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Evaluating ocular pain through MRI in patients with concussion

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Thesis

EVALUATING OCULAR PAIN THROUGH MRI IN PATIENTS WITH CONCUSSION

by

ISABELLA EMILIA CIVITARESE

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Approved by

First Reader

Karen Symes, Ph.D.
Associate Professor of Biochemistry & Cell Biology

Second Reader

Scott Holmes, Ph.D.
Associate Professor of Anesthesia
Harvard University

DEDICATION

I would like to dedicate this thesis to my mom Liz who has supported me and pushed me to achieve any and every goal I’ve sought to accomplish, my dad Giuseppe who I hope I am continuing to make proud as I pursue my dreams, and my dog Tino who is the best companion I could ever ask for. I would also like to dedicate this thesis to my boyfriend Andrew who has been unbelievably supportive during my pursuit of a Masters degree. Finally, I would like to dedicate this thesis to Dr. Scott Holmes who has gone above and beyond not only in guiding me through this project, but also in encouraging me to think more critically when delving into the world of research.

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ABSTRACT

Ocular pain is a prevalent symptom that impacts many patients of mTBI. Chronic exposure to this pain and other chronic pain symptom associated with mTBI can lead to effects on the central nervous system, however how this pain occurs is not well understood. The aim of this study was to evaluate the differences in functional connectivity between subjects with mTBI and healthy subjects at resting state, and to evaluate the differences in pain reporting between subjects with mTBI and healthy subjects. The basal ganglia, prefrontal cortex, hippocampus and occipital lobe were found to have increased connectivity in subjects with mTBI over healthy subjects in resting state conditions. Additionally, subjects with mTBI reported higher pain symptoms through PHQ, PCSS and VLSQ8 questionnaires than healthy subjects. Cohort differences demonstrated how functional connectivity and pain symptoms are altered under conditions of chronic pain from mTBI.

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LIST OF ABBREVIATIONS

ACC	Anterior Cingulate Cortex
BOLD	Blood Oxygenation Level Dependent
BU	Boston University
CT	Compound Tomography
DEQ-5	Dry Eye Questionnaire
DTI	Diffusion Tensor Imaging
fMRI	functional Magnetic Resonance Imaging
GE-EPI	Gradient Echo – Echo Planar Imaging
HPT	Heat Pain Threshold
HPTS	Heat Pain Temporal Summation Protocol
LGN	Lateral Geniculate Nucleus
MPRAGE	Magnetization Prepared Rapid Gradient Echo
mTBI	Mild Traumatic Brain Injury
NPSI-Eye	Neuropathic Pain Symptom Inventory-Eye
OPA	Ocular Photosensitivity Analyzer
OSDI	Ocular Surface Disease Index
PCS	Pain Catastrophizing Scale
PCSS	Post Concussive Symptom Scale
PFC	Prefrontal Cortex
PROMIS-29	Patient Reported Outcomes Measurement Information System
ROI	Regions of Interest

SF-MPQ2	Short-Form McGill Pain Questionnaire
SI	Primary Somatosensory Cortex
SII	Secondary Somatosensory Cortex
TRP	Transient Receptor Potential
VAS	Visual Analog Scales
VPT	Visual Photosensitivity Thresholds

I. INTRODUCTION

I.1 What Is Pain?

Pain, according to the International Association for the Study of Pain, is defined as an unpleasant sensory and emotional experience that is associated with actual and/or potential tissue damage. While there is a scientific pathway and explanation of pain, it is also a very subjective experience where every individual learns what pain is from their own experiences throughout the span of their lifetime. To some, one stimulus can be more painful than it is to other individuals. It can be considered a sensation in a part of the body that is always unpleasant and elicits an emotional response along with it. Prior to the feeling of pain, tissue injury is a common precursor however it is not always necessary for pain to be felt (Raja et al., 2020). Overall, pain also serves as a protective function for the body. For example, when touching something hot the feeling of initial pain causes one to reflexively remove their hand from the stimuli when the feeling of pain begins to prevent further pain and possible tissue damage. The feeling of pain can be considered the product of both brain and neural pathway processing, it all begins with Nociceptors. Nociceptors are nerve endings that detect harmful stimuli and lead to the sensation of pain. To start the pathway, there are three classes of Nociceptors:

1. **Thermal –** These receptors sense extreme temperatures. They are the peripheral nerve endings of small, myelinated axons which conduct action potentials upon activation by stimuli (Kandel et al., 2013).

2. **Mechanical –** These receptors detect intense pressure that is applied to the skin. Like thermal nociceptors, they are also the nerve endings of small, myelinated axons (Kandel et al., 2013).
3. **Polymodal –** These receptors respond to high intensity mechanical, chemical or thermal stimuli. These differ from mechanical and thermal as they are the nerve endings of unmyelinated axons. Due to their lack of myelination, they conduct action potentials much more slowly (Kandel et al., 2013).

Once activated by noxious stimuli, the nociceptors carry the signal to a higher order on the pathway to pain. The stimuli result in depolarizing nerve endings of afferent axons and ultimately generate action potentials when threshold is reached. The membranes of nociceptors convert thermal, mechanical, or chemical stimuli into depolarizing electrical potential. Within the membranes reside Transient Receptor Potential (TRP) channels which are ion channels that respond to the noxious stimuli. **TRPV1 channels** are expressed only by nociceptive neurons, and they permit Calcium ions to pass through the channel. Their actions are potentiated by heat (greater than 42°C), acidity (low pH) and molecules like Capsaicin. **TRPV3** channels respond to heat (greater than 33°C) and participate in the transduction of heat pain. As temperatures reach noxious levels, both V1 and V3 channels increase in activity. **TRPV2** channels are activated by very high temperatures and **TRPM8** channels are activated by cold temperatures and are involved in cold pain transduction, for example in response to menthol. **TRPA1** channels are sensitive to thermal, mechanical and chemical stimuli (McEntire et al., 2016). These channels are expressed on neuronal and non-neuronal cells.

For thermal and mechanical nociceptors, the signal is carried along Aδ fibers which results in an initial “fast” pain sensation. For polymodal nociceptors, the signal travels via C fibers and results in a secondary pain that is slow and dull. The perception of pain from noxious stimuli comes from signals in peripheral axon branches of the nociceptive sensory neurons that reside in the dorsal root ganglion or trigeminal ganglia. The central branches of these axons end in the spinal cord and the terminal branches end in the dorsal horn (Kandel et al., 2013). This process where noxious stimuli activate nerve endings is known as Transduction. Potassium, Histamine, and Serotonin (pain producing chemicals) may be released from damaged tissue cells or by blood cells that migrate out of blood vessels and into the area of tissue damage (Loeser & Melzack, 1999). These chemicals then activate primary afferent nociceptors and are converted to electrical signals that are perceived as pain. Without transduction, the body would not have the ability to feel pain (Osterweis et al., 1987).

As the message of pain continues its path to higher order centers, the next area it passes through are the Laminae. **Lamina I** is the most superficial of the dorsal horn and is found in the marginal layer. It responds to input from Aδ and C fibers from the activated nociceptors and project onto the Thalamus. **Lamina II** resides in the substantia gelatinosa, a densely packed layer containing different classes of local interneurons that respond to nociceptive inputs. **Laminae III and IV** contain a mix of local interneurons and supraspinal projection neurons. They receive input from Aβ afferent fibers which activate in response to cutaneous stimulation, specifically deflections of hair and light pressure. **Lamina V and VI** are composed of neurons that respond to a wide range noxious stimuli and project to the brain stem and thalamus. They receive their stimulation from Aδ, Aβ and C fiber nociceptors (Kandel et al., 2013). **Lamina VII** is the relay center between muscle to midbrain and cerebellum. **Lamina VIII** encompasses the spinal cord’s ventral horn and is made up of α, β, and γ neurons whose axons innervate striated or skeletal muscles (Khalid & Tubbs, 2017).

To reach the brain, nociceptive pain has five major ascending paths it can use to reach its destination.

1. **Spinothalmic Tract –** This is the most prominent tract in the spinal cord. Axons of nociception-specific, thermal and dynamic range neurons in Lamina I and V-VII of the dorsal horn. They cross the midline of the spinal cord at the origin and then ascend in the anterolateral white matter before ending in the thalamic nuclei. Here the electrical stimulation that ascends is passed onto the **Lateral Nuclear Group** which is comprised of the ventroposterior lateral, medial and posterior nucleus. This group is thought to be concerned with the processing of information about the precise location of an injury and is conveyed to consciousness as acute pain (Kandel et al., 2013).
2. **Spinoreticular Tract –** The axons of projection neurons in Laminae VII – VIII ascend the anterolateral quadrant of the spinal cord and terminate in the reticular formation and thalamus(Kandel et al., 2013). This path is receptive to stimuli of deep somatic and visceral structures (Osterweis et al., 1987).
3. **Spinomesencephalic Tract –** The axons of projection neurons in Laminae I and V, they project into the anterolateral quadrant of the spinal cord to mesencephalic

reticular formation and periaqueductal gray matter. The information transmitted can contribute to the affective component of pain (Kandel et al., 2013).

4. **Cervicothalamic Tract –** This tract runs in the lateral white matter of the upper two cervical segments of spinal cord and contains axons of neurons of the lateral cervical nucleus which receive input from the neurons of Lamina III and IV of the dorsal horn. The project across the midline, ascend in the medial lemniscus of the brain stem, and terminate in midbrain nuclei, ventroposterior lateral, and posteromedial nuclei of the thalamus (Kandel et al., 2013).
5. **Spinohypothalamic Tract –** The axons of neurons found in Laminae I, V, VIII of dorsal horn project to the hypothalamic nuclei that serve as the autonomic control center involved in the regulation of neuroendocrine and cardio responses that accompany pain syndromes (Kandel et al., 2013).

