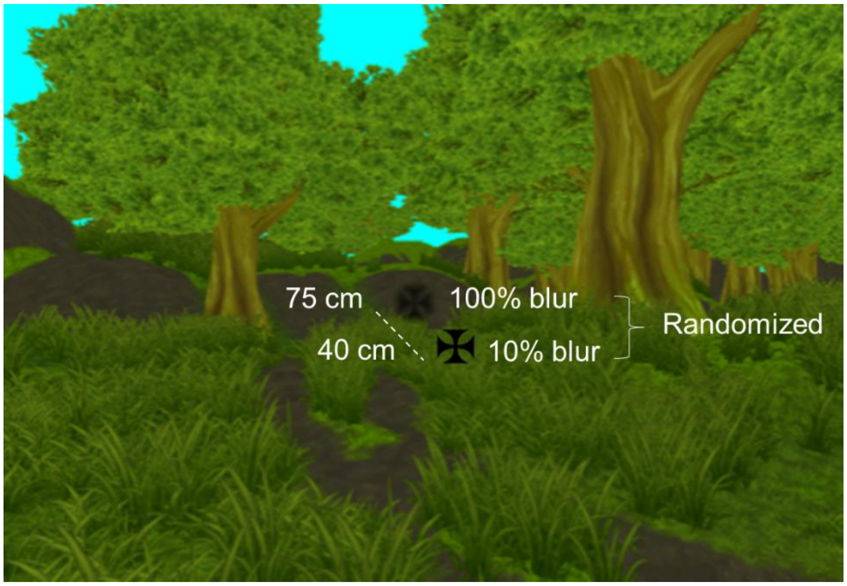


Figure 29. VR_AT testing environment and task example.

Participants are asked to judge which target has greater clarity using a Bluetooth button press device by using the bottom button to indicate target 1 (closer) and the top button to indicate target 2 (further). A standard psychometric technique, the staircase method, was used throughout each phase to manipulate difficulty based on participant responses (Treutwein, 1995). Specific adjustments to correct and incorrect responses are outlined in each phase. This test consisted of 3 sequential phases:

Phase 1: Target blur is manipulated using Unity's blur filter. In each trial one target is randomly set to 100% blur (constant target) and the other is initially set to 0% blur (variable target). If participants pass a block (4 out of 5 correct), blur in the variable target increases by 10% (e.g., goes from 0% blur to 10%). If a participant fails a block (3 out of 5 or less correct) blur in the variable target decreases by 5% (e.g., goes from 10% blur to 5%). Phase 1 ends when participants fail two blocks in a row, or if they follow a fail/pass/fail pattern. However, if participants pass two blocks of trials following a failed block, they can continue until they meet the failure criteria previously outlined. The best blur percentage achieved in the variable target is recorded.

Phase 2: Target distance is manipulated with blur held constant. Blur in the constant target (100%) and variable target (best % achieved from phase 1 – 10%) are randomly assigned in each trial to targets initially presented at 40 cm and 75 cm. If a participant passes a block, both targets are moved 10 cm closer to the participant. If a participant fails a block, both targets are moved 5 cm away from the participant. The distance between both targets (35 cm) is fixed throughout Phase 2. Phase 2 ends using the same criteria as phase 1, and the closest distance a participant achieves is recorded.

Phase 3: Blur manipulation at the closest distance. Targets are set at the closest (i.e., best) distance achieved in Phase 2, and the variable target's blur is initially set to the best blur % achieved in Phase 1. Blur increases by 10% when participants pass a block and decreases by 5% when participants fail a block. The test ends using the same criteria as Phases 1 and 2, and the final target blur percentage is recorded.

Participants were tested both binocularly and monocularly using the dominant eye to assess whether the accommodative response behaved more similarly to that of naturalistic vision in a virtual environment when binocular disparity is present, as research has shown that accommodative ability is reduced when the ability to converge on a target is removed (Campbell & Westheimer, 1960; Cumming & Judge, 1984; Koulieris et al., 2017). Therefore, we hypothesized that if performance was greater during binocular viewing, it would suggest participants' accommodative ability was preserved to a greater extent, as would be the case in the physical world.

### ***Outcome Measures***

Due to the inability to directly measure the accommodative response without a device such as an autorefractor, accommodation was inferred through behavioral measures which were predicted to reflect changes in optical power based on well-established clinical assessments (Glasser, 2006; Goss, 1992). Dependent variables were as follows: 1) Reaction time (time from the appearance of the second target to a button press), 2) Accuracy (percentage of correct trials, out of 5, in a given block), 3) Blur Percentage (the highest % of the blur filter that a participant was able to distinguish between with at-least 80% accuracy), 4) Target Distance (the closest target distance that a participant was able to distinguish between target 1 and target 2 with at-least 80% accuracy) (Figure 30). For the clinical visual assessment, standard outcome measures were used. Visual acuity was recorded as Snellen Score (e.g., 20/20) and then converted to LogMAR for statistical comparison (Holladay, 1997).

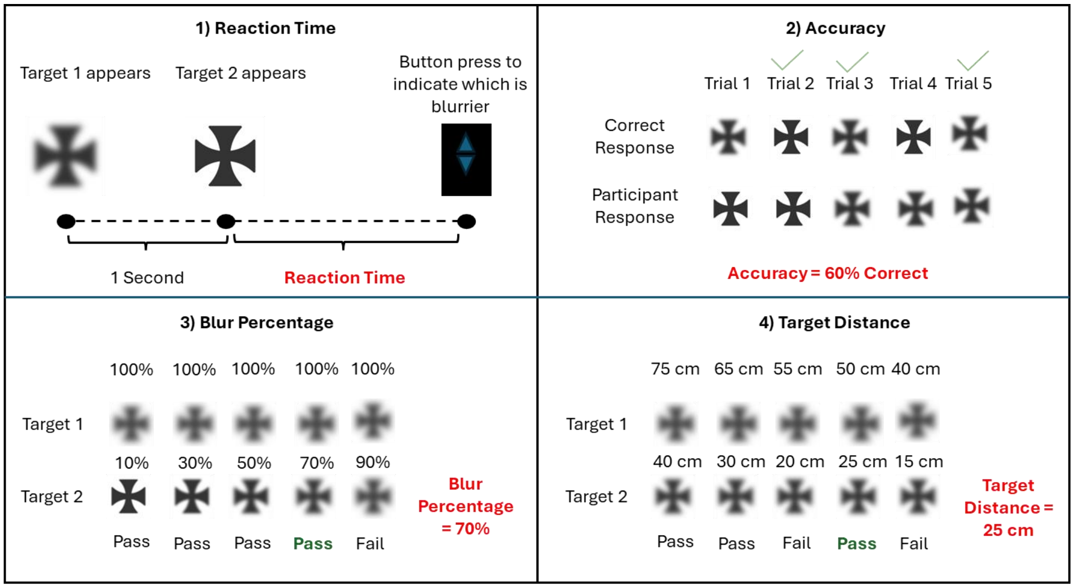


Figure 30. Diagram illustrating the VR_AT outcome measures

## Power Analysis

Our sample size was powered based on our Primary Aim 1 for several reasons. First, the research on accommodative testing in VR has traditionally focused on the feasibility of assessing oculomotor behavior in a virtual environment among healthy participants (Edgar, 2007; Hoffman et al., 2008; Kramida, 2016; Paulus et al., 2017). To our knowledge, this is the first study to investigate accommodation in virtual reality among participants with a history of concussions, making it challenging to determine an appropriate sample size based on prior research. Second, the research that currently exists on accommodative ability in VR has typically used smaller sample sizes, with sometimes as few as 5 participants (Edgar, 2007; Hoffman et al., 2008; Kramida, 2016; Paulus et al., 2017). While this has been sufficient for understanding how manipulations in VR impact accommodation, it may be less appropriate for our heterogeneous sample of both healthy participants and those with a history of concussions. Finally, our

Primary Aim 1 was focused on assessing the pupillary light reflex (PLR) in mTBI participants, which is a well-established methodology for assessing the oculomotor system following a concussion (Thiagarajan & Ciuffreda, 2015; Urosevich & Capo-Aponte, 2013). Therefore, using the PLR assessment to power our accommodation test would likely give a much more reliable estimate of an appropriate sample size compared to the reverse. As such, a total sample size of 40 (20 healthy control; 20 concussion history) was targeted.

## Statistical Analysis

Data was first processed within the custom user interface within Unity Real-Time Development Platform (Unity Technologies, San Francisco, US) to calculate and extract dependent variables for the binocular and monocular assessments (see Outcome Measures). The output from Unity was then imported into a custom script within MATLAB (MathWorks, Portola Valley, CA) and followed a standardized pipeline for managing the behavioral data. This process calculated the average reaction time and accuracy across trials and extracted the best blur percentage and blur distance achieved across each phase.

