241 Ganglion Cells: Development, axotomy, trauma
Monday, May 08, 2017 11:00 AM–12:45 PM
Exhibit/Poster Hall Poster Session
Program #/Board # Range: 1746–1769/A0357–A0380
Organizing Section: Retinal Cell Biology

Program Number: 1746 **Poster Board Number:** A0357
Presentation Time: 11:00 AM–12:45 PM
Melanopsin expression is dynamically regulated during retinal development
Katelyn Noronha, Jasmine A. Lucas, Tiffany M. Schmidt. Northwestern University, Evanston, IL.
Purpose: In addition to rod and cone photoreceptors of the outer retina, intrinsically photosensitive retinal ganglion cells (ipRGCs) constitute a third class of inner retinal photoreceptors that play roles in both image and non-image forming vision. ipRGCs express melanopsin, a photopigment that renders them intrinsically photosensitive. Each of the five ipRGC subtypes (M1 to M5) express different levels of melanopsin in adult retinas, with M1 ipRGCs expressing the most and M4 and M5 ipRGCs expressing the least. Additionally, M1 ipRGCs have been found to express two isoforms (Opn4S and Opn4L) of the melanopsin protein, while all other subtypes express only one (Opn4L). However, how ipRGCs achieve regulation of both melanopsin levels and melanopsin isoforms is unknown. We examined melanopsin expression levels in ipRGC subtypes across development to begin understand melanopsin regulation.
Methods: We assessed ipRGC number in mouse lines where different complements of the ipRGC subtypes can be immunolabeled in adulthood (Opn4Cre, Opn4-GFP, and Opn4LacZ). We then counted the total number of ipRGCs in the retina at postnatal day 2, 4, 6, 8, 14, and during adulthood. We also further examined melanopsin labeling patterns of the M4 subtype in various regions of the retina during development. We used quantitative real-time PCR to detect changes in melanopsin expression and melanopsin isoform expression in the retina during early postnatal development and adulthood.
Results: Unexpectedly, we found that M4 ipRGCs express higher levels of melanopsin in early postnatal development than in adulthood. Our qPCR results indicate that total melanopsin RNA levels, as well as melasnopsin isoform RNA levels, also fluctuate across development, before eventually settling at adult levels.
Conclusions: Our data indicate that ipRGC subtypes differentially and dynamically regulate melanopsin expression. Further investigation will examine the relative melanopsin and melanopsin isoform expression within the different subtypes of ipRGCs across development.
Commercial Relationships: Katelyn Noronha, None; **Jasmine A. Lucas**, None; **Tiffany M. Schmidt**, None

Program Number: 1747 **Poster Board Number:** A0358
Presentation Time: 11:00 AM–12:45 PM
Characterization of ipRGCs during mouse retinal development
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Purpose: Melanopsin-expressing, intrinsically photosensitive retinal ganglion cells (ipRGCs) represent a non-canonical class of photoreceptor in the mammalian retina. ipRGCs have been shown to be involved in a number of visual behaviors such as circadian photoentrainment and contrast sensitivity. The ipRGC population is made up of five different subtypes and recent evidence suggests ipRGCs influence retinal development. However, ipRGC properties and functions have not been well-characterized in the developing retina.
Methods: We utilized *in vitro* whole cell patch clamp in retinas from mice that expressed GFP under the melanopsin promoter (*Opn4-GFP)*. ipRGCs were specifically targeted using epifluoresence. We investigated the following postnatal day (P) timepoints : P6, P8, P10, P14, and adult. We measured capacitance, resting membrane potential, and input resistance. We also measured cellular response to light stimuli as well as depolarizing current injection. Neurobiotin tracer was included in pipets and after recording, tissue was fixed and immunolabled for ChAT and SMI32 for subtype identification purposes and further investigation of the morphological properties. Cells were identified based on dendritic stratification and SMI32 immunostaining.
Results: We found that ipRGCs already exhibit unique physiological properties early in development that are similar to their adult properties. To highlight this, the M1 subtype as early as P8 has no significant difference in capacitance ($p=0.305$), resting membrane potential ($p=0.375$), input resistance ($p=0.759$) or the maximum light response ($p=0.651$) in comparison to its adult counterpart. However, we find that ipRGCs undergo substantial morphological changes as they mature, reaching adult morphology sometime after P14.
Conclusions: We find that even early in postnatal retinal development, ipRGC subtypes have similar physiological properties when compared to their adult counterparts although their morphological features do not reach maturity until after eye opening (P14). Understanding these differences will provide insight into the potential influence of individual ipRGC subtypes on retinal development.
Commercial Relationships: Jasmine A. Lucas, None; **Tiffany M. Schmidt**, None
Support: T32 Training Grant in Sleep and Circadian Research

Program Number: 1748 **Poster Board Number:** A0359
Presentation Time: 11:00 AM–12:45 PM
Homer 1 protein isoforms differentially control the intracellular calcium signaling and viability of retinal neurons
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Purpose: Vision loss in optic neuropathies is characterized by the degeneration of retinal ganglion cells (RGCs). Cell death of RGCs is preceded by L-glutamate mediated excitotoxicity, subsequent cellular calcium dyshomeostasis and calcium toxicity. Control of these signaling pathways is the target of therapy development efforts. Homer proteins, in addition to their function as synaptic clustering proteins, control the intracellular free calcium ion concentration by interaction with intracellular calcium release channels. The present study tested the hypothesis that Homer 1 protein isoforms differentially control intracellular calcium signaling in RGCs and thereby affect RGC viability.
Methods: Murine RGCs were isolated and cultured. The concentration of Homer 1 protein isoforms in RGCs was altered using isoform-specific mammalian expression vectors and small interfering RNA and determined with microfluorimetry. Changes in intracellular calcium signaling were measured using calcium imaging and pharmacological control of intracellular calcium channel activity, as well as single channel electrophysiology of intracellular calcium release channels. RGC viability and changes in expression levels of Homer 1 protein isoforms were measured in response to chronic L-glutamate-mediated toxicity using cytochemical methods.

Results: RGCs express long and short isoforms of Homer 1. Expression levels change in response to chronic L-glutamate toxicity towards a higher prevalence of the short isoform when compared to vehicle controls ($p<0.01$). Knockdown of the short isoform significantly ($p<0.001$) reduced viability of RGCs by 56%, whereas knockdown of the long isoform significantly ($p<0.01$) increased the viability of RGCs by 49%. This was paralleled by statistically significantly ($p<0.01$) potentiated or attenuated calcium release from intracellular stores, respectively. Overexpression of individual isoforms had the reverse effect on calcium release from intracellular stores and viability of RGCs.
Conclusions: Isoforms of Homer 1 control calcium release from intracellular stores differentially and thereby affect cellular viability of RGCs in an isoform-specific manner as a function of cellular calcium dyshomeostasis resulting from disease processes. These mechanisms of action therefore represent novel potential targets for therapeutic intervention and drug development in optic neuropathies.
Commercial Relationships: Peter Koulen, None
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Program Number: 1749 **Poster Board Number:** A0360
Presentation Time: 11:00 AM–12:45 PM
Combining Dre and Cre recombination to identify and study mouse Retinal Ganglion Cell Types
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Purpose: We are designing mouse genetic strategies for the labeling and manipulation of specific Retinal Ganglion Cell Types. Previously we have reported the use of sequential Dre and Cre recombination employing the genetic loci of the Pou4f transcription factors to isolate individual cell populations. To improve the temporal specificity and tighten the Dre-dependency of Cre expression, we identified novel heterotopic Dre sites, and designed inversion-excision strategies targeting the Cre recombinase at the Pou4f3 locus.
Methods: Targeting constructs, ES cell targeting by homologous recombination and blastocyst injections were performed by established methodologies. The generated mouse lines were tested by crossing into available Dre and Cre and reporter lines.
Results: We report successful Dre to Cre to Cre reporter recombination resulting in RGC specific expression of the reporter. We provide neuroanatomic and molecular characterization of the lines, and apply them to RGC type characterization.
Conclusions: Gene expression profiling of neuronal cell types provides ample evidence of the broad overlap of gene expression between neuronal cell types. Therefore, genetic intersection strategies are crucial for the specific isolation of individual cell types. Strategies employing more than one recombinase can be successfully applied for specific cell type manipulation in live, otherwise intact animals.
Commercial Relationships: Tudor C. Badea, None; **Nadia Parmhans**, None; **Eileen Nguyen**, None; **Katherine Chuang**, None
Support: Intramural research program - National Eye Institute