The relay of information along these tracts is known as Transmission, the messages carried from the site of injury are delivered to brain regions that perceive the message as pain. Once received by the thalamus, the pain message has two end sites: **Ventrocaudal and Medial.** The ventrocaudal thalamus receives the nociceptive input from projecting spinal cord neurons and projects directly to the somatosensory cortex. The medial thalamus receives some indirect input from the spinal cord and major input from the region of the brain stem reticular formation where nociceptive spinoreticular neurons project and projects into the forebrain (Osterweis et al., 1987). Once the information of pain is transmitted, the pain is perceived, and pain behaviors can be elicited. Pain behaviors can include grimacing, limping, seeking medical care or verbally saying “ouch”(Loeser & Melzack, 1999).

Finally, there are several types of pain that can be experienced. First there is Transient Pain which is elicited by the activation of nociceptive transducers in the absence of any tissue damage. This type of pain evolved with the purpose to protect man from physical damage by the environment or by over stress of body tissues. It is present in everyday life and rarely a reason to seek medical treatment, for example getting an innoculation or needles to the skin (Loeser & Melzack, 1999). Then there is Inflammatory Pain which corresponds to the release of inflammatory mediators such as interleukins, prostaglandins and cytokines which sensitize nociceptive neurons (McEntire et al., 2016). This pain is adaptive, protective, and assists in the healing of injured body parts by discouraging physical contact or movement (Woolf, 2010). Neuropathic Pain or Pathological Pain is experienced from damage to the nervous system structures that relay nociceptive info to the Central Nervous System, some examples of this are Shingles or Fibromyalgia (McEntire et al., 2016). In this there exists no noxious stimuli but a substantial amount of pain exists. It can be considered as a false alarm caused by a system malfunction (Woolf, 2010). Lastly, there is Nociceptive Pain which is the pain that detects tissue damage by transducers attached to Aδ and C fibers as discussed above. This pain is elicited by from encounters with noxious stimuli including thermal, chemical and mechanical (McEntire et al., 2016).

I.2 The Ocular System and Visual Processing

In order to delve into the visual processing pathway, it is first essential to understand the anatomy of the ocular system.

Ocular Anatomy

Each eye sits in the cranium and is surrounded by the bony orbit of cranial bones. The purpose of the orbit is to protect the eye from trauma by large objects. The eyelashes detect small objects close to the eye and the eyelids close when the lashes sense danger to protect the eye from injury. The eyelids function to spread tears across the eye and the lacrimal glands, located on outer edge of the eye under the brow, produce these tears. Each eye is then attached to six extraocular muscles that function to move the eye into different positions of gaze within the cranium (Lens, 2008). The six muscles are:

1. The Superior Rectus muscle which is innervated by the Oculomotor Nerve (Cranial Nerve III)
2. The Inferior Rectus muscle which is innervated by Cranial Nerve III
3. The Medial Rectus muscle which is innervated by Cranial Nerve III
4. The Lateral Rectus muscle which is innervated by the Abducens Nerve (Cranial Nerve VI)
5. The Inferior Oblique muscle which is innervated by Cranial Nerve III
6. The Superior Oblique muscle which is innervated by the Trochlear Nerve (Cranial Nerve IV)

Each structure in the internal anatomy of each eye serves a specialized function and is composed in a way to achieve the possibility of sight. The **bulbar conjunctiva** is the mucous membrane that covers the **sclera** (whites of the eyes) of each eye and is continuous with the **palpebral conjunctiva** which lines each eye and prevents objects, like contact lenses, from disappearing behind the eye. The **episclera** lies beneath the sclera and contains a network of blood vessels that serve to nourish the sclera. The **cornea** which is the anterior portion of the eye globe is transparent in order to allow light into the eye. The cornea receives innervation from the ophthalmic branch of the trigeminal nerve. It's composed of fibers in a crisscross fashion that are kept hydrated by the corneal endothelium.

The globe of the eye itself is divided into two chambers, the **Anterior Chamber of the eye** which is contained by the anterior surface of the iris and the posterior surface of the cornea and is filled with aqueous humor. The **iris** is the structure of the eye that gives us our eye color. The iris can be thought of as a muscular shutter that regulates the size of the pupil via innervation by its dilator and sphincter muscles. The ability to regulate its shape allows the iris to prevent excess light from entering the eye and thus helps to project clear images on the retina. It is also worth mentioning that those with less pigment in their iris are often less tolerant of bright sunlight as darker pigments function as an absorber of light (Lens, 2008). The **Posterior Chamber** of the eye is contained between the posterior surface of the iris and anterior surface of the crystalline lens. The Posterior is the largest of the two chambers and is pierced by the optic nerve on the posterior of the globe. The **lens** that sits within each Posterior chamber is a transparent biconvex crystalline structure. It has no innervation and no blood supply. The lens acts to produce the image seen onto retina by allowing light to pass through it. This structure obeys the laws of optics and therefore changes shape when viewing a nearby or distant object.

Behind the lens lies the **vitreous,** a gel-like fluid that is clear and avascular and transmits the light that has passed through the cornea and then the lens. Finally, on the posterior portion of the eye lies the **retina** which is a transparent neural tissue that contains photochemicals and neurologic connections that process the light energy passing through the eye and relay it to the visual cortex for perception and integration. The retina is composed of several layers that all aid in visual processing. The **photoreceptor layer** of the retina contains the photoreceptors rods and cones. These photoreceptors convert light energy into nerve impulses that are sent further down the visual processing pathway. Rods contain the chemical rhodopsin and function for peripheral and night vision. Cones are clustered at the macula and function to provide color vision and fine vision. There are red, green and blue cones (Lens, 2008). The **bipolar layer** is the middle layer of the retina between the photoreceptor and ganglion and serves as a connection for transmission between the layers. Within the bipolar layer lie several cell types; the bipolar cells which are the main transmitters from rods and cones to the ganglion layer, amacrine and horizontal cells which function as integrators of the overall impulse transmission from receptors to visual pathway, and müller cells which structurally support the retina and nourish it. The **ganglion layer** is composed of ganglion cells which form the NFL (nerve fiber layer) cables that come together at the optic nerve head to form the optic disc on the retina (Lens, 2008). In Figure 1, we can see a visual representation of the eye anatomy structures discussed above. With the understanding of the internal anatomy of each eye, we can see how exactly each structure in the eye serves a purpose in visual processing and sending on the signal to the brain.

Figure 1

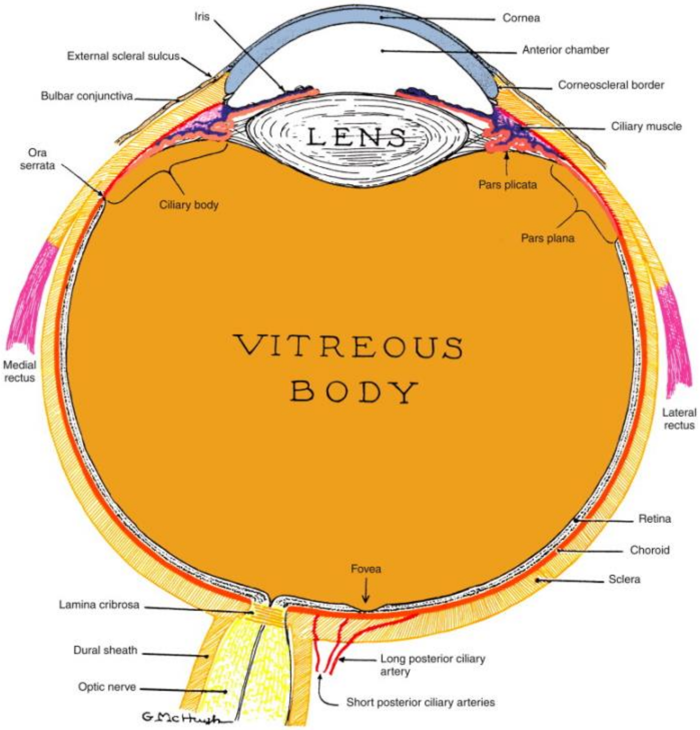

Figure 1 *Anatomy and structure of the eye labeled (Kronfeld,1943).*

Visual Processing

With an understanding of the anatomy of the eye, we can begin to understand how the “message” of sight is transmitted from the eye over to the brain. The beginning of the path starts with two parallel light rays from an object entering the eyes, these rays are refracted, inverted and directed onto the retina. If the eyes’ gaze moves to a closer object, these rays will be bent further in order to produce a focused image which is accomplished by the ciliary muscles on the lens making it become more rounded (Presland & Myatt, 2010). As discussed previously, the first cell in the pathway where light is transcribed into a neuronal signal is in the photoreceptors of the retina. This signal is then passed to amacrine and horizontal cells and then on to ganglion cells. The axons of these ganglion cells exit the retina via the optic nerve and into nasal fibers from each eye where they then cross in the optic chiasm. The chiasm is the point at which the nasal fibers of both eyes' junction over the pituitary gland. The nasal fibers join with temporal fibers and form left and right optic tracts as they exit the chiasm (Lens, 2008). Each tract terminates on the opposite side of the brain from their original eye at the **lateral geniculate nucleus** (LGN) of the thalamus. Signals from the left optic tract carry images from the right visual field and vice versa (Presland & Myatt, 2010). These fibers then leave the LGN as optic radiations, which spread out in a fanwise fashion, where they terminate in the visual cortex of the occipital lobe (Remington, 2012). The LGN is the center for complex processing by regulating the flow of visual info and sending the most important information onto the visual cortex. Each layer of the LGN receives input from a specific eye and class of ganglionic cell allowing the LGN to thoroughly process the signals it receives (Levin et al., 2025). According to a book by Remington, the magnocellular layers of the LGN most likely mediate movement direction and low spatial frequency contrast sensitivity. Whereas the parvocellular layers mediate color and high spatial frequency contrast sensitivity.