For the accommodation tests, a mixed model repeated measures ANOVA was used to address Primary Aim 3 and Secondary Aim 3. This allowed us to detect the presence of between-group differences (concussion status), within-group differences (phases of the assessment), and interaction effects. An alpha level of 0.05 was set for the main effects. If significant main effects were found, follow-up post-hoc analysis was used with Bonferroni-adjusted alpha levels to account for multiple comparisons.

Analysis was conducted for each accommodation DV listed above separately for the binocular and monocular assessments.

To address our Tertiary Aim 3, a mixed model repeated measures ANOVA was also used to determine whether there were differences across variables based on vision (binocular vs. monocular) and concussion status. For this analysis, an average value was calculated across each phase for the binocular and monocular assessments and then used for comparison.

To address Tertiary Aim 1, our statistical approach was repeated with the Concussion History Group subdivided into a Single Concussion Group (1 diagnosed concussion) and a Multiple Concussion Group (2 or more diagnosed concussions). For the concussion history subgroup analysis, significant between-group differences and subgroup x condition interaction effects were reported if detected. To determine the relationship between time since injury and performance on the VR_AT metrics, multiple regression analyses were used.

## Results

### Demographics

For the VR_AT experiment, two participants had to be removed from the original sample of forty-five due to data loss. Therefore, forty-three participants (24 females; 19 males; 26.00 ± 4.14 years [18-35 years]) completed the study. Independent samples t-tests did not show any significant differences in age between the Concussion History and Healthy Control Groups. A chi-squared test did not find significant differences in gender between both groups. However, a significant difference in gender when the groups were subdivided into the Healthy Control, One Concussion, and Multiple Concussion Groups was found ($\chi^2$(2, 43) =7.45, p=0.024). The number of concussions reported by the Concussion History Group ranged from 1 to 4 (1.75 ± 0.94 concussions) and the time since their most recent injury ranged from 0.75 to 20 years (6.56 ± 4.33 years) (Table 15). Self-reported medical history did not reveal ongoing symptoms across our Concussion History Group as a result of mTBI.

Table 15. Demographic and clinical characteristics of participants – VR_AT. (n = 43)

| **Variable** | **Healthy Control (n = 19)** | **Concussion History (n = 24)** | **p** |
|---|---|---|---|
| Age (yrs) | 25.47 ± 3.41 | 26.42 ± 4.67 | 0.465 |
| Male (%) | 4 (21%) | 11 (46%) | 0.090 |
| Female (%) | 15 (79%) | 13 (54%) | |
| Number of Concussions | 0 | 1.75 ± 0.94 | |
| Time Since Injury (yrs) | 25.47 ± 3.41 | 6.56 ± 4.33 | **< 0.001**** |

Age, number of concussions, and time since injury (mean ± standard deviation); sex (frequency). ** Indicates $p < 0.001$.

Table 16. Demographic and clinical characteristics of participants by number of concussions – VR_AT. (n = 43)

| **Variable** | **Healthy Control (n = 19)** | **Single Concussion (n = 12)** | **Multiple Concussion (n = 12)** | **p** |
|---|---|---|---|---|
| Age (yrs) | 25.47 ± 3.41 | 25.67 ± 4.18 | 27.16 ± 5.18 | 0.631 |
| Male (%) | 4 (21%) | 3 (25%) | 8 (66.6%) | **0.024*** |
| Female (%) | 15 (79%) | 9 (75%) | 4 (33.3%) | |
| Number of Concussions | 0 | 1 | 2.50 ± 0.80 | |
| Time Since Injury (yrs) | 25.47 ± 3.41 | 7.75 ± 4.43 | 5.38 ± 4.06 | **< 0.001**** |

Age, number of concussions, and time since injury (mean ± standard deviation); sex (frequency). ** Indicates $p < 0.001$.

## Clinical Visual Assessment

Independent samples t-tests did not show any significant differences in visual acuity or near visual acuity between the concussion history and Healthy Control Groups (Table 17). For the concussion history subgroup analysis, a one-way ANOVA did not show any significant differences between the Healthy Control, Single Concussion, or Multiple Concussion Groups for visual acuity or near visual acuity (Table 18).

Table 17. Clinical visual assessment results – VR AT.

| **Variable** | **Healthy Control (n = 19)** | **Concussion History (n = 24)** | **p** |
|---|---|---|---|
| Right Visual Acuity (LogMar) | 0.13 ± 0.16 | 0.15 ± 0.12 | 0.505 |
| Left Visual Acuity (LogMar) | 0.10 ± 0.10 | 0.13 ± 0.11 | 0.437 |
| Binocular Visual Acuity (LogMar) | 0.05 ± 0.10 | 0.07 ± 0.09 | 0.511 |
| Binocular Near Visual Acuity (LogMar) | 0.10 ± 0.09 | 0.10 ± 0.10 | 0.308 |
| Mean NPC Subjective Break Point (cm) | 4.38 ± 2.31 | 5.47 ± 2.66 | 0.174 |

Reported as mean ± standard deviation.

Table 18. Clinical visual assessment results by number of concussions – VR_AT.

| **Variable** | **Healthy Control (n = 19)** | **Single Concussion (n = 12)** | **Multiple Concussion (n = 12)** | **p** |
|---|---|---|---|---|
| Right Visual Acuity (LogMar) | 0.13 ± 0.16 | 0.15 ± 0.13 | 0.16 ± 0.12 | 0.791 |
| Left Visual Acuity (LogMar) | 0.10 ± 0.10 | 0.11 ± 0.11 | 0.14 ± 0.11 | 0.537 |
| Binocular Visual Acuity (LogMar) | 0.05 ± 0.10 | 0.07 ± 0.09 | 0.07 ± 0.10 | 0.808 |
| Binocular Near Visual Acuity (LogMar) | 0.10 ± 0.09 | 0.13 ± 0.10 | 0.14± 0.11 | 0.579 |
| Mean NPC Subjective Break Point (cm) | 4.38 ± 2.31 | 6.68 ± 2.74 | 4.25 ± 2.24 | **0.024*** |

Reported as mean ± standard deviation.

## Binocular VR_AT

### *Reaction Time*

Mauchly's Test of Sphericity revealed that the assumption of sphericity had been violated ($\chi^2(2) = 14.54$, p = 0.001). A repeated measures ANOVA with a Greenhouse-Geisser adjustment showed a trend towards significance for phase (F (1.53, 62.85) = 2.87, p = 0.078). No significant between-group differences or interaction effects were found for reaction time.

### *Accuracy*

Mauchly's Test of Sphericity revealed that the assumption of sphericity had been violated ($\chi^2(2) = 6.00$, $p = 0.05$). A repeated measures ANOVA with a Greenhouse-Geisser adjustment showed a significant main effect for phase (F (1.76, 71.97) = 67.27, $p < 0.001$) A follow-up post-hoc analysis with Bonferroni adjusted alpha levels revealed that accuracy was significantly higher in phase 1 (M = 0.86, SEM = 0.01) compared to phase 2 (M = 0.66, SEM = 0.02, $p < 0.001$), and phase 3 (M = 0.58, SEM = 0.02, $p < 0.001$). Accuracy was also found to be significantly higher in phase 2 compared to phase 3 ($p = 0.017$). No significant between-group differences or interaction effects were found for accuracy.

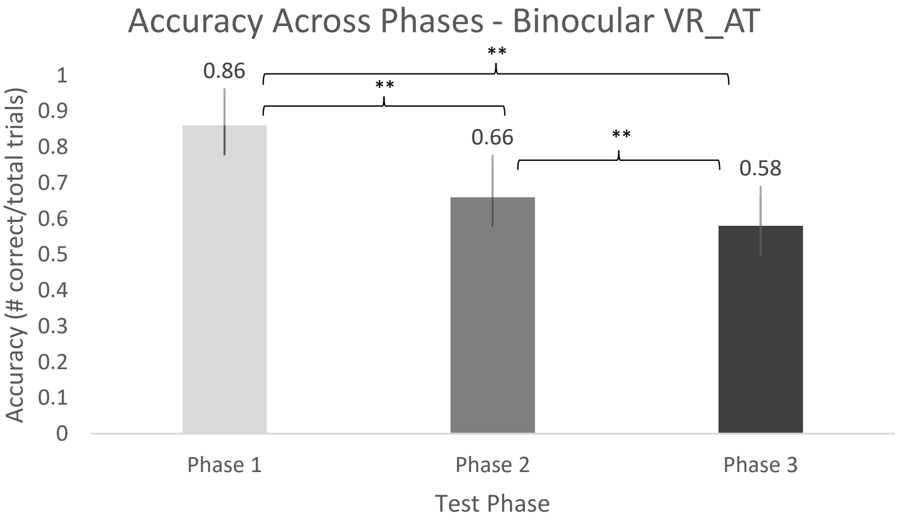


Figure 31. <u>Accuracy differences between test phases in the Binocular VR_AT condition.</u> ** Indicates $p < 0.001$.

