Histol Histopathol (2021) 36: 711-724

http://www.hh.um.es

Histology and Histopathology
From Cell Biology to Tissue Engineering

REVIEW **Open Access**

Visual deficits after traumatic brain injury

Pratheepa Kumari Rasiah[1], Ben Geier[2], Kumar Abhiram Jha[1] and Rajashekhar Gangaraju[1,2]

[1]Department of Ophthalmology, University of Tennessee Health Science Center and [2]Department of Anatomy and Neurobiology, University of Tennessee Health Science Center, Memphis, TN. USA

Summary. Traumatic brain injury (TBI) is frequently described as any head injury ceasing the brain's normal function. Anatomically, developmentally, and physiologically, the eye is deemed as an extension of the brain. Vision in TBI is underrepresented, and the number of active clinical trials in this field are sparse. Frequently, visual problems are overlooked at the time of TBI, often resulting in progressive vision loss, lengthening, and impairing rehabilitation. TBI can be either penetrative or non-penetrative, associated with degeneration of neurons, apoptotic cell death, inflammation, microglial activation, hemorrhage associated with vascular dysfunction; however, precise animal modeling that mimics the extensive visual deficits of TBI pathology remain elusive. Recent works in both the diagnostics and therapeutics fields are starting to make substantial progress in the right direction. Discussion of current advancements in TBI animal models and the recent pathophysiological findings related to the neuro-glia-vascular unit (NVU) will help elucidate novel targets for potential lines of therapeutics. Only over the past decade have newer pharmaceutical and stem cell-based treatments begun to come to light. The potency for these new lines of TBI specific curatives will be discussed along with the review of current blast-induced TBI models, providing potential directions for future research.

Key words: Retina, TBI, Blast injury, Microglia, Müller, Vision, Chronic traumatic encephalopathy, Traumatic optic neuropathy, Mesenchymal stem cells, RGC

Corresponding Author: Rajashekhar Gangaraju, Department of Ophthalmology, Anatomy and Neurobiology, Neuroscience Institute, University of Tennessee Health Science Center, 930 Madison Ave, Suite 768, Memphis, TN. 38163. USA. e-mail: sgangara@uthsc.edu

DOI: 10.14670/HH-18-315

Introduction

Traumatic brain injuries (TBI), often referred to as the silent epidemic, continue to plague approximately 50 million people each year globally (Li et al., 2020). Although the field itself has come a long way over the past few decades, there is still a large amount of work that is required if anything is to change. Substantial progress has been made in understanding the pathophysiological mechanisms underlying this condition, yet effective diagnostics and treatments are still in their infancy. TBIs are induced via external forces applied to the head, which can have a plethora of different downstream neurological repercussions. Whether it be loss of consciousness, speech difficulties, cognitive deficits, or chronic headaches, the manners in which symptoms manifest all depend on the severity of the TBI that includes mild, moderate, and severe types. While the mild form is characterized by loss of consciousness for a few seconds to minutes, the moderate form involves loss of consciousness for a few hours to a few weeks. Unlike mild to moderate TBI, the more severe cases characterized by prolonged coma are often better recognized and treated in intensive care units due to the comorbidity with physical injuries.

Known as mild traumatic brain injury (mTBI), concussions are the most common type of TBI, affecting 1.6 to 3.8 million individuals each year (CDC). Concussions occur when an individual receives a direct blow to the head or body, resulting in a rapid head movement that disrupts the brain (Galgano et al., 2017). Estimates suggest that close to one-third of athletes have sustained undiagnosed concussions, implying the rate of occurrence is higher than reported (Meehan et al., 2013). Concussions tend to gain the most attention due to their high prevalence as well as the strong linkage between mTBI and chronic traumatic encephalopathy (CTE) though further research is needed to draw definitive conclusions (Aldag et al., 2017). Developing due to repeated blows to the head, CTE is associated with cognitive dysfunction, motor disturbances, emotional dysregulation, and more. In a recent study that analyzed the autopsy cases of 300 athletes and 450 non-athletes,

the authors determined that a total of 42 individuals met the pathological criteria of CTE; 27 of the patients were athletes while 15 were non-athletes, with American football players displaying the highest prevalence (Bieniek et al., 2020). In addition to a sports-related injury, according to the Defense and Veterans Brain Injury Center (DVBIC) of the Department of Defense, a staggering 440,720 U.S. military service members sustained TBI in 2000-2020, of which 82.4% of them were classified as mTBI (Dod worldwide numbers for TBI, 2020).

In 2014 alone, the United States reported approximately 2.87 million TBI-related emergency department visits, hospitalizations, and deaths. Of that number, falls (52%) and motor vehicle accidents (20%) made up the majority of TBI hospitalizations (Prevention, 2019). Moreover, these visits in themselves can serve as a double-edged sword for the individual suffering from the TBI. Not only do individuals have to deal with the physical ramifications of TBI but in some instances need the financial support to continue to live everyday life. Due to the high rates of TBI occurrences, this puts great financial strain on the global economy, with estimates reaching 400 billion USD spent combating this silent epidemic each year (Maas et al., 2017).

The clinical signs of TBI in human subjects vary from subject to subject. TBI cases often refer to two events, the primary insult and the secondary insult. Primary insult/injury is a reference to the initial acute mechanical damage inflicted to the individual. This can come in the form of intracranial hemorrhages, hematomas (epidural or subdural), diffuse axonal injury (DAI), or cerebral contusions. Secondary insult/injury refers to the delayed physiological repercussions that arise as a result of the primary insult. Cerebral edema, increased levels of inflammatory cytokines, excitotoxicity, and ischemia are some of the pathophysiological examples of secondary injury (Greve and Zink, 2009). The multitude of combinations of macro/microstructural abnormalities paired with downstream negative physiological cascades is why TBIs are so challenging to treat. It is because of this the nosology behind this condition has become tangled. For a detailed description of the major clinical and pathological features of TBI, including the visual signs and symptoms associated with the condition, readers are encouraged to refer to the review by Armstrong (Armstrong, 2018).

Unfortunately, the current status of diagnostics and treatments are lacking. Still used today in assessing TBIs, the Glasgow Coma Scale (GCS) serves as an efficient noninvasive method for approximating the severity of a TBI. Yet, its lack of precision requires the utilization of CT and MRI imaging to assess damage for severe cases. Usage of these imaging techniques serves more as a method to determine whether surgical intervention is necessary. If surgery is not required, patients are either monitored or sent home to recover (National Academies of Sciences, 2019). Outside of preventative measures to help stabilize the patient, any neurological damage sustained during the primary event can rarely be reversed. Consequently, treatment for TBIs is limited, consisting of treating symptoms as they arise as opposed to the condition itself.

In the decades since neurotrauma research first began, over 30 clinical trials for potential TBI therapeutics have failed (Ng and Lee, 2019). This speaks to the enigmatic nature of TBI. Although the main challenge faced by the scientific community stems from the uniqueness of each case, breaking it down into clades, this problem becomes more manageable. One such area of focus that is often overlooked is the visual system (Evans et al., 2018). It is estimated that approximately 50% of the cerebral activity is dedicated to visual processing. Thus, visual deficits are a well-known side effect related to TBIs. Unfortunately, visual problems are often unheeded during the initial treatment of the injury, lengthening, and impairing rehabilitation. The unbiased affliction of TBIs to all age groups further exemplifies the need for efficacious treatments.

Clinical trials

At the time of this review, there are over 300 open clinical trials (Clinicaltrials.gov) that describe TBI or trauma, with approximately 56 of these incorporating the term "visual." This suggests that vision in TBI is underrepresented. Most open studies either suggest rehabilitative efforts or the use of a device to bring about better outcomes in TBI. For example, a prospective open trial, "Rehabilitation of Visual Attention Following mTBI" (NCT02719964) set out to evaluate visual attention deficits in U.S. military service members after different cognitive rehabilitation programs on improving attention complaints in mTBI. Currently, there is growing interest in identifying if TBI predisposes subjects to increased risk of dementia. In accordance with this assumption, a case-controlled observational clinical trial, "Contributions of mTBI to Neurodegeneration Due to Chronic Traumatic Encephalopathy (CTE) and Alzheimer's Disease (AD)" (NCT04124029) aims to study subjects' memory, brain wave activity, brain structure and proteins that can be elevated after brain injury and in dementia. Such studies will help determine the future incidence of dementia after TBI. Although not directly related to visual deficits of TBI, "Neurologic Stem Cell Treatment Study (NEST)", an open-label non-randomized clinical trial (NCT02795052) aims to study whether the usage of bone marrow-derived stem cells (BMSC) will provide improvement in neurologic function for patients with certain neurologic conditions. This is the only approved study that aims to determine the efficacy of stem cell therapies via intravenous and intranasal routes for a variety of different neurological conditions, including TBI. We expect these open-label trials will provide a great deal of data concerning the safety of MSC use. Among the trials that were completed, a double-blinded placebo-controlled randomized study of N-Acetyl

Cysteine (NAC) conducted on active duty service members demonstrated beneficial effects on the severity and resolution of sequelae after blast-induced mTBI (NCT00822263) (Hoffer et al., 2013). On the other hand, The Citicoline Brain Injury Treatment Trial (COBRIT), a phase 3, double-blind, randomized clinical trial (NCT00545662) did not result in improvement of functional and cognitive status in TBI patients. Due to no differences between Citicoline, an endogenous substance offering potential neuroprotective properties, and the placebo, the study was terminated (Zafonte et al., 2012). Despite the multiple attempts, clinical trials so far have not produced a successful new therapeutics for neuroprotection in TBI, possibly because the effects of TBI are heterogeneous compared to other clinical diseases. In accordance with this, future clinical studies have been suggested to incorporate the rehabilomics framework, which incorporates biomarker-based examination and simultaneous treatment of neurologic and non-neurologic conditions that likely impact function and rehabilitation outcomes (Wagner and Kumar, 2019). Additional recommendations include leveraging the advances in imaging and information technology (Cox et al., 2019) that can capture both structural as well as real-time changes in biomarkers that will likely inform designed trials for a successful outcome in TBI trials.