Once the signal is read by the LGN, it exits as an optic radiation to the Primary Visual Cortex of the occipital lobe. The cortex is located by the calcarine fissure of the skull, the fissure divides the cortex into the upper portion (cuneus gyrus) and lower portion (lingual gyrus) (Remington, 2012). Two functions of the visual cortex are contour analysis of vision and binocular vision. The cortex also connects with the **superior colliculus** which is involved with visual orientation, foveation and saccadic eye movements. It integrates the visual and sensory info it receives to drive reflexive behaviors (Levin et al., 2025). The visual pathway is a complex system, as visualized in Figure 2, with many essential pieces to pass a signal onto higher processing centers to understand what our eyes are seeing. To perceive what our eyes see and process what it is would not be possible without this system's ability to communicate within itself and onto higher brain centers.

Figure 2

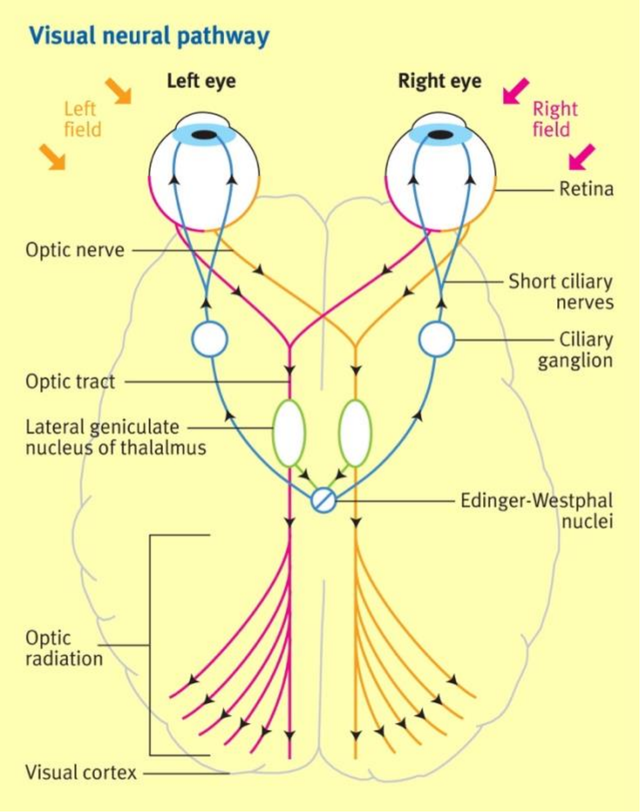

Figure 2 *Diagram of the visual pathway (Levin et al., 2025)*

I.3 Pain in the Brain

While there is much known about the brain, its anatomy and mechanisms, there is still much about its functions and mechanisms of operation that are still widely unknown to us. The way in which signals of pain are transmitted from nociceptors to the brain via pathways like the spinothalamic tract, as was discussed previously in the Pain chapter, has been discovered. What many researchers and scientists are in the process of discovering is how exactly these signals cause changes in the brain that translate to the perception of pain. What structures of the brain communicate? How do these structures signal to one another that pain is being experienced by the individual? In this chapter we will explore some of the potential methods in which the brain structures connect to one another to relay pain and which structures may be responsible for this connection.

It has been postulated by numerous studies that some main contenders of this communication network include the primary somatosensory cortex, the thalamus, the insula, anterior cingulate cortex, prefrontal cortex and amygdala.

Primary and Secondary Somatosensory Cortices

The primary somatosensory cortex (SI) is located on the posterior surface of the central sulcus. This cortex is involved in deciphering the location and character of a sensory stimulus and more finely deciphering the identity of an object by touch. The SI has been postulated to be a structure that is involved with pain perception. In a study examined in the *Neuroimaging of Pain,* an injury or lesion in SI resulted in a loss of capacity for specification of the location and intensity for the nociceptive stimulus (Saba, 2017). The secondary somatosensory cortex (SII) is found in the inferior posterior central gyrus and is believed to be involved with identifying the location of pain and understanding the characteristics of the pain stimuli (Saba, 2017). This cortex has an outsized sensory representation for the areas of the hands, feet, and face because these areas contain such a high number of nerve endings (Ambron, 2022). In another book *The Brain and Pain* by Ambron, they suggest the SI and SII form the somatosensory system which is responsible for perceiving pain and localizing its origin source.

Thalamus

The Thalamus is a structure within each brain hemisphere that receives inputs from all sensory neurons except those of olfaction. We perceive pain from the thalamus when second order axons in the thalamus activate their third order neurons in the thalamus. The thalamus then relays that pain is being perceived to other structures of the brain (Ambron, 2022).

Insula

The insula is a structure that comes up across the board in pain relay studies of the brain. Given its constant occurrence in studies, it is clear this structure is a huge contender in understanding how the brain relays the perception of pain. In *Neuroimaging of Pain,* Saba states that the insula, when stimulated, produces emotional sensations of fear. This conclusion was supported from a study performed where injury to the insula resulted in an absence of emotional responses to nociceptive stimuli. The lack of emotional response leads to aid the understanding that emotion is an immense contributor to how we experience pain. Anticipation and expectation of pain as we know it can greatly affect the way we perceive pain from past experiences with it or with similar stimuli from the course of our lives. Additionally, in the *Brain and Pain* they state that the insula seems to be attuned to information about an injury or lesion and is activated by noxious stimuli, when stimulated electrically the sensation of pain was produced in the subject. Finally, in a study from Brooks et al. where they studied how pain perception results from nociception, they found higher connectivity through fMRI in the insula when the subjects were actively paying attention to or anticipating pain. This result suggested the insula is a region that monitors the state of the body for changes in homeostasis or pain (Brooks & Tracey, 2005).

Anterior Cingulate Cortex

The Anterior Cingulate Cortex (ACC) resides beneath the surface of the anterior part of the cingulate gyrus. The ACC is believed to be a major mediator of the awareness of sensations in the body allowing us to be aware that we are in pain. Additionally, the cross-talk between the thalamus and the ACC aid in making us aware of injury and pain (Ambron, 2022). In the study by Pogatzki-Kahn et al., they postulated that the ACC is involved with the motivational-affective component of the pain matrix and works in association with the thalamus and amygdala to perceive pain (Esther M. Pogatzki-Zahn et al., 2010).

Prefrontal Cortex

The Prefrontal Cortex (PFC) is part of our limbic system and functions in emotion regulation as well as motivational-affective aspect of pain, like the ACC (Saba, 2017). It is located in the frontal region of the frontal lobe of the brain (Ambron, 2022). The PFC plays a major role in learning and predicting errors meaning that over time and from life experiences the PFC learns painful scenarios and functions to prevent future painful situations.

Amygdala

The amygdala is located in the temporal lobe of the brain in front of the hippocampus. This is the center for our emotions and is a serious pain pathway contender as it can have a major effect on the way we perceive pain. Due to the fact it has thalamic connections, it receives signals from the nociceptive pathway and makes it a part of the pain pathway (Ambron, 2022). It has been postulated to affect how we remember certain experiences in life as painful and then in the future when these experiences are visited again fear or anticipation emotions accompany the experience of pain and increase the perception of pain. For example, as a child when receiving a vaccine and it hurt or shocked you, it becomes encoded as a painful experience and as an adult the experience of getting a vaccine will evoke the same emotions in the amygdala (Ambron, 2022). According to the study performed by Pogatzki-Zahn et al., the amygdala plays a critical role in cognitive-emotional processing of pain and aids in antinociception. Additionally, in their study they found a positive correlation between the subject’s perceived pain intensity and bilateral activation of the amygdala following the incision. This finding suggested that the amygdala has an integratory function of nociception and anxiety following an incision (Esther M. Pogatzki-Zahn et al., 2010).

Integration of the Pain Matrix Contenders

While understanding how each structure functions in the pain matrix independently, it is also essential to try and comprehend how these structures connect with one another to aid in the perception of pain and influence the intensity at which we perceive it. Across fMRI studies of the brain in pain, more sensitive subjects to pain showed increased brain connectivity in the SI, ACC and PFC suggesting they feel pain more intensely due to their increased sensitivity (Saba, 2017). This finding was further supported in the study by Brooks et al., where they found that subjects who were highly sensitive to pain exhibited more frequent and robust activation of the SI, ACC and PFC than subjects less sensitive to pain. Additionally, an interesting finding by Brooks et al., was that sensitive subjects not only showed activation bilaterally in the insula, ACC, brainstem and cerebellum when they were perceiving pain but also when they discovered a loved one was experiencing pain. This suggested that empathetic subjects experienced activation of the affective components in the pain matrix. This is a significant finding because it adds to the understanding that pain is a truly multifaceted experience as it is not just the pain matrix that contributes to the perception of pain but the subject's emotional state and past experiences with pain that can alter the intensity of the pain they feel. In a study from Holmes et al., where they examined if a surgical incision activated parts of the sensory discriminative pain system in subjects with ankle injury, their results showed that the amygdala aligned with an increase in fear of pain and increase of pain reporting. Furthermore, they found that subjects with neuropathic pain reported higher levels on self-report measures for fear of pain, pain catastrophizing and screening tools that were associated with pain intensity and disability (Holmes et al., 2020). This suggests the same idea, that emotional state and past perceptions of pain of the subject truly influences the perception or intensity of pain being presently experienced.