### *Blur Percentage*

A repeated measures ANOVA showed a significant main effect for phase (F (2, 82) = 91.78, p < 0.001). A follow-up posthoc analysis with Bonferroni adjusted alpha levels revealed that the best blur percentage achieved was significantly higher in phase 1 (M = 0.69, SEM = 0.02) compared to phase 2 (M = 0.59, SEM = 0.02, p < 0.001) and phase 3 (M = 0.63, SEM = 0.02, p < 0.001).

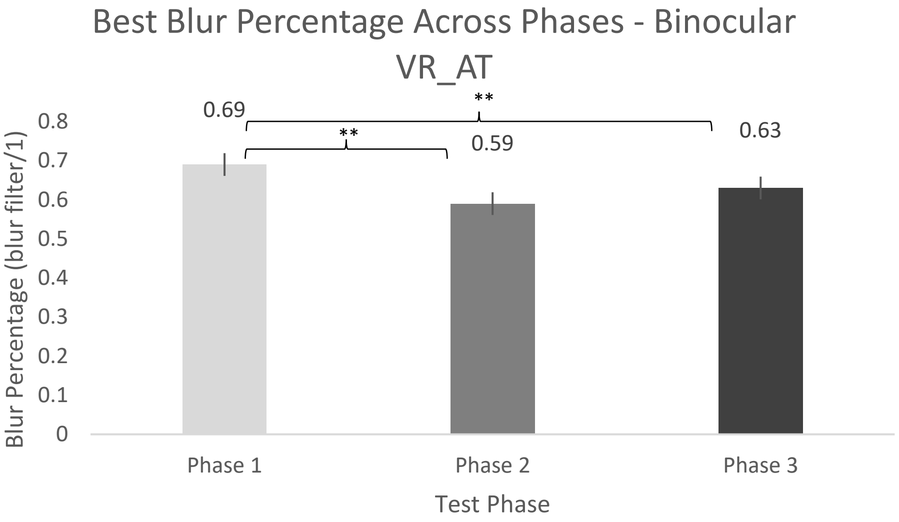


Figure 32. <u>Best blur percentage differences between test phases in the Binocular VR_AT condition.</u> ** Indicates p < 0.001.

A significant interaction between phase and concussion status was also found (F = (2, 82) = 3.15, p = 0.048). A follow-up post-hoc analysis with Bonferroni adjusted alpha levels did not reveal significant differences between the healthy control and concussion groups at any phase. No other significant effects were found for blur percentage.

### *Target Distance*

A repeated measures ANOVA showed a significant main effect for phase (F (1, 41) = 49.16, $p < 0.001$). A follow-up post-hoc analysis revealed that the closest target distance achieved in phase 2 (M = 28.37, SEM = 1.67) was significantly closer than in phase 1 (M = 40.00, SEM = 0.00, $p < 0.001$). No significant between-group differences or interaction effects were found for target distance.

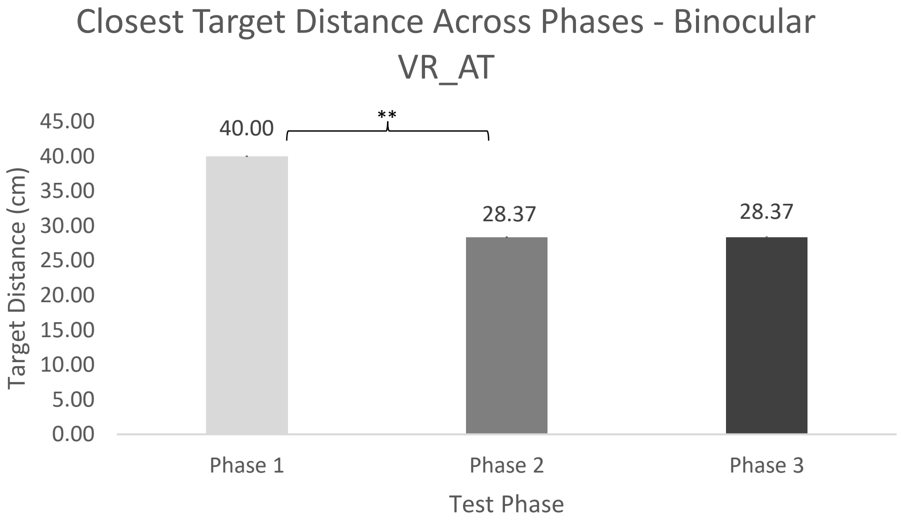


Figure 33. Closest target distance differences between test phases in the Binocular VR_AT condition. ** Indicates $p < 0.001$.

## Binocular VR_AT Concussion History Subgroup Analysis

No additional significant within-group differences, between-group differences, or interaction effects were found across the binocular VR_AT variables for the concussion history subgroup analysis.

## Binocular VR_AT Time-Since-Injury Analysis

The multiple regression analysis revealed that time since injury did not predict task performance across the Binocular VR_AT variables.

Table 19. Binocular VR_AT Variables - results across phases by participant status.

| Binocular | Healthy Control | | | Concussion History | | |
|---|---|---|---|---|---|---|
| Variable | Phase 1 | Phase 2 | Phase 3 | Phase 1 | Phase 2 | Phase 3 |
| **Reaction Time (seconds)** | 1.03 ± 0.50 | 0.94 ± 0.26 | 0.96 ± 0.27 | 1.08 ± 0.45 | 0.92 ± 0.27 | 0.98 ± 0.44 |
| **Accuracy (# correct/total trials)** | 0.85 ± 0.03 | 0.68 ± 0.14 | 0.58 ± 0.11 | 0.86 ± 0.04 | 0.64 ± 0.15 | 0.57 ± 0.14 |
| **Blur Percentage (blur filter/1)** | 0.69 ± 0.16 | 0.59 ± 0.16 | 0.65 ± 0.16 | 0.69 ± 0.11 | 0.59 ± 0.11 | 0.61 ± 0.12 |
| **Target Distance (cm)** | 40.00 ± 0.00 | 27.37 ± 11.35 | 27.37 ± 11.35 | 40.00 ± 0.00 | 29.37 ± 10.35 | 29.37 ± 10.35 |

Reported as mean ± standard deviation.

## Monocular VR_AT

### *Reaction Time*

Mauchly's Test of Sphericity revealed that the assumption of sphericity had been violated ($\chi^2(2) = 53.49$, $p < 0.001$). A repeated measures ANOVA with a Greenhouse-Geisser adjustment showed a significant main effect for phase (F (1.15, 47.19) = 4.39, p = 0.036). A follow-up post-hoc analysis with Bonferroni adjusted alpha levels revealed a trend towards phase 1 (M = 1.01, SEM = 0.11) being significantly larger than phase 3 (M = 0.87, SEM = 0.05, p = 0.063). No significant between-group differences or interaction effects were found for reaction time.

### *Accuracy*

Mauchly's Test of Sphericity revealed that the assumption of sphericity had been violated ($\chi^2(2) = 7.33$, $p = 0.026$). A repeated measures ANOVA with a Greenhouse-Geisser adjustment showed a significant main effect for phase (F (1.71, 70.24) = 84.86, p

< 0.001). A follow-up post-hoc analysis with Bonferroni adjusted alpha levels revealed that accuracy was significantly higher in phase 1 (M = 0.86, SEM = 0.01) compared to phase 2 (M = 0.75, SEM = 0.02, p < 0.001), and phase 3 (M = 0.59, SEM = 0.02, p < 0.001). Accuracy was also found to be significantly higher in phase 2 compared to phase 3 (p <0.001).

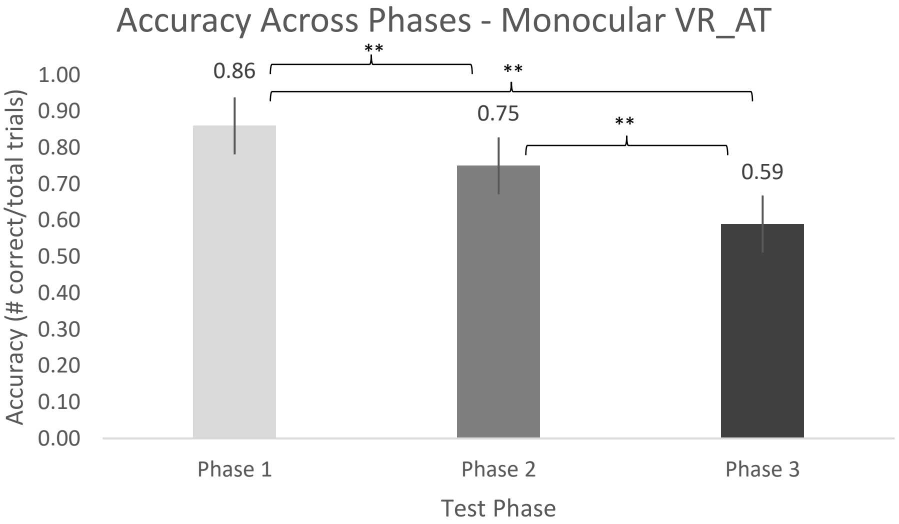


Figure 34. <u>Accuracy differences between test phases in the Monocular VR_AT condition.</u>
** Indicates p < 0.001.