Preclinical studies

In consideration of the heterogeneous nature of TBI, a variety of preclinical animal models of TBI have been developed. Among these, the most widely studied models include: fluid percussion injury (FPI), controlled cortical impact injury (CCI), weight drop–impact acceleration injury, and blast injury. For an excellent overview of these models, including their strengths and weaknesses, readers are referred to the review paper by Xiong and colleagues (Xiong et al., 2013). Since visual deficits have been studied extensively in blast-induced TBI models, we will emphasize blast-induced TBI studies further (Table 1). Additionally, we will cover

Table 1. Commonly used animal models to study visual deficits of TBI.

Mode1B2:H16	Species	Single/ Multi Blast	Pressure (psi)	Focal/ Diffuse Blast	Outcome	Author/Year
Blast	Mouse	Single	20	Diffuse	Loss of RGC, retinal thinning and declined visual function	Dutca et al., 2014
Blast	Mouse	Single	4	Diffuse	Axonal degeneration preceeds memory, motor, and visual based tasks	Yin et al., 2016
Blast	Rat	Single	80	Diffuse	Increased ERG amplitudes & reduction in contrast sensitivity for both eyes. As well as increased retinal thickness and GFAP levels	Allen et al., 2018
Blast	Mouse	Single	20-60	Focal	20-psi blasts does not produce deficits. 25-40 psi yields only motor deficits. 50-60 psi produced decreased visual acutiy and contrast sensitivity as well as brain damage	Guley et al., 2016
Blast	Mouse	Single	50	Focal	Focal cranial blast results in ipsilateral retinal microglial activation, axonal injury and poor contrast sensitivity	Guley et al., 2019
Blast	Mouse	Single	50	Focal	Loss of neuronal cells in GCl, increased GFAP, microglial polarization, endothelial activation, decreased visual acuity and contrast sensitivity	Jha et al., 2018
Blast	Rat	Single	~19	Diffuse	Increased plasma levels of IPA in the plasma at 1 and 4 h post blast. Deecreased visual acuity, ERG amplitudes, cellular voids in the outer nuclear layer and increased GFAP within 8 days post blast	Arun et al., 2020
Blast	Mouse	Single	50	Focal	Increased extracellular glutamate, decreased GS/GLAST and increased AQP4 in retina accompanied by decreased ERG amplitudes	Jha et al., 2021
Blast	Mouse	Multi	43.5	Diffuse	Retina displayed activation of Muller glia, loss of photoreceptor cells, and increased levels of phosphorylated tau (Thr231 & Thr181)	Mammadova et al., 2017
Blast	Mouse	Multi	5, 20	Diffuse	Low levels of blast preconditioning helped preserve RGC structure/function	Harper et al., 2019
Blast	Mouse	Multi	50	Focal	Consussive but not subconcussive multiple focal air blasts increased pathology in retina, optic nerve, and oculomotor nucleus, as well as drove microglial shift from M2 to M1 state. Neurodegeneration evident over 14 months	Honig et al., 2019
Ocular Blast	Mouse	Single	23.6, 26.4, 30.4	Focal	Retinal damage, decreased visual acuity, corneal edema, cornela abrasions, and optic nerve avulsion	Hines-Beard et al., 2012
Ocular Blast	Mouse	Single	26	Focal	Drop in visual acuity in contralateral eye with -10% retinal cell death	Bricker-Anthony and Rex, 2015
Ocular Blast	Mouse	Single	26	Focal	Retina and optic nerve exhibited increased caspase-1 & RIPK1/3 in inner retina with increased caspase-1 in starburst amacrine cells and drop in visual acuity	Bricker-Anthony et al., 2014
Ocular Blast	Mouse	Multi	15	Focal	Increased glial presence in optic nerve as well as levels of Il-1a and Il-113 in the ON & retina	Bernardo-Colon et al., 2019
Ocular Blast	Mouse	Multi	15, 26	Focal	Quick successive non-damaging insult resulted in stronger axonal degeneration compared to daily blows	Vest et al., 2019

various experimental therapeutics that have been tested in various TBI models with special emphasis on the impairment of the integrity of visual systems following blast-induced TBI. For an excellent overview of our current understanding of the pathology and molecular mechanisms of visual deficits in TBI, readers are referred to the review by Sen (2017).

A growing literature supports the findings that over 78% of ocular injuries are a result of modern conflict and are associated with blast-related ammunition (McMaster and Clare, 2021). Although advances in body armor significantly reduced the incidence of bodily injury, the most vulnerable parts such as the brain, neck, and face, in particular, the eyes, are susceptible to a higher rate of injury. Additional concerns include repetitive exposure to blast injuries or the secondary wave exposure resulting in additive or synergistic influence on primary blast; inability to provide medical attention in the 'golden hour' thus delaying time-sensitive critical injuries are shown to complicate blast-related TBI outcomes. We and others have developed animal models of blast TBI that recapitulate the real-world scenario to better understand the pathophysiology. Two major categories of blast TBI models are being studied: 1) whole head blast injury and 2) focal blast injury. In the latter category, focal injury may be on the brain or eye that results in a focal injury in the absence of a bodily injury that will help address precise molecular pathophysiological mechanisms of TBI without comorbidities. Finally, several studies have developed animal models in which multiple repetitive blast injuries were more effective at demonstrating long-term neurodegeneration over the single blast models.

Harper and colleagues have used a pressurized enclosed blast chamber fitted with a Mylar membrane to create a blast wave resulting in loss of retinal ganglion cells (RGCs), retinal thinning accompanied by declined visual function as assessed by Pattern-evoked electroretinography (PERG) starting at 2 weeks lasting until 16 weeks after injury (Dutca et al., 2014). Interestingly, early axonal degeneration in this model was shown to be the likely key driver of subsequent neurobehavioral complications of blast-mediated TBI (Yin et al., 2016). One limitation of this model is that the entire head is involved in blast wave exposure. To this end, Rex and colleagues have developed a blast injury model in which blast waves were provided to the left eye directly, while the head, body, and contralateral eye were protected from blast injury. Interestingly, blast mice developed corneal edema, optic nerve avulsion, decreased visual acuity, and intraocular pressure (IOP) about 4 weeks post-blast injury suggesting that primary blast exposure alone is sufficient to induce severe and permanent damage to the eye and to the retina (Hines-Beard et al., 2012). While the contralateral eye is shielded from the over-pressure air-wave, the authors detected vision loss in the contralateral eye due to blunt trauma. This model system can be used to explore mechanisms and functional outcomes of both blast and blunt ocular trauma, a frequent scenario experienced by military personnel during combats (Bricker-Anthony and Rex, 2015). Although the direct ocular blast model did not develop RGC loss, a remarkable increase in markers of oxidative stress, inflammation and necroptosis was observed in the inner retina about 4 weeks post-blast injury (Bricker-Anthony et al., 2014). While most studies performed evaluated short term consequences of blast injury up to 4 to 8 weeks post-blast, Pardue and colleagues have developed a rat model of single blast injury using a modified shock tube device to the head and followed up long-term for 8 months to assess retinal structure and function deficits (Allen et al., 2018). Interestingly, a single blast injury could have long-lasting deficits with increased ERG amplitudes, delayed implicit times, decreased visual contrast sensitivity accompanied by thickened retina with gliotic changes (Allen et al., 2018). This study provides compelling reasoning to advocate for a more comprehensive retinal examination that would include ERG, SD-OCT, and visual function testing in subjects several months after exposure to such a blast injury.

Contrary to the aforementioned studies that involve whole body explosive blast, Reiner and colleagues have developed a focal cranial blast model that replicates mTBI in the absence of any bodily injury to selectively study the pathophysiological mechanisms of blast injury (Heldt et al., 2014; Guley et al., 2016). In this air-blast mouse model, a single 50-psi focal cranial air blast produces small, transient deficits in motor function and widespread axonal injury but no overt damage to the brain or the eye. Interestingly, blast pressure wave crossing the skull associates with stretch, stress, and shear forces resulting in injured axons, as is evident by the presence of swollen axonal bulbs in the optic nerve. As a result of increased microglia that have been activated by molecules released from the RGC axons, visual deficits over 4 to 11 weeks after a primary injury have been observed. Despite the eyes being shielded from the blast, this model demonstrates retinal injury in the form of reductions in ERG amplitudes, upregulation of glial fibrillary acidic protein (GFAP) at 30 days (Guley et al., 2019), and thinning of the inner retina in the ipsilateral eye by 11 weeks (Reiner et al., 2015). Although early microglial activation leading to premature microglial aging and dysfunction plays an important role in the pathogenic process, using the same focal cranial blast model, we have recently identified changes in Müller cells accompanied by increased excitatory extracellular glutamate levels in the retina (Jha et al., 2021). Unlike other models that have shown changes in the Caspase levels and consequent apoptosis in the inner retina, only focal changes of retinal ganglion cell layer (GCL) with loss of neuronal or vacuolated cells in blast group retina were observed with focal cranial blast model (Fig. 1). It is likely that the observed damage might have been caused by retinal inflammation (Jha et al., 2019) and oxidative stress as indicated by increased DNA-RNA damage markers (Fig. 2) or

decreased synaptophysin levels in the inner nuclear layer (INL), resulting in structure-function alterations involved in the regulation of synaptic plasticity (Fig. 3). Taken together, these studies support that the focal cranial blast model closely represent a closed-head mTBI that develops widespread loss of axons in the optic nerve, retinal thinning, glial activation, extensive retinal inflammation and oxidative stress to demonstrate significant neurodegeneration and loss of vision (Guley et al., 2016; Jha et al., 2018, 2021; Honig et al., 2019).

