These abstracts are licensed under a Creative Commons Attribution-NonCommercial-No Derivatives 4.0 International License. Go to http://iovs.arvojournals.org/ to access the versions of record.
**Purpose:** Blue light is an identified risk factor for age-related macular degeneration (AMD). Using a custom-made illumination system delivering 10 nm-wide illumination bands within the blue-green range, we recently showed that the narrow range 415-455 nm was the most toxic for A2E-loaded RPE cells (Arnault et al., 2013). To further understand the mechanisms involved in AMD, we investigated the impact of light on the expression of VEGF and its receptors in A2E-loaded RPE cells under illumination in the blue-green range.

**Methods:** Primary culture of porcine retinal pigment epithelium cells (RPE) were incubated for 6 hours with A2E and then exposed for 15 hours to 10 nm-wide illumination bands centered from 390 to 520 nm in 10 nm increments; one additional band centered at 630 nm was used as a light control. Light irradiances were normalized with respect to the natural sunlight reaching the retina after being filtered by the ocular structures. These conditions were selected because they induce oxidative stress in RPE cells but remain below the maximal cell death induction threshold. VEGF, VEGF-R1 and VEGF-R2 mRNA contents were quantified by qPCR after light exposure, results were normalized to the ribosomal gene 18S. VEGF protein content was quantified by ELISA in cell lysates and in cell culture medium.

**Results:** In A2E-loaded RPE cells exposed to blue-violet light, VEGF mRNA expression decreased significantly while VEGF-R1 mRNA expression increased. VEGF-R2 was less expressed than VEGF-R1 and was not affected by light exposure, except in RPE cells exposed to 400nm without A2E in which an increase was observed. Secreted and intracellular VEGF protein expression increased in the presence of A2E. VEGF protein expression levels decreased in A2E-loaded-RPE cells and the culture medium when exposed to blue-violet light.

**Conclusions:** Using our in vitro model of AMD and our custom-made illumination system, we showed that the expression of the VEGF and its receptors could be modified by light exposure at the mRNA level but also at the protein level. We have also confirmed that A2E is able to induce VEGF production and secretion in RPE cells. Surprisingly, blue-violet light, which we previously found to induce oxidative stress and apoptosis, tend to decreases VEGF production. These results suggest that blue light toxicity might contribute preferentially to the development of dry AMD rather than to the complications of wet AMD.

**Commercial Relationships:** Melanie MARIE, None; Pauline GONDOUIN, None; Coralie BARRAU, Essilor International (E); Thierry VILLETTE, Essilor International (E); Denis COHEN-TANNOUDJI, Essilor International (E); Jose A. Sahel, None; Serge A. Picaud, Essilor International (C), Essilor International (F)

**Program Number:** 5860 Poster Board Number: B0453
**Presentation Time:** 11:30 AM–1:15 PM

**Age-related beta-Synuclein alters the p53/MDM-2 pathway and induce apoptosis within Brain-Microvascular Endothelial Cells in-vitro**

**Michael Böhm**, 1, 2; Katrin Brockhaus, 1; Harutyn Melkonyan, 1; Klaus-Peter Steuhl, 1; Solon Thanos, 1, 3; 1Department of Ophthalmology, Essen University Hospital, Essen, Germany; 2Institute for Experimental Ophthalmology, School of Medicine, Westfalian Wilhelms-University Muenster, Muenster, Germany.

**Purpose:** Increased beta-synuclein (SNCB) has been previously described within the aging visual system including neuroretina and primary visual cortex. SNCB functions as physiological antagonist of alpha-synuclein (SNCA), which is involved in neurodegenerative diseases like Parkinson’s and Alzheimer’s disease. How ever, the exact function of SNCB is already unknown. Brain-microvascular endothelial cells (BMECs) maintain physiological homeostasis and support studies about the blood-brain barrier within the neurovascular unit. Aim of this study was elucidate the age-dependend role of SNCB within endothelial cells of the neurovascular unit of rats.

**Methods:** BMECs were isolated from cortices of 5-9 days old Sprague-Dawley rats and were cultured with different concentrations of recombinant SNCB (rSNCB) up to 72h. Viability (MTT), apoptosis (TUNEL) and expression levels of SNCA, members of the Poshophilase D2 (PLD2) - p53 - MDM-2 - p19-ARF pathway, response to AKT, and stress mediated factors like HMOX and NOX4 were studied by using immunohistochemistry (IF), Western blot...
**Results**: BMECs revealed a decreased viability and increased apoptosis after SNCB exposure. Decreased protein- and mRNA expression levels of SNCA have been found. IF and WB analysis indicate an inhibition of Akt together with an elevation of PLD2, activation of p53, endeored intracellular MDM2 translocation and elevated p19-ARF. More over, an elevation of PLD2 activity in SNCB exposed BMECs has been found. Alterations of HMOX and Nox4 indicate a stress related response of BMECs exposed to SNCB.

**Conclusions**: The presented results indicate a distinct effect of SNCB on BMECs in-vitro. Due to a p53 mediated and Akt independent apoptosis together with a stress mediated response of BMECs may be induced by increasing SNCB with age in the neurovascular unit. Further studies on the molecular mechanisms based on age-dependent role of SNCB may help to increase understanding about neurodegenerative diseases. Supported by the German Research Foundation (DFG, BO 4556/1-1)

**Commercial Relationships**: Michael Böhm, Novartis Pharma GmbH, Nürnberg, Germany (R); Katrin Brockhaus, None; Harutyun Melkonyan, None; Klaus-Peter Steuhl, None; Solon Thanos, Novartis Pharma GmbH, Nürnberg, Germany (R)

**Support**: Grant of the German Research Foundation (DFG Grant BO 4556/1-1)

**Program Number**: 5862 Poster Board Number: B0454

**Presentation Time**: 11:30 AM–1:15 PM

**Can the retina be used as a reliable mirror to evaluate changes occurring in the Alzheimer’s brain?**

António F. Ambrósio 1, 2, Samuel Chiquita 1, 2, Catarina Neves 1, 2, Rafael Carecho 1, 3, Filipa Baptista 1, Elisa Campos 1, 3

1 Center for Neuroscience and Cell Biology, University of Coimbra, Portugal, Coimbra, Portugal; 2 CN.CIBIL Consortium, Coimbra, Portugal; 3 Center for Neuroscience and Cell Biology, University of Coimbra, Coimbra, Portugal.

**Purpose**: Diagnosis of Alzheimer’s disease (AD) is difficult and relies on expensive and invasive methods. Since many AD patients experience early visual problems, the retina is an appealing source of information for AD diagnosis. We intend to clarify whether the retina is early affected and could be considered a reliable mirror of the brain alterations of an AD mouse model.

**Methods**: Male triple transgenic (3xTg-AD; AD model) and age-matched wild-type (WT; C57BL/6J/129S) mice (4 and 8 months old; early time points) were used to evaluate retinal structural and functional changes by optical coherence tomography (line and circular scans), electoretinography (ERG; scotopic, photopic, and photopic flicker) and pattern ERG (PERG). Retina, hippocampus and total cortex homogenates (4 months old mice) were used to evaluate synaptic loss and glial reactivity by Western blot.

**Results**: At 8 months, but not at 4 months, there was a significant reduction of retinal thickness of 3xTg-AD mice compared with WT, assessed with line scans (179.96±1.07 µm vs 188.60±2.34 µm) and circular scans (176.49±1.28 µm vs 186.75±2.36 µm). This reduction was detected in several layers, including ganglion cell layer. At 4 and 8 months there was also an increased (p<0.05) scotopic and photopic b-wave amplitude and photopic flicker amplitude (1st and 2nd harmonic) in 3xTg-AD mice. No differences were detected in PERG recordings between 3xTg-AD and WT mice.

At 4 months, there was a significant increase in the levels of amyloid beta (Aβ) and p-Tau in the hippocampus (287.97±57.23% and 198.77±39.89% of WT, respectively) and total cortex (220.23±37.70% and 166.10±15.62% of WT, respectively), but not in the retina, of 3xTg-AD mice. The levels of β-secretase and choline acetyltransferase were not affected in the three regions analyzed. There was a significant increase of glial fibrillary acidic protein, but only in the hippocampus of 3xTg-AD mice (212.18±27.01% of WT). The synaptic proteins synaptophysin and syntaxin were unchanged in these three regions, with the exception of syntaxin levels (increased in the cortex of 3Tg-AD mice).