A significant interaction between phase and concussion status was also found (F (1.71, 70.24) = 4.60, p = 0.017). A follow-up post-hoc analysis with Bonferroni adjusted alpha levels revealed that accuracy was significantly lower in the Healthy Control Group (M = 0.55, SEM = 0.03) compared to the Concussion History Group (M = 0.62, SEM = 0.02, p = 0.042) in phase 3. No other significant effects were found for accuracy.

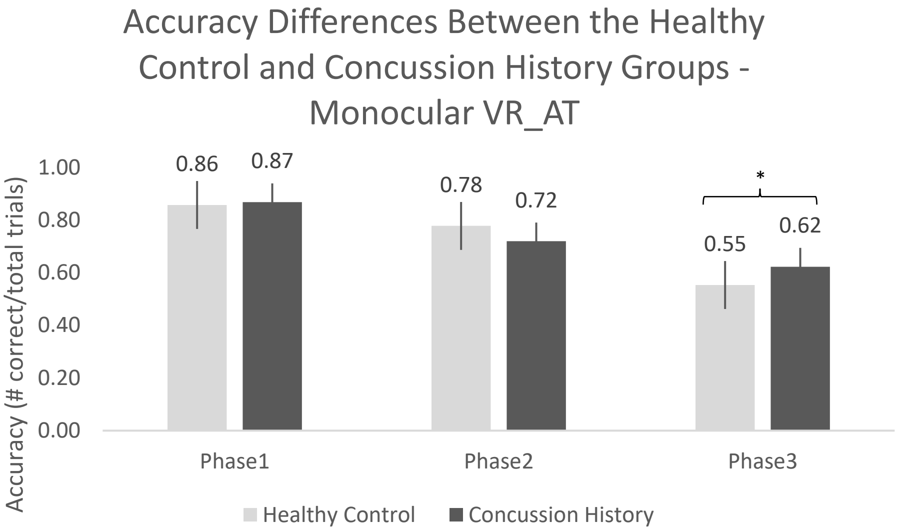


Figure 35. Accuracy differences between the Healthy Control and Concussion History groups across phases in the Monocular VR_AT condition. * Indicates $p < 0.05$.

### *Blur Percentage*

A repeated measures ANOVA showed a significant main effect for phase (F (2, 82) = 40.25, $p < 0.001$). A follow-up posthoc analysis with Bonferroni adjusted alpha levels revealed that the best blur percentage achieved was significantly higher in phase 1 (M = 0.70, SEM = 0.02) compared to phase 2 (M = 0.60, SEM = 0.02, $p < 0.001$) and phase 3 (M = 0.66, SEM = 0.02, $p = 0.025$).

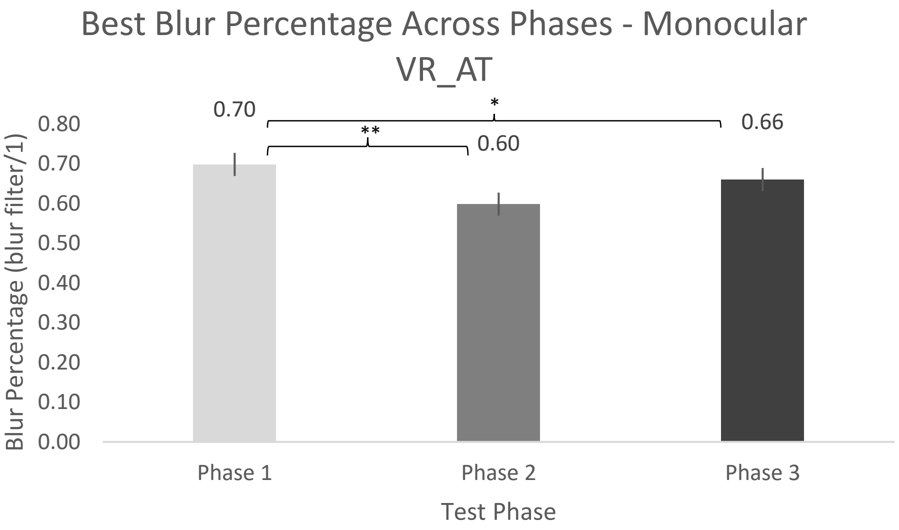


Figure 36. Best blur percentage differences between phases in the Monocular VR_AT condition. * Indicates $p < 0.05$, ** indicates $p < 0.001$.

A significant interaction between phase and concussion status was also found (F (2, 82) = 4.04, p = 0.021). A follow-up post-hoc analysis with Bonferroni adjusted alpha levels did not reveal significant differences between the healthy control and concussion groups at any phase. No other significant effects were found for blur percentage.

***Target Distance***

A repeated measures ANOVA showed a significant main effect for phase (F (1, 41) = 140.51, $p < 0.001$). A follow-up post-hoc analysis with Bonferroni adjusted alpha levels revealed that the best target distance achieved in phase 2 (M = 22.05, SEM = 1.51) was significantly closer than in phase 1 (M = 40.00, SEM = 0.00, $p < 0.001$).

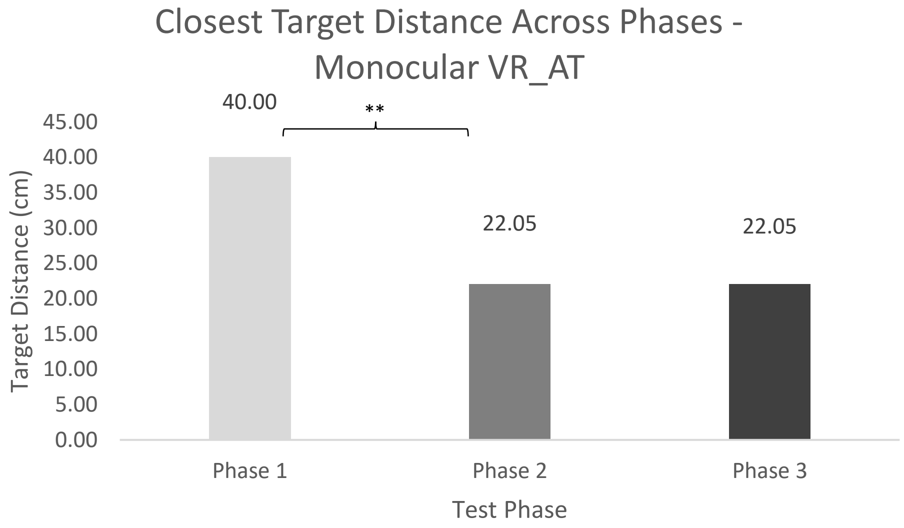


Figure 37. Closest target distance differences between phases in the Monocular VR_AT condition. ** Indicates p < 0.001.

A significant interaction between phase and concussion status was also found (F (1, 41) = 4.94, p = 0.032). A follow-up post-hoc analysis with Bonferroni adjusted alpha levels revealed that the closest target distance achieved by the Healthy Control Group (M = 18.68, SEM = 2.26) was significantly closer compared to the Concussion History Group (M = 25.42, SEM = 2.01, p = 0.032) in phase 2 and 3. No other significant effects were found for target distance.

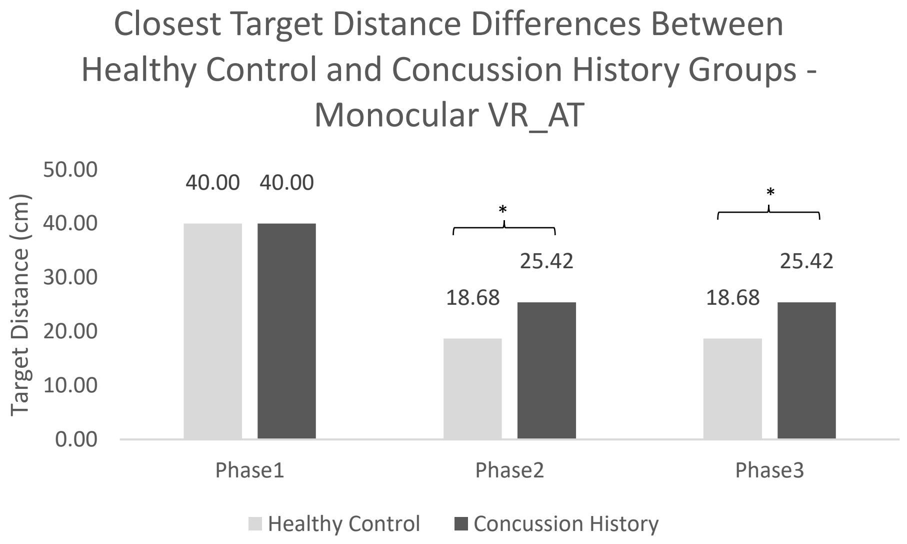


Figure 38. Closest target distance differences between the Healthy Control and Concussion History groups across phases in the Monocular VR_AT condition. * Indicates $p < 0.05$.