It has been shown that repetitive blast-mediated TBI results in cumulative visual deficits. A prospective longitudinal evaluation of military breacher training subjects demonstrated that exposure to repetitive low-level primary blasts might have detrimental effects on visual field sensitivity. This suggests that even a low-level primary blast has the potential to produce occult eye injury (Capó-Aponte et al., 2015). To address this repetitive blast-mediated TBI, several animal models were developed. Tzekov and colleagues developed a repetitive blast model with an inter-concussion interval of 48 hours over a 10-day period delivered using an electromagnetic controlled impact device and assessed 10-13 weeks after injury resulted in optic nerve damage and 67% loss of RGCs (Tzekov et al., 2014). This model was further improved by changing the impact depth from

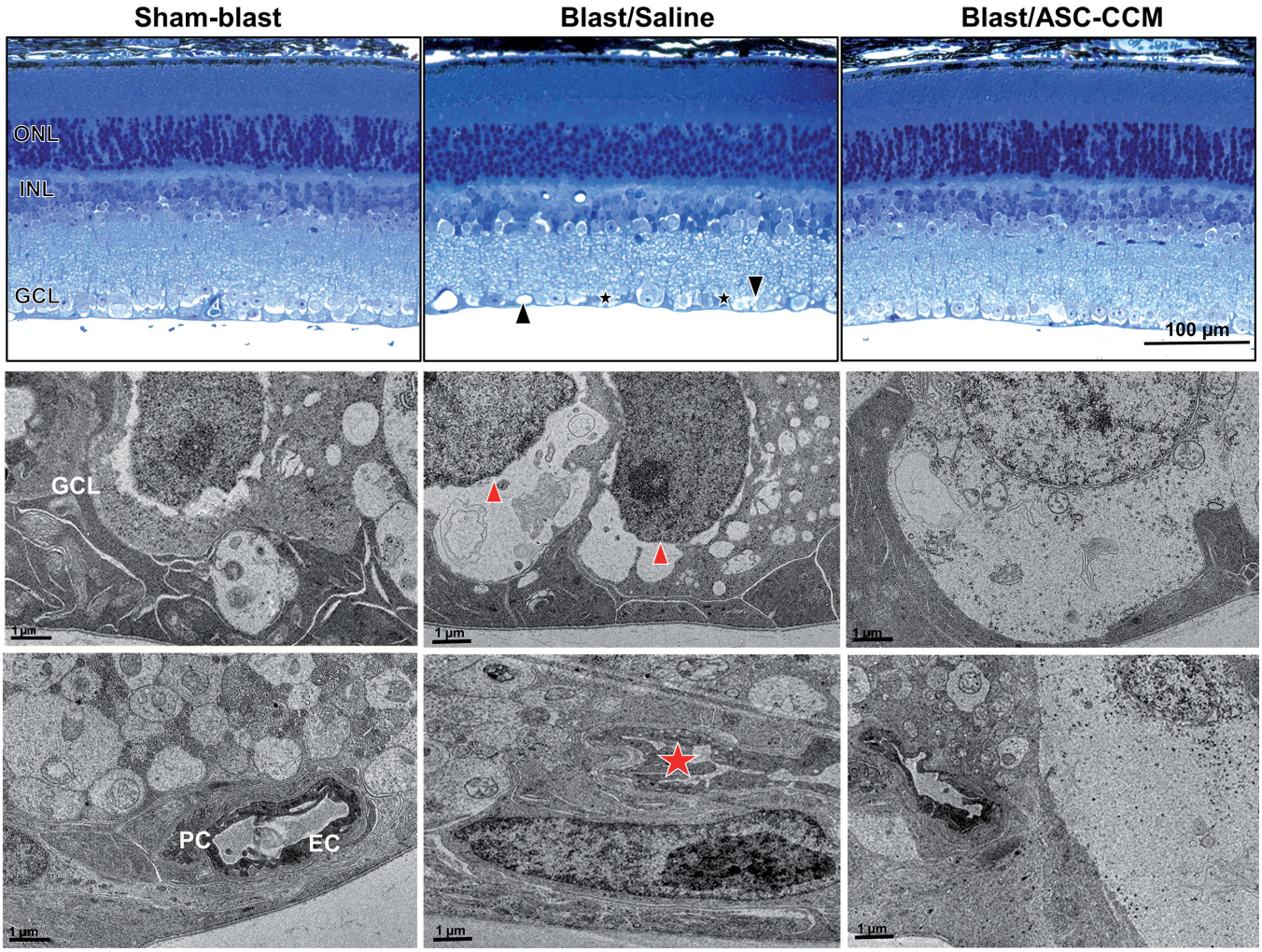

Fig. 1. Morphological and ultrastructural changes in the retina after focal cranial blast injury. Retinal tissue 4 weeks post-blast injury fixed in 2% paraformaldehyde and 2.5% glutaraldehyde in 0.1 M PB, pH 7.3 embedded in Araldite CY212. Thin sections (1 μm) stained in toluidine blue were imaged with Lionheart™ FX Automated Microscope (Biotek US., Winooski, VT) revealing focal loss of neuronal cells (star) and many vacuolated spaces (arrowheads) in GCL in the blast mice retina. (Data reproduced from Jha et al., Int. J. Mol. Sci., 2018). Thin sections (70-80 nm) were stained with uranyl acetate and lead citrate and viewed under a Morgagni 268D transmission electron microscope (FEI Company, Eindhoven, The Netherlands). Sham-blast mice retina shows a normal appearance of the RGC layer and intact vasculature. Blast mice retina shows vacuolated cytoplasmic space around the nucleus, dark and shrunken nuclei (arrowheads), and altered microvasculature (star) in the RGC layer. Intravitreal delivery of adipose tissue-derived MSC secretome (ASC-CCM) provided right after blast injury shows near-normal morphology 4 weeks post-blast injury. Data displayed is a representation of 3 animals/group. PC: pericyte; EC: endothelial cell; GCL: ganglion cell layer.

1 to 1.5 mm, which resulted in degenerative changes in the entire visual pathway, including nerve fiber damage in the optic tract, optic chiasm, and optic nerve (Das et al., 2019). Mammadova and colleagues have used a shock wave head injury model to deliver 300 kPa (43.5 psi) blast wave pressure for three consecutive days resulting in inflammation as evidenced by glial cell activation, neuronal loss, and an increase in phosphorylated tau in the retina 1-month post-injury (Mammadova et al., 2017). Interestingly, the authors did

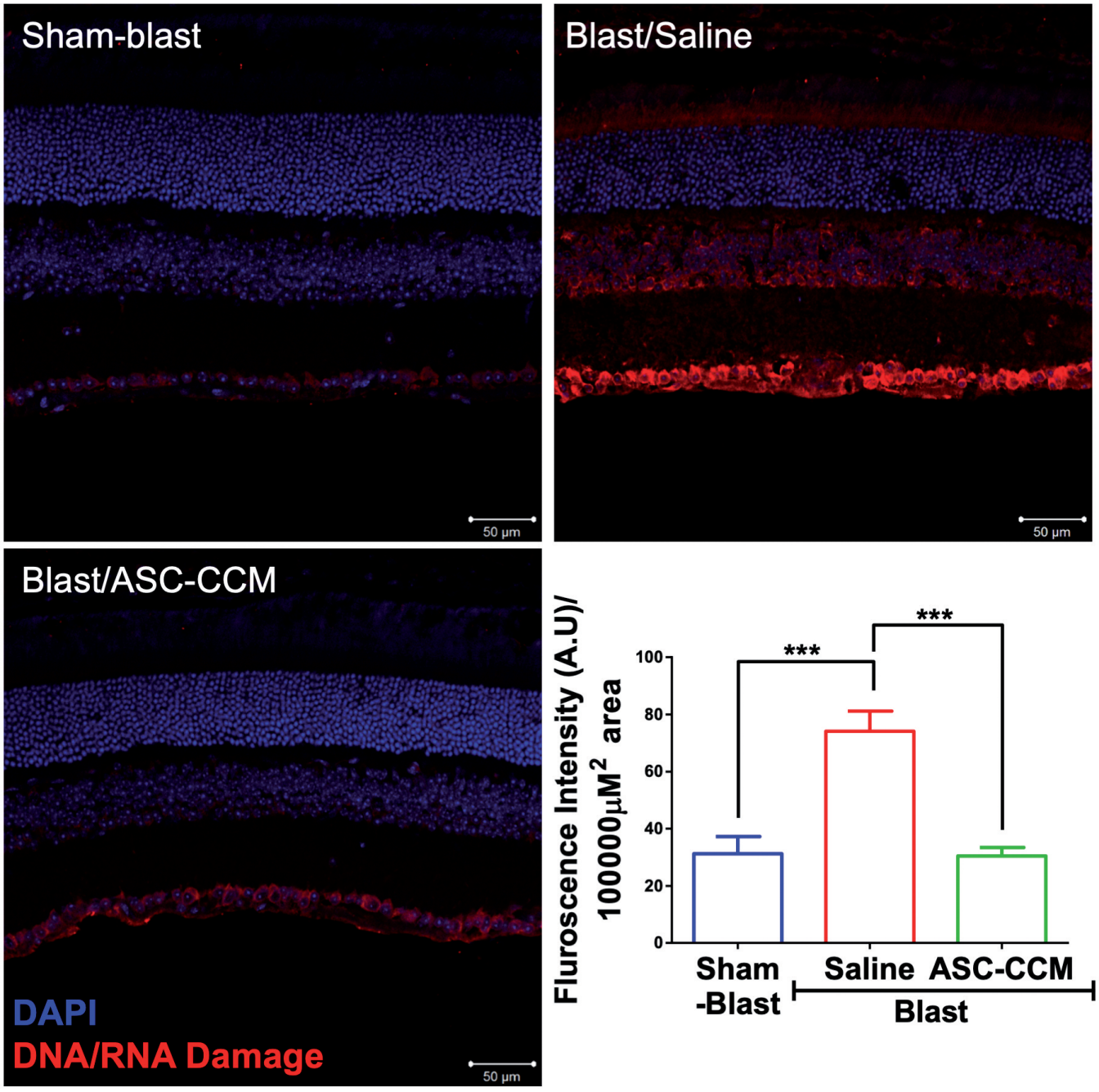

Fig. 2. Increased oxidative stress, as evidenced by DNA and RNA adducts in the retina of focal cranial blast injury. Blast mice retina shows increased immunostaining for Anti-DNA/RNA Damage antibody [15A3] (ab62623) as compared to sham-blast mice retina. Intravitreal delivery of adipose tissue-derived MSC secretome (ASC-CCM) provided right after blast injury shows a near-normal pattern of immunostaining for DNA and RNA adducts. Quantitative analysis of Anti-DNA/RNA Damage antibody-positive area throughout the retina is represented is from 4 animals/group at 4 weeks post-blast injury. One-way ANOVA, Bonferroni posthoc test. *** $p<0.001$.