**Conclusions**: This study shows that at early stages (4 and 8 months) the brain (hippocampus and/or cortex) of 3xTg-AD mice is more affected than the retina, but the retina is also affected, and so changes in the retina might be used for AD diagnostics.

**Support**: Neuroscience Mantero Belard Prize 2015 (Santa Casa da Misericórdia; Ref: MB-1049-2015; FCT, Portugal, PEst-UID/NEU/04539/2013, FEDER-COMPETE.

**Program Number**: 5863 Poster Board Number: B0455

**Presentation Time**: 11:30 AM–1:15 PM

**Multimodal investigation in a pediatric population affected by retinal disorders**

Lucia Ziccardi 1, Daniela Giannini 2, Giuseppe Lombardo 3, sebastiano serrao 4, Paolo Esposito Veneruso 5, Adriano Magli 6, Vincenzo Parisi 7, Matteo Bertelli 8, Marco Lombardo 9

1 Neuroophthalmology Unit, Bietti Eye Foundation IRCCS, Rome, Italy; 2 Fondazione Bietti, Rome, Italy; 3 CNR-IPCF, Messina, Italy; 4 GLMA Eyecare Center, Naples, Italy; 5 Ophthalmology, University of Salerno, Salerno, Italy; 6 MAGI’s Lab, Rovereto, Italy.

**Purpose**: To investigate the functional and microstructural retinal features in a pediatric population with retinal disorders including inherited dystrophies, by using a multimodal instrumental methodological approach.

**Methods**: Thirteen children (mean age 11.23±3.76 years, ranging from 7 to 18 years) with retinal disorders including Best’s macular dystrophy (BMD), retinitis pigmentosa (RP), Stargardt’s disease (STGD), X-linked retinoschisis (XLSR), cone-rod dystrophy (CRD), fovea plana and juvenile macular drusen were recruited in the study. Six children (age 11.2±2.1 years, ranging from 7 to 15 years) were enrolled as controls. All subjects underwent high-resolution retinal imaging using a flood-illumination adaptive optics (AO) retinal camera, combined scanning laser ophthalmoscope (SLO) and spectral domain-optical coherence tomography (SD-OCT). Functional measurements were acquired by testing visual acuity (BCVA), contrast sensitivity function (CSF), Goldmann visual field or automated perimetry, full-field and multifocal electroretinography (mERG).

**Results**: The combined analysis of the cone mosaic and the outer retinal layers in AO and SD-OCT images respectively, provided significant information on the integrity of the photoreceptor mosaic in children. The functional measurements, especially if combined with mERG ring analysis, permitted to detect and establish a diagnosis of a wide spectrum of inherited retinal dystrophies. The clinical diagnosis was confirmed by genetic testing. The AO analysis demonstrated cone loss with respect to controls in STGD, RP and BMD eyes. In the cases with fovea plana and juvenile macular drusen, AO imaging was reliable to detect small entity changes of the cone mosaic. SD-OCT and mERG analysis showed abnormalities of the integrity of the retinal layers in children with XLRS.

**Conclusions**: The multimodal approach for assessing retinal microstructures and function in children affected by degenerative these abstracts are licensed under a Creative Commons Attribution-NonCommercial-No Derivatives 4.0 International License. Go to http://iovs.arvojournals.org/ to access the versions of record.
There was a significant difference in the ERG and pVEP.

Joubert syndrome (JS) is a rare autosomal recessive disease allows detecting pathological retinal changes, at cellular level, with high accuracy.

**Commercial Relationships:** Lucia Ziccardi, None; Daniela Giannini, None; Giuseppe Lombardo, None; sebastiano serrao, None; Paolo Esposito Veneruso, None; Adriano Magli, None; Vincenzo Parisi, None; Matteo Bertelli, None; Marco Lombardo, None

**Program Number:** 5864 Poster Board Number: B0456
**Presentation Time:** 11:30 AM–1:15 PM

**Light-evoked properties of compound action potentials of the optic nerve**
Sarah Davis, Christopher L. Passaglia. Biomedical Engineering, University of South Florida, Tampa, FL.

**Purpose:** The health of the eye is commonly assessed via the electroretinogram (ERG), a noninvasively recorded signal that reflects the summed electrical response of retinal neurons to a light stimulus. The aim of this study was to isolate the subcomponent of the signal produced by action potentials fired by retinal ganglion cells and to characterize how the compound action potential (CAP) waveform depends on the light stimulus.

**Methods:** Long Evans and Brown Norway rats (male, 300-400g, 6-8 months old) were anesthetized with ketamine and xylazine. The optic nerve was exposed via an incision along the supraorbital ridge or a craniotomy at Bregma, and a bipolar electrode was inserted into the dura of the nerve. Electrodes were placed on the cornea to record the ERG and in the posterior skull to record the visual evoked potential (VEP). Animals were placed in darkness for 15 minutes, and a series of full-field LED flashes were then delivered to the eye under dark and light-adapted conditions. The flashes were 10ms in duration, spaced 3s apart, and varied logarithmically in intensity (0.0001 to 1.25 log cd s/m²).

**Results:** The full-field flash CAP recorded from the optic nerve differed noticeably in waveform from the ERG and VEP, consisting of several positive waves that decreased in latency, increased in amplitude, and increased number with stimulus intensity. For the brightest flashes tested, the CAP had 8 ± 2 peaks on average, which correlated in time with oscillatory potentials in the ERG. The mean amplitude and latency of the first peak had a mean latency of 37±7 ms and mean amplitude of 22±11 µV. No change in CAP waveform was detected under dark- and light-adapted conditions.

**Conclusions:** Light-evoked CAPs can be recorded from the optic nerve of rats. The signal has multiple peaks that presumably reflect the synchronous firing of populations of nerve fibers. As such, the light-evoked CAP offers a direct measure of optic nerve health in animal models of ocular disease.

**Commercial Relationships:** Sarah Davis; Christopher L. Passaglia, None
**Support:** R21 EY023376 R01 EY027037

**Program Number:** 5865 Poster Board Number: B0457
**Presentation Time:** 11:30 AM–1:15 PM

**FUNCTIONAL AND GENETIC FINDINGS IN PATIENTS AFFECTED BY JOUBERT SYNDROME: RETINAL DYSTROPHY IS CONNECTED WITH DEFINITE CAUSATIVE GENES**
Caterina Toma1, Giulio Ruberto2, Enza M. Valente3 4, Sabrina Signorini5, Chiara Bertone6, Mauro Antonini7, Paolo Emilio Bianchi8 9. 1Department of Ophthalmology, IRCCS Policlinico S.Matteo, University of Pavia, Pavia, Italy; 2Department of Ophthalmology, IRCCS Policlinico S.Matteo, Pavia, Italy; 3Department of Medicine and Surgery, Section of Neurosciences, University of Salerno, Salerno, Italy; 4Neurogenetics Unit, IRCCS Santa Lucia Foundation, Rome, Italy; 5Unit of Child Neurology and Psychiatry, Centre of Child Neuro-ophthalmology, C. Mondino National Neurological Institute, Pavia, Italy.

**Purpose:** Joubert syndrome (JS) is a rare autosomal recessive or X-linked non-progressive congenital ataxia, characterized by a peculiar malformation of the brainstem and cerebellar vermis. Over 30 genes are known to cause JS, all involved in the structure or functioning of the primary cilium. Visual system anomalies and oculomotor system defects are relatively frequent in JS, especially in association with specific gene defects, such as AHI1 and CEP290. Aim of the present study was to assess the ability of electrotetrogrammetry (ERG) and patterned visual evoked potentials (pVEP) to detect functional retinal and visual pathway anomalies in JS patients with or without retinal dystrophy.

**Methods:** In this retrospective study, 44 patients who met diagnostic criteria for JS were included. Every patient underwent a complete neuro-ophthalmological evaluation as well as repeated ERG and pVEP recordings. Genetic testing was performed in 40 patients by means of a next-generation-sequencing based targeted resequencing of 120 ciliopathy-related genes, including all known JS genes. We compared quantitative and qualitative data of patients with (n=25) and without (n=19) retinal dystrophy, and attempted to correlate ophthalmologic data with genetic results. A control ERG and pVEP normative group present in our database was compared with the JS patients.