## Monocular VR_AT Concussion History Subgroup Analysis

### ***Target Distance***

A repeated measures ANOVA showed a significant main effect for the concussion history subgroup (F (2, 40) = 3.62, p = 0.036). A follow-up post-hoc analysis with Bonferroni adjusted alpha levels revealed that the closest target distance achieved by the Healthy Control Group (M = 25.79, SEM = 1.49) was significantly closer compared to the Single Concussion Group (M = 32.22, SEM = 1.87, p = 0.031).

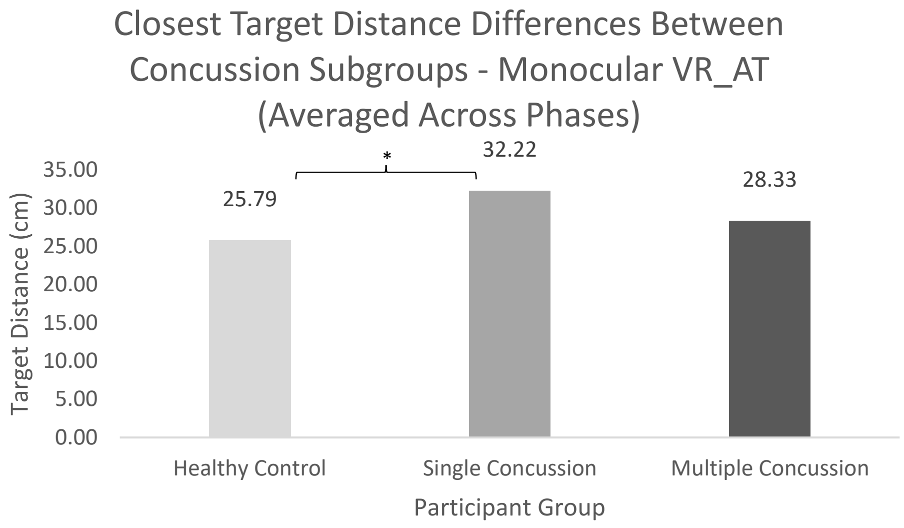


Figure 39. Closest target distance differences across phases between concussion subgroups in the Monocular VR_AT condition. * Indicates $p < 0.05$.

No additional significant within-group differences, between-group differences, or interaction effects were found across the monocular VR_AT variables for the concussion history subgroup analysis.

## Monocular VR_AT Time-Since-Injury Analysis

### ***Accuracy***

A multiple regression found that time since injury significantly explained the variance in accuracy across participants in Phase 3 of the Monocular VR_AT test (F (1, 43) = 5.73, $p < 0.021$, $R^2 = 0.12$). The regression coefficient (B = -0.004) indicated that an increase in 1 year since a concussion decreased accuracy, on average, by 0.4%. The multiple regression analysis revealed that time since injury did not predict task performance across any other Monocular VR_AT variables.

### *Age*

A follow-up multivariate regression analysis using age and time since injury as independent variables found that age was not a significant predictor of accuracy but did trend toward significance in Phase 3 (F (1, 43) = 3.83, p = 0.057).

## Binocular VS Monocular Analysis

### *Reaction Time*

No significant within-group differences between-group differences, or interaction effects were found for reaction time.

### *Accuracy*

A repeated measures ANOVA showed a significant main effect for vision (F (1, 41) = 5.61, p = 0.023). Accuracy was found to be significantly larger in the Monocular VR_AT (M = 0.73, SEM = 0.01) compared to the Binocular VR_AT (M = 0.70, SEM = 0.01). No significant between-group differences or interaction effects were found for accuracy.

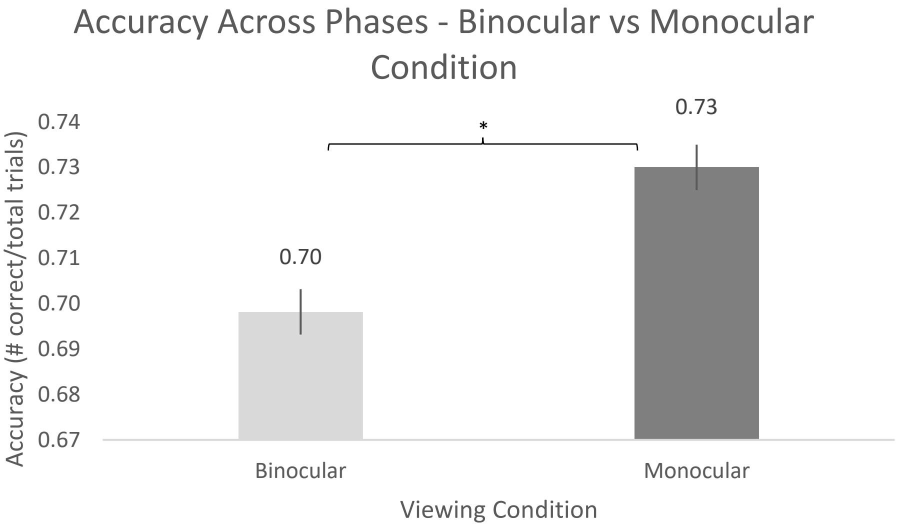


Figure 40. Accuracy differences between the Binocular and Monocular conditions in the VR_AT. * Indicates $p < 0.05$.

### *Blur Percentage*

No significant within-group differences between-group differences, or interaction effects were found for blur percentage.

### *Target Distance*

A repeated measures ANOVA showed a significant main effect for vision (F (1, 41) = 10.01, p = 0.003). The closest target distance was found to be significantly closer in the Monocular VR_AT (M = 28.68, SEM = 1.23) compared to the Binocular VR_AT (31.60, SEM = 1.09). No significant between-group differences or interaction effects were found for target distance.

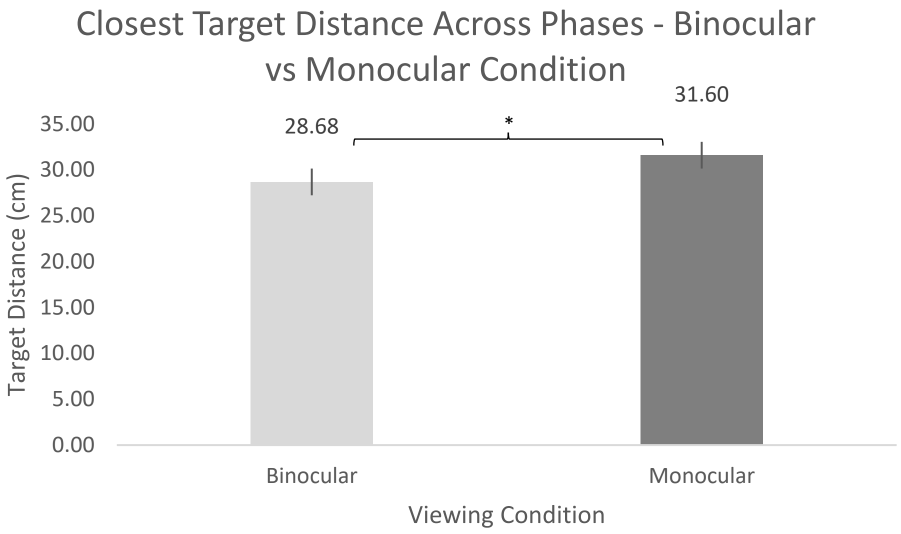


Figure 41. Closest target distance differences between the Binocular and Monocular conditions in the VR_AT. * Indicates $p < 0.05$.