not find any significant changes in cognitive or motor function resulting in speculation that the retina may be more sensitive to injury as compared to CNS and may serve as the sensitive indicator of low-level injury due to blast wave pressure (Mammadova et al., 2017). Harper and colleagues investigated the effects of multiple blast waves that were separate by 1 hour or 1 week on RGC function, finding no difference in single blast mice. However, if mice were preconditioned with low-level blast exposure, their RGCs were significantly protected from subsequent higher-intensity blasts (Harper et al., 2019). Contrary to this line of observation, Reiner and colleagues observed that repeated exposure to subconcussive insults (four air blasts one week apart to the left side of the mouse cranium) resulted in worse outcomes than concussive (single blast) air blasts

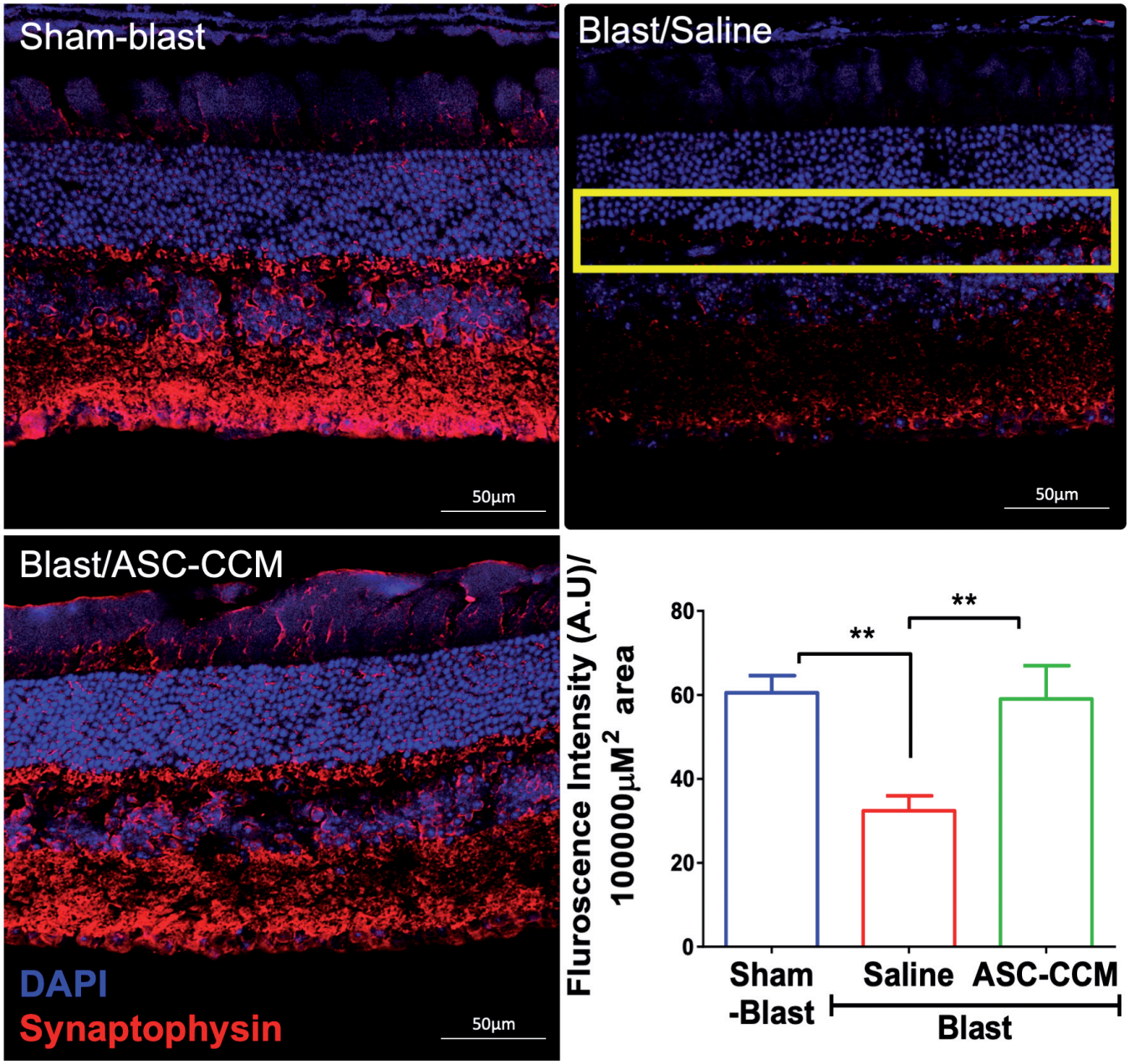

Fig. 3. Synaptic changes in the retina of focal cranial blast injury. Blast mice retina shows decreased immunostaining for synaptophysin levels as compared to sham-blast mice retina. Intravitreal delivery of adipose tissue-derived MSC secretome (ASC-CCM) provided right after blast injury shows a near-normal pattern of immunostaining for synaptophysin. Quantitative analysis of synaptophysin positive area at the OPL is represented from 5 animals/group at 4 weeks post-blast injury using Anti-Synaptophysin antibody [SY38] (ab8049). One-way ANOVA, Bonferroni posthoc test. ** $p<0.01$.

delivered to the head of mice. The authors followed up the behavioral assessment via characterization of microglia morphology in the hippocampus 14 months after the delivery of the injury, resulting in microglial activation and dysfunctional endothelium accompanied by memory loss (Honig et al., 2020). Whether such long-term repeated air blasts will have any effect on the retina and subsequent visual dysfunction is unknown. To this end, using a rotational acceleration model of repetitive head injury (closed head injury by mechanically engineered rotational acceleration; CHIMERA) Desai and colleagues were able to document decreased visual evoked potential with no changes in electroretinography (Desai et al., 2020). Interestingly, increased visual dysfunction in this model was correlated with immunoreactive increases in both GFAP and Iba-1 in the optic tract. This observation led to the speculation that inflammation in the affected brain region conveys signals for impaired vision.

Distinct from these repeat blast studies, Rex and colleagues have identified indirect traumatic optic neuropathy (ITON), a condition that is commonly associated with TBI that is distinct from neuropathologies such as glaucoma. In this eye-directed air-blast exposure model, a transient increase in intraocular pressure was observed with subsequent RGC death and axon degeneration in the optic nerve resulting in a significant reduction in visual function (Bernardo-Colón et al., 2019). Subsequently, the same group has used their closed-system ocular neurotrauma model to deliver bursts of 15 or 26-psi overpressure with an inter injury interval of 1 min or less. This resulted in synergistic axon damage, whereas mild repeated trauma at longer inter injury intervals caused supplementary damage (Vest et al., 2019). Taken together, these studies suggest that repeated blast injuries, whether they are directed to ocular tissue or the brain, increase the susceptibility of axons in the optic nerve to greater damage to the visual system.

Experimental therapeutics

Given that the eye is a continuation of CNS, TBI related visual deficits are initiated by primary axonal injury and brain damage resulting in progressive neuroinflammatory cascades and neurodegeneration. Though the exact processes and probable mechanisms are elusive, trauma produced optic nerve damage, RGC loss, sub-retinal hemorrhage, photoreceptor degeneration, thinning of the retina, and subsequent visual dysfunction have been the primary target for experimental therapeutics. The therapeutic approaches reported thus far address the accompanying degenerative cascades and improvement of visual functional response, not the primary insult itself. However, the loss of RGC, retinal detachment, and other consequences are seemingly irreversible.