Program Number: 1750 **Poster Board Number:** A0361
Presentation Time: 11:00 AM–12:45 PM
Retinal ganglion cell death is attenuated by a PI3K activating synthetic peptide
Enrique J. De La Rosa[1], A. M. Hernandez-Pinto[1], M. Marchena[2], J. L. M. San-Martin[2], P. Fernandez[4], F. de Pablo[1], M. Morales[3]. [1]Cell & Molecular Medicine, Centro de Investigaciones Biologicas, Madrid, Spain; [2]ProRetina Therapeutics, Noain, Spain; [3]Neuroscience Institute, UAB, Barcelona, Spain; [4]Sciences, UNED, Madrid, Spain.
Purpose: Retinal ganglion cell (RGC) damage and loss is a common trait of several retinal diseases, including glaucoma. RGC loss, leading to visual impairment, seems to be mediated by programed cell death. Therefore, activation of survival pathways, such as that of PI3K/Akt, is a potential therapeutical target to delay retinal degeneration. The aim of this study is to test in retinal explants the effect of PTD4-PI3KAc, a synthetic peptide combining a PI3K activator and a membrane transductor, on NMDA-induced RGC excitotoxicity and death.
Methods: Activation of PI3K was by the PTD4-PI3KAc peptide, which consists in a transduction domain, PTD4 (Tyr-Gly-Arg-Lys-Lys-Arg-Arg-Gln-Arg-Arg-Arg), fused to a phosphopeptide containing the intracellular phosphorylated domain of the PDGF receptor C-terminus (Gly-Ser-Asp-Gly-Gly-pTyr-Met-Asp-Met-Ser). PTD4-PI3KAc effect was monitored by measuring Akt phosphorylation in the SH-SY5Y human cell line as well as by determining survival of NMDA-treated rat hippocampal neurons in culture. C57BL/6 mouse retinal explants were cultured 48 h in the presence of 50 μM NMDA alone or together with IGF-I (10 nM) or PTD4-PI3KAc (25 μM). RGC death was determined by TUNEL.
Results: PTD4-PI3KAc treatment of SH-SY5Y cells induced an 162%-increase of phospho-AKT(S473) levels in comparison with the control peptide. Further, PTD4-PI3KAc reduced by 34% the death of NMDA-treated rat hippocampal neurons. NMDA treatment of retinal explants induced a consistent RGC death that was reduced by 80% in the presence of PTD4-PI3KAc and by 88% in the presence of IGF-I.
Conclusions: PTD4-PI3KAc attenuates excitotoxic RGC death and may provide a useful drug candidate for the treatment of retinal degenerative diseases.
Commercial Relationships: Enrique J. De La Rosa, ProRetina Therapeutics (F), ProRetina Therapeutics (I); **A. M. Hernandez-Pinto**, None; **M. Marchena**, ProRetina Therapeutics (E); **J. L. M. San-Martin**, ProRetina Therapeutics (E); **P. Fernandez**, None; **F. de Pablo**, ProRetina Therapeutics (I); **M. Morales**, Spineup (P), Spineup (I), ProRetina Therapeutics (F)
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Program Number: 1751 **Poster Board Number:** A0362
Presentation Time: 11:00 AM–12:45 PM
αA-crystallins attenuate endoplasmic reticulum (ER) stress in retinal neurons but not in glia
Anne Ruebsam, Angela M. Myers, Patrice E. Fort. Ophthalmology and Visual Sciences, University of Michigan, Ann Arbor, MI.
Purpose: αA-crystallins are highly upregulated by ganglion cells and Müller glial cells in the retina of diabetic animals and humans, cell types in which they are protective *in vitro*. Increased ER stress, a pathway in which chaperones such as αA-crystallins play a central role, contributes to the pathophysiology of diabetes. We also showed that aA-crystallins phosphosphorylation on S/T148 is reduced during diabetes but its impact on the specific molecular mechanisms by which αA-crystallin promotes survival remain unclear. Thus we tested the hypothesis that phosphorylation of αA-crystallins on

S/T148 regulates retinal cell survival by attenuation of serum starvation induced ER stress.
Methods: Differentiated R28 cells, a model of retinal neurons, as well as rMC-1, a Müller glial cell line, were transfected with plasmids encoding either wild-type (WT), phosphomimetic (148D), or non phosphorylatable mutants (148A) of αA-crystallin on the 148 residue in 3 independent experiments. Markers of the 3 unfolded protein response (UPR) branches, IRE1, ATF6 and PERK, were analyzed by a combination of transcriptomic, immunoblot and immunostaining methods under normal or metabolic stress conditions (1% FBS). Treatment with the ER stress inducer tunicamycin served as positive control.
Results: Levels of phosphorylated eIF2α and total ATF4 were reduced in αA-crystallin-WT (285±12%;1059±20%) and further in αA-148D mutant (261±16%;684±18%) transfected neurons compared to the EV control (400±20%;2065±2%) upon serum starvation. This protective effect was not seen when cells were transfected with αA-148A (380±28%;1531±37%). While αA-crystallin and its phosphorylation regulates the PERK-associated branch, no effect could be detected in the 2 other branches. In glial cells an increase in ER stress upon serum starvation could be detected, but with no significant difference between the EV and αA-crystallin transfected cells (p-eIF2a level: 337±22% for EV; 302±19% αA-WT; 305±25% αA-148D; 330±40% αA-148A).
Conclusions: αA-crystallins protect retinal neurons through attenuation of the UPR, thus contributing to a novel mechanism of action of αA-crystallins. This effect is abolished by specific alteration of their phosphorylation profile mimicking what is seen during diabetes with a decrease in phosphorylation on the 148 phosphosite. αA-crystallins also protect Müller glial cells, but likely by a different mechanism.
Commercial Relationships: Anne Ruebsam, None; **Angela M. Myers**, None; **Patrice E. Fort**, None
Support: Research Fellowship from Deutsche Forschungsgemeinschaft "RU 2020/2-1"

Program Number: 1752 **Poster Board Number:** A0363
Presentation Time: 11:00 AM–12:45 PM
AAV2-GRP78-mediated gene therapy prevents retinal neuronal injury via downregulation of Tau oligomers
Wei Liu[1, 2], Yonju Ha[1], Shuang Zhu[1], Hua Liu[3], Nisha Azhar[1], Julia E. Gerson[4], Massoud Motamedi[3], Rakez Kayed[4], Wenbo Zhang[1, 5].
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Purpose: Retinal ganglion cell (RGC) death following axonal injury that occurs in traumatic optic neuropathy or glaucoma causes irreversible vision loss. Currently, there is no proven effective therapy to prevent RGC death in such conditions. This study aims to determine whether RGC can be protected from death by eliminating endoplasmic reticulum (ER) stress with adeno-associated virus (AAV)-mediated gene therapy and to investigate the mechanisms underlying its beneficial effects
Methods: Optic nerve crush (ONC) model was used to study RGC death after axonal injury, in which the optic nerve was crushed 2 mm behind the eye globe. Infection efficiency and location of different AAV serotypes in mice retinas were tested *in vivo* using green fluorescent protein as a marker after intravitreal injection. AAV2 was selected to introduce GRP78 gene into the retina. ER stress signaling molecules, tau oligomers and RGC injury were examined by immunohistochemistry or Western blot
Results: Among AAV1, 2, 5, 6, 8 and 9, AAV2 was the most efficient serotype that infects and delivers genes to RGCs. At 3 days after ONC, there was a marked increase in ER stress and RGC apoptosis. GRP78 is a molecular chaperon that enhances protein folding and controls the activation of ER stress pathways. GRP78 overexpression not only attenuated ER stress but also reduced RGC apoptosis by 64.4% ($n=5$, $p<0.05$). At 7 days after ONC, there was 58.8% RGC loss in mice receiving intravitreal AAV2-null and such loss was reduced to 35.7% in mice receiving AAV2-GRP78 ($n=5$, $p<0.05$). RGC function, as indicated by the positive scotopic threshold responses of electroretinography, was prominently reduced at 7 days after ONC, which was significantly prevented by AAV2-GRP78. Since protein aggregation is increased during ER stress and aggregated proteins such as tau oligomers are key players in neurodegenerative diseases, we examined tau oligomers in the retina and found that AAV2-GRP78 alleviated ONC-induced increase in tau oligomers. Blocking tau oligomers with a novel tau oligomer monoclonal antibody (TOMA) significantly attenuated ONC-induced RGC loss.
Conclusions: These data indicate that gene therapy with AAV2-GRP78 or immunotherapy with TOMA offer novel therapeutic approaches to prevent RGC loss after axonal injury. The effect of AAV2-GRP78 is partially mediated by the reduction of tau oligomer formation
Commercial Relationships: Wei Liu, None; **Yonju Ha**, None; **Shuang Zhu**, None; **Hua Liu**, None; **Nisha Azhar**, None; **Julia E. Gerson**, None; **Massoud Motamedi**, None; **Rakez Kayed**, None; **Wenbo Zhang**, None
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Program Number: 1753 **Poster Board Number:** A0364
Presentation Time: 11:00 AM–12:45 PM
Neuroprotective efficacy of topical ocular delivery of trabodenoson in a rodent model of anterior ischemic optic neuropathy
Cadmus C. Rich[1], David Albers[1], Rebecca Fawcett[2], Yan Guo[2], Zara Mehrabyan[2], Rudolf Baumgartner[1], Steven L. Bernstein[2].
[1]Ophthalmology, Inotek Pharmaceuticals, Chelmsford, MA; [2]Ophthalmology, University of Maryland School of Medicine, Baltimore, MD.
Purpose: Purpose: To evaluate the neuroprotective potential of ocular (topical) delivery of trabodenoson on the retina and optic nerve (ON) in a rodent model of anterior ischemic optic neuropathy (rAION).
Methods: *Methods*: Male Long-Evan rats (200-300 g) received twice daily ocular (topical) delivery of 3% trabodenoson (n=13) or vehicle (n=13) in both eyes starting 3 days prior to rAION induction and every day thereafter out to 21 days post induction. rAION was induced in right eye (OD) of anesthetized rats 30 sec following an intravenous injection of rose bengal dye (2.5 mM) by ON illumination (500 um spot size, 11 sec duration) via frequency-doubled Nd-YAG laser (532 nm) as previously described (Touitou et al (2013) *IOVS* 54, 7402). The left eye (OS) from each animal served as the control eye. Oscillation potentials (OPs) were recorded bilaterally 2-3 weeks post induction. Animals were euthanized 30 days post induction and perfused with paraformaldehyde. Retinal ganglion cells (RGCs) in post-fixed retinas were immunostained