In Ambron's book, they proposed a manifestation of pain in the brain as follows; an individual is suffering or in a stressful state, inputs are received from the ACC to the amygdala and then onto higher brain centers like the hypothalamus, this activates autonomic nerves which result in a physical change in the individual like sweating, increased heartrate and tear production which signal to the individual the intensity of pain that is being experienced. Within the structures of the pain matrix, it was proposed that communication between the thalamus and ACC make us aware of an injury, the ACC and Insula together determine whether the sensation of pain needs our attention, and the Insula and PFC work in conjunction to subjectively evaluate the injury based on our knowledge, the context, and circumstances of the injury (Ambron, 2022). In the study by Pogatzki-Zahn et al., they postulate that the SI, SII and insula together are important for the sensory-discriminative perception of pain as they found the insula showed activation with negative emotions and overall unpleasantness and that activation of the somatosensory cortex depends on pain intensity (Esther M. Pogatzki-Zahn et al., 2010).

Related to these findings, a curious discovery by Ambron and Pogatzki-Kahn et al. that could be an area of further study would be that distraction showed a decreased perception of pain. Ambron found that hurting from an injury is a normal experience but can be greatly modified by pain matrix structures. For example, a reward can diminish the perception of pain but fear can make the pain much worse (Ambron, 2022). Pogatzki-Kahn et al. found that there was a negative correlation between thalamic activation to a noxious stimulus during distraction which suggested that the thalamus is involved in pain modulation through attention. This would be an interesting avenue to further explore with pain management to discover ways that pain perception can be diminished and visualizing through fMRI what structures of the pain matrix decline in connectivity during distraction.

I.4 Functional MRI

Functional Magnetic Resonance Imaging (fMRI) is a revolutionary tool that has significantly aided the field of neuroscience, providing a non-invasive technique to visualize the connectivity of the human brain. By detecting changes in blood oxygenation levels, fMRI provides researchers insights into the functioning of the brain while individuals engage in various cognitive, sensory, or motor tasks. fMRI has become the choice for many researchers and physicians in the field of neuroscience because it is a safe, non-invasive and repeatable technique. It does not provide a risk of radiation to the patient allowing the possibility for multiple scans over a long period of time with no harm to the patient’s health. This technique has become instrumental in research and clinical settings as it allows us to advance our understanding of brain function, disorders and neurological conditions.

fMRI is a subset of MRI, which produces images based off the properties of mobile hydrogen nuclei that are within water molecules. fMRI specifically detects changes in blood oxygenation-level in a method know as BOLD (Gore, 2003). These changes arise when neuronal connectivity occurs following a change in the state of the brain during a stimulation event or when performing a task. A task or stimulation event causes synaptic and electric activities in regions of the brain which in return triggers an increase in cerebral blood flow and volume, and cerebral metabolic rate of oxygen and glucose (Kim & Bandettini, 2010). fMRI detects these changes caused by neural connectivity and results in the imaging we see during fMRI. The inference made by researchers when using fMRI is that an increase in cerebral metabolic rate of oxygen reflects the regions of increased neuronal connectivity because of the increased demand for oxygen in that brain region (Mier & Mier, 2015). With this inference, when investigating how certain tasks or emotions stimulate the brain, the brain regions that show an increase in cerebral metabolism of oxygen signals that region is involved in the processing of the stimulation.

The BOLD signal detected by fMRI relies on the oxygenation of the blood in the brain region of interest. The longevity of signals used to produce MRI images is dependent on the uniformity of the magnetic field within the region which is most uniform when blood is oxygenated. While performing a task or under stimulation, the brain region of interest is consuming more oxygen during processing which therefore means more oxygenated blood is replacing deoxygenated in that region relative to regions not involved in the task or stimulation. The regions not involved have magnetic fields that are less uniform meaning there is a greater mixture of differing signal frequencies that arise from the region and therefore causes the fMRI signal to decay more rapidly (Gore, 2003). In essence, fMRI takes advantage of the differences in magnetism between oxygenated and deoxygenated hemoglobin in blood to produce its images. When hemoglobin in the blood becomes deoxygenated, it transitions to a paramagnetic state, causing the electrons to lose their uniform spin. This disruption prevents the fMRI from detecting the signal effectively. The ratio of deoxygenated to oxygenated hemoglobin defines the intensity of the fMRI signal (Casey et al., 2002). Overall, the order of events that lead to a BOLD fMRI image begins with a task that increases neural connectivity and an increase in blood flow within a brain region which results in an increased blood oxygenation. This in turn results in a decreased deoxyhemoglobin and causes an increase in fMRI signal and results in a BOLD fMRI image.

The duration and frequency of the BOLD signal can be affected by the type of stimulus used and the duration of stimulus. Typically, fMRI experiments involve subjects being asked to perform behavioral tasks and a comparison is made between the levels of connectivity in brain regions during the tasks. The application for fMRI has grown beyond experimental measures in research. In clinical applications, fMRI is used to map sensory and motor function brain regions. In neurology applications, fMRI has been used to study brain regions in patients recovering from stroke. In psychiatry applications, fMRI has been used to determine neurological bases for cognitive deficits and aberrant behaviors (Gore, 2003). fMRI has also been implemented in studying the pain pathway and in identifying what regions of the brain process pain. In a study by Reddan and Wager, they used fMRI to model pain. In this study, they utilized fMRI to determine brain representations of pain and developed biological markers for processes related to pain in the brain. They determined that activation in fMRI demonstrated the thalamus, the anterior and posterior cingulate cortex, the insula, the amygdala, the somatosensory pathways and the periaqueductal gray were associated with subject reports of increased pain (Reddan & Wager, 2018). The findings of this study demonstrate the unique value of fMRI as it can visualize the exact regions in the brain that are associated in the pain pathway. Given that the pain pathway within the brain is still not completely understood, studies such as this can lend to understanding exactly what occurs when an individual experiences pain and perceives it as pain.

An additional study done by Valet et al. examined how distraction during pain affected the brain regions involved in pain through fMRI. They noted that focusing attention on the noxious stimuli showed fMRI activation within the prefrontal cortex, the anterior cingulate cortex and the thalamus. When the subject's attention was distracted from the noxious stimuli, fMRI showed activations in the insular cortex shift from the anterior portion to the posterior portion. A takeaway from their study was that distraction tasks reduce the subjective pain sensation and decreased associated cerebral pain activation (Valet et al., 2004). This technique demonstrated additional novelty in the use of fMRI as researchers could visualize how distraction techniques altered the neuronal connectivity of the pain associated brain regions.

fMRI has shown to be a vital tool not only in its strengths of being a safe, repeatable means of scanning patients in comparison to methods like Computed Tomography (CT) scans and X-rays, but also a novel tool as it can allow researchers and clinicians to visualize neuronal connectivity. In relation to the understanding of pain, it can provide a means to determine exactly what occurs when an individual senses pain and how they perceive the pain that is felt. Future studies could even include revisiting fMRI with the purpose of inventing a more revolutionary technique that could solve the question behind “what is pain’?

I.5 Specific Aims

Experiencing chronic pain post-mTBI is a common symptom for many patients. Currently the way pain manifests itself is not fully understood, but is the subject of much research. Enduring the effects of chronic pain for a long period of time can result in psychological and neurological effects While ocular pain is a common symptom in patients with mTBI it is not understood what regions of the brain are responsible for the evolution of this pain. Delving into this topic may shed light on the functional connectivity behind this symptom and aid to fortify the understanding behind the perception of pain and the brain regions involved.

This study looks to examine ocular pain in subjects with mTBI and to compare the differences in functional connectivity between subjects with mTBI and Healthy Controls. Collection of pain questionnaire data and fMRI scans from Healthy Controls and subjects with mTBI was the basis of this study.

Aim 1: Evaluate the extent to which subjective pain reporting (PCSS, PCS, PHQ and VLSQ8) is impacted in a cohort of subjects with mTBI relative to a cohort of healthy subjects. This aspect is of interest to see how concussion leads to the perceptions of ocular pain. We predict that those in the mTBI cohort will report elevated pain compared to healthy subjects.

Aim 2: Determine the extent to which functional connectivity at a whole brain level was different between a cohort with mTBI and a cohort of healthy controls .We predict increased functional connectivity in areas related to pain in the brain for the mTBI cohort.

II. METHODS AND MATERIALS

The following study was approved by the Institutional Review Board (IRB) and included the involvement of collaborators to ensure expertise in all areas of the data collection and analysis process.

II.1 Overview

This study involved 31 subjects in total, 25 subjects belonging to the Control cohort and 6 subjects belonging to the mTBI cohort. Subjects underwent 6-hour study visits at 2 Brookline Place which was comprised of screening, obtaining consent/assent, ocular assessment, quantitative sensory testing, corneal microscope imaging, completion of a questionnaire, and a 1-hour MRI scan session.