A number of studies have used the optic nerve crush injury model that closely mimics TBI to study the protection strategies to rescue dying RGCs. Due to their inherent capacity to support regeneration, cell-based therapies such as mesenchymal stem cells (MSC) are shown to regenerate RGC's and injured axons (Mead and Scheven, 2015). *In vitro*, axotomized RGC's co-cultured with human dental pulp stem cells (DPSC), human bone marrow-derived mesenchymal stem cells (BMSC), and human adipose-derived stem cells (ASC) have successfully shown to impart protection. Interestingly, *in vivo* transplantation of DPSCs or BMSCs delivered intravitreally after an optic nerve crush injury promoted the survival of Brn-3a$^+$ RGCs and axon regeneration. This finding was independently confirmed by Mesentier-Louro et al. (2014), who demonstrated improved Tuj1- or Brn3a-positive cells in retinas and optic nerve regeneration post intravitreally injected BMSCs. Recent studies have focused on paracrine factors secreted by MSC's inclusive of extracellular vesicles loaded with mRNA, regulatory miRNA, anti-inflammatory biologics, and several trophic agents. Intravitreally delivered BMSC secreted exosomes have been shown to promote RGC survival after TON (Mead and Tomarev, 2017). Krüppel-like factor 4 (KLF4), a conserved zinc finger-containing transcription factor, has been linked to axon regeneration. Accordingly, Cui and colleagues reported KLF4 knockdown *in vivo* significantly enhances ciliary neurotrophic factor-induced axon regeneration of RGCs after optic nerve crush via the inhibition of JAK-STAT3 signaling (Cui et al., 2017). Although the above studies discussed have a positive outcome in terms of RGCs protection *in vivo*, the limitation of the optic nerve crush model being an indirect feature of TBI related retinal damage should be considered. To this end, we have used our focal cranial blast model to demonstrate a significant rescue in neuronal cells in the RGC layer using intravitreally delivered adipose tissue-derived MSC secretome (Fig. 1) (Jha et al., 2018). Harper and colleagues used a novel class neuroprotective compound P7C3-S243 in their shockwave blast model. The compound appears to show a visual function rescue in the latent period that can be related to protection against functional degeneration of RGC (Dutca et al., 2014). PERG deficits observed one-week post TBI injury showed a temporary deficit during the latent period and resolved by 4 weeks post-blast injury. Treatment with P7C3-S243 displayed improved provocative PERG response throughout the 16 week recovery period. However, loss of RGC during the chronic post-TBI period due to significant dendritic retraction or ganglion cell rearrangement seems to be permanent. This suggests that early RGC preservative intervention is crucial to mitigate long-term visual impairments post-TBI.

Given that optic nerve injury is the most common event after TBI, the associated axonal damage, oxidative stress and inflammation that arise from it have become the next target for TBI related visual deficits. Erythropoietin (EPO), a known neuroprotective agent that inhibits neuronal apoptosis, was tested in a single 26psi over-pressure air-wave ocular blast model.

Systemic treatment of EPO significantly reduced cell death in optic nerve axons, reduced glial reactivity, and protected against oxidative stress only when delivered post-blast (up to 3 weeks after injury). Alternatively, EPO significantly increased cell death if it was administered before or soon after the primary insult (Bricker-Anthony et al., 2017). Because of its multifaceted role in protecting against axon degeneration and loss, the FDA-approved acetylcholinesterase inhibitor, Galantamine was tested in the repeat ocular blast model of TON. Provided in the blast mice drinking water, Galantamine successfully protected against axon degeneration and mitigated visual function deficits by dampening oxidative stress and inflammation (Naguib et al., 2020). Increased histone methyltransferase, G9a has been linked to downregulation of antioxidant mechanisms. Through activation of the Nuclear factor E2-related factor 2 (Nrf2)-antioxidant response element signaling pathway, it manages to regulate the transcription of antioxidant genes. Gupta and colleagues have tested UNC0638, a known G9a inhibitor in a CCI-TBI model to restore RGC and optic nerve axon loss (Gupta et al., 2019). Animals receiving UNC0638 showed a significant reduction in the methylation pattern of H3K9Me2 in the retina and optic nerve. Inhibition of G9a with UNC0638 demonstrated improvement of antioxidant response, restored RGC death, protection from optic nerve degeneration, and improved retinal thickness. Enhanced DNA binding activity of Nrf2 as elicited by UNC0638 is instrumental in neuroprotection, axonal preservation and epigenetic modifications (Gupta et al., 2019). Lysophosphatidic acid (LPA), a bioactive metabolite of phospholipids known to be elevated in biofluids after injury has recently been identified as a therapeutic target in a rat model of blast-induced ocular injury. Intravenous administration of anti-LPA antibody (but not the isotype-matched control antibody) one hour post blast exposure significantly alleviated GFAP in the retina, improved visual acuity and amplitudes for both a and b-waves 7-8 days post blast injury (Arun et al., 2020). Though the authors were unable to correlate the observed benefits to decreased retinal levels of LPA, it is essential that future studies focus on LPA species in order to link pro-inflammatory activities of LPA with the direct promotion of neurodegeneration within the retina.

Microglia are the immune watchdog of the injured retina. In accordance with this, several studies have targeted microglia as the therapeutic target for TBI related retinal damage. Reiner and colleagues in their focal cranial blast model showed upregulation of activated microglia in the retina paired with a decrease in visual acuity and contrast sensitivity in blast mice. Cannabinoid type-2 (CB2) receptor inverse agonist SMM-189 when provided daily beginning 2 hours post-blast, reduced axon injury, and microglial abundance with a bias towards M2 state at three days. Furthermore, it mitigated axon loss, contrast sensitivity deficits, microglial abundance, and Müller cell GFAP upregulation over a month after blast injury (Guley et al., 2019). This study provided the first clue that microglial polarization may mediate the observed visual deficits of TBI. Consequently, FDA-approved estrogen receptor drug, Raloxifene was tested in the same model. Interestingly, not only Raloxifene delivered daily for two weeks after blast but also when delayed until 48 h after TBI, it was effective in polarizing microglia as well as able to reverse visual injury after brain trauma (Honig et al., 2019). Following these studies, we have shown that adipose tissue-derived MSC secretome treatment attenuates the retinal inflammation by suppressing the activation of the pro-inflammatory cascade, favoring the polarization of microglia from the M1 towards the M2 phenotype both *in vitro* and in blast injured retina (Jha et al., 2018). To better understand which of the pro-inflammatory molecules subsequent to microglial polarization play a role in the retina, as a next logical step, pharmacological blocking of IL-1 pathway or STAT3 signaling was explored. Blockade of IL-1 pathway using IL-1R antagonist, Anakinra prior to blast injury protected RGC loss, decreased microglial activation, and improved PERG response four weeks post-blast injury (Evans et al., 2020b). Although the effects of Anakinra were significant in this study, the protection against RGC damage is partial, suggesting the IL-1 pathway alone may not be involved in observed outcomes. This was confirmed by genetic mice lacking IL-1 pathway components providing partial rescue on visual outcomes (Evans et al., 2020a). Exogenous cytokine IFNγ and TNF-α priming of ASC's can stimulate the secretion of TNF-stimulated gene 6 protein (TSG-6), an anti-inflammatory protein known to polarize microglia. Intravitreal delivery of ASC secretome containing TSG-6 but not the secretome lacking TSG-6 mitigated microglial and endothelial activation, GFAP immunoreactivity, and improved visual function at 4 weeks after blast injury (Jha et al., 2019). Not only were the TSG-6 effects mediated via upregulation of STAT3 phosphorylation in microglia, but it was also seen in endothelial cells. This suggests multifaceted cell-mediated pro-inflammatory mechanisms likely operative in the complex pathophysiological mechanism of TBI associated visual deficits.

Immune cell dysregulation in TBI as a consequence of neuroinflammation is well documented (Needham et al., 2019). The recruitment of T-cells into the injured brain, the loss of blood-brain barrier, and chemokine production have all been linked to inflammation and microglial activation. Whether such mechanisms are operative in the retina is an emerging question. Among the CC chemokine receptor-ligand pair, CCR6-CCL20 has gained attention due to their association with TBI (Das et al., 2011). Mohapatra and colleagues using CCR6 knockout mice demonstrated the critical role of the CCR6-CCL20 pathway in retinal degeneration using a repetitive TBI injury model. In this model, the observed RGC loss, increased microglial activation with increased CCL20 expression in retina could be

significantly attenuated in homozygous adult CCR6 knockout mice. Additional studies using Pioglitazone (PG), a PPARγ agonist that negatively regulates CCL20 or a neutralizing antibody to CCL20, demonstrated a similar reduction in retinal pathology, further confirming the importance of the CCR6-CCL20 axis (Das et al., 2019). Interestingly, neutralization of CCL20 in the animal model reduced infiltrating CD3 positive T cells suggesting a link to immune cell response in the retina following repetitive TBI. Although this phenomenon needs further investigation, such immune infiltration in the retina has been documented recently in ischemic retinal pathologies (Khanh Vu et al., 2020), and blocking such pathways seems quite adequate for visual function recovery (Tang et al., 2020).

Breakdown of the blood-retina barrier (BRB) and the role of Müller cells in the retina of mTBI is another active area of recent research. Injury to retinal Müller glial cells may likely have an effect on retinal neuron synaptic activity because of their role in the uptake of glutamate; fluid transport in the inner retina through their association with retinal blood vessels leading to edema, neurodegeneration, and loss of vision (Bringmann et al., 2006, 2009). The overall functioning of Müller glia resides on the glutamate homeostasis, which is briefly maintained by glutamate-aspartate transporter (GLAST) and glutamine synthetase (GS). The tightly regulated glutamate-glutamine cycle is overridden in a blast injured retina, leading to increased free glutamate levels (Jha et al., 2021). Interestingly, adipose tissue-derived MSC secretome provided intravitreally into the focal cranial blast model right after the injury provided signals that support homeostatic Müller cell function via glutamate uptake and decreased aquaporin-4 to protect retinal synapses as evidenced by increased synaptophysin levels that are critical for maintaining vision (Fig. 3) and (Jha et al., 2021). Though Müller cell gliosis in TBI is likely involved in tissue remodeling, maintaining Müller cell health seems neuroprotective. Future studies are needed to test this hypothesis in order to further explore the neuro-glia interaction and breakdown of BRB in visual deficits of TBI (Fig. 4).