**Results:** There was a significant difference in the ERG and pVEP recordings between patients with retinal dystrophy and patients without retinal dystrophy. The analysis for repeated ERG and pVEP measures demonstrated a reduction in the “a” and “b” waves amplitudes and in pVEP amplitudes in JS retinal dystrophic subjects respect the non dystrophic. Pathogenic biallelic mutations in a known JS gene were identified in 30 (75%) pts, including 12 out of 22 (55%) without and all 18 patients (100%) with retinal dystrophy. Interestingly, we did not find any genetic overlap among the two groups: retinal dystrophy was associated to mutations in AHI1, CEP290, INPP5E, OFD1 and TMEM67, while patients without retinal dystrophy carried mutations in C5Orf42, CC2D2A, RPRGRPL1, KIF7, NPHP1, TCTN1 and TMEM237 genes.

**Conclusions:** ERG and pVEP provide useful functional information about retinal involvement characteristics and visual pathways in subjects affected by Joubert syndrome.

**Commercial Relationships:** Caterina Toma, None; Giulio Ruberto, None; Enza M. Valente, None; Sabrina Signorini, None; Chiara Bertone, None; Mauro Antonini, None; Paolo Emilio Bianchi, None
**Program Number:** 5866 Poster Board Number: B0458  
**Presentation Time:** 11:30 AM–1:15 PM  
**Novel Method to Assess the Function of Neurons and Glia Reveals Signal Transmission Potentiation during Rod Degeneration in Retinitis Pigmentosa Model**  
**Frans Vinberg,** Ophthalmology and Visual Sciences, Washington University in St. Louis, St Louis, MO.

**Purpose:** Eye diseases or external stress such as excessive light exposure can cause global changes in the function and structure of various retinal neurons and glia. Quantitative determination of the complex pathophysiological events in intact retinal networks is challenging. Here I develop a novel method to study the functional state of distinct cell types in healthy and diseased retina affected by autosomal dominant P23H mutation by using *Ex Vivo* Electretoretinography.

**Methods:** *Ex vivo* ERG responses to flashes of light were recorded from intact WT and Rho<sup>WT/P23H</sup> (P23H) mouse retina explants. ON bipolar cell (R<sub>p</sub>) and Müller cell (R<sub>m</sub>) components were isolated by subtracting light responses recorded after blocking these components by DL-AP4 and Barium, respectively, from those recorded before application of the blockers. Photoreceptor responses (a wave) were isolated in the presence of both DL-AP4 and Barium. The functional state of the rods, bipolar cells and Müller cells were analyzed by plotting the respective components as a function of flash intensity, rod response amplitude (R<sub>p</sub>) and the area under the pharmacologically isolated a-waves (A), respectively.

**Results:** The maximal R<sub>p</sub> was 400 µV in control mice and decreased to 250 µV in 1 mo and to 140 µV in 3 mo P23H mice while the flash intensity producing half-maximal response did not change in P23H mutants. Surprisingly, bipolar cell response did not decline in 1 mo P23H mice. Moreover, the photoreceptor input needed for the half-maximal bipolar cell response (R<sub>p</sub><sub>1/2</sub>) was actually decreased >2-fold in 1 mo P23H mice showing the increased sensitivity of rod – bipolar cell signaling at the onset of rod degeneration. At 3 mo P23H mice bipolar cell response declined but the R<sub>p</sub><sub>1/2</sub> remained significantly smaller as compared to WT mice. Müller cell responses behaved linearly as a function of A with a steeper slope up to the range where photoreceptors started to saturate and shallower slope beyond that.

**Conclusions:** A new method was used to reveal a potentiation of rod-bipolar cell signaling in response to slow rod photoreceptor degeneration in the P23H mice. In the future I will use this method to determine the functional state of rod/cone-bipolar cell signaling and Müller cells in various models of retinal disease including retinitis pigmentosa and diabetic retinopathy.

**Commercial Relationships:** Frans Vinberg, None  
**Support:** NIH Grant K99EY026651

---

**Program Number:** 5867 Poster Board Number: B0459  
**Presentation Time:** 11:30 AM–1:15 PM  
**A pro-diabetic diet triggers early functional and structural changes in the rat retina**  
**Elisa Vidal,<sup>1</sup> Elise Lalarme,<sup>1</sup> Laurence Decoq<sup>1</sup>, Marie-Annick Maire<sup>2</sup>, Jeaninne Lherminier<sup>4</sup>, Magalie Thierry<sup>2</sup>, Alain M. Bron<sup>2</sup>, Catherine P. Creuzot Garcher<sup>1</sup>, Niyazi Acar<sup>1</sup>, Lionel Bretillon<sup>1</sup>,  
**University of Burgundy Franche-Comte, Eye & Nutrition Research Group, Dijon, France;  
**1Horus Pharma laboratories, Saint Laurent du Var, France;  
**2Animalerie Experimentale, CSGA, UMR1324 INRA, 6265 CNRS, University of Burgundy Franche-Comte, Dijon, France;  
**3Dimacell – Cell Imaging Platform Cell Imaging Platform, INRA, University of Burgundy Franche-Comte, Dijon, France;  
**4Department of Ophthalmology, University Hospital, Dijon, France.**

**Purpose:** Diabetic retinopathy (DR) is the leading cause of blindness in industrial countries before the age of 50 years. The early consequences of diabetes on the retina are nevertheless poorly known. We therefore aimed at characterizing the early effects of a high fructose and high fat diet on the function and structure of the rat retina.

**Methods:** Male Brown Norway rats (6 weeks of age) were fed for 8 days (n=16), 4 weeks (n=16) and 12 weeks (n=8) with 60% fructose+10% lipid rich diet (HFHF), or a standard chow (n=8). At each time point, intraperitoneal insulin tolerance test (ITT-0.5 U/ml) and intraperitoneal glucose tolerance test (GTT-2g/kg body weight) were carried out. Blood was collected to measure insulin during GTT. Flicker (8Hz), scotopic and photopic single flash electroretinograms (ERG) were recorded from both eyes. At the time of euthanasia, blood was collected to measure glycemia, plasma circulating cytokine levels. Rats were enucleated and the ocular globes were processed for electron microscopy in Epon resin in 86nm-thick sections after counterstaining with uranyl acetate and lead citrate.

**Results:** At the three time points, HFHF diet increased fasting glycemia (+20% for 8 days, +12% for 4 weeks, +6.5% for 12 weeks) as compared to standard chow diet (p<0.01). Moreover, HFHF feeding induced a significant increase in plasma glucose in ITT (p<0.05) and in GTT (p<0.05), with an elevated insulin response at 12 weeks compared to control group. Our data highlighted a partial loss of cone sensitivity to light in rats fed for 4 weeks with HFHF as revealed by 8Hz Flicker ERG (A=0.5 log(I)). However, no significant effect of HFHF was reported on scotopic and photopic single flash ERG. Finally, structural changes were observed, such as deposition of amorphous material between Bruch’s membrane and choriocapillaris in rats fed for 12 weeks with HFHF.

**Conclusions:** The consumption of high fructose and high fat diet triggered deregulation of glucose metabolism, loss of cone sensitivity and ultrastructural changes in the retina. These findings are consistent with epidemiological data reporting color vision impairment at early stages of diabetes type 2 in human retina, and lipid deposits in Bruch’s membrane at early stages of DR.