II.2 Recruitment Methods

Ophthalmology department patients at Boston Children's Hospital were approached during the time of their clinical visits and given the option of participating in the study. Patients were referred to our study by physicians Dr. Aparna Raghuran OD, PhD, Assistant Professor of Ophthalmology, and Dr. Emily Wiecek OD, PhD, Instructor of Ophthalmology. Participants were additionally recruited from Boston Children's Hospital Sports Medicine clinical databases using medical chart reviews under the supervision of Dr. William Meehan (Director of The Micheli Center for Sports Injury Prevention, Clinical Effectiveness Research Center and Director of Research, Sports Medicine) from the Sports Concussion Clinics under the direction of Dr. Michael O'Brien (Director of Sports Concussion Clinics). With patient permission, the names and phone numbers of interested individuals were given to study personnel and/or they received contact information for the study coordinator and reached out to study personnel. Clinicians at Spaulding Rehabilitation passed out research brochures and hung flyers in approved waiting rooms. IRB approved flyers were also posted in the waiting rooms of clinics, at schools, local YMCAs, libraries, and similar community-based centers (with permission). The study was also able to be found on the “Find a Clinical Trial” section of Boston Children’s Hospital external website. Tabling events at 2 Brookline Place lobby were done to raise awareness of the research study. Flyers and brochures with information regarding the study were available. Finally, word-of-mouth was an additional recruitment method utilized.

An initial phone call interview was conducted to determine if subjects met the study’s basic requirements. Subjects were also screened. If no obvious health problems were present, subjects were scheduled for a physical exam and interview with staff with their parent/guardian present. Upon arriving at the research location, parents/guardians gave permission for their child (<18 years old) to participate in the research, and the volunteer provided written informed consent if 12 years or older, or assent if under 12 years old.

II.2.1 Inclusion and Exclusion Criteria

Potential participants were identified from a large database of pediatric post-traumatic headache patients who expressed interest in participating in the study.

<u>Concussion Patients without Photophobia Inclusion Criteria:</u>

- Age 10-21 years old
- Receiving medical care for a concussion diagnosis without report of photophobia

- English speaking ability sufficient to comprehend consent and study procedures (with parental assistance)
- Ability to tolerate corneal microscopy procedure
- Ability to lie still for a 60-minute MRI session
- MRI compatible

Concussion Patients with Photophobia Inclusion Criteria:

- Age 10-21 years old
- Receiving medical care for a concussion with report of photophobia
- English speaking ability sufficient to comprehend consent and study procedures (with parental assistance)
- Ability to tolerate corneal microscopy procedure
- Ability to lie still for a 60-minute MRI session
- MRI compatible

Healthy Subjects Inclusion Criteria:

- Age 10-21 years old
- English speaking ability sufficient to comprehend consent and study procedures (with parental assistance)
- No history of recent concussion
- Ability to tolerate corneal microscopy procedure
- Ability to lie still for a 60-minute MRI session
- MRI compatible

Exclusion Criteria for all:

- Claustrophobia
- Pregnant or nursing
- Weight/size incompatible with the limit of the MRI table and/or head coil
- Magnetic implants or metal-containing tattoos on their chest or above, or other MRI contraindications
- Significant medical history, including:
    - Current DSM-5 axis I psychiatric disorders except depression
    - Chronic pain unrelated to concussion
    - Corneal surgeries (exception of LASIK and PRK)
    - Active ocular surface infection/inflammation
    - Previous history of dry eye disease or diabetes
    - Seizures
    - Brain Tumor
    - Cerebrovascular accident
    - Neurological disease aside from concussion
    - HIV-AIDs
- Prescription medications that include eye dryness as a recognized side effect, including:
    - Psychostimulants
    - Dopaminergic or anti-dopaminergic agents, including:
        - Antipsychotics

        - Mood stabilizers
        - Antidepressants with prominent catecholaminergic effects (tricyclic amines, bupropion, mirtazapine, venlafaxine, and duloxetine)
        - Non-steroidal anti-inflammatory drugs
    - Allergy to eyedrops/lubrication used in the study:
        - Proparacaine
        - Genteel lubricating ointment

II.3 Data Collection

II.3.1 Ocular Health Assessment

Subjects with mTBI underwent a comprehensive eye exam and binocular vision clinical workup before enrollment in the study. The workup included tear break up time and Schrimer strips to assess the tear film, and a slit lamp/fundus exam to determine whether photophobia can be attributed to pathology not specific to mTBI. Results of the exam were used as part of the study and accessed from the participants health records by study staff only after consent has been completed.

Control participants underwent a similar ocular surface work up as part of their study visit, including assessment of dry eye symptoms and signs, to verify ocular health.

II.3.2 Questionnaires

Self-report questionnaires were administered to participants to assess symptoms in a multi-dimensional manner including:

- Demographics, Current Medications, and Comorbidities: In-house questionnaires designed to inventory demographic, medication, and comorbidity information, to subsequently assess as factors in pain/photophobia development and maintenance. These questions were asked as part of the screening process, as some medications and comorbidities are exclusionary, but were recorded and included in the analysis. Age, but not date of birth, were recorded in the demographic form
- Pain - Photophobia Intensity Rating: Assesses current eye pain and light sensitivity now, and the average and most intense experienced over the past week, on a 0-100 Visual Analog scale. These questions will be asked as part of the screening process but will be recorded and included in the analyses. Ratings will be asked for both right and left eye to determine existence/extent of pain laterality.
- Pain History Questionnaire: An in-house questionnaire designed to inventory the existence and duration of non-eye pain in the body (e.g., lower back pain).
- Pain Catastrophizing Scale and Pain Catastrophizing Scale for Children: The PCS questionnaire assesses negative thinking related to pain, which can affect the experience of pain. Subjects read and evaluated 13 pain related items on a 5-point scale (Crombez et al., 2003).
- Visual Light Sensitivity Questionnaire 8: assesses and reports the presence and intensity of photophobia (Lovell & Collins, 1998)
- Post Concussive Symptom Scale (PCSS): The questionnaire is 21-items with a scoring range of 0-6. 0 being none, 1-2 being mild, 3-4 being moderate and 5-6

being severe. It is often used as part of neuropsychological assessment in the diagnosis and treatment of concussion.

II.3.3 MRI Imaging

Subjects completed 1-hour of MRI imaging. Using a 3 Tesla Siemens Trio scanner (Erlangen, Germany) anatomical scans were taken in the form of a high-resolution 3D whole-brain anatomical scan (Magnetization Prepared Rapid Gradient Echo [MPRAGE]). Functional Gradient Echo – Echo Planar Imaging (GE-EPI) scans were also acquired to measure BOLD signal fluctuations with: (1) an event-related design with a light vs. dark visual stimulus presentation; and (2) resting state where participants were asked to remain still, clear their minds, keep their eyes open and not fall asleep. Diffusion Tensor Imaging (DTI) was acquired using an 80-direction protocol with FOV: 256x256 mm.

The visual stimulus protocol consisted of intermittent presentations of two visual conditions: an OFF condition, which featured a white fixation cross on a black background; and an ON condition, a featureless slide with a pure white background. The subject was presented with nine episodes of sustained bright light, each lasting 6 s. The scanner environment was kept dark during the entire experiment, with only a screen providing intermittent brief illumination. At the end of each session, the subject was asked to rate any light-evoked pain intensity and unpleasantness they experienced during the scan/visual stimulation protocol (ON condition) on a 0-100 numeric rating scale. Subjects received one drop of Proparacaine hydrochloride during the scanning session. The MRI protocol involved 5 separate scans:

1. Registration/baseline scan
2. Resting state scan (dark screen)
    a. Asked for NRS pain intensity and unpleasantness ratings (0-100) after the scan
3. Artificial tears scan (light and dark screens)
    a. Prior to scan start, one drop of artificial tears was given to each eye
    b. Asked for NRS pain intensity and unpleasantness ratings (0-100) after the scan
4. Proparacaine scan (light and dark scenes)
    a. Prior to scan start, one drop of Proparacaine was given to each eye
    b. Asked for NRS pain intensity and unpleasantness ratings (0-100) after the scan
5. Structural scan (black screen)

II.3.4 CONN Toolbox with MATLAB

CONN Toolbox is a computer software program used to compute, analyze and visualize functional connectivity by processing fMRI scans. Scans were imported from a secured RCFS folder through Boston Children's Hospital and converted to be compatible with computer software.

ROI-to-ROI analyses were conducted in this study to identify clusters within the fMRI scans of participants that showed significant differences between regions in the mTBI cohort and the Healthy Control. CONN was used to analyze the resting state of both the control cohort and the mTBI cohort. CONN produced ROI-to-ROI connectivity matrices based on these analyses which displayed the level of functional connectivity through z-scores between each pair of ROIs.

III. Results

III.1 Participant Characteristics

Subjects included a total number of 35 participants, 27 withing the Healthy Control group, 4 within the Photophobia Negative group, and 4 within the Photophobia Positive group. 15 participants were male, 18 participants were female and 2 participants identified as non-binary.

III.1.1 Demographics

Demographics for all subjects were collected including race, age, and gender identity (Figure 3, Figure 4, and Table 1). The Healthy control cohort had 27 participants, 14 female, 12 male, and 1 who identified as non-binary. The Photophobia negative cohort had 4 participants, 3 male and 1 female. The Photophobia positive cohort had 4 pariticipants, 3 female and 1 who identified as non-binary. The breakdown of each cohort's gender demographics are graphed in Figure 3.