Conclusions and future directions

Vision in TBI is underrepresented, and the number of active clinical trials in this field are sparse. TBI is a chronic neuroinflammatory condition; however, to date, no mouse model of TBI recapitulates all

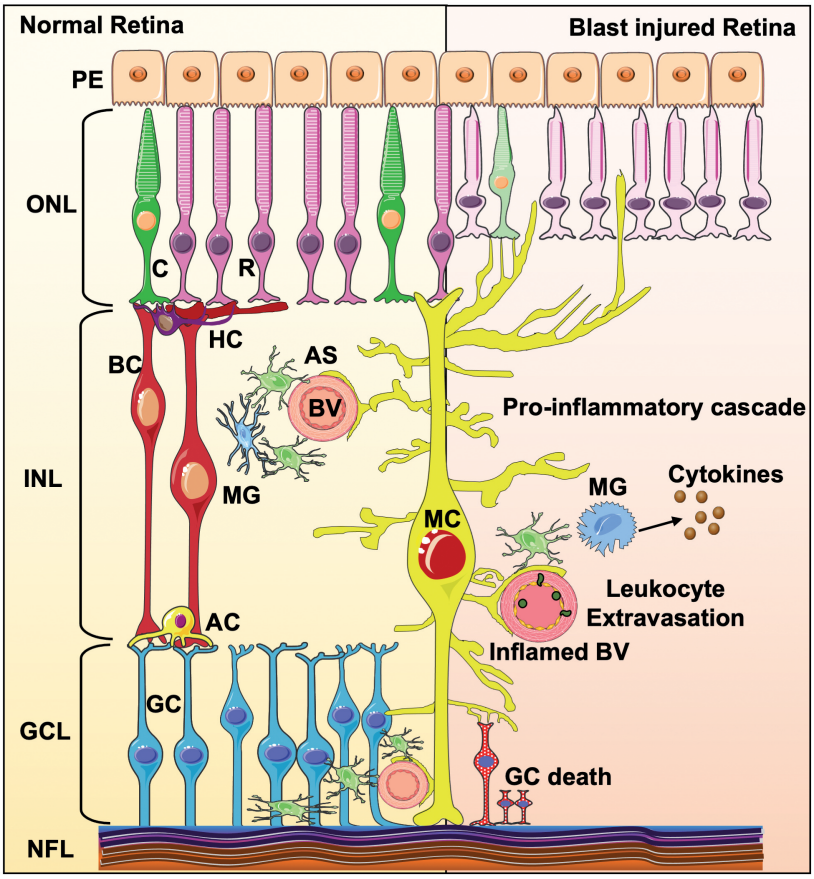

Fig. 4. Neuro-glial interaction with blood vessels in the retina forms the retina's neurovascular unit that maintains tight retinal blood barrier integrity in a healthy retina is disrupted in TBI. Although the sequence of events is unclear, extensive microglial activation, Müller cell dysregulation, and endothelial dysfunction culminate in oxidative stress and pro-inflammatory milieu after TBI dampens the retinal blood barrier integrity resulting in vicious cycles of neurodegeneration as evidenced by loss of RGC and photoreceptors leading to loss of vision. Drawings are not to scale. Created with content from Servier Medical Art (https://smart.servier.com) under Creative Commons Attribution 3.0 Unported license (https://creativecommons.org/licenses/by/3.0/). ONL: Outer nuclear layer; INL: Inner nuclear layer; GCL: Ganglion cell layer; NFL: Nerve fiber layer; HC: Horizontal cell; AC: Amacrine cell; BC: Bipolar cell; PE: Pigmented Epithelium; R:Rods; C:Cones; MC: Muller cells; GC: Ganglion cells; BV: Blood Vessel; MG: Micro Glia; AS: Astrocytes.

pathophysiological events that are shown in a human TBI. Visual deficits have been studied extensively in blast-induced TBI models; however, the methodologies to induce blast concussions across laboratories are heterogeneous in nature (BIRCO, 2019). Both the anatomical locations and pressure ranges (~4psi to 80psi) of the blasts vary across studies, ultimately contributing to differences in pathological outcomes. Repetitive blast injuries are expectedly more damaging than single blast events; however, it is unclear as to why preconditioned low-level blasts can help protect from subsequent blast injuries. Several studies support the use of noninvasive methods in the assessment of visual function in TBI studies as these correlate well with changes detected by morphometric, histological, and immunohistochemical methods in animal models. Regardless of research outcomes, the need for new and modified therapies post-TBI associated with neurodegeneration is unmet. Significant progress has been achieved in developing pharmaceutical based drugs and stem cell-based biologics against visual deficits in TBI. Future studies aiming to develop validated clinical biomarkers in appropriate animal models will likely help translate the designed trials for successful outcomes in TBI experimental therapeutics. Towards this end, blast mTBI models do not develop appreciable vascular defects to study loss of blood-retinal barrier (BRB), a fundamental physiological function of the neuro-glia-vascular unit (NVU) of the retina. Large animal models of neurotrauma may likely mimic human physiology better, however, the logistics of developing such models may pose more challenges than opportunities. With increasing knowledge that is emerging, both genetic and environmental factors have been associated with neurotrauma, we expect there will be more studies addressing the genetic associations and mechanisms by which they elicit such an influence so better therapeutics can be designed. Rapid advances in computational modeling such as artificial intelligence, or machine learning, are expected to spearhead the development of novel diagnostics and evidence-based treatments.

Acknowledgements. Authors wish to acknowledge Dr. Reiner laboratory (Anton Reiner, PhD, UTHSC) for the help with focal cranial blast model studies. This work is supported by grants from the Department of Defense (W81XWH-16-1-0761), and unrestricted funds from Research to Prevent Blindness to R.G. The funders played no role in the conduct of the study, collection of data, management of the study, analysis of data, interpretation of data, or preparation of the manuscript.

Competing interests. RG is a co-founder and hold equity in Cell Care Therapeutics Inc., whose interest is in the use of adipose-derived stromal cells in visual disorders. None of the other authors declare any financial conflicts.

References

Aldag M., Armstrong R.C., Bandak F., Bellgowan P.S.F., Bentley T., Biggerstaff S., Caravelli K., Cmarik J., Crowder A., DeGraba T.J., Dittmer T.A., Ellenbogen R.G., Greene C., Gupta R.K., Hicks R., Hoffman S., Latta R.C. 3rd, Leggieri M.J. Jr., Marion D., Mazzoli R., McCrea M., O'Donnell J., Packer M., Petro J.B., Rasmussen T.E., Sammons-Jackson W., Shoge R., Tepe V., Tremaine L.A. and Zheng J. (2017). The biological basis of chronic traumatic encephalopathy following blast injury: a literature review. J. Neurotrauma. 34, S26-S43.

Allen R.S., Motz C.T., Feola A., Chesler K.C., Haider R., Ramachandra Rao S., Skelton L.A., Fliesler S.J. and Pardue M.T. (2018). Long-term functional and structural consequences of primary blast overpressure to the eye. J. Neurotrauma. 35, 2104-2116.

Armstrong R.A. (2018). Visual problems associated with traumatic brain injury. Clin. Exp. Optom. 101, 716-726.

Arun P., Rossetti F., DeMar J.C., Wang Y., Batuure A.B., Wilder D.M., Gist I.D., Morris A.J., Sabbadini R.A. and Long J.B. (2020). Antibodies against lysophosphatidic acid protect against blast-induced ocular injuries. Front. Neurol. 11, 611816.

Bernardo-Colón A., Vest V., Cooper M.L., Naguib S.A., Calkins D.J. and Rex T.S. (2019). Progression and pathology of traumatic optic neuropathy from repeated primary blast exposure. Front. Neurosci. 13, 719.

Bieniek K.F., Blessing M.M., Heckman M.G., Diehl N.N., Serie A.M., Paolini M.A. 2nd, Boeve B.F., Savica R., Reichard R.R. and Dickson D.W. (2020). Association between contact sports participation and chronic traumatic encephalopathy: a retrospective cohort study. Brain Pathol. 30, 63-74.

BIRCO. (2019). Identifying blast injury knowledge gaps. Https://blastinjuryresearch.Amedd.Army.Mil/index.Cfm/news_and_highlights/identify_knowledge_gaps. Accessed December 28, 2020.

Bricker-Anthony C. and Rex T.S. (2015). Neurodegeneration and vision loss after mild blunt trauma in the c57bl/6 and dba/2j mouse. PLoS One 10, e0131921.

Bricker-Anthony C., Hines-Beard J. and Rex T.S. (2014). Molecular changes and vision loss in a mouse model of closed-globe blast trauma. Invest. Ophthalmol. Vis. Sci. 55, 4853-4862.

Bricker-Anthony C., D'Surney L., Lunn B., Hines-Beard J., Jo M., Bernardo-Colon A. and Rex T.S. (2017). Erythropoietin either prevents or exacerbates retinal damage from eye trauma depending on treatment timing. Optom. Vis. Sci. 94, 20-32.

Bringmann A., Pannicke T., Grosche J., Francke M., Wiedemann P., Skatchkov S.N., Osborne N.N. and Reichenbach A. (2006). Muller cells in the healthy and diseased retina. Prog. Retin. Eye Res. 25, 397-424.

Bringmann A., Iandiev I., Pannicke T., Wurm A., Hollborn M., Wiedemann P., Osborne N.N. and Reichenbach A. (2009). Cellular signaling and factors involved in muller cell gliosis: neuroprotective and detrimental effects. Prog. Retin. Eye Res. 28, 423-451.

Capó-Aponte J.E., Jurek G.M., Walsh D.V., Temme L.A., Ahroon W.A. and Riggs D.W. (2015). Effects of repetitive low-level blast exposure on visual system and ocular structures. J. Rehabil. Res. Dev. 52, 273-290.

Cox Jr. C.S., Juranek J. and Bedi S. (2019). Clinical trials in traumatic brain injury: cellular therapy and outcome measures. Transfusion 59, 858-868.