for Brn3a and quantified by stereology. ON tissue was either evaluated by immunolabeling for intact neurofilaments (SMI312) and inflammatory cells (IBA1), or fixed with glutaraldehyde and prepared for analysis by transmission electron microscopy (TEM).
Results: *Results*: Electrophysiological results reveal that rAION reduced OPs in vehicle-treated eyes by approximately 50% of contralateral, control eyes. Comparatively, trabodenoson treatment significantly improved this rAION-induced reduction in OPs (33% of the control eyes, p=0.037). Preliminary results from RGC layer stereological cell counts in vehicle-treated eyes (n=6) indicate Brn3a-stained cells were reduced approximately 50%, whereas Brn3a-stained cells in trabodenoson-treated eyes (n=6) were only reduced by 33% (p=0.07 vs vehicle). Neurofilament immunolabeling of ON as well as TEM ultrastructural analysis suggests axonal preservation in the ON from trabodenoson-treated eyes as compared to vehicle treatment.
Conclusions: *Conclusions*: The electrophysiological results support a putative protective role for trabodenoson treatment in preserving RGC-inner retinal function in the rAION model. Preliminary results indicate that twice daily, ocular (topical) delivery of trabodenoson attenuates RGC loss and may preserve ON axonal fibers, as compared to vehicle treatment in this rodent model of NAION.
Commercial Relationships: Cadmus C. Rich, Inotek Pharmaceuticals (S); **David Albers**, Inotek Pharmaceuticals (E); **Rebecca Fawcett**, None; **Yan Guo**, None; **Zara Mehrabyan**, None; **Rudolf Baumgartner**, Inotek Pharmaceuticals (S); **Steven L. Bernstein**, None

Program Number: 1754 **Poster Board Number:** A0365
Presentation Time: 11:00 AM–12:45 PM
Extracellular vesicles purified from porcine vitreous positively regulate retinal ganglion cell axon survival and growth
Yolandi van der Merwe[1, 2], Anne Faust[2, 3], Bianca Leonard[2, 3], Mark Curtis[2, 4], Apoorva Kandakatla[2, 3], Kevin C. Chan[1, 2], Michael Steketee[2, 4]. [1]Department of Bioengineering, University of Pittsburgh, Pittsburgh, PA; [2]Department of Ophthalmology, University of Pittsburgh, Pittsburgh, PA; [3]McGowan Institute for Regenerative Medicine, University of Pittsburgh, Pittsburgh, PA; [4]Center for Neuroscience, University of Pittsburgh, Pittsburgh, PA.
Purpose: Retinal injury can lead to retinal ganglion cell (RGC) axon degeneration and ultimately RGC death due to the default healing response in the CNS. Extracellular vesicles (EV) are emerging as naturally-derived therapeutics with the potential to modulate the default healing response by positively regulating the phenotypes of multiple cell types simultaneously. EVs are released from all cells and carry unique bioactive cargoes that reflect the state of the cell from which they are released, which during injury or disease tends to potentiate inflammatory signaling and secondary trauma. We hypothesize EVs derived from healthy porcine vitreous can increase RGC survival and neurite growth *in vitro* and neuroprotect injured RGCs *in vivo*
Methods: Vitreous vesicles (VV) were isolated from collagenase treated porcine vitreous by differential centrifugation, visualized by transmission electron microscopy (TEM), analyzed for exosome markers by flow cytometry, and miRNA content by sequencing. *In vitro*, primary RGCs from P3 Sprague-Dawley rats were cultured with VVs. After 3 days, viability and neurite growth were analyzed. *In vivo*, intraocular pressure was elevated to 130mmHg for 60 minutes via anterior chamber perfusion with or without VV injection 24 hours after injury. After 7 days, RGC survival was analyzed by TUNEL
Results: TEM showed relatively uniform VVs, ranging in size from 10-30 nm (Fig. 1). Flow cytometry showed VVs were positive for exosome markers, including CD63 but not CD81. *In vitro*, fluorescent VVs were internalized within minutes into RGC cell bodies, neurites, and growth cones. VVs increased RGC viability and neurite growth (Fig. 1). *In vivo,* intravitreally injected VVs were internalized by glial and RGCs and RGC survival is being analyzed. VVs contain various miRNAs known regulate cellular differentiation and apoptotic signaling
Conclusions: Porcine VVs are uniform in size, have exosome markers, and carry unique miRNA cargoes. I*n vitro*, VVs increase primary RGC survival and growth. *In vivo,* VVs integrate into multiple layers of the retina. We are currently investigating the *in vivo* effects of VVs on RGC survival in injury models and the roles that VV miRNAs play in regulating RGC survival and growth. These initial studies indicate VVs hold promise as biologically derived neuroprotective therapeutics for retinal injury

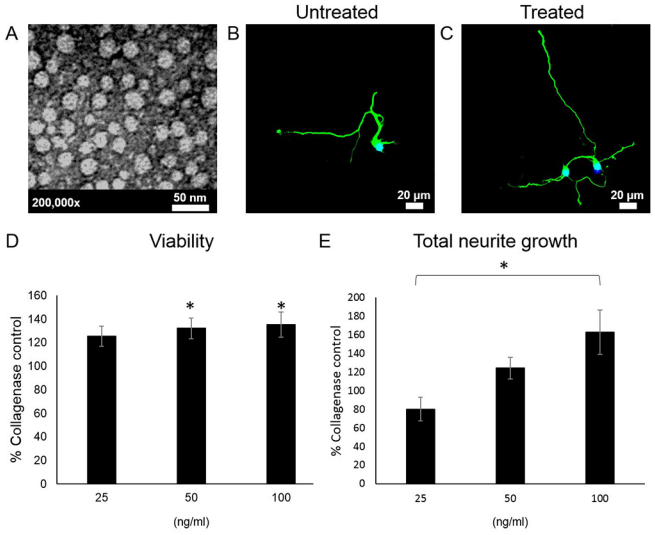

Figure 1. A. TEM image of adult VVs isolated with collagenase. B. Representative image of βIII-tubulin stained RGC cultured with media. C Representative image of βIII-tubulin stained RGC cultured with adult VVs plus collagenase. D. Viability data. E. Total neurite growth data. *$p < 0.05$

Commercial Relationships: Yolandi van der Merwe, None; **Anne Faust**, None; **Bianca Leonard**, None; **Mark Curtis**, None; **Apoorva Kandakatla**, None; **Kevin C. Chan**, None; **Michael Steketee**, None
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Program Number: 1755 **Poster Board Number:** A0366
Presentation Time: 11:00 AM–12:45 PM
Wnt signaling promotes retinal ganglion cell survival and axonal regeneration in mouse models of optic nerve injury
Amit Patel[1], Kevin Park[2], Abigail Hackam[2]. [1]Ophthalmology, University of California San Diego, San Diego, CA; [2]Bascom Palmer Eye Institute, University of Miami, Miami, FL.
Purpose: Optic neuropathies are a family of diseases that result in axonal damage of retinal ganglion cells (RGCs). As part of the central nervous system, the axons do not self-regenerate, leading to RGC cell death and vision loss. The canonical Wnt/β-catenin signaling pathway is known to regulate axon growth and neurite extension in

the developing mammalian nervous system. In this study, we tested the hypothesis that Wnt signaling could be repurposed to increase RGC survival and induce axonal growth in the adult optic nerve after injury.
Methods: We tested the role of Wnt signaling in axonal regeneration using the optic nerve crush model in transgenic Wnt reporter mice. Recombinant Wnt3a protein was intravitreally injected into the mouse eye, in order to induce Wnt signaling. Optic nerves were crushed at the same time as injection to induce axonal injury. Optic nerves were labelled and collected at 2 and 4 week timepoints after injury to assess for regeneration. RGC survival and function were measured by retinal flatmount counting and pattern electroretinography, respectively. To test the mechanism of Wnt signaling changes in the axonal regeneration, we examined the Stat3 pathway using floxed Stat3 mice.
Results: We found that Wnt3a induced Wnt signaling in RGCs and resulted in significant axonal regrowth past the lesion site when measured at two and four weeks post-injury ($p<0.05$, n=6). Furthermore, Wnt3a-injected eyes showed increased survival and function of RGCs and significantly higher PERG amplitudesfollowing optic nerve injury compared to the control ($p<0.05$, n=3-4). Additionally, Wnt3a-induced axonal regeneration and RGC survival was associated with activation of Stat3 signaling ($p<0.05$, n=4), and knocking out Stat3 in the floxed mice led to reduced Wnt3a-dependent axonal regeneration and RGC survival ($p<0.05$, n=5).
Conclusions: These findings show a novel role for Wnt signaling in the adult retina as a neuroprotective and regenerative mechanims for RGCs and their axons following optic nerve injury. These results may lead to the development of novel therapies for optic neuropathies and axonal injury related diseases.
Commercial Relationships: Amit Patel, None; **Kevin Park**, None; **Abigail Hackam**, None