**Commercial Relationships:** Elisa Vidal, Horus Pharma Laboratories (E); Elise Lalarme, None; Laurence Decoq, None; Marie-Annick Maire, None; Jeaninne Lherminier, None; Magalie Thierry, Horus Pharma Laboratories (E); Alain M. Bron, None; Catherine P. Creuzot Garcher, None; Niyazi Acar, None; Lionel Bretillon, None  
**Support:** Horus Pharma Laboratories; Regional Council of Burgundy France (PARI Agral 1) and FEDER (European Funding for Regional Economical Development)

---

These abstracts are licensed under a Creative Commons Attribution-NonCommercial-No Derivatives 4.0 International License. Go to [http://iovs.arvojournals.org/](http://iovs.arvojournals.org/) to access the versions of record.
Methods: Pregnant female Himalayan mice (OCA-1B) were treated daily with 8 mg/kg oral nitisinone (n=7) or vehicle (n=7) beginning at day 10 of pregnancy. Treatment continued after delivery and weaned pups additional month. Visual function was evaluated using Direct coupled electoretinogram (DC-ERG), Optokinetic response (OKR) and Optomotor response (OMR). Standard and DC-ERGs were performed on mice anesthetized with intraperitoneal injection of ketamine/xylazine. OMR was assessed by measuring reflexive head movements of unrestrained mice tracking a rotating virtual cylinder displaying a sine wave grating. OKR was performed on head fixed mice and eye movement recorded using an infrared reflective mirror, while displaying stimuli similar to the OMR. For retinal development; FACS/Rhodopsin test was used to determine cell population in the retina and specifically the rod population and Alexa Fluor Conjugated Cholera toxin B injection was used to show distribution of ganglion cell projections to the brain.

Results: Results: Treated Himalayan mice showed increase in fur and eye melanin pigment. While a- and b- waves of treated mice were close to wild type (WT) mice, the DC-ERG showed c-wave amplitude for untreated mice and WT was approx. 0.7 mV and 1.0 mV respectively. Intriguingly, this difference could be reversed by nitisinone treatment as the treated mice had close to 1.0 mV. The OMR/OKR showed that vision did not improve. The FACS/rhodopsin showed that there was 3-5 % increase in rods. All brain targets were correctly innervated with comparable numbers of ipsilateral and contralateral ganglion cell axons in the treated and untreated mice.

Conclusions: Conclusions: Prenatal treatment of Himalayan mice with nitisinone results in measurable changes in ERG responses and rod numbers of mice, but did not lead to a demonstrable change in visual behavior or axonal routing. These data will help in future studies in understanding the mechanism of RPE-mediated retinal development and visual function.

Commercial Relationships: Ighovie F. Onojafe, None; Friedrich Kretschmer, None; Kiyoharu Miyagishima, None; Congxiao Zhang, None; Haohua Qian, None; Tudor C. Badea, None; Brian P. Brooks, None

Program Number: 5869 Poster Board Number: B0461
Presentation Time: 11:30 AM–1:15 PM
Visual Motor Response of a Transgenic Retinitis Pigmentosa Zebrafish Model
Logan Ganzen1, 2, Chi Pui Pang1, Mingzhi Zhang1, Motokazu Tsujikawa1, Yik Fai Leung2, 3
1 Purdue University Interdisciplinary Life Sciences Program, Purdue University, West Lafayette, IN; 2Department of Biological Sciences, Purdue University, West Lafayette, IN; 3Department of Ophthalmology and Visual Sciences, Chinese University of Hong Kong, Kowloon, Hong Kong; 4Joint Shantou Eye Center, Shantou University & the Chinese University of Hong Kong, Shantou, China; 5Department of Ophthalmology, Osaka University Graduate School of Medicine, Osaka, Japan; 6Purdue Institute for Integrative Neuroscience, Purdue University, West Lafayette, IN.

Purpose: Retinitis Pigmentosa (RP) affects approximately 1 in 4000 individuals globally, and there are currently no effective treatment options available. To identify new drugs, we optimized a visual-behaviour assay, termed visual-motor response (VMR) (Emran et al., 2008), around a transgenic zebrafish carrying a truncated human rhodopsin transgene (Tg(rho:Rsa.RHI_Q344X)). This line also carried a Tg(-3.7rhom:EGFP) reporter for rod visualization. The Q344X larvae experiences significant rod degeneration by 7 days post-fertilization (dpf) (Nakao et al., 2012).

Methods: To assess the vision of the Q344X zebrafish, the VMR assay was run under a dim-light condition based on recorded rod b-waves in larval fish (Moyano et al., 2013) and the minimum cone activation threshold in mice (Cachafeiro et al., 2010). Specifically, Q344X and control larvae at 7dpf were placed into a 96-well plate and acclimated to a dim-light source (1.80±0.03 cm²) for 1 hour. The VMR was tracked and quantified during light offset. The total distance travelled was averaged and analyzed at one second post-stimulus. Retinas were dissected from Q344X and control larvae and whole-mounted to validate the rod degeneration in the Q344X model.

Results: We found that the Q344X larvae displayed an attenuated VMR (0.121 ± 0.041 cm²) to the control larvae (0.2751 ± 0.038 cm²). (Two-Sample T-test; p-value=4.619e-14, N=19). Analysis of whole-mounted retinae indicated significant rod degeneration at 7dpf compared with controls (Control: 87 rods/retina, Q344X: 9.3 rods/retina, Welch’s Two-Sample T-test p-value=1.4e-4). It is unlikely that the cones of the zebrafish contributed to this VMR since the light intensity of the assay was below the cone detection threshold of mice. As the only apparent difference between the two groups of larvae is significant rod degeneration, it can be concluded that the behavioral phenotype was a result of the degeneration.

Conclusions: These results suggest that the attenuated Q344X VMR is a result of the rod degeneration. This behavioral phenotype can be utilized to screen chemical libraries to identify compounds that ameliorate the rod degeneration. Compounds that prevent degeneration are expected to result in a significant increase in VMR in response to the dim-light stimulus.

Commercial Relationships: Logan Ganzen, None; Chi Pui Pang, None; Mingzhi Zhang, None; Motokazu Tsujikawa, None; Yik Fai Leung, None

Support: NIH Grants TL1 TR001107 and UL1 TR001108

Program Number: 5870 Poster Board Number: B0462
Presentation Time: 11:30 AM–1:15 PM
Taurine deficiency induces retinal inflammation
Wahiba Hadj Said1, É Dubus1, Stéphane Fouquet1, Sauleine Sanglier2, Diego Garcia-Ayuso2, Maria P. Villegas-Perez2, Jose A. Sahel1, 3, Serge A. Picaud1, 3
1 Sorbonne Universités, UPMC Univ Paris 06, INSERM, CNRS, Institut de la Vision, Paris, France; 2Departamento de Oftalmología, Facultad de Medicina, Universidad de Murcia, Murcia, Spain and Instituto Murciano de Investigación Biosanitaria- Hospital Virgen de la Arrixaca (IMIB-Arrixaca), Murcia, Spain; 3CHNO des Quinze-Vingts, DHI Sight Restore, INSERM-DHOS CIC, Paris, France.

Purpose: We recently showed that taurine deficiency induced the selective and concomitant degeneration of cone photoreceptors and retinal ganglion cells (RGC) (Hadj-Said et al., IOVS 2016; 57:4692-4703). We investigate here if the degenerative process may trigger retinal gliosis with microglial activation and their migration into the photoreceptor layers.

Methods: We used the taurine transporter (Tau-T) inhibitor, guanidoethane sulfonate (GES), to induce taurine depletion. Retinas were dissected as wholemounts and immunolabelled for Iba1 (microglia) and PNA (cones). The retinal distribution of microglia was studied using 3D images from confocal microscopy. Retinal inflammation was also examined on retinal sections to define the relationship of microglial cells to apoptotic cells (Tunnel Assay).

Results: In retinal sections, microglial cells were often observed in the outer layer (ONL) in GES-treated mice whereas they were not found in the same layer in non-treated mice. This abnormal distribution of microglial cells in the ONL was also clearly detectable.

These abstracts are licensed under a Creative Commons Attribution-NonCommercial-No Derivatives 4.0 International License. Go to http://iovs.arvojournals.org/ to access the versions of record.
on retinal flatmounts. This flatmount strategy enabled us to generate a systematic counting approach to quantify the drastic ongoing inflammation so that we can then assess therapeutic treatments. Furthermore, these observations provide a more integrated examination of the whole microglial populations and its relationship to blood vessel or degenerating cone photoreceptors. The complete cell morphology is also better appreciated showing the cell budding, which is another evidence of the microglial activation.

**Conclusions:** This study demonstrates that taurine depletion causes microglial activation and migration and establishes a relation of cone loss and microglial migration to the ONL of retina. These results question if the retinal inflammation is consecutive to the photoreceptor degeneration from GES-treated mice.