Cui D.-M., Zeng T., Ren J., Wang K., Jin Y., Zhou L. and Gao L. (2017). Klf4 knockdown attenuates tbi-induced neuronal damage through p53 and jak-stat3 signaling. CNS Neurosci. Ther. 23, 106-118.

Das M., Leonardo C.C., Rangooni S., Pennypacker K.R., Mohapatra S. and Mohapatra S.S. (2011). Lateral fluid percussion injury of the

brain induces CCL20 inflammatory chemokine expression in rats. J. Neuroinflammation 8, 148.

Das M., Tang X., Han J.Y., Mayilsamy K., Foran E., Biswal M.R., Tzekov R., Mohapatra S.S. and Mohapatra S. (2019). CCL20-CCR6 axis modulated traumatic brain injury-induced visual pathologies. J. Neuroinflammation 16, 115-115.

Desai A., Chen H. and Kim H.Y. (2020). Multiple mild traumatic brain injuries lead to visual dysfunction in a mouse model. J. Neurotrauma. 37, 286-294.

Dod worldwide numbers for tbi. (2020). Https://health.Mil/about-mhs/oasdha/defense-health-agency/research-and-development/traumatic-brain-injury-center-of-excellence/dod-tbi-worldwide-numbers. Accessed February 03, 2021.

Dutca L.M., Stasheff S.F., Hedberg-Buenz A., Rudd D.S., Batra N., Blodi F.R., Yorek M.S., Yin T., Shankar M., Herlein J.A., Naidoo J., Morlock L., Williams N., Kardon R.H., Anderson M.G., Pieper A.A. and Harper M.M. (2014). Early detection of subclinical visual damage after blast-mediated tbi enables prevention of chronic visual deficit by treatment with p7c3-s243. Investig. Ophthalmol. Vis. Sci. 55, 8330-8341.

Evans L.P., Newell E.A., Mahajan M., Tsang S.H., Ferguson P.J., Mahoney J., Hue C.D., Vogel E.W. 3rd, Morrison B. 3rd, Arancio O., Nichols R., Bassuk A.G. and Mahajan V.B. (2018). Acute vitreoretinal trauma and inflammation after traumatic brain injury in mice. Ann. Clin. Transl. Neurol. 5, 240-251.

Evans L.P., Boehme N., Wu S., Burghardt E.L., Akurathi A., Todd B.P., Newell E.A., Ferguson P.J., Mahajan V.B., Dutca L.M., Harper M.M. and Bassuk A.G. (2020a). Sex does not influence visual outcomes after blast-mediated traumatic brain injury but il-1 pathway mutations confer partial rescue. Investig. Ophthalmol. Vis. Sci. 61, 7.

Evans L.P., Woll A.W., Wu S., Todd B.P., Hehr N., Hedberg-Buenz A., Anderson M.G., Newell E.A., Ferguson P.J., Mahajan V.B., Harper M.M. and Bassuk A.G. (2020b). Modulation of post-traumatic immune response using the il-1 receptor antagonist anakinra for improved visual outcomes. J. Neurotrauma. 37, 1463-1480.

Galgano M., Toshkezi G., Qiu X., Russell T., Chin L. and Zhao L.R. (2017). Traumatic brain injury: Current treatment strategies and future endeavors. Cell transplant. 26, 1118-1130.

Greve M.W. and Zink B.J. (2009). Pathophysiology of traumatic brain injury. Mt. Sinai. J. Med. 76, 97-104.

Guley N.H., Rogers J.T., Del Mar N.A., Deng Y., Islam R.M., D'Surney L., Ferrell J., Deng B., Hines-Beard J., Bu W., Ren H., Elberger A.J., Marchetta J.G., Rex T.S., Honig M.G. and Reiner A. (2016). A novel closed-head model of mild traumatic brain injury using focal primary overpressure blast to the cranium in mice. J. Neurotrauma. 33, 403-422.

Guley N.M., Del Mar N.A., Ragsdale T., Li C., Perry A.M., Moore B.M., Honig M.G. and Reiner A. (2019). Amelioration of visual deficits and visual system pathology after mild TBI with the cannabinoid type-2 receptor inverse agonist SMM-189. Exp. Eye Res. 182, 109-124.

Gupta R., Saha P., Sen T. and Sen N. (2019). An augmentation in histone dimethylation at lysine nine residues elicits vision impairment following traumatic brain injury. Free radic. Biol. Med. 134, 630-643.

Harper M.M., Woll A.W., Evans L.P., Delcau M., Akurathi A., Hedberg-Buenz A., Soukup D.A., Boehme N., Hefti M.M., Dutca L.M., Anderson M.G. and Bassuk A.G. (2019). Blast preconditioning protects retinal ganglion cells and reveals targets for prevention of neurodegeneration following blast-mediated traumatic brian injury. Investig. Ophthalmol. Vis. Sci. 60, 4159-4170.

Heldt S.A., Elberger A.J., Deng Y., Guley N.H., Del Mar N., Rogers J., Choi G.W., Ferrell J., Rex T.S., Honig M.G. and Reiner A. (2014). A novel closed-head model of mild traumatic brain injury caused by primary overpressure blast to the cranium produces sustained emotional deficits in mice. Front. Neurol. 5, 2.

Hines-Beard J., Marchetta J., Gordon S., Chaum E., Geisert E.E. and Rex T.S. (2012). A mouse model of ocular blast injury that induces closed globe anterior and posterior pole damage. Exp. Eye Res. 99, 63-70.

Hoffer M.E., Balaban C., Slade M.D., Tsao J.W. and Hoffer B. (2013). Amelioration of acute sequelae of blast induced mild traumatic brain injury by n-acetyl cysteine: a double-blind, placebo controlled study. PloS One 8, e54163-e54163.

Honig M.G., Dorian C.C., Worthen J.D., Micetich A.C., Mulder I.A., Sanchez K.B., Pierce W.F., Del Mar N.A. and Reiner A. (2020). Progressive long-term spatial memory loss following repeat concussive and subconcussive brain injury in mice, associated with dorsal hippocampal neuron loss, microglial phenotype shift, and vascular abnormalities. Eur. J. Neurosci. 00, 1-36.

Honig M.G., Del Mar N.A., Henderson D.L., Ragsdale T.D., Doty J.B., Driver J.H., Li C., Fortugno A.P., Mitchell W.M., Perry A.M., Moore B.M. and Reiner A. (2019). Amelioration of visual deficits and visual system pathology after mild TBI via the cannabinoid type-2 receptor inverse agonism of raloxifene. Exp. Neurol. 322, 113063.

Jha K.A., Pentecost M., Lenin R., Klaic L., Elshaer S.L., Gentry J., Russell J.M., Beland A., Reiner A., Jotterand V., Sohl N. and Gangaraju R. (2018). Concentrated conditioned media from adipose tissue derived mesenchymal stem cells mitigates visual deficits and retinal inflammation following mild traumatic brain injury. Int. J Mol. Sci. 19, 2016.

Jha K.A., Pentecost M., Lenin R., Gentry J., Klaic L., Del Mar N., Reiner A., Yang C.H., Pfeffer L.M., Sohl N. and Gangaraju R. (2019). TSG-6 in conditioned media from adipose mesenchymal stem cells protects against visual deficits in mild traumatic brain injury model through neurovascular modulation. Stem Cell Res. Ther. 10, 318.

Jha K.A., Gentry J., Del Mar N.A., Reiner A., Sohl N. and Gangaraju R. (2021). Adipose tissue-derived mesenchymal stem cell concentrated conditioned medium alters the expression pattern of glutamate regulatory proteins and aquaporin-4 in the retina after mild traumatic brain injury. J. Neurotrauma. (in press).

Khanh Vu T.H., Chen H., Pan L., Cho K.S., Doesburg D., Thee E.F., Wu N., Arlotti E., Jager M.J. and Chen D.F. (2020). CD4(+) T-cell responses mediate progressive neurodegeneration in experimental ischemic retinopathy. Am. J. Pathol. 190, 1723-1734.

Li C., Li W., Liu H., Zhang Y., Chen G., Li Z. and Wang Q. (2020). An activatable NIR-II nanoprobe for *in vivo* early real-time diagnosis of traumatic brain injury. Angew Chem. Int. Ed. Engl. 59, 247-252.

Maas A.I.R., Menon D.K., Adelson P.D., Andelic N., Bell M.J., Belli A., Bragge P., Brazinova A., Büki A., Chesnut R.M., Citerio G., Coburn M., Cooper D.J., Crowder A.T., Czeiter E., Czosnyka M., Diaz-Arrastia R., Dreier J.P., Duhaime A.-C., Ercole A., van Essen T.A., Feigin V.L., Gao G., Giacino J., Gonzalez-Lara L.E., Gruen R.L., Gupta D., Hartings J.A., Hill S., Jiang J.-y., Ketharanathan N., Kompanje E.J.O., Lanyon L., Laureys S., Lecky F., Levin H., Lingsma H.F., Maegele M., Majdan M., Manley G., Marsteller J., Mascia L., McFadyen C., Mondello S., Newcombe V., Palotie A., Parizel P.M., Peul W., Piercy J., Polinder S., Puybasset L., Rasmussen T.E., Rossaint R., Smielewski P., Söderberg J., Stanworth S.J., Stein M.B., von Steinbüchel N., Stewart W.,