Program Number: 1756 **Poster Board Number:** A0367
Presentation Time: 11:00 AM–12:45 PM
Inhibition of Primary Cilia Resorption Increases Retinal Ganglion Cell Survival After Axotomy
Brian Choi, Philippe M. D'Onofrio, Paulo D. Koeberle. Surgery, University of Toronto, Toronto, ON, Canada.
Purpose: Primary cilia inhibit cell division but it is unknown whether they can hault the abortive cell cycle re-entry exhibited by RGCs (retinal ganglion cells) during apoptosis. Aurora Kinase A, HDAC6 (histone deacetylase 6), Plk1 (Polo-like kinase 1), HEF1 (Human enhancer of filmanentation 1), and Nde1 (neurodevelopment protein 1) are proteins involved in the dissolution of the primary cilia and are elevated at various stages of the cell cycle. This study investigates the hypothesis that inhibiting the pathways responsible for ciliary resorption can prevent apoptotic cell cycle re-entry in RGCs after optic nerve transection.
Methods:
Adult Sprague-Dawley rats received intraorbital optic nerve transections. At 3 and 8 days postaxotomy, animals received 4uL intraocular injections of an Aurora A kinase inhibitor I (AAi; N=6) or Tubastatin A (TBA; N=4), an inhibitor of HDAC6, delivered at 10mM or 20 mM concentrations respectively. To silence Plk1, HEF1, and Nde1 expression, Adeno-associated virus serotype 2 (AAV2) vectors were intraocularly injected 1 week before axotomy to express shRNAs against these proteins (N=4 each). At 14 days postaxotomy RGCs were imaged by immunofluorescence directed against RBPMS (RNA Binding Protein with Multiple Splicing) and survival was quantified from fixed, flat-mounted retinas. Data was analyzed using a one-way ANOVA followed by Tukey's post-hoc test in order to identify statistically significant differences between control and experimental groups. To examine the effects of cilia disruption in RGCs, Ift88 (intraflagellar transport) shRNAs were used to interfere the trafficking system responsible for cilia maintenance. Survival after cilia knockdown was quantified 7 days after axotomy.
Results: RGC cilia were significantly shorter and in lesser amounts in axotomized retinas compared to normal. At 14 days postaxotomy retinas treated with AAi, TBA, or shRNAs had significantly higher RGC density compared to controls. Ift88 shRNA treated retina's had significantly reduced RGC survival compared to those treated with scrambled control shRNA.
Conclusions: Our findings suggest that the primary cilia may play a role in keeping RGCs in a post mitotic state. Interfering with the pathways involved in cilia resorption appears to halt apoptotic cell cycle re-entry. These findings support a novel role of the primary cilium in regulating the survival of RGCs after injury.
Commercial Relationships: Brian Choi, None; **Philippe M. D'Onofrio**, None; **Paulo D. Koeberle**, None
Support: CIHR 119309

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Presentation Time: 11:00 AM–12:45 PM
ERK1/2 Induces RGC Degeneration via Necroptosis Following Optic Nerve Axotomy and Crush
Philippe M. D'Onofrio[1, 2], Brian Choi[1, 2], Paulo D. Koeberle[1, 2]. [1]Department of Rehabilitation Science, University of Toronto, Toronto, ON, Canada; [2]Surgery, University of Toronto, Toronto, ON, Canada.
Purpose: Extracellular signal-related kinases (ERKs) 1 and 2 are highly expressed in the brain and are involved in apoptosis signaling. However, a possible role in necroptosis remains poorly understood. We tested the hypothesis that ERK1/2 is implicated in RGC necroptosis via interactions with receptor interacting protein kinase 3 (RIP3), and that inhibition of ERK1/2 will block necroptotic cell death. This was assessed *in vitro* in N1E-115 cells and *in vivo* in retinal ganglion cells (RGCs) using the optic nerve transection model.
Methods: N1E-115 cell death was induced by ODQ toxicity (50μM/100 μM), whereas adult female Sprague-Dawley rats (N=4/treatment) were used for optic nerve crush and transection procedures. *In vivo* treatments were administered via intra-ocular injection into the vitreous chamber, and results were asessed 14d after axotomy and 21d after crush. Protein levels and interactions were quantified by western blot and immunoprecipitation; localization was assessed via immunohistochemistry on cryosectioned tissue. RGC survival was quantified by retinal flat-mounting followed by immunohistochemistry and visual counting of surviving cells. ANOVA followed by Tukey's post-hoc test was used for statistical analysis.
Results: Administration of RIP1 inhibitor increased the number of RGCs post-axotomy (1100 RGCs/mm^2) and increased the number of axons extending beyond the damage site post-optic nerve crush (2-fold). RIP3 inhibitor significantly increased the number of RGCs post-axotomy (900 RGCs/mm^2). ERK1/2 activation was maximal between 12 hours and 2 days post-axotomy and co-immunoprecipitation of ERK1/2 demonstrated RIP3 interaction, suggesting a role in necroptosis initiation. Blocking the ERK1/2 - RIP3 binding site increased survival *in vitro* and *in vivo* (1000 RGCs/mm^2). Additionally, levels of p-MLKL, a crucial signaling molecule in necroptosis progression, were reduced in the ganglion cell layer following ERK1/2 inhibition.
Conclusions: Our results indicate that ERK1/2 plays a role in the initiation of necroptosis via the activation of RIP3 and leading to MLKL activation, an essential step in necroptosis progression. This

supports the hypothesis of necroptosis as an alternative mechanism to apoptosis in RGCs following optic nerve transection and crush damage. It also suggests that it may be an alternative mechanism in other forms of RGC, as well as CNS, neuron death.
Commercial Relationships: Philippe M. D'Onofrio; **Brian Choi**, None; **Paulo D. Koeberle**, None
Support: CIHR 119309

Program Number: 1758 **Poster Board Number:** A0369
Presentation Time: 11:00 AM–12:45 PM
Primary blast overpressure causes pathological changes on retina and optic nerve in a rat model
J. David Rios[1], Sandra Becera[2], Josianne Babineaux[1], Peter Johnson[1], Peter Edsall[1], William Elliott[1], Nathan Wienandt[2], Ammon W. Brown[2], Brian Lund[1]. [1]Ocular Trauma Research Task Area, U.S. Army Institute of Surgical Research, JBSA Fort Sam Houston, TX 78234-7767, Burlington, MA; [2]Comparative Pathology Support Task Area, U.S. Army Institute of Surgical Research, JBSA Fort Sam Houston, TX 78234-7767, San Antonio, TX.
Purpose: To determine the effect of primary blast exposure on retinal and optic nerve in an experimental shock-tube blast animal model.
Methods: Anesthetized adult male Long-Evan rats were placed in the target area of a compressed air-driven shock tube with their left sides facing the oncoming shock wave. Exposure groups experienced blast waves with peak overpressure of 123 ± 4 kPa and a positive phase duration of 3.34 ± 0.05 ms. The overpressure specific impulse was 148 ± 3 kPa-ms. Unexposed rats or shams were included as controls. The rats were euthanized while under anesthesia at 48 h post blast wave exposure. The retina and optic nerves tissues were subjected to immunohistochemistry, immunofluorescence, western and ELISA analysis for caspase-3, glial fibrillary acidic protein (GFAP), and aquaporin-4 (AQP4) expression, and TUNEL. Tissues were evaluated for relative levels of positive signals compared to unexposed controls.
Results: Caspase-3 activation was observed in the retina and optic nerve of both the right and left eyes at 48 hour post blast exposure. However, a higher level of caspase-3 expression was observed in the left eye as compared to the contralateral eye and respective controls. Activated caspase-3 was detected preferentially in the inner nuclear layer and ganglion layer. GFAP was also expressed throughout the inner nuclear layer and ganglion layer. There was also increased AQP4 expression and retinal thickness on the left eye at 48 h post blast. In the optic nerve, the highest level in GFAP expression was detected on the left side and optic chiasm. A significant increase in TUNEL cells was observed through the retina and optic nerve preferentially in the left eye at 48 h post-injury.
Conclusions: Primary blast exposure resulted in retinal and optic nerve damage manifested by the increased expression of proteins involved in edema, gliosis, and apoptosis. The injury response was greater on the side facing the incident blast wave, and therefore subject to the reflected as well as the overpressure from blast wave. These findings support potential acute injury mechanisms in which increased water permeability, microglial activation, and apoptosis are caused by primary blast exposure. This experimental shock-tube blast animal model may be useful for analyzing the effect of therapeutic interventions on retinal damage due to primary blast waves.
Commercial Relationships: J. David Rios, None; **Sandra Becera**, None; **Josianne Babineaux**, None; **Peter Johnson**, None; **Peter Edsall**, None; **William Elliott**, None; **Nathan Wienandt**, None; **Ammon W. Brown**, None; **Brian Lund**, None