**Commercial Relationships:** Wahiba Hadj Said; Élisabeth Dubus; None; Stéphane Fouquet; None; Sauleine Sanglier; None; Diego García-Ayuso; None; Maria P. Villegas-Perez; None; Jose A. Sahel; None; Serge A. Picaud; None

**Program Number:** 5871 **Poster Board Number:** B0463 **Presentation Time:** 11:30 AM–1:15 PM

**Nonlinear Mixed Effects Modeling of Electroretinography (ERG) b-wave Latency for Whole Eye Transplantation**

Richard A. Bilionick 1,2, Valeria L. Fu 1, Lin He 3, 4, Chiaki Komatsu 4, Maxine R. Miller 1, Ian Rosner 1, Wendy Chen 1, Jila Noorikolouri 1, 4, Kia M. Washington 3, 4. Ophthalmology, University of Pittsburgh School of Medicine, Pittsburgh, PA; Biostatistics, University of Pittsburgh Graduate School of Public Health, Pittsburgh, PA; Children’s Hospital of Pittsburgh, Pittsburgh, PA; Plastic Surgery, University of Pittsburgh School of Medicine, Pittsburgh, PA; Plastic, Aesthetic and Craniofacial Surgery, First Affiliated Hospital of Xi’an Jiaotong University, Xi’an, China; Veterans Administration Pittsburgh Healthcare System, Pittsburgh, PA.

**Purpose:**

The goal was to estimate the difference in ERG b-wave latency for transplanted eyes vs the fellow/naive eye.

**Methods:**

6 rats were studied. Right eyes were transplanted. At 12 week timepoint, rats were dark-adapted in a dark box overnight. Animals were anesthetized and were prepared for ERG recording in a light-sealed room. Recording electrodes gently contacted the corneal surface of eyes. A subdermal needle electrode served as common reference while another subdermal needle electrode was inserted at the base of the left leg. A Ganzfield delivered light stimuli with various stimulus strengths following different programmed dark adaption and light adaption protocols. Log b-wave latency responses were sigmoidal, so a nonlinear mixed effects model was used. R environment for statistical computing was used. 4 parameter logistic function was used to fit the b-wave log latency as a function of log light intensity with parameters: 1) A – asymptote as light intensity goes to -infinity, 2) B - asymptote as light intensity goes to +infinity, 3) $X_{\text{mid}}$ – inflection point for light intensity, and 4) $S$ – scale = difference between $X_{\text{mid}}$ and light intensity where response is 75% of distance from A to B asymptotes. Larger S = shallower slope. Fixed effects were A, B, $X_{\text{mid}}$ and S for the left/naive eye and their differences with right eyes. Random effects (RE) were included for rats for $X_{\text{mid}}$ ($\sigma_{\text{mid}}$) and for eyes for B ($\sigma_{\text{eye}}$). Fixed effects (FE) describe typical effect while REs describe rat/eye-specific effects.

**Results:**

There were no consistent detectable OD ERG responses for rat 1. Model results shown in Table 1. Left half of table shows FE estimates in log space. Where possible, FE values were converted to original scale and shown on right half of table. Bottom part of table shows RE estimates and residual standard error. Compared to left/naive eyes, sigmoidal curve for transplanted right eyes had 1) similar asymptote A, 2) lower asymptote B, 3) higher inflection point $X_{\text{mid}}$, and 4) lower scale S. Difference in inflection points was statistically significant (0.0810, P<0.0001). FE + RE are shown in Figure 1.

**Conclusions:** The b-wave latency showed clear sigmoidal patterns for transplanted and left eyes. For transplanted/right eyes, however, the decrease in latency occurred later and bottomed out at a lower level than for the naive/left eye. Rate of decrease was greater for the transplanted/right eyes.

<table>
<thead>
<tr>
<th>Log Scale</th>
<th>Original Scale</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lower Estimate</td>
<td>Upper</td>
</tr>
<tr>
<td>Lower Estimate</td>
<td>Upper</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Fixed Effects</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>$A_{\text{OD}}$</td>
<td>5.0315</td>
</tr>
<tr>
<td>$A_{\text{OD}}$</td>
<td>4.5519</td>
</tr>
<tr>
<td>$A_{\text{OD}}$</td>
<td>-0.0689</td>
</tr>
<tr>
<td>$B_{\text{OD}}$</td>
<td>3.8605</td>
</tr>
<tr>
<td>$B_{\text{OD}}$</td>
<td>3.0894</td>
</tr>
<tr>
<td>$B_{\text{OD}}$</td>
<td>-0.3742</td>
</tr>
<tr>
<td>$X_{\text{mid}}$</td>
<td>-5.1663</td>
</tr>
<tr>
<td>$X_{\text{mid}}$</td>
<td>-2.9549</td>
</tr>
<tr>
<td>$X_{\text{mid}}$</td>
<td>1.5641</td>
</tr>
<tr>
<td>$X_{\text{mid}}$</td>
<td>0.9014</td>
</tr>
<tr>
<td>$X_{\text{mid}}$</td>
<td>0.7565</td>
</tr>
<tr>
<td>$S_{\text{OD}-\text{OD}}$</td>
<td>-0.6287</td>
</tr>
<tr>
<td>$S_{\text{OD}-\text{OD}}$</td>
<td>-3.3517</td>
</tr>
<tr>
<td>$X_{\text{mid}}$</td>
<td>-1.4103</td>
</tr>
</tbody>
</table>

Random Effects

$\sigma$ | 0.0636 | 0.0709 | 0.0792 |

Residual Error

$\text{FE}$ = 0.1221 | 0.2772 | 0.2640

**Table 1:** Parameter estimates from four-parameter logistic nonlinear mixed effects model for b-wave latency (ms) as a function of light intensity (cd/m$^2$).

**Figure 1:** b-wave latency (ms) as a function of light intensity (cd/m$^2$) for each eye and rat. OD eyes were transplanted. Solid sigmoidal curves denote the estimated fixed effect and dashed sigmoidal curves denote the rat/eye specific prediction (fixed effect + random effects). Horizontal solid lines denote the asymptote A (upper) and B (lower). Vertical solid lines denote the inflection point $X_{\text{mid}}$ (left) and the inflection point plus scale parameter B + S (right).

**Commercial Relationships:** Richard A. Bilionick, None; Valeria L. Fu, None; Lin He, None; Chiaki Komatsu, None; Maxine R. Miller, None; Ian Rosner, None; Wendy Chen, None; Jila Noorikolouri, None; Kia M. Washington

**Support:** Office of the Assistant Secretary of Defense for Health Affairs under Award No. W81XWH- 14-1-0421, VA Pittsburgh Healthcare Administration; Eye and Ear Foundation (Pittsburgh, Pennsylvania); and Research to Prevent Blindness (New York, New York)

These abstracts are licensed under a Creative Commons Attribution-NonCommercial-No Derivatives 4.0 International License. Go to http://iovs.arvojournals.org/ to access the versions of record.
Development of an acoustic-driven model of traumatic brain injury (TBI) in order to measure changes in the zebrafish retina and central visual pathways
Salvatore L. Stella¹, Dillon McDevitt¹, Lance Sommer², Yunsung Kim², Mark Stahl³, ¹Neural and Behavioral Sciences, Penn State University College of Medicine, Hershey, PA; ²Neurology, Penn State University College of Medicine, Hershey, PA.

**Purpose:** The retina serves as a window into the brain and this conduit is comprised of retinal ganglion cell axons, which combine to form the optic nerve and central visual pathways. Vision loss following head trauma, affects 20-40% of all people who suffer brain injuries. The causes of post-traumatic vision loss are varied and include direct ocular, nerve, or brain injury, but the mechanisms behind this neurodegeneration are unknown. Animal models are needed that can recapitulate the pathological cascade of human TBI. The aim of this study is to develop a novel vertebrate model of TBI in zebrafish in which multiple biological readouts can be evaluated.

**Methods:** TBI was elicited in zebrafish following anesthesia with MS-222, and placed in a mesh inside a tank above an underwater speaker. An acoustic mass was delivered in the form of sound pressure via the speaker to the zebrafish. Visual behavior to light and swimming reflexes were analyzed in both larvae and adults using a Zebrabox. Structural changes were mapped with a pan neuronal marker (elav) driven by GFP or with immunohistochemical detection of phosphorylated ERK as a readout of neural activity in the retina and brain. Western blots and immunohistochemistry were used to measure chronic traumatic encéphalopathy (e.g., tau, TDP-43) to quantify changes in protein levels in the retina and brain.