Figure 3 - Gender

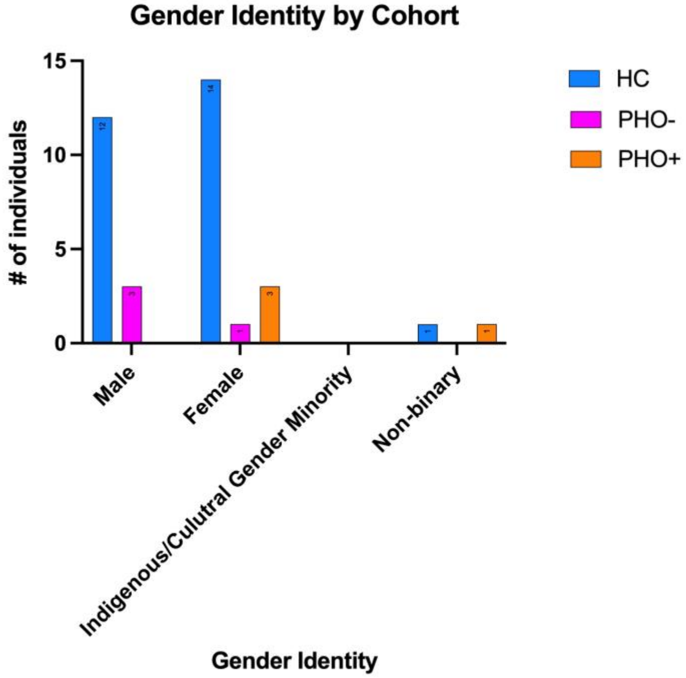

Figure 3 Gender. *Presents the gender distribution of subjects by cohort.*

The Healthy control cohort was comprised of 25 White individuals, 5 Latino/Hispanic individuals, 4 Asian individuals, 1 American Indian/Alaska Native individual, 1 Black/African American individual, and 1 White/Indian individual. The Photophobia negative cohort was comprised of 4 White individuals and 1 Asian individual. The Photophobia positive cohort was comprised of 4 white individuals. The race demographics for each cohort can be visualized in Figure 4. As for age, the mean age for Healthy Control was 17.89 years old, for Photophobia Negative the average age was 18.35 years old, and for Photophobia Positive it was 15 years old. These results can be seen in Table 1.

Figure 4 - Race

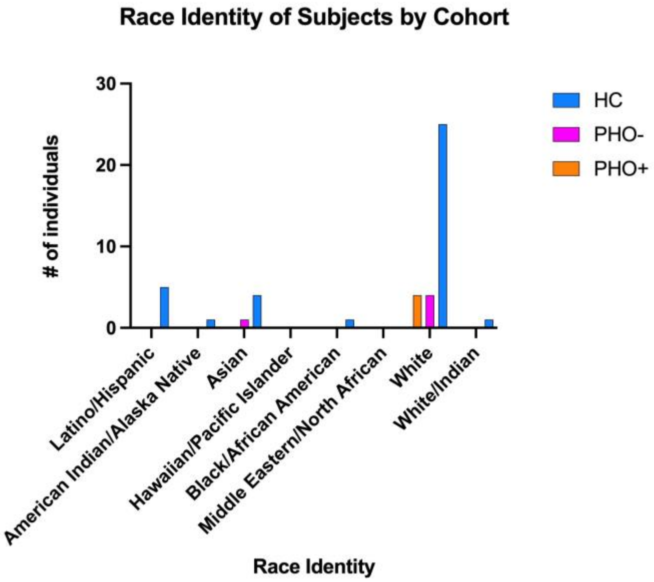

Figure 4 Race. *Presents the race distribution of project subjects by cohort*

Table 1 - Age

Age	Healthy Control	Photophobia Negative	Photophobia Positive
Minumum	10	16	13
Maximum	21	21	17
Mean	17.89	18.25	15
SD	3.06	2.22	1.63

Table 1 Age. *Age distribution of project subjects by cohort. Maximum, Minimum, Mean and Standard Deviation of age of each cohort are displayed***.**

III.1.2 Behavioral Data

Data was collected from participants through questionnaires. The questionnaires used were the PCS, VLSQ8, PCSS, and Pain History Questionnaire. Pain History, PCS, PCSS, and VLSQ8 data results were calculated from 34 of 39 participants due to loss of data.

Table 2 - Pain History Questionnaire

Pain History Questionnaire	Healthy Control	Photophobia Negative	Photophobia Positive
Do you currently experience persistent pain that has been present for the last 3 months or more?	25 No 1 Yes	3 No 0 Yes	0 No 4 Yes
Burning Mouth Syndrome	0 Ever 0 Now	0 Ever 0 Now	0 Ever 0 Now
Headaches	1 Ever 0 Now	0 Ever 1 Now	1 Ever 3 Now
Migraines	0 Ever 0 Now	0 Ever 0 Now	1 Ever 2 Now
Temporomandibular Disorder	0 Ever 0 Now	0 Ever 0 Now	0 Ever 1 Now
Trigeminal Neuralgia Pain	0 Ever 0 Now	0 Ever 0 Now	0 Ever 0 Now
Arthritis	0 Ever 0 Now	0 Ever 0 Now	0 Ever 0 Now
Low Back Pain	0 Ever 0 Now	0 Ever 0 Now	0 Ever 0 Now
Burn Pain	0 Ever 0 Now	0 Ever 0 Now	0 Ever 0 Now
Cancer Pain	0 Ever 0 Now	0 Ever 0 Now	0 Ever 0 Now

Diabetic Neuropathy Pain	0 Ever	0 Ever	0 Ever
	0 Now	0 Now	0 Now
Fibromyalgia	0 Ever	0 Ever	0 Ever
	0 Now	0 Now	0 Now
Muscle Pain	1 Ever	0 Ever	2 Ever
	0 Now	1 Now	1 Now
Sciatica	0 Ever	0 Ever	0 Ever
	0 Now	0 Now	0 Now
Shingles	0 Ever	0 Ever	0 Ever
	0 Now	0 Now	0 Now
Post-surgical Pain	0 Ever	0 Ever	0 Ever
	0 Now	0 Now	0 Now
Tendonitis	0 Ever	0 Ever	0 Ever
	0 Now	0 Now	0 Now
Chronic Fatigue	1 Ever	1 Ever	1 Ever
	0 Now	0 Now	0 Now
Irritable Bowel	1 Ever	0 Ever	0 Ever
	0 Now	0 Now	0 Now
Interstitial Cystitis	0 Ever	0 Ever	0 Ever
	0 Now	0 Now	0 Now

Table 2 Pain History Questionnaire Results. *The table displays the responses by participants per cohort to the question of the Pain History Questionnaire. They replied either with ever having said symptom or experiencing said symptom at the time of data collection.*

The Pain History Questionnaire, depicted in Table 2, was created in house as a measure to inventory the existence of non-eye pain in the body of each participant Ever (at one point in their life) and Now (at the time of the data collection). The symptoms reported by participants were Headaches, Migraines, Temporomandibular Disorder, Muscle Pain, Chronic Fatigue and Irritable Bowel. Notably, for each reported pain symptom except Irritable Bowel, participants with mTBI reported having the symptom at the time of data collection. A majority of symptoms reported by pariticipants were localized centrally.

Table 3 – Mean and Standard Deviation

	Cohort	N	Mean	Std Dev
VLSQ8 Total	HC	26	1.192	3.406
	mTBI	7	8.429	6.079
PCSS Total	HC	24	1.333	4.229
	mTBI	7	25.00	28.821
PCS A&C Total	HC	26	8.654	9.566
	mTBI	7	22.286	16.183

Table 3 *Mean and Standard Deviation by Cohort for each pain questionnaire***.**

Table 4 - t-Test

t-Test	t	df	One-Sided p	Two-Sided p
Pain Catastrophizing Scale (Adult & Child)	-2.131	7.167	.035	.070
VLSQ8	-3.024	7.045	.010	.019
Post Concussion Symptom Scale	-2.166	6.076	.036	.073

Table 3 t-Test Results. *The t-Test displays the correlation of pain questionnaires PCS, VLSQ8, and PCSS. The t-Test shows significance for VLSQ8 and PCSS, but not PCS.*

Data from the pain metrics questionnaires were analyzed using SPSS and found to show significant differences ($p < 0.05$) between cohorts in the VLSQ8 and PCSS (Table

3). The PCSS questionnaire allowed participants to score each post concussion symptomson a range from 0-6. The t-Test result (Table 4) showing a significance for p-value indicates that between the Healthy Cohort and the mTBI cohorts (PHO+/-) there was a significant difference in scores, individuals with mTBI scored more intense post concussion symptoms than those in Healthy Control. On average, the Healthy Control total scored a 1.333 and the mTBI cohort total scored a 25.

The VLSQ8 scale was used to score the subjects' intensity of photophobia they were experiencing. Overall, the results (Table 3) showed higher scores for indivduals in the mTBI cohort indicating more intensity of photophobia experienced. The t-Test data (Table 4) also showed a significance in p-value for VLSQ8 indicating a major difference in photophobia intensity between Healthy Control and mTBI cohorts (PHO+/-), with mTBI cohorts experiencing more intense photophobia than those in Healthy Control. On average, the Healthy Control cohort total scored 1.192 and the mTBI cohort total scored 8.429.

While PCS scale was not statistically significant in differences between Healthy Control and mTBI cohorts, on average Healthy Controls total scored 8.654 and mTBI cohort total scored 22.286 (Table 3).