Program Number: 1759 **Poster Board Number:** A0370
Presentation Time: 11:00 AM–12:45 PM
Necroptosis is responsible for RGC but not photoreceptor degeneration after blunt ocular trauma
Chloe N. Thomas[1], Adam Thompson[1], Ann Logan[1, 2], Richard J. Blanch[1, 2], Zubair Ahmed[1]. [1]Neuroscience and Ophthalmology, Institute of Inflammation and Ageing, University of Birmingham, Edgbaston, Birmingham, United Kingdom; [2]University Hospitals Birmingham, NIHR Surgical Reconstruction and Microbiology Centre, Birmingham, United Kingdom.
Purpose: Blunt ocular trauma causes commotio retinae and traumatic optic neuropathy (TON), characterised by photoreceptor and retinal ganglion cell (RGC) degeneration, respectively. Photoreceptor and RGC death occurs at and around the injury site, contributing to potentially irreversible visual loss. TON can be direct, where the optic nerve is crushed or cut, or more commonly indirect, where brain or ocular injury is associated with secondary RGC degeneration. Receptor interacting protein-1 (RIP-1) is a cell death protein associated with regulated necrosis, termed necroptosis. We hypothesised that RIP-1 contributes to retinal degeneration after blunt ocular trauma and its inhibition would reduce retinal cell death.
Methods: Anaesthetised adult Lister hooded rats were subjected to bilateral ballistic ocular trauma. RIP-1 expression was assessed by western blots in whole retinal lysate at 5, 24 and 48 hours after injury. RIP-1 activity was inhibited by unilateral intravitreal injection of Necrostatin-1 at 0 and 7 days post injury, with contralateral PBS control. Animals were euthanized at 14 days and RGC survival assessed by Brn3a and RBPMS counts and photoreceptor survival by outer nuclear layer (ONL) thickness, on sagittal retinal sections.
Results: Retinal full-length RIP-1 protein expression increased at 48 hours after injury (ANOVA; $p<0.01$, post-hoc Tukey; $p<0.01$). RIP-1 inhibition by intravitreal injection of Necrostatin-1 protected RGC [Brn3a (GEE; $p<0.01$) and RBPMS (GEE; $p<0.05$) counts], at the centre of the injury site (student t-test $p<0.01$), but did not affect ONL thickness (GEE; $p>0.05$), compared to PBS control.
Conclusions: RIP-1 mediates a proportion of RGC death in blunt ocular trauma towards the centre of the injury site, but does not mediate photoreceptor degeneration.

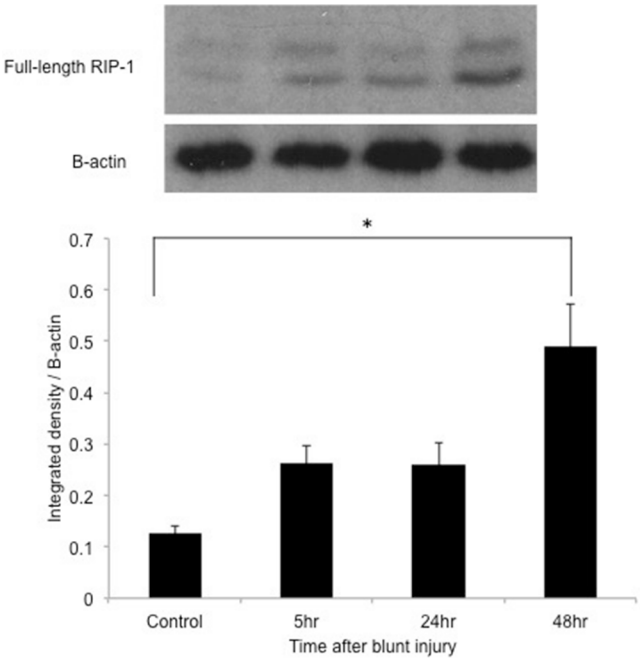

Figure 1. On western blots, full-length RIP-1 protein expression increases at 48 hours after injury (ANOVA; p<0.01, post-hoc Tukey p<0.01).

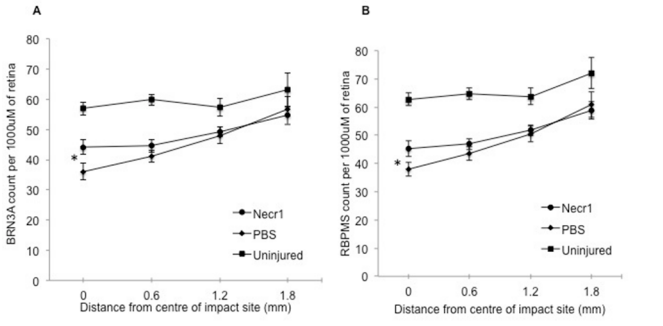

Figure 2. Necrostatin-1 protected RGC in **(A)** Brn3a (GEE; p<0.01) and **(B)** RBPMS (GEE; p<0.05) counts, compared to PBS control. RGC were protected at the centre of the injury site (0 mm) in Brn3a and RBPMS counts (student t-test p<0.01). n=8

Commercial Relationships: Chloe N. Thomas, None; **Adam Thompson**, None; **Ann Logan**, None; **Richard J. Blanch**, None; **Zubair Ahmed**, None
Support: Fight for Sight PhD Studentship (Grant code: 1560/1561)

Program Number: 1760 **Poster Board Number:** A0371
Presentation Time: 11:00 AM–12:45 PM
Arginase 2 deletion prevents optic nerve crush-induced retinal degeneration
Ruth B. Caldwell[2, 1], Zhimin Xu[2, 1], S. Priya Narayanan[2, 1], Tahira Lemtalsi[2, 1], Chintan Patel[1], Esraa Shosha[1], William Caldwell[3]. [1]Vascular Biology Center, Medical College of Georgia, Augusta, GA; [2]VA Medical Center, Augusta, GA; [3]Pharmacology & Toxicology, Medical College of Georgia, Augusta, GA.
Purpose: We have shown previously that deletion of the arginase 2 (A2) gene reduces neurovascular injury and improves retinal ganglion cell (RGC) survival in a model of ischemia/reperfusion injury. The aim of this study was to determine the role of A2 expression in traumatic retinal injury following optic nerve crush (ONC).
Methods: ONC was produced in the left eye of wild-type (WT) or A2 knockout ($A2^{-/-}$) mice by incising the limbal conjunctiva, deflecting the intraocular muscles and clamping the optic nerve (2 mm from the eyeball, 3 sec). The right eye served as sham control. Loss of ganglion cell layer (GCL) neurons and RGCs and microglial activation were assessed in retinal whole mounts labeled for the neuronal marker (NeuN), RCG marker (Brn3a) and microglia/macrophage marker (Iba1). Cell death, glial activation, and retinal thinning were assessed by TUNEL assay, GFAP immunostaining, and H&E staining of retinal sections, respectively. Molecular mechanisms were examined by western blotting and quantitative RT-PCR.
Results: At 7 days after ONC, the number of NeuN positive neurons in the GCL of the WT retinas was decreased by 60% as compared to the sham controls. This ONC-induced neuronal loss was diminished in the $A2^{-/-}$ retinas ($p<0.05$). Brn3a-positive RGCs were significantly protected by A2 deletion ($p<0.05$). Significant retinal thinning was also seen in WT retinas following ONC, and this was blocked in $A2^{-/-}$ mice ($p<0.01$). Cell death studies showed an increase in TUNEL positive cells in the GCL after ONC in the WT retinas, and this was attenuated by A2 deletion. Activation of glial and microglial cells was elevated in WT retinas after ONC, and this was markedly reduced by A2 deletion. Western blotting showed that ONC also induced significant increases in levels of phosphorylated P38 and FASL in WT retinas compared with sham controls but not in the $A2^{-/-}$ mice ($p<0.01$). Quantitative RT-PCR showed that pro-inflammatory mediators (IL1β, ICAM1, MCP1, and NOX4) were upregulated following ONC in the WT retinas, and this was abrogated by A2 deletion.
Conclusions: Deletion of A2 limits ONC-induced neurodegeneration and glial activation by a mechanism involving decreases in retinal inflammation. These data demonstrate that A2 plays an important role in ONC-induced retinal damage. Blockade of A2 activity may offer a therapeutic strategy for preventing vision loss induced by traumatic retinal injury.
Commercial Relationships: Ruth B. Caldwell, None; **Zhimin Xu**, None; **S. Priya Narayanan**, None; **Tahira Lemtalsi**, None; **Chintan Patel**, None; **Esraa Shosha**, None; **William Caldwell**, None
Support: VA Merit Review Award I01BX003221; NIH Grant EY011766