**Results:** We found a significant decrease in locomotion (p < 0.05) and mean velocity (p < 0.05) following TBI treatment in both adults and larvae. GFP labeling of axons visualized damage to both visual and non-visual targets in the zebrafish brain. p-ERK labeling showed a loss of neuronal activity in TBI treated zebrafish. Our results also indicate that significant changes occur in proteins related to chronic traumatic encéphalopathy in both the retina and the brain (tau and TDP-43).

**Conclusions:** Taken together, this novel form of acoustic generated TBI in zebrafish has the potential to be applied to both larval and adult zebrafish as a viable model that can recapitulate TBI damage in humans. This approach provides a stepping stone and tool for deciphering the long term visual changes that occur in both the retina and the brain following TBI.

**Commercial Relationships:** Salvatore L. Stella, None; Dillon McDevitt, None; Lance Sommer, None; Yunsung Kim, None; Mark Stahl, None

**Support:** H. G. Barsumian, M.D. Memorial Fund and George L. Laverty Foundation.
The pattern electroretinogram (pERG) response reflects, for comparison. Using amplitudes and implicit times of P and N waveform components, a cluster analysis was performed to evaluate sensitivity of each test.

**Results:** Sensitivity and specificity were 66.7% and 75.0%, respectively, for conventional pERG, and were both 100% for ppERG. The distance-from-healthy-mean measure used in the cluster analysis was nearly the same for normal and patient groups for the pERG test (mean +/- SD = 7.1+/-.2.3 vs. 7.7+/-.2.9), but provided strong separation for the ppERG test (6.7+/-.1.9 vs. 27.2+/-.11.0). Proof of concept for sector stimulation in healthy eyes was demonstrated.

**Conclusions:** High-luminance peripheral-field pattern stimulation shows promise as a test with high sensitivity to glaucoma, with possible advantage for early-stage disease. Sector stimulation may exploit the asymmetric damage associated with early glaucoma, and better correlate with retinal nerve fiber layer thickness measurements at the optic disk.
**Purpose:** The Goto-Kakizaki (GK) rat is a spontaneously occurring, polygenic, non-obese model of Type II diabetes that develops impaired insulin secretion at 2 weeks and fasting hyperglycemia at 4 weeks. Previously, we showed that retinal deficits in the GK rat appeared by 4 weeks and preceded cognitive and exploratory behavior deficits. Here, we followed GK and Wistar rats longitudinally for 8 months to assess long term changes in retinal and cerebral function and retinal vasculature.

**Methods:** In male and female GK rats and Wistar (W) controls, glucose and insulin tolerance tests (hyperglycemia and insulin resistance) were performed at 1, 2, and 8 months of age. Electoretinogram (ERG, retinal function) and Y-maze (spatial alternation for cognitive function and number of entries for exploratory behavior) were performed monthly from 1-8 months. Retinas from rats euthanized at 8 months were assessed for vascular pathology.

**Results:** GK rats exhibited significant glucose intolerance and insulin resistance beginning at 1 month of age (p < 0.001 for both) and persisting to 8 months (p < 0.001 for both). GK rats showed significant increases in a-wave and b-wave amplitudes across all time points (p < 0.001 for both) and flicker amplitudes beginning at 5 months (p < 0.05), as well as significant delays in flicker implicit time beginning at 1 month (p < 0.01). GK rats also showed significant deficits in spatial alternation (p < 0.01) and exploratory behavior (p < 0.001) beginning at 6 and 2 months, respectively. Retinas from 8-month-old GK rats did not show any increase in the number of degenerate (acellar) retinal capillaries compared to that in nondiabetic controls.

**Conclusions:** GK rats exhibited retinal function deficits by 1 month of age, with cerebral function deficits appearing later, raising the question of associative pathology in human subjects. Vascular pathology was not observed in the GK rat even by 8 months. Future analysis will include optical coherence tomography (OCT, retinal structure), functional hyperemia (retinal vascular function), and assessment of vascular pathology at 12 months. The time course of retinal deficits in Type II diabetes suggests that early retinal screening in human subjects using appropriate methods, such as ERG, may facilitate clinical interventions before patients develop symptomatic DR and cognitive deficits.

**Commercial Relationships:** Rachael S. Allen, None; Andrew Feola, None; Cara T. Motz, None; Amy Ottensmeyer, None; Peter M. Thulle, None; Timothy S. Kern, None; Machelle T. Pardue, None

**Support:** This material is based upon work supported by the Department of Veterans Affairs (Rehabilitation R&D Service Merit Award (E0951-R) and Research Career Scientist Award (C9257) to MTP), Research to Prevent Blindness (Emory), NIH NEI P30EY06360 (Emory), and Foundation Fighting Blindness.

---

**Program Number:** 5877

**Poster Board Number:** B0469

**Presentation Time:** 11:30 AM–1:15 PM

**Ion Channel Properties of Cells Expressing Two Different Types of Channelrhodopsin Genes**

Hiroshi Tomita1,2, Eriko Sugano1, Kitako Tabata1, Yoshito Watanebe1, Taku Ozaki1, Makoto Tamai1

1Chemistry and Biological Sciences, Iwate University, Morioka, Japan; 2Clinical Research, Innovation and Education Center, Tohoku University Hospital, Sendai, Japan.

**Purpose:** Optogenetic technologies are expected to be applicable for clinical use in restoring vision. However, the degree of recovered visual function is highly dependent on the function of the chosen optogenetic gene. To investigate the effect on ion channel properties of cells expressing two different types of channelrhodopsin genes, we established a cell line expressing both a modified Volvox-derived channelrhodopsin gene (mVChR1) and channelrhodopsin-2 (ChR2).

**Methods:** An expression plasmid including ChR2 gene (pChR2-IRESPuro) was electroporated into cultured HEK293 cells. Following the establishment of HEK-Chr2 cell line, the linearized pAAV-mVChR1V vector was electroporated into stable transfectant HEK-Chr2 cells. Venus-positive cells were sorted using a cell sorter as cells stably expressing the ChR2 and mVChR1 genes (HEK-Chr2+mVChR1). Photocurrents of these cell lines were recorded under whole-cell patch clamping of isolated cells. To investigate the effect on visual function of dual expression of genes with different wavelength sensitivities, we transduced a mVChR1 gene via an adenov-associated virus vector into transgenic rats harbouring the Chr2 gene in retinal ganglion cells. The native photoreceptor cells were degenerated by an intraperitoneal injection of N-methyl-N-nitrosourea prior to transduction of mVChR1.

**Results:** The results of patch clamp recordings indicated that increased photocurrent mediated by dual gene expression was observed in wavelength ranges such as 550 and 600 nm, in which Chr2 did not exhibit effective functioning. In the Chr2 transgenic...
rats, visually evoked potentials were clearly detectable in spite of native photoreceptor function abolishment; however the responses were limited to within blue wavelengths. In contrast, the limited wavelength sensitivities were improved by the additional transduction of mVChR1, which exhibited sensitivities to green and red. 

**Conclusions:** The transductions of dual genes encoding channelrhodopsins that exhibit different wavelength sensitivities represents a promising candidate method to expand and to enhance rescued wavelength sensitivities in blind subjects.

**Commercial Relationships:** Hiroshi Tomita; Eriko Sugano, US8754048 (P); Kitako Tabata, None; Yoshito Watanabe, None; Taku Ozaki, None; Makoto Tamai, None

Support: AMED Grant No.15lm0103007j0004, JSPS Grant Nos. 16H05485, 16K15729 and 16K11314

---

**Program Number:** 5879 Poster Board Number: B0471

**Presentation Time:** 11:30 AM–1:15 PM

**The Influence of Dark and Light Adaptation on Phosphenе Thresholds Elicited with DTL Electrodes**

renan R. memória, Santana José Galdino Souza, Rodrigo Jorge, Andre Messias. Ophthalmology, FMRP-USP, Ribeirão Preto, Brazil.

**Purpose:** To investigate the influence of dark, and light adaptation using 4 different chromatic backgrounds (white; blue; red and amber), on phosphenе thresholds (PT) elicited with DTL electrodes.