III.2 Functional Connectivity Differences Between Cohorts

Through processing of fMRI images with CONN Toolbox, 3 clusters of interest were identified. The first cluster (1/325) with Statistic F(2,28) =14.96 and p-unc 0.000038 included structures such as the Pallidum, Putamen, the Supracalcarine Cortex and the Cuneal Cortex. The second cluster (2/325) with statistics F(2,28) = 11.57 and p-unc 0.000217 included structures such as the Hippocampus, Parahippocampal Gyrus, the Frontal Orbital Cortex and Inferior Frontal Gyrus. The third cluster (3/325) with statistics F(1,29) = 16.30 and p-unc 0.000362 included the left and right Paracingulate Gyri.

Figure 5 – Functional Connectivity Cluster 1

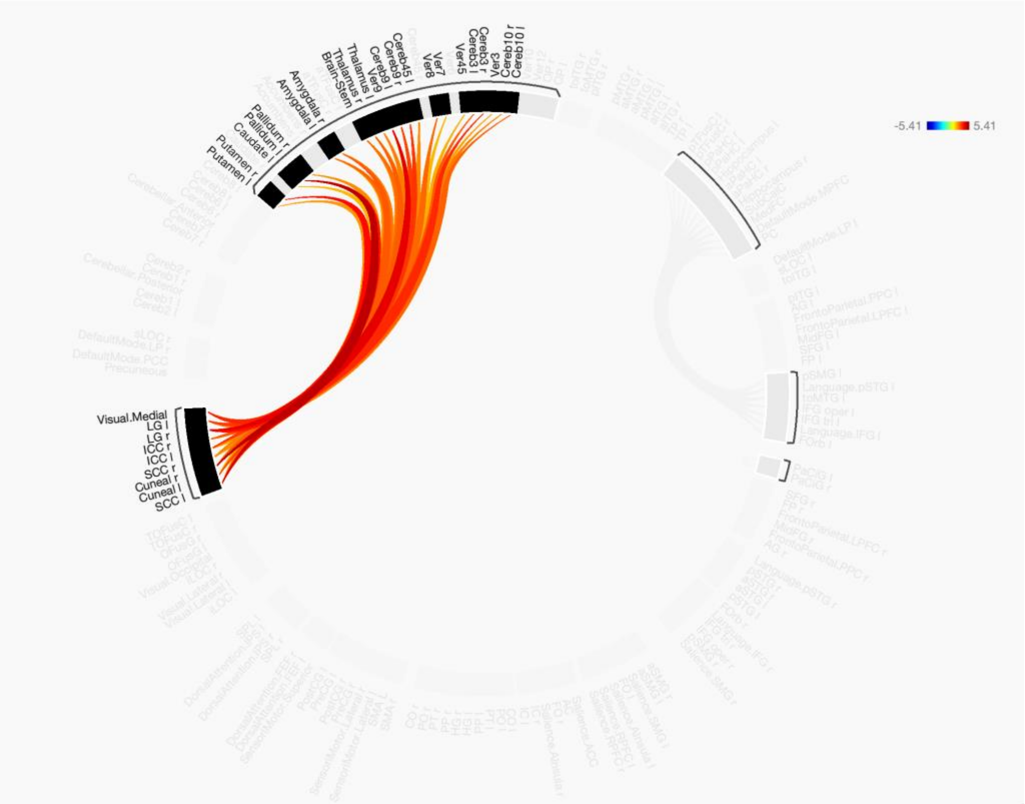

Figure 5 Functional Connectivity Cluster 1. *Graphic that displays the ROIs of Cluster 1 which displayed more activation in the mTBI (PHO+/-) cohort over the Healthy Control cohort.*

Figure 6 Functional Connectivity Cluster 2

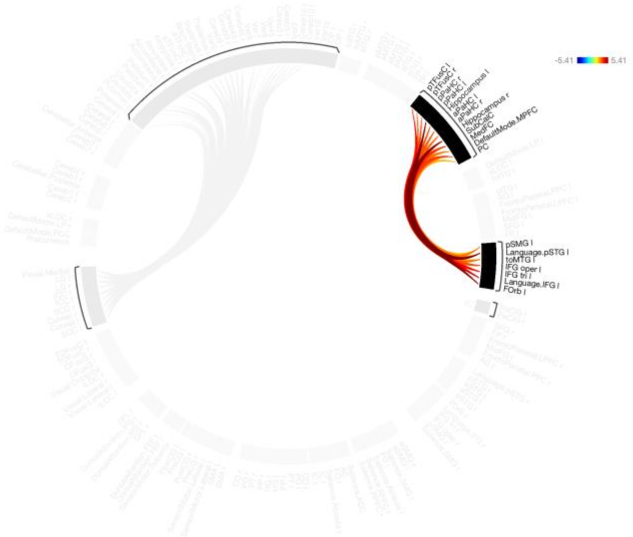

Figure 6 Functional Connectivity for Cluster 2 Graphic displays the functional connectivity of Cluster 2 where mTBI (PHO+/-) cohort exhibited more activation in regions versus Healthy Control Cohort.

Figure 7 Functional Connectivity Cluster 3

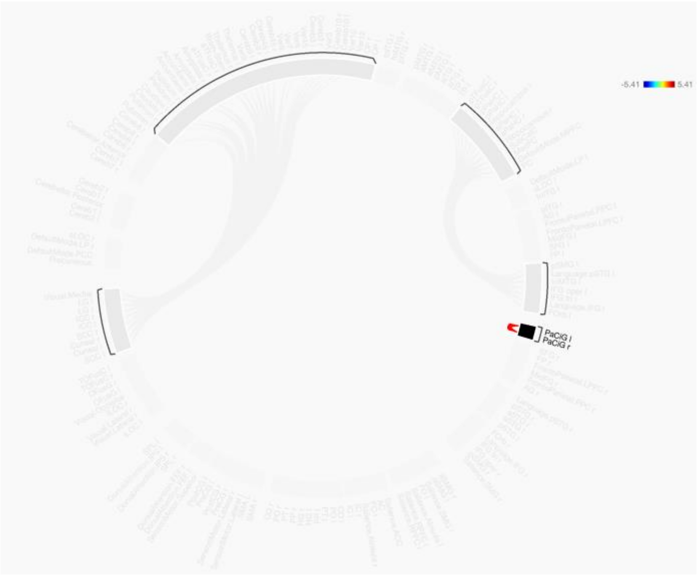

Figure 7 Functional Connectivity of Cluster 3 Graphic displays the functional connectivity of Cluster 3 where mTBI (PHO+/-) cohort exhibited more activation in regions versus Healthy Control Cohort.

IV. DISCUSSION

The effects of ocular pain on persons with mTBI were examined in this study against healthy controls. Using fMRI analysis and analysis of pain focused questionnaires, differences between the Healthy Control cohort and mTBI cohort were observed in regards to pain experience and functional connectivity. Our results from subject responses of the pain questionnaires indicated an elevated reporting of pain symptoms for individuals in the mTBI cohort versus individuals in the Healthy Control cohort. Individuals with mTBI scored significantly higher on the VLSQ8 questionnaire and PCSS questionnaire versus individuals of Healthy Control which indicates an increased perception in pain that is centrally located. The VLSQ8 questionnaire gave subjects the means to rate their eye sensitivity to light. Given the elevated scale ranking for subjects with mTBI, these results suggest that those with mTBI experience ocular pain and photophobia compared to healthy controls. The results of the PCSS questionnaire agree with this as subjects from the mTBI cohort also scored significantly higher ratings compared to Healthy Controls. The PCSS contains symptoms relating to ocular pain such as light sensitivity.

The Pain History Questionnaire (PHQ) displayed an elevated number of responses to “Now” or “Ever” by individuals with mTBI for symptoms: Headaches, Migraines, Temperomandibular Disease, Muscle Pain, and Fatigue. The elevated responses to these pain history symptoms, light sensitivity and post concussive symptoms for subjects with mTBI suggests an increase in pain that is centrally located in these individuals. Given these individuals experienced mTBI, these results are in line with reports from previous literature that connect the inflammation resulting from a concussion to the generation of pain symptoms. In an article by Patterson et al., they explore the specific neuroinflammatory responders to mTBI that cause an inflammatory cascade in the brain (Patterson & Holahan, 2012). With inflammation from mTBI, pain occurs. Additionally, in a study by Portanova et al., they examined factors that are involved in the development for chronic pain after mTBI. In their study, they found that patients with mTBI showed greater pain symptoms than those with more severe TBI and a higher prevalence of chronic pain. In concurrence with our results from pain questionnaires, their findings showed an increase of reporting of headache, sleep disturbance, fatigue, irritability, forgetfulness and poor concentration 1 year post mTBI injury (Portanova et al., 2021). The findings from these studies support our own findings in that those in the mTBI cohort reported more centrally located pain symptoms, like migraine and headache, confirming a correlation between mTBI and increased ocular pain perception.

These pain symptoms of headache, migraine and light sensitivity are further supported by the results of the fMRI images collected for this study. The results of the fMRI analysis discovered 3 clusters of significance in functional connectivity. The ROIs that showed increased connectivity in the mTBI cohort over the Healthy Control cohort included the cuneal cortex, supracalcarine cortex, pallidum and putamen in Cluster 1, the inferior frontal gyrus, frontal orbital cortex, hippocampus and parahippocampal gyrus in Cluster 2, and the paracingulate gyrus in Cluster 3. From Cluster 1, the pallidum and putamen are structures that comprise the basal ganglia (Crosson et al., 2002), the cuneal cortex and supracalcarine cortex are structures of the occipital lobe (Johns, 2014). From

Cluster 2, the parahippocampal gyrus is a structure of the temporal lobe (Van Hoesen et al., 2000), and the inferior frontal gyrus and frontal orbital cortex are structures belonging to the prefrontal cortex (Petrides & Pandya, 2004). From Cluster 3, the paracingulate gyrus is a structure of the prefrontal cortex(Petrides & Pandya, 2004). These structures have been linked, in previous literature, to playing a role in the perception of pain.

