Young Researcher Vision Camp

An international Career building Symposium

2019

Castle Wildenstein
Leibertingen
Germany

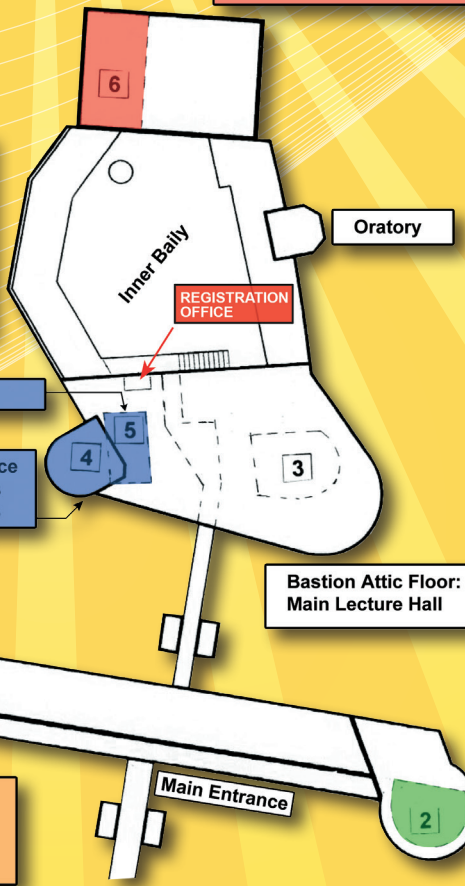
www.vision-camp.eu

Jugendherberge
88637 Burg Wildenstein

Manor House
Upper Floor: 30/31/33/34/35
Attic Floor: 38/39/40

Lounges:

1 West Tower
2 East Tower
3 Alleyway Bastion: Casemate
4 Commander's Office: Former Chapel
5 Bastion: Common Hall
6 Manor House



Bastion: 49

Commander's Office
Upper Floor: 52/53
Attic Floor: 54/55

West Tower Entrance at the right
Ground Floor: 84/85
Upper Floor: 96/97/98/100

Bastion Attic Floor:
Main Lecture Hall

West Tower Entrance at the left
Basement: 77/78
Upper Floor: 89/90/91
Attic Floor: 93/94

East Tower
1st Upper Floor: 63/64/65/66/67/68/69
2nd Upper Floor: 73/74
Attic Floor: 75/76

Contact:

Jugendherberge Burg Wildenstein
88637 Leibertingen-Wildenstein
Tel: +49 7466-411
Fax: +49 7466-417
E-Mail: info@jugendherberge-burg-wildenstein.de

www.leibertingen-wildenstein.jugendherberge-bw.de

Preamble

Dear Colleagues,
Dear Participants of the Young Researcher Vision Camp 2019,

The aim of this camp is to give young investigators (MSc and PhD students, young MDs and post-docs) an opportunity to present themselves and their work to fellow researchers, to allow them to build and strengthen personal networks in an international environment.

Take the time for professional and social networking
Take the time for new views on career paths to shape your future
Take the time to bridge the gap between basic and clinical research
Take the time to revive a medieval castle

ENJOY THE YOUNG RESEARCHER VISION CAMP

Thomas Wheeler-Schilling
on behalf of the organising committee (in alphabetical order)
Sigrid Diether
Norbert Kinkl
Alexander Leube
Arne Ohlendorf
Francois Paquet-Durand
Vera Schmid
Timm Schubert

FRIDAY, JULY 12TH, 2019

Each scientific session consists of 4 talks a 10min; total 60 min per session; including introduction (up to 3 min) and discussion