Table 20. Monocular VR_AT Variables - results across phases by participant status.

| Monocular | Healthy Control | | | Concussion History | | |
|---|---|---|---|---|---|---|
| **Variable** | **Phase 1** | **Phase 2** | **Phase 3** | **Phase 1** | **Phase 2** | **Phase 3** |
| **Reaction Time (seconds)** | 1.00 ± 0.78 | 0.87 ± 0.39 | 0.81 ± 0.27 | 1.20 ± 0.66 | 0.97 ± 0.39 | 0.93 ± 0.35 |
| **Accuracy (# correct/total trials)** | 0.86 ± 0.02 | 0.78 ± 0.12 | 0.55 ± 0.12 | 0.87 ± 0.03 | 0.72 ± 0.16 | 0.62 ± 0.10 |
| **Blur Percentage (blur filter/1)** | 0.70 ± 0.11 | 0.60 ± 0.11 | 0.64 ± 0.13 | 0.69 ± 0.12 | 0.59 ± 0.12 | 0.68 ± 0.15 |
| **Target Distance (cm)** | 40.00 ± 0.00 | 18.68 ± 7.78 | 18.68 ± 7.78 | 40.00 ± 0.00 | 25.42 ± 11.22 | 25.42 ± 11.22 |

Reported as mean ± standard deviation.

## Discussion and Conclusion

This study investigated the feasibility of eliciting and testing visual accommodation in VR by manipulating blur cues, focus distance, and viewing conditions (binocular vs. monocular), and measuring participants' ability to distinguish between targets. Without technology such as an autorefractor, the goal of the VR_AT was to indirectly assess participants' accommodation by requiring them to detect differences between targets based on known cues that drive accommodation (Campbell &

Westheimer, 1959; Ward, 1987). We found evidence for performance differences across the three phases of VR_AT tests, with changes in accuracy, blur percentage, and target distance found for both binocular and monocular conditions. Notably, accuracy was worse in the Binocular VR_AT compared to the Monocular VR_AT and we found that participants tended to perform better during monocular viewing compared to binocular viewing. We also examined the impact of concussion history on accommodation response, inferred through our VR_AT assessment. We found that the closest distance that participants were able to accurately distinguish between targets was significantly closer in the Healthy Control Group compared to the Concussion History Group during monocular viewing conditions. However, we also found that healthy participants had worse accuracy in Phase 3 of the Monocular VR_AT. Lastly, our regression analysis found that accuracy decreased over time as patients recovered from their injuries.

It was hypothesized that target blur and distance changes would stimulate the accommodative response needed to accurately judge the difference between targets. Accuracy was found to differ across phases as the task became more difficult, with reductions in accuracy accompanying both targets moving closer in the visual field and reducing the difference in blur between targets. As distance and blur are well-established drivers of accommodation, our findings support that accommodation played a significant role in task performance (Cumming & Judge, 1984; Glasser, 2006; Mays & Gamlin, 1995; Myers & Stark, 1990). However, a major limitation to our VR-based assessment is that accommodation had to be inferred since an objective measure such as an autorefractor was not utilized. Therefore, the influence of vergence cannot be discounted as a driver of task performance during binocular testing, as shared pre-motor neural pathways connecting vergence and accommodation (i.e., the vergence-accommodation crosslink) produces synkinesis between both oculomotor responses (Cumming & Judge, 1984; Glasser, 2006; Mays & Gamlin, 1995; Myers & Stark, 1990).

Therefore, to help clarify the influence of vergence and accommodation on task performance, binocular and monocular test conditions were incorporated. The monocular condition minimized vergence by limiting disparity as a driver of the near response, which allowed us to estimate whether vergence or accommodation had a greater relative influence over the task (Cumming & Judge, 1984; Koulieris et al., 2017; Ward, 1987). In contrast, the inclusion of vergence during binocular viewing has been shown to increase accuracy and gain of accommodation in VR, which leads us to assume that performance would be improved during binocular testing (Koulieris et al., 2017). Contrary to our hypothesis, our results suggest that task performance, as measured through accuracy and the best (closest) target distance at which participants could effectively discriminate between targets, was superior in the Monocular VR_AT. Based on our current understanding of how disparity cues impact accommodation, removing disparity should lead to reduced accommodative response proportional to accommodative demand, which has also been documented in VR environments (Horwood & Riddell, 2008; Koulieris et al., 2017). Therefore, the attenuated accommodation ability during the monocular viewing condition should have led to a decreased ability to effectively distinguish the clarity of the two targets, which was not the case in our study.

One potential hypothesis is that the influence of the vergence-accommodation conflict during the binocular condition had a more powerful, negative impact on task performance compared to the monocular condition, which effectively erased any added benefit of having a disparity cue (Hoffman et al., 2008; Kramida, 2016; Paulus et al., 2017; Zabels et al., 2019). Prior research has found that inaccurate visual cues during VR viewing led to increased target identification time, depth estimation, and reduced stereo acuity, which aligns closely with the demands of our task (Hoffman et al., 2008). As such, despite producing a reduced accommodative response, participants were not forced to contend with incongruent blur and disparity signals as the eyes attempted to focus on the nearby display lens while the HMD created the perception of targets at different depths.

Our VR_AT test found some evidence that oculomotor performance was impacted by a history of concussions, but results were conflicting due to cases in which healthy control participants performed worse in certain aspects of the test. On one hand, the healthy control participants were able to distinguish between targets at closer distances during the monocular viewing conditions, supporting past research which shows that concussion patients frequently have difficulty tracking targets closer in the visual field (Cooper & Jamal, 2012; Duprey et al., 2017; Fraser & Mobbs, 2021; Kawata et al., 2016; Pearce et al., 2015). mTBI patients have been found to have receded NPC and reduced accommodative amplitude, which would make focusing on closer and closer targets challenging (Cooper & Jamal, 2012; Duprey et al., 2017; Fraser & Mobbs, 2021; Kawata et al., 2016; Pearce et al., 2015). In contrast, target accuracy was found to be significantly higher in the Concussion History Group during the Monocular VR_AT test, which contradicts the well-established negative effects of mTBI on the accommodation system and the previously stated finding (Fraser & Mobbs, 2021; Green et al., 2010; Raghuram et al., 2021).

Finally, our regression analysis found that accuracy in the Monocular VR_AT decreased by 4% for each year following a concussion, which would suggest that more recovery time would lead to decreased ability to distinguish between visual targets with differing degrees of blurriness. We attempted to rule out aging as a potential explanation for why accuracy would be reduced over time through a multivariate regression, which found that age was not a significant predictor of task performance. However, it should be noted that age did trend towards significance in Phase 3, where a significant, negative relationship between recovery time and accuracy was found. The impact of aging on accommodation is well established, with deficits in accommodative amplitude typically appearing among patients in their early forties (Breinin et al., 1973; Sun et al., 1988). While the average age of our participants was approximately 26, we did recruit up to the age of 35, which is when patients may begin experiencing lens hardening, weakening of ciliary muscles, and other factors that progress into presbyopia (Breinin et al., 1973; Sun et al., 1988). Notably, changes in accommodation, such as slowed dynamic accommodation and reduced accommodation amplitude, have been noted in subjects over the age of 30 and it is possible these could have been a confounding factor in our VR_AT test. Therefore, while our analysis suggests aging is not the source of declining accuracy over time, it may be worth investigating further to see if the negative relationship between accuracy and time since injury would disappear if a younger patient population was targeted (or if the relationship would be stronger in older individuals).

It is unclear why concussion participants would be more successful at distinguishing between two targets that varied in visual clarity, especially during monocular but not binocular viewing conditions. Prior research suggests that accuracy should be greater during binocular viewing, as accommodation and vergence responses would be stronger due to the preserved disparity cue between the right and left eye through the vergence-accommodation crosslink (Horwood & Riddell, 2008; Koulieris et al., 2017; Kramida, 2016; Zabels et al., 2019). Prior research has shown that concussions can produce changes in accommodative amplitude, velocity, and ratio of response to demand, as well as reduced performance in clinical assessments such as the push-up and pull-away tasks (Fraser & Mobbs, 2021; Green et al., 2010; Raghuram et al., 2021). Therefore, the findings do not align with our initial hypothesis that accuracy would be reduced due to impairments in accommodation that are likely to accompany mTBI.

One possible explanation is that the impact of concussions on the vergence and accommodation of neural pathways is more likely to impact structures after accommodation-convergence and convergence-accommodation (AC/CA) crosslinks (Bharadwaj et al., 2020; Mays & Gamlin, 1995; Myers & Stark, 1990; Searle & Rowe, 2016). This would mean that the fast vergence and accommodation controllers which integrate the primary signals for each reflex (disparity and blur respectively) remain intact or recover more quickly, which would explain why results in the binocular assessment are similar between groups (Bharadwaj et al., 2020; Mays & Gamlin, 1995; Myers & Stark, 1990; Searle & Rowe, 2016). However, if concussion patients have a damaged CA crosslink or tonic accommodation controller, which integrates the vergences signal further in the near reflex feedback loop, it could explain why target distance changes led to worse performance in that group. Recent evidence suggests that this exact scenario is possible in patients with spasms of the near reflex (SNR), where vergence and pupil responses remain intact, but evidence points to an affected tonic accommodation integrator (Bharadwaj et al., 2020). While our participants were not screened for SNR, it has been found in patients with a history of concussions and may persist for years after an injury occurs (Chan & Trobe, 2002; Knapp et al., 2002).