Program Number: 1761 **Poster Board Number:** A0372
Presentation Time: 11:00 AM–12:45 PM
Retinal microgliosis, functional sequelae, and traumatic brain injury (TBI) in an impact concussion mouse model
Lee E. Goldstein[1, 4], Olga Minaeva[1, 2], Mark W. Wojnarowicz[1], Juliet A. Moncaster[1], Andrew M. Fisher[1, 4], Erich S. Franz[1, 4], Ivana Arellano[2], R D. Ferguson[7], Mircea Mujat[7], Bertrand R. Huber[5, 6], Anne B. Fulton[2, 3], David G. Hunter[2, 3], James D. Akula[2, 3]. [1]Molecular Aging and Development Laboratory, Boston University School of Medicine, Boston, MA; [2]Dept. of Ophthalmology, Boston Children's Hospital, Boston, MA; [3]Dept. of Ophthalmology, Harvard Medical School, Boston, MA; [4]College of Engineering, Boston University, Boston, MA; [5]Boston University School of Medicine, Boston, MA; [6]VA Boston Healthcare System, Boston, MA; [7]Biomedical Optics, Physical Sciences, Inc, Andover, MA.
Purpose: Head trauma is a potent stimulus for brain inflammation, a neurotrauma hallmark that is characterized by reactive microgliosis, infiltration of peripheral monocytes, and pro- and anti-inflammatory molecules. These responses can either facilitate recovery or trigger sequelae. Activation and interaction of specific immune cells and phenotypes are determinative of temporal pacing and outcome of brain recovery after concussive head injury.
Methods: We utilized Ccr2RFP/Cx3cr1GFP mice (Jackson Laboratory) to enable immune cell visualization by class

(microglia, monocyte), origin (peripheral, brain, retina), morphology, and location. We used a new closed-head impact injury mouse model (Tagge *et al.*, submitted) that recapitulates key features of human concussion. Experimental injury was conducted without anesthesia (approved IACUC protocol) to enable quantitative neurological assessment (Boston University Concussion Scale, BUCS; *ibid.*). We evaluated histopathology, ultrastructural analysis, blood-brain/retinal barrier function, immune cell imaging, and electrophysiology. We also used an adaptive optics fluorescence scanning laser ophthalmoscope (AO-fSLO) with OCT imaging (Physical Sciences, Inc.) and electroretinography (ERG).
Results: Head-injured mice exhibited transient contralateral hemiparesis, truncal ataxia, impaired balance, and abnormal locomotion that recapitulated concussion in humans. Impact injury induced axonopathy, blood-brain barrier disruption, reactive astrocytosis and microgliosis, and peripheral monocyte infiltration. Concussive signs did not correlate with structural endpoints. Before injury, Ccr2RFP/Cx3cr1GFP mice showed normal cellular distribution and morphological phenotype of resting microglia in retina and brain. We observed significant increase in microglia in retina and brain post-injury. Microgliosis included reactive phenotype transformation with overlapping ramifications and perivascular changes. ERG analysis (see Akula *et al.*) showed attenuated photoreceptor, postreceptor, and inner retinal responses after head injury.
Conclusions: Closed-head impact injury is associated with reactive inflammatory responses and sequelae in both retina and brain. The retina can be used as a "brain proxy" for noninvasive diagnosis, prognosis, staging and monitoring of neuroinflammation after closed-head injuries.
Commercial Relationships: Lee E. Goldstein, Rebiscan (P), Rebiscan (C); **Olga Minaeva**, None; **Mark W. Wojnarowicz**, None; **Juliet A. Moncaster**, None; **Andrew M. Fisher**, None; **Erich S. Franz**, None; **Ivana Arellano**, None; **R D. Ferguson**, Physical Sciences, Inc (E); **Mircea Mujat**, Physical Sciences, Inc (E); **Bertrand R. Huber**, None; **Anne B. Fulton**, None; **David G. Hunter**, Rebiscan (I), Rebiscan (P); **James D. Akula**, None
Support: DoD W81XH-14-1-0592, Massachusetts Lions Eye Research Fund, Boston Children's Hospital Ophthalmology Foundation, Private Foundations

Program Number: 1762 **Poster Board Number:** A0373
Presentation Time: 11:00 AM–12:45 PM
Closed globe trauma activates sterile inflammation in the retina
Adrienne Clark, Alexandra Bernardo, Tonia S. Rex. Vanderbilt Eye Institute, Vanderbilt University Medical Center, Nashville, TN.
Purpose: Our goal was to determine if ocular trauma activates sterile inflammation in the retina.
Methods: We exposed one eye of an anesthetized C57Bl/6 mouse to a burst of overpressure air. Sham mice were anesthetized and placed into the air blast system, but were not exposed to the overpressure air. At multiple time-points after injury tissue was collected for immunohistochemistry, Western blot analysis, and multiplex ELISA to assess activation of sterile inflammation.
Results: In the C57Bl/6 mouse, cell death, axon degeneration, and vision loss are first detectable at 1 month after eye trauma in our model. Sterile inflammation can be initiated by the alarmin, Il-1a. Levels of IL-1a were increased at 2 weeks and further increased at 4 weeks after injury as compared to shams. The caspase that mediates sterile inflammation is caspase-1. Levels of caspase-1 were detected in Chat-positive amacrine cells beginning as early as 3 days after ocular trauma and total retina levels of total and cleaved caspase-1 were also increased after injury. The extent of retina with caspase-1 immunolabeling increased over time after injury from a focal area at 3 days to throughout the retina at 1-month. Activation of IL-1R by IL-1a or IL-1b induces increases in IL-1b and IL-18, which are both cleaved into their active forms by caspase-1. Both IL-1b and IL-18 were increased at 1-month after injury as compared to shams.
Conclusions: Prior to detection of neurodegeneration and vision loss in the retina there was an increase in the pro-inflammatory cytokine and "alarmin", IL-1alpha. In addition, increases in cleaved caspase-1 and Il-1b and IL-18 point towards a role of sterile inflammation after eye trauma. Interestingly the caspase-1 labeling was specific to the Chat-positive, i.e. starburst, amacrine cells. Thus these neurons, rather than glial/immune cells may be mediating this response to injury. Future studies are needed to determine if these secreted pro-inflammatory cytokines are responsible for the cell death, axon degeneration, and vision loss detected after ocular trauma in our model.
Commercial Relationships: Adrienne Clark, None; **Alexandra Bernardo**, None; **Tonia S. Rex**, None
Support: NIH Grant EY022349, DoD Grant W81XWH-13-1-0048, DoD Grant W81XWH-15-1-0096

Program Number: 1763 **Poster Board Number:** A0374
Presentation Time: 11:00 AM–12:45 PM
Long-term functional and structural consequences of primary blast injury to the eye
Cara T. Motz[1], Rachael S. Allen[1], Andrew Feola[2, 1], Kyle Chesler[2], Raza Haider[1], Sriganesh Ramachandra Rao[3], Lara Skelton[4], Steven J. Fliesler[3, 4], Machelle T. Pardue[1, 2]. [1]Center for Visual and Neurocognitive Rehabilitation, Atlanta VA Medical Center, Atlanta, GA; [2]Biomedical Engineering, Georgia Institute of Technology, Atlanta, GA; [3]Ophthalmology, Biochemistry, & Neuroscience Program, SUNY- University at Buffalo, Buffalo, NY; [4]Research Service, VA Western NY Healthcare System, Buffalo, NY.
Purpose: Patients with primary blast injury (PBI) often present with delayed visual deficits of unknown etiology. In this study, we evaluated longitudinal changes in retinal function and morphology following acoustic blast exposure, using a rat model of PBI.
Methods: Adult male Long-Evans rats (N=10-12/group) were exposed to a single acoustic blast (63 kPa, 195 dB-SPL) at 3.5 mo of age, using a shock tube device. The blast was directed at the right eye only, perpendicular to the body axis, with only the head exposed. Non-blasted, age-matched rats served as controls. Animals were tested longitudinally at 2, 4, 6 and 8 mo post-blast for: retinal function with electroretinography (ERG); contrast sensitivity (CS) and spatial frequency (SF) thresholds with optokinetic tracking (OKT); and retinal structure with spectral domain-optical coherence tomography (SD-OCT). At 8 mo post-blast, animals were sacrificed and tissues were taken for future biochemical and immunohistochemical analyses. Statistical analysis: repeated measures two-way ANOVA (significance: $p<0.05$).
Results: CS thresholds were consistently decreased in both eyes of blast-exposed animals vs. controls across time ($p<0.001$), and the blasted eyes had significantly lower CS thresholds than the contralateral eyes (*e.g.,* at 8 mo post-blast: $\Delta = 45\%$; $p<0.001$). Blasted eyes also showed a 20% decrease in SF thresholds compared to contralateral and control eyes ($p<0.001$), at all time points. ERG scotopic a- and b-wave amplitudes were substantially *greater* ($p<0.001$) and b-wave implicit times were increasingly delayed (*e.g.*, 18% at 8 mo; $p<0.001$) in blast-exposed rats, relative to controls. Similar trends were observed for photopic flicker responses. SD-OCT showed *increased* total retinal thickness, relative to controls, in both eyes of blast-exposed rats across all time points