**Methods:** PT were psychophysically determined using a staircase method in 27 healthy subjects after 30 min dark adaptation, and after 5 min adaptation to 4 different full-field light backgrounds: red, amber, blue and white (in this order, all at 10 photopic cd/m²). DTL electrodes were used, and stimuli were generated (20 Hz, square waves) using an electric stimulation system (Okuvision - Germany). DTL electrodes were used, and stimuli were generated (20 Hz, square waves) using an electric stimulation system (Okuvision - Germany). ColorDome – Diagnosys LLC). DTL electrodes were used, and stimuli were generated (20 Hz, square waves) using an electric stimulation system (Okuvision - Germany). ColorDome – Diagnosys LLC). DTL electrodes were used, and stimuli were generated (20 Hz, square waves) using an electric stimulation system (Okuvision - Germany). ColorDome – Diagnosys LLC). DTL electrodes were used, and stimuli were generated (20 Hz, square waves) using an electric stimulation system (Okuvision - Germany). ColorDome – Diagnosys LLC). DTL electrodes were used, and stimuli were generated (20 Hz, square waves) using an electric stimulation system (Okuvision - Germany). ColorDome – Diagnosys LLC). DTL electrodes were used, and stimuli were generated (20 Hz, square waves) using an electric stimulation system (Okuvision - Germany). ColorDome – Diagnosys LLC). DTL electrodes were used, and stimuli were generated (20 Hz, square waves) using an electric stimulation system (Okuvision - Germany). ColorDome – Diagnosys LLC).

**Results:** Mean PT found after dark-adaptation was statistically significant higher (P < 0.05) than after light-adaptation, no significant differences were found between red, amber and blue, but PT after white background adaptation was significantly lower than with the other colors. The mean PT in the dark was 316.9 ± 8.0 μA; 185.4 ± 8.1 μA; and 170.7 ± 5.8 μA for red, amber, blue and white background respectively (Fig. 1).

**Conclusions:** These results suggest that the human retina requires higher electrical current to elicit visualization of phosphenes when adapted to darkness, and also, that light adaptation with a white background appear to reduce PT more than red, amber or blue backgrounds.

---

These abstracts are licensed under a Creative Commons Attribution-NonCommercial-No Derivatives 4.0 International License. Go to http:// iovs.arvojournals.org/ to access the versions of record.
of 250 ± 9.6 μV (n = 8) was abolished in Kir7.1 shRNA injected mice.

Conclusions: Changes in subretinal space K+ concentration in light is restored to normal levels by RPE Kir7.1 channels. Knocking down Kcnj13 in the RPE using shRNA results in loss of Kir7.1 transcript and a severely reduced ERG in mice. Absence of Na+3 response further confirmed the loss of functional RPE cells. In both SVD and LCA, a loss of Kir7.1 channel function occurs, and as with the loss of Kcnj13 expression in our model, directly affects ERG outcome which can serve to non-invasively measure therapeutic results.

Commercial Relationships: Bikash R. Pattnaik, None; Pawan K. Shahi, None; Bryce Aul, None; Akshita Pattnaik, None; Yu Chang, None; De-Ann M. Pillers, None

Support: NH grant EY24995

Program Number: 5881 Poster Board Number: B0473
Presentation Time: 11:30 AM–1:15 PM

Microperimetry as a Screening Test for Hydroxychloroquine Toxicity
Naheed W. Khan, Husam Alghanem, Leslie M. Niziol, David Musch, Thiran Jayasundera. Ophthalmology and Visual Science, Kellogg Eye Center, University of Michigan, Ann Arbor, MI.

Purpose: Compare retinal function measured using multifocal electroretinogram (mfERG) with microperimetry (MP) to investigate the utility of MP as a surrogate screening test for hydroxychloroquine (HCQ) retinopathy.

Methods: Retinal function tests were compared between 25 patients referred for evaluation of HCQ retinopathy after ≥ 5 years of HCQ use and 42 normal controls. The 3 innermost central rings of mfERG and MP responses at approximately equivalent distances from the fovea were compared (mfERG - R1:0°-1°, R2:1°-4°, R3:4°-8°) and (MP - MR1: 1°, MR2: 3°, MR3: 5°). mfERG ring ratios were calculated as the average response density of R1, R2 or R3 divided by the mean of R5. Average retinal sensitivity of each MP ring was calculated. Pearson correlations (r) were used to test for a linear association between mfERG and MP measures in HCQ patients and t-tests were used to compare mfERG and MP measures between HCQ patients and normal controls. Gold standard HCQ toxicity was defined as an mfERG ring ratio >2 standard deviations (SD) below the mean observed in the normal sample. A similar calculation was used to define toxicity with MP measures. Sensitivity and specificity to detect HCQ toxicity with MP measures compared to mfERG were calculated, including 95% Wilson confidence intervals (CI).

Results: 46 eyes of 25 HCQ patients and 52 eyes of 42 normal controls were evaluated. HCQ cases were on average 56.6 years old (SD=10.7). They were treated on average for 11.1 years (SD=6.3), with a mean exposure to HCQ of 1440 g (SD=860). mfERG R2 and R3 ring ratios were positively correlated with their corresponding MP ring averages (both r=0.55, P<0.001). mfERG R2 and R3 ratios of HCQ cases were significantly lower than controls (R2: P=0.0105, R3: P=0.0003). All three MP rings for HCQ patients were significantly lower than those of controls (P<0.0001). 2 HCQ patients (4%) were categorized as having toxicity from mfERG R1 ratio, 6 (13%) from R2 ratio, 13 (28%) from R3 ratio, and 14 (30%) if detected by any ring ratio. Sensitivity of MP to detect HCQ toxicity compared to mfERG was 100% (CI:34-100%) for MR1, 100% (CI:61-100%) for MR2, 85% (CI:58-96%) for MR3, and 86% (CI:60-96%) over all rings. Specificity was 61%, 55%, 55%, and 53%, respectively.

Conclusions: MP shows high sensitivity to detect HCQ toxicity when compared to mfERG. However, the low specificity suggests that MP use will classify many healthy individuals as having HCQ toxicity.

These abstracts are licensed under a Creative Commons Attribution-NonCommercial-No Derivatives 4.0 International License. Go to http:// iovs.arvojournals.org/ to access the versions of record.
**Program Number:** B0475
**Presentation Time:** 11:30 AM–1:15 PM
**Visual acuity and optical flow in primate retinal ganglion cells treated with an optogenetic vision restoration strategy using ChrimsonR**

Himanshu Akolkar1, Gregory Gauvain1, Romain Caplette2, Deniz Dalkara1, Celine Jaillard3, Jose A. Sahel4, Didier Pruneau4, Serge A. Picaud1, Ryad Benosman1.


**Purpose:** Visual restoration has been shown to be a success in blind mice following photoreceptor degeneration by expressing microbial opsins in retinal ganglion cells (RGCs). Investigating the clinical translation of this optogenetic therapy, we recently showed that the AAV2.7m8–ChrimsonR-tdTomato viral construct can induce significant response in peri-foveal retinal ganglion cells of non-human primates. We have further investigated the performance of this optogenetic vision restoration strategy in terms of visual acuity of the retinal ganglion cells.

**Methods:** We performed ex-vivo multi-electrode array recordings from the peri-foveal retina of non-human primates under a high resolution light stimulation setup using digital micromirror display (DMD). RGCs were stimulated with bars of different sizes (25um and 50um) moving at 4 different orientations (0, 45 90, 135 w.r.t the DMD). We then compared cells responses to these moving bar stimuli in order to determine the minimum length of bar required for the cells to respond and estimate the direction of the bars. We further stimulated the retinal ganglion cells with different letters (E, X, T, P) and shapes (Circle and Square) of different sizes (55, 110, 227, 340.5um) moving over the retina at different angles at a speed of 11 degree/sec.

**Results:** We used a spatio-temporal event-based optical flow algorithm (already published) to compute the flow from the recorded spiking activity in the RGC population during moving bar stimuli to estimate the orientation, speed and direction of the motion of the bar. Individual RGCs could detect motion of small bars (25um) moving at a speed of 2.27mm/sec (equivalent to 11 deg/sec). The RGC population could estimate the orientation of the bar using algorithm upon an accuracy of 86.76 ± 2.05% (n=3). For letters and shapes, we computed the ability of the RGC population to discriminate stimuli of different sizes. Using information theory analysis, we found that the RGC population could discriminate letters and shapes of greater than 110 um on the retina, which is above the limit of legal blindness.