The lack of consistent differences due to concussion status in our study may be attributable to the heterogeneity in patient profiles, with time since injury ranging from 0.75 to 20 years. As we did not specifically recruit acute and subacute participants, the impact of mTBI on the accommodation system could have resolved over time, as many symptoms have been shown to resolve several days/weeks after injury (Cheever et al., 2018; Laker, 2011; Scorza & Cole, 2019). Further, while accommodation deficits have been studied in chronic mTBI patients, time since injury has generally been more tightly constrained than in our study (Wiecek et al., 2021). Another possibility is that a significant portion of our participants were simply never affected by accommodation deficits as a result of mTBI(s). While accommodative insufficiency is present in as many as 50% of concussion patients, our study was limited by not specifically targeting those with reported accommodation deficits, as is the case of certain studies (Fraser & Mobbs, 2021; Green et al., 2010; Master et al., 2016; Raghuram et al., 2021). Therefore, future research should aim to address these limitations by focusing on testing acute and subacute mTBI participants and those with reported accommodation deficits to investigate whether differences can be detected using VR technology in a more tightly controlled patient population with homogenous injury characteristics.

Despite being unable to find consistent differences between the control and the Concussion History Groups, our VR_AT test did find some evidence to support the ability of a VR assessment to elicit and indirectly infer visual accommodation. Namely, we found that task performance differed based on target blur and target distance across test phases and that there was an interaction of blur and distance which made the last testing conditioning more challenging. Blur is well established as the primary driver of visual accommodation, meaning that clarity of the visual target should produce a stimulus-dependent oculomotor response (Cambell && Westheimer, 1959; Cumming & Judge, 1984; Glasser, 2006; Ward, 1987). While we were unable to directly measure accommodative response, our findings support the ability of VR to recreate clinical tests, which use subjective judgments of moving targets that elicit a blur response (e.g., accommodative push-up test) (Fraser & Mobbs, 2021; Glasser, 2006; Goss, 1992; León et al., 2012).

In conclusion, our results suggest the ability of the VR_AT assessment to detect concussion-related deficits to accommodation is conflicting. However, our study shows promising results for the capability of VR HMDs to elicit and indirectly measure accommodative response through subjective judgments of targets in a VR environment. In part, this is not surprising given the well-established challenges eliciting and measuring accommodative response in VR due to vergence-accommodation conflict and our heterogenous participant profile (Hoffman et al., 2008; Koulieris et al., 2017; Kramida, 2016; Paulus et al., 2017). Therefore, future research should continue to explore the utility of accommodation testing in VR, especially as technology improves to allow for more naturalistic viewing conditions that address current limitations in HMDs.

# CHAPTER 5

# DISCUSSION

The results of our study suggest that VR HMDs can elicit and measure pupillary responses to light stimuli, vergence of the eyes, and infer accommodation based on behavioral responses. This novel approach to studying the oculomotor system supports the construct validity of HMDs for assessments of the near reflex. Across tests, we found that oculomotor responses predictably changed based on manipulations in the VR environment, often producing response patterns that matched expected outcomes based on prior studies using physical stimuli and matching our hypotheses based on past research of oculomotor behavior in the physical world. However, we only found limited support that outcomes significantly differed in participants with a history of concussions, especially among our heterogeneous patient population that varied based on time since injury and were asymptomatic. Notably, auditory pupil response, divergence fusion, and monocular accommodation testing appeared to be the most sensitive to potential deficits as a result of chronic mTBI. However, results were generally limited to a small number of outcome measures, and in some cases, findings were conflicting, with healthy participants performing worse in certain measures.

## Review of Specific Aims

The diagnosis, management, and treatment of oculomotor symptoms and disorders that accompany mTBI have become a major area of focus among clinicians who work with concussion patients. Vision disorders are common across concussion patients, with as many as half or more of all those diagnosed experiencing some form of visual or oculomotor dysfunction (Master et al., 2016; Ventura et al., 2016). Estimates suggest that over 1 million concussion cases occur each year and represent an economic burden of over 60 billion dollars (Humphreys et al., 2013; Langlois J A et al., 2004). Therefore, there is a significant need to develop valid and reliable concussion assessment tools to improve patient outcomes.

The near reflex triad represents a key function of the oculomotor system, which allows us to achieve and maintain focus on targets moving depth (Mays & Gamlin, 1995; Myers & Stark, 1990; Rucker et al., 2019). It consists of pupillary constriction, the medial rotation of the eyes to prevent binocular disparity (convergence), and a change in lens shape to increase power and keep an object in focus (accommodation) as a target moves closer in the visual field (Mays & Gamlin, 1995; Myers & Stark, 1990; Rucker et al., 2019). All aspects of the near reflex triad are well-established to be impacted by concussions, with pupillary light sensitivity, convergence insufficiency, and accommodative dysfunction, commonly reported symptoms of mTBI (Ciuffreda et al., 2017; Cooper & Jamal, 2012; Duprey et al., 2017; Green et al., 2010; Kawata et al., 2016; Podolak et al., 2019; Raghuram et al., 2021; Urosevich & Capo-Aponte, 2013). However, while individual components of the near reflex have been well-studied, there is currently no gold-standard comprehensive assessment. Further, clinical tools and assessments that are commonly used to assess the near reflex may present challenges due to prohibitive costs, lack of access to a trained professional, and lack of portability.

To address these concerns, this study was designed to assess the viability of a VR HMD with built-in VOG to elicit and measure each aspect of the near response. With the growing popularity of VR technology in popular culture, it presents a unique avenue for offering virtual medical care that is easily accessible to a variety of patient populations and locations across the globe. With concussion patients often facing physical, psychological, and financial challenges, VR has the potential to improve access and compliance to necessary care that could improve patient outcomes. Therefore, the purpose of our novel approach to studying the near reflex in participants with a history of concussion was to not only improve our understanding of the oculomotor and visual deficits associated with mTBI but also expand our understanding of how VR technology could be used as a tool to diagnose and treat concussions.

## Summary of Results

Forty-five young adults (29 females, 16 males; Age M = 26.18, SD 4.18 years [18-35 years]) participated in the study. Participants were divided between a healthy control (n = 21) and a Concussion History Group (n = 24). Our VR-based assessment of pupillary light reflex (VR_PLR) found strong evidence for the ability of VR HMDs to elicit and measure the PLR predictably to stimuli of varying intensity and duration. Additionally, we found strong evidence that our VR_VA assessment can elicit and measure the vergence response to a target moving in depth through convergence, divergence, vergence holding, and vergence tracking. The results of our VR_AT assessment were less conclusive given that we are unable to directly measure accommodation with a standard VR HMD. While we found stimulus-dependent differences in test scenarios that require a functioning accommodation system, improved performance in monocular testing conditions contradicts our hypothesis and the body of literature on accommodation and VR. Finally, we did not find clear evidence for differences in the near reflex triad between the Healthy Control Group and the Concussion History Group across our VR assessments. Follow-up analyses examining the time since injury and the number of past injuries did not provide clear evidence for concussion-related changes in oculomotor function.

## Aim 1

Our results did not support our Primary Hypothesis 1, as we did not find significant differences in the PLR between participants with a history of concussions and healthy participants. The lack of differences across PLR metrics is inconsistent with the literature on the effects of concussion on pupillary response. Notably, there is strong evidence to suggest mTBI patients have slower (peak and average constriction and dilation velocity), delayed (constriction latency and dilation recovery), and reduced (maximum and minimum diameter, constriction amplitude) across the subacute, acute, and chronic recovery periods (Ciuffreda et al., 2017; Podolak et al., 2019; Thiagarajan & Ciuffreda, 2015; Truong & Ciuffreda, 2016; Urosevich & Capo-Aponte, 2013). While studies have examined the PLR in participants with a longer recovery window (i.e., at least 1 year post-injury in the case of Thiagarajan & Ciuffreda (2015) our study used participants who had experienced a concussion as far as 20 years before data collection. Additionally, our study recruited participants who reported being asymptomatic for both visual and other mTBI-related symptoms during medical screening. This meant that any potential oculomotor deficits may have been resolved by the time of testing if they were even present, to begin with. While studies have investigated chronic, visually normal, and stable mTBI patients (e.g., Thiagarajan & Ciuffreda, 2015), the greater variability in our participant’s time since injury compared to past research likely limited our ability to detect differences between groups. Therefore, future research would likely benefit from stricter inclusion criteria to limit heterogeneity between participants and perform testing at a time when deficits are less likely to have fully recovered.