(5% at 8 mo; p<0.01), with inner nuclear layer and inner/outer segment layers being the major contributors.
Conclusions: PBI can manifest with functional and structural changes to the retina that are counter-intuitive and atypical of common retinal diseases. Here, blast exposure compromised visual function, yet ERG amplitudes were supranormal, with delayed implicit times, suggesting disruption of inhibitory signaling pathways. Rather than degenerative thinning, retinal thickness of blast-exposed eyes was *greater* than normal. The underlying mechanisms for these changes remain to be determined.
Commercial Relationships: Cara T. Motz; **Rachael S. Allen**, None; **Andrew Feola**, None; **Kyle Chesler**, None; **Raza Haider**, None; **Sriganesh Ramachandra Rao**, None; **Lara Skelton**, None; **Steven J. Fliesler**, None; **Machelle T. Pardue**, None
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Program Number: 1764 **Poster Board Number:** A0375
Presentation Time: 11:00 AM–12:45 PM
Neuroprotective effect of enriched environment on experimental optic neuritis
Marcos Luis Aranda, Maria Florencia Gonzalez Fleitas, Hernan Hugo Dieguez, Maria Ines Keller Sarmiento, Monica Chianelli, Pablo Sande, Damian Dorfman, Ruth E. Rosenstein. Human Biochem/Sch of Med, University of Buenos Aires, Buenos Aires, Argentina.
Purpose: Optic neuritis (ON) is an inflammatory, demyelinating, and neurodegenerative condition of the optic nerve, which might induce permanent vision loss. Enriched environment involves housing conditions which stimulate social interaction, physical, cognitive and explorative activity, and exert beneficial effects on the brain in many central nervous system disorders. However, its therapeutic potential during neuroinflammation remains less understood. The aim of this work was to examine the effect of enriched environment (EE) on optic nerve and retinal damage induced by experimental ON.
Methods: Bacterial lipopolysaccharide (LPS) or vehicle were microinjected into the optic nerve from adult male *Wistar* rats. Immediately after injections, one group of animals was housed in EE, and another group remained in standard environment (SE) for 21 days. Visual pathway function (visual evoked potentials (VEPs), and pupillary light reflex (PLR)), anterograde transport from the retina to the superior colliculus(after an intravitreal injection of cholera toxin b-subunit), microglial/macrophages reactivity (Iba-1 and ED1-immunoreactivity), astrocytosis (glial fibrillary acidic protein (GFAP) immunohistochemistry), demyelination (luxol fast blue staining), axons (phosphorylated neurofilament heavy immunoreactivity (pNFH) andtoluidine blue staining), retinal ganglion cells (RGC, Brn3a-immunoreactivity), and optic nerve lipid peroxidation (thiobarbituric acid reactive substances) were assessed in vehicle- and LPS-injected optic nerves from animals housed in SE or EE.
Results: EE housing completely prevented the decrease in VEPs and PLR, and anterograde transport induced by experimental ON. In addition, EE prevented the decreased pNFH-immunoreactivity microglia/macrophage reactivity, astrocytosis, demyelination, and axon and RGCloss induced by experimental ON. In SE, LPS-injected optic nerves displayed oxidative damage which was prevented by EE housing. When EE housing started at 4 days post-injection of LPS, a preservation of VEPs and PLR was observed.
Conclusions: These data support that EE housing preserved visual functions and reduced neuroinflammationof the optic nerve.
Commercial Relationships: Marcos Luis Aranda, None; **Maria Florencia Gonzalez Fleitas**, None; **Hernan Hugo Dieguez**, None; **Maria Ines Keller Sarmiento**, None; **Monica Chianelli**, None; **Pablo Sande**, None; **Damian Dorfman**, None; **Ruth E. Rosenstein**, None

Program Number: 1765 **Poster Board Number:** A0376
Presentation Time: 11:00 AM–12:45 PM
Single molecule fluorescent in situ hybridization in the mouse retina
Michael Thomsen[1], Mayuri Viswanathan[1], Samer Hattar[1, 2]. [1]Biology, Johns Hopkins University, Baltimore, MD; [2]Neuroscience, Johns Hopkins University, Baltimore, MD.
Purpose: High-throughput transcriptomic data sets often yield hundreds of interesting candidate genes for further study, but confirmation of this data in vivo often relies on methods of in situ hybridization that are time-consuming and technically challenging. To facilitate rapid, robust screening of gene targets from transcriptomic studies, we have adapted a protocol for single molecule fluorescent in situ hybridization (smFISH) for use in whole-mount mouse retinas. smFISH requires less than half the time of conventional in situ hybridization protocols and permits single transcript resolution and quantitation.
Methods: We used computational methods to design a library of 20-30mer oligonucleotide probes that tile >100 mRNA sequences of interest with minimal off-target binding. Each probe was flanked by universal primer sequences and gene-specific orthogonal priming sequences that permit amplification of probe sets for a single gene from the library. The total library of >12,000 oligos was chemically synthesized and suspended in a small volume of TE buffer. Probe sequences for each gene were amplified from the library using several rounds of PCR with gene-specific primers. Following amplification of probes for each gene, antisense fluorescent oligonucleotide probes were generated by PCR with fluorophore-coupled primers. Freshly dissected mouse retinas were quartered and lightly fixed in 4% PFA then permeabilized in 0.5% TritonX-100/PBS. Following permeabilization retinas were incubated overnight with fluorescent probes in hybridization buffer. After hybridization, retinas were washed several times in 10% formamide/2XSSC, mounted, and imaged immediately.
Results: smFISH robustly labeled transcripts in the ganglion cell layer (GCL) of mouse retinas with little background fluorescence. Individual transcripts for more than 20 genes were visualized and quantified.
Conclusions: We have optimized a method of smFISH for rapid and reliable detection of individual mRNA transcripts in the mouse retina. This method has significant advantages over conventional in situ hybridization including shortened protocol time, single transcript resolution, and improved reliability.
Commercial Relationships: Michael Thomsen, None; **Mayuri Viswanathan**, None; **Samer Hattar**, None
Support: GM076430 and EY024452

Program Number: 1766 **Poster Board Number:** A0377
Presentation Time: 11:00 AM–12:45 PM
Overturning the role of Math5 in Retinal Ganglion Cell Specification
Justin Brodie-Kommit[1], Hope Shi[2], Fuguo Wu[4], Tiffany M. Schmidt[3], Xiuqian Mu[4], Joshua H. Singer[2], Samer Hattar[1, 5]. [1]Biology, Johns Hopkins University, Baltimore, MD; [2]Biology, University of Maryland, College Park, College Park, MD; [3]Neurobiology, Northwestern University, Evanston, IL; [4]Ophthalmology, University at Buffalo, Buffalo, NY; [5]Neuroscience, Johns Hopkins School of Medicine, Baltimore, MD.
Purpose: Retinal ganglion cells (RGCs) are the sole conduits of light information to the brain. RGC differentiation occurs by retinal progenitor cells expressing transcription factors in a developmentally temporal fashion, including Math5 (Atoh7). Math5, a bHLH transcription factor, is thought to be necessary for RGC specification; since Math5 null mice lack 95% of RGCs. A recent publication, however, used lineage tracing to show that a subset of RGCs do not express Math5. Therefore, we re-examined the Math5 null mouse line on a background that prevents RGC death by knocking out the proapoptotic gene, Bax. Mutations in Math5 are responsible for multiple retinal pathologies, including arPHPV where the fetal hyaloid vasculature fails to regress and the retinal vasculature does not form, occurring in both humans and mice. Understanding its role in retinal development is key to understanding the development of the retina.
Methods: We utilized Math5$^{-/-}$, Bax$^{-/-}$, and the Math5-tTA;tetO-Pou4f2-Isl1 mouse lines combined with multielectrode array recordings and immunofluorescence staining to analyze RGC and retinal development.
Results: Surprisingly, we found that even in the absence of Math5 and Bax, substantial numbers of retinal progenitor cells differentiate into RGCs and express RGC specific genes such as Brn3a, Brn3b, Isl1, and RBPMS. These rescued RGCs fail to exit the eye and form an optic nerve, yet by multielectrode array recordings, RGCs from Math5/Bax double knockout animals displayed ON, OFF and ON/OFF rod/cone light responses. We also found that even when RGC numbers in Math5/Bax double knockout animals are mostly restored, the retinal vasculature deficits were not rescued. To test whether Math5 or the optic nerve is required for the formation of the retinal vasculature we used a published mouse line where Brn3b and Islet1 are ectopically expressed from the Math5 promoter in the absence of Math5. This rescues RGC numbers and optic nerve in the absence of Math5, and surprisingly the hyaloid regression was observed and the retinal vasculature was mostly restored.
Conclusions: These data suggest that a subset of RGCs could be specified in the absence of Math5 and that Math5 is required for RGC differentiation in that subset by preventing apoptosis. These data also suggest that rescuing the optic nerve and RGC numbers, but not RGC numbers alone, is required for hyaloid regression and the following retinal vasculature development.
Commercial Relationships: Justin Brodie-Kommit, None; **Hope Shi**, None; **Fuguo Wu**, None; **Tiffany M. Schmidt**, None; **Xiuqian Mu**, None; **Joshua H. Singer**, None; **Samer Hattar**, None
Support: NIH Grant GM076430 and NIH Grant EY024452