In regard to the basal ganglia, whose structures of the pallidum and putamen were ROIs that appeared to show significantly higher connectivity in the mTBI cohort than the Healthy Control, it has been studied in previous literature in its connection to pain processing and perception. In an article by Borsook et al. they examined the connections between the basal ganglia and other brain regions with regard to pain. In their study they found that damage or blood loss to the lenticular nucleus which is comprised of the pallidum and putamen, the two basal ganglia structures identified in our study for Cluster 1, can result in pain. The basal ganglia has connections with other brain regions, like the prefrontal cortex, hippocampus and somatosensory cortices, which are involved in motor control, behavior, attention and learning. These connections are relevant to the perception of pain because the response to pain involves motor, cognitive, emotional and sensory processing. Their findings also supported an increase in connectivity within the pallidum and putamen to mechanical and cold allodynia stimuli (pain from a non-painful stimulus). Specifically, the putamen showed increased connectivity in chronic and acute pain conditions which they found correlated to an increase in volume of the putamen. They hypothesized this increase in volume could be due to a continuous drive for activation by pain stimuli in chronic/acute pain conditions (Borsook et al., 2010). Their findings support ours as the mTBI cohort experienced chronic pain and displayed increased connectivity in the basal ganglia from fMRI analysis which suggests that there is a connection between pain perception in these individuals that is perceived via the basal ganglia.

The prefrontal cortex (PFC) was a second location that showed significant increase in activation in individuals with mTBI over Healthy Controls. This finding is supported by previous literature that highlights the PFC as being an integral part in pain processing. Ong et al. studied PFC's role in pain processing and found that pain intensity activates the ventral pathway from the insular cortex to the PFC and that spatial aspects of pain activate the dorsal path from the posterior parietal cortex to the PFC (Ong et al., 2019). Ong et al.'s findings support our finding of increased activation of the PFC regions in our fMRI analysis as a pathway for pain perception. An additional finding by Ong et al. was that atrophy of the PFC gray matter in combination with reduced white grey matter integrity and connectivity to the basal ganglia was found in patients who had chronic complex regional pain syndrome. While this finding is not in relation to patients with mTBI, it suggests a possible avenue for further research in the topic of our study in regard to changes in brain volume of regions affected by mTBI.

The third region of most significant difference between mTBI and Healthy Control cohorts was the hippocampus which is a region that is associated with memory and learning within the brain and involved in the generation of episodic memory (D. Burman, 2023). Mutso et al. explored the abnormalities of hippocampal functioning in individuals with chronic pain. Their hypothesis stated that chronic pain could be considered a state of continuous learning through which aversive emotional associations were made with normal events in life due to the fact individuals are in a persistent state of chronic pain. Additionally, their results demonstrated decreased neurogenesis in the presence of chronic neuropathic pain which suggested that hippocampal learning and memory may be critical in chronic pain (Mutso et al., 2012). These findings suggest that our results of increased connectivity in the hippocampus alongside functional connectivity in the basal ganglia and PFC, known pain processing regions, could signify that patients mTBI who are experiencing persistent pain could be learning everyday tasks or normal events as painful. The idea of chronic pain being seen as a continuous state of learning was reiterated in a study by Barroso et al. where they examined the hippocampus and its involvement with memory and learning in individuals with chronic pain. They establish that these individuals with chronic pain repeatedly associate negative mood and aversive emotional states to new pain memory traces. Additionally, they state the hippocampus to be a key piece for the development of chronic pain because its structural and functional characteristics could predispose individuals to developing chronic pain (Barroso et al., 2021). This study suggests not only that the hippocampus is involved in pain, but the functions of the hippocampus could lead to the experience of chronic pain. Therefore, the increase of functional connectivity within the hippocampus alongside the basal ganglia and PFC of our individuals with mTBI could suggest that the pain processing occurring in the basal ganglia and PFC could be endcoded in the hippocampus as a painful memory or a learned pain experience.

The occipital lobe strucutures of the cuneal cortex and supracalcarine cortex were structures that showed significant connectivity in the mTBI cohort over the Healthy Controls. While there is not a significant amount of research linking the occipital lobe to chronic pain or ocular pain specifically, we can suggest a relationship due to the fact our subjects with mTBI scored higher on the VLSQ8 scale for light sensitivity and due to the significant connectivity of other brain regions related to pain. Further research would need to be done to determine whether the occipital lobes plays a role in pain perception like the structures of the basal ganglia, PFC, and hippocampus.

Overall, our results suggest there could be a neurological pathway that causes ocular pain in individuals with mTBI. The results of the VLSQ8, PCSS and PHQ being significantly higher for those in the mTBI cohort support the presence of ocular pain in our mTBI cohort over Healthy Control. The fMRI results indicating greater connectivity in the basal ganglia, PFC, hippocampus and occipital lobe point towards ROIs that could be involved in this neurological pathway. This study took initial steps towards identifying the possible presence of a neurological pathway that results in ocular pain in patients with mTBI. Our findings indicate that further research would be essential in discovering such a pathway with the goal to establish a possible medical intervention that could treat ocular pain in individuals with mTBI.

IV.1 Further Research

From previous literature, two directions were most pronounced that could be avenues for further research for ocular pain in patients with mTBI. The first being studies on how volume of pain related brain regions are affected in chronic pain. The studies done by Mutso, Borsook, and Ong all discuss the differences in volumes of the brain regions in subjects who have chronic pain conditions. For example, Ong found that atrophy of the Ventro-mPFC gray matter along with reduced white matter integrity and connection to the basal ganglia was found with patients who had chronical complex regional pain syndrome (Ong et al., 2019). In our data collection, we did collect the information to determine volume measurements of brain ROIs, but this was beyond the scope of our current study. This could be an area of interest for our purposes as studying how chronic pain from mTBI affects brain volumes in pain regions could give additional discoveries on the pain processing pathway and its relation to ocular pain more specifically.

The second direction that could be explored in further research is the impact of mental health on experiencing ocular pain. In the previous study mentioned by Portanova et al. exploring the factors that can lead to persistent pain, they delved into the effects of mental health on chronic pain perception. They found that those with PTSD and depression along with TBI had the highest risk of experiencing chronic pain. They believed this was due to the fact that pain made these individuals more aware of their mental challenges and exacerbated the chronic pain they experienced. They suggested treating the mental health challenges alongside treating the pain could be most beneficial in improving patient outcomes (Portanova et al., 2021). Our study excluded participants with comorbidities, including mental health conditions, which could be a potential area for future research. These findings could be an interesting avenue to explore within our own study as mental health data was not analyzed and could highlight a relationship between patients with mTBI and ocular pain experienced.

IV.2 Limitations

The effects of mTBI on chronic pain, specifically ocular pain, were observed by studying those with confirmed mTBI history. Using pain questionnaires and fMRI scans, the psychological and functional effects were compared between subjects with mTBI and Healthy Controls. It is noted that our sample size used in the study was a limitation. Ideally, a larger sample size would be preferable for this data collection in order to compare ROIs of connectivity in participants with mTBI over Healthy Controls with more reliability.

A second limitation was the use of fMRI in this study as it has weaknesses in spatial and temporal resolution (Glover, 2011). Spatial resolution as it can cause spatial distortion which can lead to decreased signal detection in smaller regions of the brain that could be ROIs needed to study for our purposes. Temporal resolutions is also limited in fMRI as the neuronal connectivity it is capturing occurs at a much faster rate than the fMRI can capture fully. To increase the temporal resolution in data collection, pairing fMRI with EEG (electro-encephalography) could be a possible avenue to obtaining improved temporal resolution. EEG is a technique used to compare neural connectivity over time and has excellent temporal resolution (Hogendoorn et al., 2015). Integrating EEG with fMRI in future studies could be advantageous for where fMRI is limited in temporal resolution.

V. CONCLUSION

The functional effects of ocular pain were studied as a symptom of chronic pain in subjects with mTBI versus Healthy Control subjects. Questionnaires for patient-reported pain symptoms and light sensitivity as a result of mTBI injury showed significant effects on the experience of pain. fMRI scans and analyses were done to study the functional connectivity of subjects with mTBI and Healthy Control subjects. These analyses suggested evidence of ocular pain due to the effects of mTBI chronic pain. Increased connectivity of ROIs associated with the pain processing pathway, basal ganglia and PFC, in conjunction with the results of increased pain and presence of light sensitivity in patients with mTBI suggest there could be a correlation between these areas of the brain and ocular pain in patientswith mTBI.

Additionally, these findings suggest there could be a learning aspect of pain that involves the hippocampus where individuals with mTBI may learn normal events in their lives as painful memories due to being in a constant state of pain.

These findings suggest a need for further research as there is a lack of research overall on the symptom of ocular pain and its specific pathway in pain processing within the brain. Photosensitivity and pain post-mTBI are common symptoms for patients with mTBI and suggests a need to understand the workings behind how this pain occurs in the hopes for future research to discover a means of intervention for these patient.

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