Our VR_PLR protocol was the first to recreate a dynamic PLR assessment used in mTBI participants within a virtual environment. Our testing conditions closely matched the methodology used in past research by Urosevich & Capo-Aponte (2013), Thiagarajan & Ciuffreda (2015), and others, which have shown stereotypical response patterns to light stimuli of varying intensity and duration. A major goal of this study was to investigate whether the VR_PLR could elicit and measure pupillary responses similarly to those found across dynamic pupillometry studies. To this end, we found strong evidence that VR HMDs with built-in VOG can serve as a dynamic pupilometer. We found a stereotypical dynamic pupil response profile seen in Figure 6, with a stable pre-stimulus baseline diameter, short delay following the introduction of a light stimulus followed by rapid constriction to a minimum diameter, and, finally, a gradual recovery toward baseline (Ciuffreda et al., 2017). Across variables, we found the strongest pupillary responses to light stimuli that were the most intense and longest duration (Bright Step) compared to stimuli that were less intense (Dim Step), shorter duration (Bright Pulse), or both (Dim Pulse). Responses to the Bright Step condition were categorized by significant differences in pupil diameter (minimum, maximum, final, and response amplitude) and velocity (peak and average constriction and dilation velocity), supporting prior research and aligning with the characteristic pupil response profile seen in physical environments (Ciuffreda et al., 2017; Podolak et al., 2019; Thiagarajan & Ciuffreda, 2015; Truong & Ciuffreda, 2016; Urosevich & Capo-Aponte, 2013).

Taken together, the significance of our study demonstrates the vast potential VR HMDs have for assessing the PLR, as well as opening the door for investigating other disorders that impact the pupillary system. While we were unable to support our hypothesis that pupillary responses in the Concussion History Group would be reduced, receded, slowed, and delayed, we found strong evidence that VR HMDs can elicit and measure pupillary responses in a manner that aligns with established pupillometric devices. More research is needed with participants who report ongoing PLR deficits following a mTBI to determine whether our test can detect the presence of altered pupillary responses in the chronic concussion period.

## Aim 2

Despite using a comprehensive battery of vergence assessments that attempted to recreate the demands of traditional tests used in past research, we were unable to find consistent differences based on concussion status to confidently support our Primary Hypothesis 2. There is extensive evidence to suggest that concussions cause deficits in vergence response subacute, acute, and chronic phases (Kawata et al., 2016; Pearce et al., 2015; Raghuram et al., 2021; Yaramothu et al., 2019). However, we were only able to detect significant differences in the Divergence Fusion test, with results often conflicting.

For instance - The relationship between IPD and target position was stronger in the Concussion History Group, but the time since injury analysis showed that the strength of the relationship also increased with greater recovery time. Given the lack of other significant differences based on concussion status, this may be an artifact of the VR environment and testing conditions. It is important to note that numerous participants had difficulty fusing the target during this assessment, which suggests the dynamics of positive fusional vergence in VR may need to be studied further to fully understand how HMDs impact the divergence response.

We found strong evidence across our four vergence tests that both convergence and divergence can be elicited and measured in VR environments in a predictable manner that mimics distance-dependent responses seen in the physical world (Scheiman et al., 2003). Namely, there was a strong, significant positive correlation between IPD and target position in the VR_NPC assessment, suggesting that greater convergence responses were seen to a closer target (and vice versa). These findings were supported across all other assessments, which showed a distance-dependent response of pupil position based on target distance. While responses match stereotypical response profiles, there were clear differences in overall vergence response distances between the physical and virtual worlds, with an average gap of approximately 10 cm. Differences are likely due to the well-known vergence-accommodation conflict caused by VR HMDs, which would alter the ratio of vergence to accommodative response to a target moving in-depth in the virtual world (Hoffman et al., 2008; Kramida, 2016; Mohamed Elias et al., 2019; Paulus et al., 2017; Zabels et al., 2019).

Overall, our findings suggest that VR HMDs can elicit and measure vergence in a predictable manner that mimics the response patterns seen in the physical world. Our study provides evidence for the utility of VR technology to be used as part of oculomotor assessments, but more research is needed to fully understand how the near reflex is impacted by the unique viewing conditions presented in a virtual environment. However, vergence deficits were not detectable in our participants. Given that our study focused on the chronic concussion period, and our participants reported being asymptomatic, vergence deficits may not have been present or could have been resolved by the time testing occurred. Therefore, future research should focus on testing participants with vergence deficits using more tightly controlled recovery timelines, to better understand whether a VR vergence assessment is sensitive to chronic concussion-related changes.

## Aim 3

Our VR_AT assessment found conflicting results, which may make it difficult to definitively support our Primary Hypothesis 3. On one hand, the closest distance that participants were able to distinguish which target was artificially blurred was closer in healthy participants compared to the Concussion History Group in monocular viewing conditions. This aligns with the well-established impact of concussions on accommodative amplitude, reducing the ability of participants to focus on a nearby target (Fraser & Mobbs, 2021; Green et al., 2010; Raghuram et al., 2021). Conversely, we also found that accuracy during our monocular test conditions was worse in healthy participants. Our regression analysis demonstrated that time with injury was inversely related to accuracy, which reduced by 4% for each year following a mTBI. Age was examined as a potential covariate but was only found to trend toward significance as a predictor of accuracy in Phase 3. Therefore, while age plays a role in declining accommodative ability over time, we cannot conclusively say it explains why accuracy would be reduced as participants' recovery time increased.

To our knowledge, there is no evidence to suggest that mTBI confers some sort of advantageous adaptation to accommodation that would improve accuracy. Because there were no differences in clinical tests of visual acuity or NPC between groups, we can only speculate as to the cause of these differences. It is possible that persistent deficits in the tonic accommodation integrator, which incorporates the CA crosslink signal, would uniquely impact performance judging near targets in participants with a history of concussions, as seen in those with spasms of the near reflex (Bharadwaj et al., 2020; Chan & Trobe, 2002; Knapp et al., 2002).

We found that monocular viewing conditions were more likely to elicit significant differences between groups compared to binocular viewing. Both accuracy and the closest distance that participants were able to distinguish between targets were significantly better in the monocular condition, which conflicts with our initial hypothesis that binocular conditions would improve performance through greater accommodation and vergence ability. While accommodation is minimized in VR, binocular viewing has been shown to elicit a stronger vergence and accommodation response when measured objectively through an autorefractor (Koulieris et al., 2017). Therefore, the most likely explanation is that by removing the disparity signal in the monocular viewing condition, we minimized the impact of the vergence accommodation conflict, allowing for more optimal task performance.

The design of our VR_AT assessment attempted to recreate standard clinical accommodation tests in a VR environment. We found evidence that manipulations to target distance and blur may elicit changes in accommodation and vergence demand in VR. Namely, significant differences between phases were found for accuracy, blur percentage, and target distance. This suggests participants were required to use the near reflex to judge differences in target blur, and that the task became more difficult when targets were either closer in the visual field or clarity/blurriness was closely matched. While we cannot objectively verify accommodation occurred, the requirements of our test are similar to that of an accommodative push-up used in clinical settings (Koslowe et al., 2010).

Overall, our findings should be interpreted with caution but are a promising first step toward using VR HMDs as a means of clinical accommodation testing. As technology improves, to both resolve the vergence-accommodation conflict and allow for the integration of objective measurement tools, more research will need to verify how accommodation responds to manipulations in VR environments.

## Limitations

Our study recruited a diverse range of mTBI participants, with a wide range of recovery times spanning over 20 years. Though it is clear chronic PLR, vergence, and accommodations deficits can persist throughout the recovery timeline (i.e., subacute, acute, and chronic phases), the heterogenous injury characteristics of our participants likely contributed to our lack of consistent findings between groups (Cheever et al., 2018; J. McDevitt et al., 2016; Kawata et al., 2016; Raghuram et al., 2021; Thiagarajan & Ciuffreda, 2015; Wiecek et al., 2021; Wright et al., 2017). As we did not specifically recruit participants with chronic oculomotor deficits and our patient population did not report symptoms at the time of testing, the long duration between injury and our study meant that any deficits present may have naturally recovered as commonly found across other concussion symptoms (Comeau & Pfeifer, 2019; Laker, 2011; Scorza & Cole, 2019). Further, while oculomotor deficits are common in patients following a concussion, it is possible that our sample never experienced any to begin with following their injury(s). While we had predicted our tests would be sensitive to detect underlying deficits related to the near reflex triad among participants with a history of concussion, these limitations presented significant potential challenges that should be addressed in future studies.

## Conclusion

The results of this study suggest that the PLR, vergence, and accommodation can be elicited and measured using VR HMDs. To our knowledge, this is the first study that created a comprehensive battery of assessments to examine each aspect of the near reflex using VR technology. While we did not find conclusive evidence of differences between participants with a history of concussions and healthy participants with the current protocol, these findings may help open the door for the future development of VR-based clinical tests.

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