**Conclusions:** These results provide an estimate of the visual performance possible in blind subjects that will undergo optogenetic vision restoration therapy with ChrimsonR-TdTomato. They suggest our optogenetic strategy could restore a visual ability above legal blindness.

**Commercial Relationships:** Himanshu Akolkar, None; Gregory Gauvain, None; Romain Caplette, None; Deniz Dalkara, Gensight Biologics (C), Gensight Biologics (P); Celine Jaillard, None; Jose A. Sahel, Pixium (P), Pixium (I), Gensight Biologics (F), Gensight Biologics (C), Chronocam (I), Chronocam (P), Pixium (C), Chronocam (C), Gensight Biologics (P), Gensight Biologics (I); Didier Pruneau, Chronocam (I), Gensight Biologics (I), Gensight Biologics (E); Serge A. Picaud, Gensight Biologics (F), Gensight Biologics (C), Chronocam (I), Pixium (C); Ryad Benosman, Chronocam (C), Gensight Biologics (C), Gensight Biologics (I), Pixium (C)

---

**Program Number:** B0476
**Presentation Time:** 11:30 AM–1:15 PM
**A neuroprotective effect of HIF inhibitor topotecan in a murine model of retinal ganglion cell degeneration**

Hiromitsu Kunimi1,2, Yukhiro Miwa1, Yusaku Katada1,2, Kazuo Tsubota1, Toshiohide Kurishara1,2.

1Department of Ophthalmology, Keio University School of Medicine, Tokyo, Japan; 2Laboratory of Photobiology, Keio University School of Medicine, Tokyo, Japan.

**Purpose:** Therapeutic opportunities against the retinal ganglion cell (RGC) degeneration such as glaucoma are limited to lowering intraocular pressure to date. Recent studies show that hypoxia-inducible factor (HIF) plays roles including neurodegeneration in retinal diseases. We hypothesize that a HIF inhibitor topotecan may have a neuroprotective effect to the retinal ganglion cell degeneration, and examined the effect electrophysiologically using a murine N-methyl-D-aspartic acid (NMDA)-induced RGC degeneration model.

**Methods:** Intraperitoneal injections of vehicle or topotecan (0.625mg/kg/day) in 8 week-old C57/B6J were performed for 4 weeks (Control group; n=5, Topotecan group; n=6, respectively). Flash visual evoked potentials (VEPs) were measured on the last day of the drug injection for each group. On the same day, all mice were injected NMDA intravitreally (10mM in 1ul) in bilateral eyes. The effect of topotecan was evaluated by comparing changes of VEP amplitudes 24-hours after NMDA injections.

**Results:** The VEP amplitudes (P1-N1) showed no significant difference (p=0.50) between the groups after 4 week drug injections (Control group; 127±35μV, Topotecan group; 117±32μV, respectively). Topotecan group showed a significantly (p<0.01) lower decrease in amplitudes (31±24μV) compared to control group (59±24μV) whereas both groups exhibited amplitude decreases after NMDA injections.

**Conclusions:** An administration of topotecan significantly suppressed a decrease of VEP amplitudes induced by intravitreal NMDA injections in mice. These results suggested that HIF inhibition may have therapeutic effects against RGC degeneration.

**Commercial Relationships:** Hiromitsu Kunimi, None; Yukhiro Miwa, None; Yusaku Katada, None; Kazuo Tsubota, None; Toshiohide Kurishara, None

---

**Program Number:** B0477
**Presentation Time:** 11:30 AM–1:15 PM
**Characteristics of patients with post-geniculate pathology and homonymous ganglion cell layer thinning**

Morgan Godin, Mays El-Dairi. Ophthalmology, Duke University, Durham, NC.

**Purpose:** Homonymous ganglion cell layer (GCL) thinning has been reported in homonymous hemianopia caused by post geniculate pathology and is presumed due to retrograde trans-synaptic degeneration. We observed that this finding is not universal. In this study we looked at patient variables that are associated with this finding.

**Methods:** A retrospective review was performed on all patients seen at Duke from 1/1/2010 to 12/1/2015. Relevant patients were identified by the ICD code for homonymous hemianopsia then matches were screened for patients who had macular SD-OCT (Heidelberg) and HVF 24-2 SITA Fast protocol on the same date. Patients were also required to have appropriate neuro-imaging to identify geniculate vs. pre-geniculate pathology. Data collected included: age, sex, pathology, location of pathology (occipital, temporal, and/or parietal and thalamic involvement vs. not), lesion type (ischemic vs. non-ischemic), homonymous GCL thinning on
OCT vs. not, OCT timing after insult (<2 years or ≥ 2 years), center sparing on HVF vs. not. Chi square analyses of patients with and without homonymous GCL thinning were performed in order to determine whether significant differences in the aforementioned characteristics existed between these groups. Excluded were eyes with ocular pathology that might affect GCL or RNFL. All MRI scans and segmented macular OCT were reviewed in a masked fashion. **Results:** Included were 32 subjects, age 41.88 ± 27.3 years. Homonymous thinning of GCL was correlated with pathology older than 2 years ($p = 0.03$). Homonymous thinning was not statistically dependent on age at diagnosis ($p = 0.64$), lesion location (occipital/temporal/parietal, $p = 0.36, 0.30, 0.96$), thalamic involvement ($p = 0.12$), or lesion type (ischemic vs. non-ischemic, $p = 0.40$). Homonymous thinning was independent of difference in average RNFL between eyes ($p = 0.84$). There was also no association between homonymous thinning on OCT and center sparing on HVF ($p = 0.89$).

**Conclusions:** Post-geniculate pathology associated with development of homonymous GCL thinning was mostly dependent on the duration of lesion. Further prospective studies with serial follow-up might help elucidate the development of this finding.

**Program Number:** 5886  **Poster Board Number:** B0478  **Presentation Time:** 11:30 AM–1:15 PM  **Initial Proof-of-Concept of Photoacoustic Neural Stimulation; A Potential Approach to Retinal Stimulation: Preliminary In Vitro Study**

**Purpose:** There is prior work to provide “virtual vision” for blind patients via electrical or ultrasonic stimulation. Here we propose initial proof-of-concept for a novel photoacoustic neuromodulation (PANM) approach. This *in vitro* study, indicate predicted neural responses that suggest a potentially promising approach for eventual stimulation of retinal neurons in blind patients.

**Methods:** For this preliminary proof-of-concept study, the experimental procedures consists of three steps: 1) localized excitation using a pulsed laser delivery to an absorptive material placed under the plate containing neurons, 2) cell stimulation by photoacoustic pressure, and 3) fluorescence quantification of the resulting change in membrane potential. A plate harboring hippocampal neuronal cells was situated above the absorptive media which generates the photoacoustic pressure by the laser excitation using 1064-nm fiber-coupled laser with 1mJ energy per pulse (redENERGY G4-20W-Z type, SPI Lasers UK Ltd., United Kingdom). The membrane potential change of neurons loaded with a fast-acting membrane potential sensitive fluorescent dye (FLIPR) was monitored over 300 seconds. The PANM was performed for 5 seconds at the 60-second time point with at varying pulse repetition rates, i.e., 1kHz and 2kHz. Identically treated sham animals received no PANM. The membrane potential change was normalized at 10 seconds in each case.

**Results:** The sham and PANM groups had comparable membrane potentials at the 60-second time point (prior to PANM), i.e., 1.07 ± 0.02 and 1.05 ± 0.02, respectively. However, at 300 seconds, the membrane potential change in PANM treated groups showed a statistically significant and dose dependent increase in depolarization; PANM treated groups at 1 kHz and 2 kHz PRF respectively, equal 1.3 ± 0.05 and 1.53 ± 0.44 as compared to shams at 1.21 ± 0.06. While there is baseline drift in the sham the change between PANM treated groups was significant.

**Conclusions:** Based on these preliminary findings, we are investigating the potential for photoacoustic retinal stimulation as a potential strategy to achieve virtual vision in blind patients.