Program Number: 1767 **Poster Board Number:** A0378
Presentation Time: 11:00 AM–12:45 PM
Roles of Tbr1 in retinal ganglion cell subtype formation
Chai-An Mao[1], Ye Long[1], Christophe Whitaker[1], Lian-Ming Tian[1], Harry Liu[2], Ping Pan[1], Tudor C. Badea[3], Jan Parker-Thornburg[4], William H. Klein[5], Stephen L. Mills[1], Stephen C. Massey[1]. [1]Ophthalmology & Visual Science, U.T. Health Science Center Houston, Houston, TX; [2]Rice University, Houston, TX; [3]NEI/NIH, Bethesda, MD; [4]Genetics, The University of Texas MD Anderson Cancer Center, Houston, TX; [5]Systems Biology, The University of Texas MD Anderson Cancer Center, Houston, TX.
Purpose: The developmental and molecular mechanisms controlling the formation and maturation of the diverse RGC subtypes remains unclear. Here, we provide evidence showing that T-box transcription factor T-brain 1 (Tbr1) is expressed in a subset of RGCs in mouse retinas and may play key roles in the formation and maintenance of two morphologically and functionally distinct RGC subtypes.
Methods: Molecular tools (ISH, IF, and qRT-PCR) were used to detect the spatiotemporal expression patterns of Tbr1 in developing and mature mouse retinas. To determine Tbr1-expressing RGC subtypes, we generated Tbr1^{CreERT2}:Pou4f1^{CKOAP} and Tbr1^{CreERT2}:Ai9 mouse lines and conducted sparse-labeling and dye-filling in Tbr1-expressing RGCs, respectively. Alkaline phosphatase staining on the tamoxifen-activated Tbr1^{CreERT2}: Pou4f1^{CKOAP} retinas was used to reveal the overall Tbr1+ RGC subtypes. NBT dye-filling in individual Tbr1+ RGCs in tamoxifen-activated Tbr1^{CreERT2}:Ai9 retinas was used to examine the detailed dendritic morphologies of these RGCs. Furthermore, we conducted loose patch-clamp recording on individual Tbr1+ RGCs to determine their light-responses. To determine the central projection of Tbr1+ RGCs, we examined the GFP signal in brain sections from Tbr1^{TauGFP} mice.
Results: We detected Tbr1 expression in a subset of RGCs, starting from E14.5. By sparse labeling and dye-filling, we revealed two morphologically distinct Tbr1+ RGC subtypes, including the JAM-B RGC and a RGC subtype with symmetric dendritic ramification pattern. The terminal dendrites of both RGC subtypes stratify slightly above the ChAT OFF band in the IPL. The second RGC subtype exhibited sluggish OFF response, and preferred medium size spots with both positive and negative contrast. They are not direction-selective RGCs, and showed ON responses to annuli. The GFP signals in Tbr1^{TauGFP} brain sections were detected in superior colliculus and dorsal lateral geniculate nucleus.
Conclusions: In summary, we have demonstrated that Tbr1 expression marks two RGC subtypes, suggesting that RGC subtypes may be established by intrinsically programmed transcriptional regulatory mechanism. These mouse lines will be used to study the developmental timing and relationship of these two RGC subtypes during retinogenesis. Furthermore, Tbr1^{flox} allele will be used to determine whether and how Tbr1 regulates the formation and maintenance of these two RGC subtypes.
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Neural Cell Degeneration in Human Retinas May Explain Visual Dysfunction in Parkinson Disease
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Purpose: Diverse studies have shown the existence of visual alterations in Parkinson disease, but how it affects different retinal cell types remains unknown. This study aims to characterize some of the cellular changes that occur in the retina of Parkinson disease donors. Concretely, we looked for the presence of phosphorylated α-synuclein, the impairment of the dopaminergic system and the loss of cells in the ganglion cell layer.
Methods: Whole eyes were obtained postmortem from Parkinson disease (PD) and control healthy donors (C). Immunohistochemistry using the avidin-biotin-complex method or fluorescence labeling was performed. Flat wholemount retinas were stained using an antibody raised against α-synuclein phosphorylated at serine 129 (p-synuclein; N=10 PD, 12 C) and structures accumulating the protein were analyzed. A score based on the amount of staining was given to each retina and compared to its brain score using the Spearman correlation test. Antibodies against tyrosine hydroxylase (N=9 PD, 4 C) and calretinin (N=9 PD, 4 C) were also employed. Morphology, density and number of synaptic contacts of dopaminergic and AII amacrine cells were established. Cells in the ganglion cell layer were stained with Hoescht (N=4 PD, 4 C) and cell density was calculated. T-tests were used for statistical analysis.
Results: P-synuclein staining was found in ganglion cells, axons, somas, dendrites and abnormal structures. The density of p-synuclein-immunoreactive structures present in the retina significantly correlated with that found in ten standard brain regions ($R=0.849$; $p<0.001$). A significant decrease of dopaminergic cell density was found in Parkinson disease (PD=10.43±2.04, C=18.64±1.06; $p<0.001$) while no differences were found in AII amacrine cell density. Synaptic contacts between dopaminergic and AII cells were also reduced. Finally, a significant loss of cells in the ganglion cell layer was detected ($p<0.05$).
Conclusions: Accumulation of p-synuclein, impairment of the dopaminergic system and loss of cells in the ganglion cell layer may explain the visual alterations detected in Parkinson disease and could be employed as biomarkers of the presence or progression of the disease. These findings indicate that the retina may be a useful model with which to study the neurodegenerative mechanisms underlying Parkinson disease.
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Down Stream Targets in the OA1 Signaling Cascade Critical for Retinal Axon Navigation
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Purpose: Ocular albinism type1, caused by mutations in the *OA1* gene, is characterized by abnormalities in the retinal pigment epithelial (RPE) cells' melanosome development and in the crossing of the optic axons at the brain's optic chiasm. Exactly how the OA1, a G protein coupled receptor product of the *OA1* gene, exerts its effects on the retinal ganglion cells (RGCs) to influence the routing of the optic axons remains unsolved. In the Oa1 knockout ($Oa1^{-/-}$) mouse, a model for the disease, there is a 24% reduction in the number of ipsilateral axons compared to the B6/NCrl control mouse. This leads to aberrant stereospecific vision. Here, we studied how the cAMP response element binding protein (Creb) may contribute to the axonal routing errors.
Methods: Western blots were used to compare the levels of phosphorylated Creb (pCreb) and doublecortin (Dcx) in the posterior eyecups of developing control B6/NCrl and $Oa1^{-/-}$ mice while immuno-histochemistry allowed us to determine the spatiotemporal expression patterns of pCreb, Creb binding protein (Crebbp) and Dcx in the eyes during the critical developmental stages.
Results: Western blot and immuno-histochemistry results showed a decrease in pCreb and its co-activator, Crebbp, in both the embryonic and post embryonic eyes of $Oa1^{-/-}$ mice compared to those of control B6/NCrl mice. pCreb and Crebbp expression was seen in both the RPE and the retina. Control B6/NCrl mice also showed strong immuno-reactivity for the microtubule binding protein, Dcx, in immature cells and cell processes of the E14.5 retinas, a stage when retinal cells are not yet organized in different layers. In contrast, the $Oa1^{-/-}$ mouse retina showed reduced expression of Dcx in developing eyes.
Conclusions: These data provide evidence that Creb and its down-stream target gene, Dcx, may be involved in early retinal neurogenesis and suggest that reduced levels of Dcx in the absence of Oa1 (in $Oa1^{-/-}$ mice) may affect the routing of RGC axons. Future studies will establish the link between Oa1-Creb-Dcx signaling in developing mouse eyes.
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