Steyerberg E.W., Stocchetti N., Synnot A., Te Ao B., Tenovuo O., Theadom A., Tibboel D., Videtta W., Wang K.K.W., Williams W.H., Wilson L., Yaffe K., Adams H., Agnoletti V., Allanson J., Amrein K., Andaluz N., Anke A., Antoni A., van As A.B., Audibert G., Azaševac A., Azouvi P., Azzolini M.L., Baciu C., Badenes R., Barlow K.M., Bartels R., Bauerfeind U., Beauchamp M., Beer D., Beer R., Belda F.J., Bellander B.-M., Bellier R., Benali H., Benard T., Beqiri V., Beretta L., Bernard F., Bertolini G., Bilotta F., Blaabjerg M., den Boogert H., Boutis K., Bouzat P., Brooks B., Brorsson C., Bullinger M., Burns E., Calappi E., Cameron P., Carise E., Castaño-León A.M., Causin F., Chevallard G., Chieregato A., Christie B., Cnossen M., Coles J., Collett J., Della Corte F., Craig W., Csato G., Csomos A., Curry N., Dahyot-Fizelier C., Dawes H., DeMatteo C., Depreitere B., Dewey D., van Dijck J., Đilvesi Đ., Dippel D., Dizdarevic K., Donoghue E., Duek O., Dulière G.-L., Dzeko A., Eapen G., Emery C.A., English S., Esser P., Ezer E., Fabricius M., Feng J., Fergusson D., Figaji A., Fleming J., Foks K., Francony G., Freedman S., Freo U., Frisvold S.K., Gagnon I., Galanaud D., Gantner D., Giraud B., Glocker B., Golubovic J., Gómez López P.A., Gordon W.A., Gradisek P., Gravel J., Griesdale D., Grossi F., Haagsma J.A., Håberg A.K., Haitsma I., Van Hecke W., Helbok R., Helseth E., van Heugten C., Hoedemaekers C., Höfer S., Horton L., Hui J., Huijben J.A., Hutchinson P.J., Jacobs B., van der Jagt M., Jankowski S., Janssens K., Jelaca B., Jones K.M., Kamnitsas K., Kaps R., Karan M., Katila A., Kaukonen K.-M., De Keyser V., Kivisaari R., Kolias A.G., Kolumbán B., Kolundžija K., Kondziella D., Koskinen L.-O., Kovács N., Kramer A., Kutsogiannis D., Kyprianou T., Lagares A., Lamontagne F., Latini R., Lauzier F., Lazar I., Ledig C., Lefering R., Legrand V., Levi L., Lightfoot R., Lozano A., MacDonald S., Major S., Manara A., Manhes P., Maréchal H., Martino C., Masala A., Masson S., Mattern J., McFadyen B., McMahon C., Meade M., Melegh B., Menovsky T., Moore L., Morgado Correia M., Morganti-Kossmann M.C., Muehlan H., Mukherjee P., Murray L., van der Naalt J., Negru A., Nelson D., Nieboer D., Noirhomme Q., Nyirádi J., Oddo M., Okonkwo D.O., Oldenbeuving A.W., Ortolano F., Osmond M., Payen J.-F., Perlbarg V., Persona P., Pichon N., Piippo-Karjalainen A., Pili-Floury S., Pirinen M., Ple H., Poca M.A., Posti J., Van Praag D., Ptito A., Radoi A., Ragauskas A., Raj R., Real R.G.L., Reed N., Rhodes J., Robertson C., Rocka S., Røe C., Røise O., Roks G., Rosand J., Rosenfeld J.V., Rosenlund C., Rosenthal G., Rossi S., Rueckert D., de Ruiter G.C.W., Sacchi M., Sahakian B.J., Sahuquillo J., Sakowitz O., Salvato G., Sánchez-Porras R., Sándor J., Sangha G., Schäfer N., Schmidt S., Schneider K.J., Schnyer D., Schöhl H., Schoonman G.G., Schou R.F., Sir Ö., Skandsen T., Smeets D., Sorinola A., Stamatakis E., Stevanovic A., Stevens R.D., Sundström N., Taccone F.S., Takala R., Tanskanen P., Taylor M.S., Telgmann R., Temkin N., Teodorani G., Thomas M., Tolias C.M., Trapani T., Turgeon A., Vajkoczy P., Valadka A.B., Valeinis E., Vallance S., Vámos Z., Vargiolu A., Vega E., Verheyden J., Vik A., Vilcinis R., Vleggeert-Lankamp C., Vogt L., Volovici V., Voormolen D.C., Vulekovic P., Vande Vyvere T., Van Waesberghe J., Wessels L., Wildschut E., Williams G., Winkler M.K.L., Wolf S., Wood G., Xirouchaki N., Younsi A., Zaaroor M., Zelinkova V., Zemek R. and Zumbo F. (2017). Traumatic brain injury: Integrated approaches to improve prevention, clinical care, and research. Lancet Neurol. 16, 987-1048.

Mammadova N., Ghaisas S., Zenitsky G., Sakaguchi D.S., Kanthasamy A.G., Greenlee J.J. and West Greenlee M.H. (2017). Lasting retinal injury in a mouse model of blast-induced trauma. Am. J. Pathol. 187, 1459-1472.

McMaster D. and Clare G. (2021). Incidence of ocular blast injuries in modern conflict. Eye (Lond). (in press).

Mead B. and Scheven B.A. (2015). Mesenchymal stem cell therapy for retinal ganglion cell neuroprotection and axon regeneration. Neural Regen. Res. 10, 371-373.

Mead B. and Tomarev S. (2017). Bone marrow-derived mesenchymal stem cells-derived exosomes promote survival of retinal ganglion cells through mirna-dependent mechanisms. Stem Cells Transl. Med. 6, 1273-1285.

Meehan W.P. 3rd, Mannix R.C., O'Brien M.J. and Collins M.W. (2013). The prevalence of undiagnosed concussions in athletes. Clin. J. Sport Med. 23, 339-342.

Mesentier-Louro L.A., Zaverucha-do-Valle C., da Silva-Junior A.J., Nascimento-Dos-Santos G., Gubert F., de Figueirêdo A.B., Torres A.L., Paredes B.D., Teixeira C., Tovar-Moll F., Mendez-Otero R. and Santiago M.F. (2014). Distribution of mesenchymal stem cells and effects on neuronal survival and axon regeneration after optic nerve crush and cell therapy. PLoS One 9, e110722.

Naguib S., Bernardo-Colón A., Cencer C., Gandra N. and Rex T.S. (2020). Galantamine protects against synaptic, axonal, and vision deficits in experimental neurotrauma. Neurobiol. Dis. 134, 104695.

National Academies of Sciences E., and Medicine; Health and Medicine Division; Board on Health Care Services; Committee on the Review of the Department of Veterans Affairs Examinations for Traumatic Brain Injury. (2019). In: Evaluation of the disability determination process for traumatic brain injury in veterans. Washington (DC). 23-56.

Needham E.J., Helmy A., Zanier E.R., Jones J.L., Coles A.J. and Menon D.K. (2019). The immunological response to traumatic brain injury. J. Neuroimmunol. 332, 112-125.

Ng S.Y. and Lee A.Y.W. (2019). Traumatic brain injuries: Pathophysiology and potential therapeutic targets. Front. Cell. Neurosci. 13, 528.

Prevention C.f.D.C.A. (2019). Tbi: Get the facts. In, 4/5/2019 ed. US Centers for Disease Control and Prevention.

Reiner A., Heldt S.A., Presley C.S., Guley N.H., Elberger A.J., Deng Y., D'Surney L., Rogers J.T., Ferrell J., Bu W., Del Mar N., Honig M.G., Gurley S.N. and Moore B.M., 2nd. (2015). Motor, visual and emotional deficits in mice after closed-head mild traumatic brain injury are alleviated by the novel cb2 inverse agonist smm-189. Int. J. Mol. Sci. 16, 758-787.

Sen N. (2017). An insight into the vision impairment following traumatic brain injury. Neurochem. Int. 111, 103-107.

Tang Y., Xiao Z., Pan L., Zhuang D., Cho K.S., Robert K., Chen X., Shu L., Tang G., Wu J., Sun X. and Chen D.F. (2020). Therapeutic targeting of retinal immune microenvironment with CSF-1 receptor antibody promotes visual function recovery after ischemic optic neuropathy. Front. Immunol. 11, 585918.

Tzekov R., Quezada A., Gautier M., Biggins D., Frances C., Mouzon B., Jamison J., Mullan M. and Crawford F. (2014). Repetitive mild traumatic brain injury causes optic nerve and retinal damage in a mouse model. J. Neuropathol. Exp. Neurol. 73, 345-361.

Vest V., Bernardo-Colon A., Watkins D., Kim B. and Rex T.S. (2019). Rapid repeat exposure to subthreshold trauma causes synergistic axonal damage and functional deficits in the visual pathway in a mouse model. J. Neurotrauma. 36, 1646-1654.

Wagner A.K. and Kumar R.G. (2019). TBI rehabilomics research: Conceptualizing a humoral triad for designing effective rehabilitation

interventions. Neuropharmacol. 145, 133-144.

Xiong Y., Mahmood A. and Chopp M. (2013). Animal models of traumatic brain injury. Nat. Rev. Neurosci. 14, 128-142.

Yin T.C., Voorhees J.R., Genova R.M., Davis K.C., Madison A.M., Britt J.K., Cintrón-Pérez C.J., McDaniel L., Harper M.M. and Pieper A.A. (2016). Acute axonal degeneration drives development of cognitive, motor, and visual deficits after blast-mediated traumatic brain injury in mice. eNeuro 3, ENEURO.0220-16.2016.

Zafonte R.D., Bagiella E., Ansel B.M., Novack T.A., Friedewald W.T., Hesdorffer D.C., Timmons S.D., Jallo J., Eisenberg H., Hart T., Ricker J.H., Diaz-Arrastia R., Merchant R.E., Temkin N.R., Melton S. and Dikmen S.S. (2012). Effect of citicoline on functional and cognitive status among patients with traumatic brain injury: Citicoline brain injury treatment trial (cobrit). JAMA 308, 1993-2000.

Accepted February 18, 2021