until latest 16:00	Arrival & Registration (for details see 'How to get there')
16:25 - 16:30	Welcome
16:30 - 17:00	**KEYNOTE LECTURE I** **"EMERGING THERAPIES FOR INHERITED RETINAL DYSTROPHIES"** Hendrik Scholl, IOB, Basel, Switzerland
17:00 - 18:00	**THE MYFUN-SESSION** **SESSION 1 – "MYFUN AND MYOPIA RESEARCH"** Chair: Arne Ohlendorf • Vahid Pourreza Ghoushchi "Does myopia affect accommodation" • Miguel Garcia "Peripheral 2D image quality metrics" • Pablo Sanz Diez "Accommodative response following contrast adaptation" • Andrea Carrillo Aleman "Why might reading make myopic"
18:00 - 19:00	**SESSION 2 – "IMAGING THE EYE"** Chair: Christina Schwarz • Rowena Schultz, Universitätsklinikum Jena, Jena, Germany "As time goes by – fluorescence lifetime imaging ophthalmoscopy of the ocular fundus" • Niklas Domdei, University Hospital Bonn, Germany "Photoreceptor-targeted psychophysics with adaptive optics scanning laser ophthalmoscopy" • Juan Mompeán Esteban, Laboratorio de Óptica, Universidad de Murcia, Murcia, Spain "High Performance Computing for OCT images of cataractous eyes" • Clara Pfäffle, Institute of Biomedical Optics, University of Lübeck, Germany "Optophysiology in the living human eye"
19:00 - 19:30	**KEYNOTE LECTURE II** **"SIGNAL PROCESSING IN THE RETINA"** Jeffrey Diamond, NIH, Bethesda, USA
19:45 - open end	Open-air Barbecue
21:00 – 22:00	[Optional] Evening Tour with Wild Life Ranger in the Valley Danube (separate registration at desk necessary) [Optional] Evening Tour through the Medival Castle and its hidden Secrets (separate registration at desk necessary)

SATURDAY, JULY 13TH, 2019

Each scientific session consists of 4 talks a 10min; total 60 min per session; including introduction (up to 3 min) and discussion

06:00 - 07:00	Early morning exercises
07:00 - 08:00	Breakfast
08:00 - 09:00	**SESSION 3 – "THE RPE AND ITS ROLE IN VISION"** Chair: Helen May-Simera • Daniela Intartaglia, Telethon Institute of Genetics and Medicine, Pozzuoli, Italy "The light-responsive miR-211 regulates RPE cell clearance via Ezrin targeting" • Elora Vanoni, Vision Institute, INSERM, Paris, France tba • Sandra Schneider, Johannes Gutenberg University, Mainz, Germany "Possible role of epithelial-to-mesenchymal transition underlying retinal pigment epithelium phenotype in cilia mutant mice" • Monica Fernandes Freitas, University College London, London, UK "Rescuing photoreceptors and RPE function in Bardet-Biedl Syndrome"
09:00 - 10:00	**THE TRANSMED SESSION** **SESSION 4 – "CELL DEATH MECHANISM IN RETINAL DEGENERATION"** Chair: Francois Paquet-Durand • Giuditta Dal Cortivo, University of Verona (Italy) "Effects of missense mutations in the GUCA1A gene associated with cone-rod dystrophy and perturbation of second messenger homeostasis in photoreceptors" • Arianna Tolone, University of Tübingen (Germany) "New treatments for hereditary photoreceptor degeneration: Testing cyclic nucleotide analogues on organotypic retinal explant cultures" • Michel Rasmussen, Ophthalmology, Lund University (Sweden) "Potential novel cGMP binding partners in mouse retina may be relevant in photoreceptor degeneration" • Soumaya Belhadj, University of Tübingen (Germany) "Molecular imaging of neurodegenerative processes in the retina"
10:00 - 10:30	Coffee Break
10:30 - 11:30	**THE SWITCHBOARD SESSION** **SESSION 5 – "RETINAL CIRCUITS IN THE HEALTHY AND DISEASED RETINA"** Chair: Timm Schubert • Klaudia Szatko, Institute for Ophthalmic Research/CIN, University of Tübingen "Chromatic processing in mouse retinal ganglion cells" • Maj-Britt Hölzel, Netherlands Institute for Neuroscience, Amsterdam "A retinal origin of congenital nystagmus" • Sabrina Duda, Department of Neuroscience, University of Oldenburg "Alpha ganglion cells of the guinea pig retina" • Marili Korympidou, Institute for Ophthalmic Research/CIN, University of Tübingen "Disentangling the computation of direction selectivity in starburst amacrine cell dendrites"

11:30 - 12:30	**SESSION 6 – "PHOTORECEPTOR DEGENERATION AND THERAPEUTIC APPROACHES"** Chair: Jerome Roger • Pasquale Pensieri, Institute of Biology of Valrose (iBV), Nice (France) "Role of Otx2 transcription factor in the adult retina photoreceptors." • Najata Ait-Ali: (Institut de la vision) "The RdCVF metabolic signaling and its origin." • Diana Garcia-Garcia: Neuro-PSI (Orsay) "Retinal regeneration and inflammation: role of YAP in Muller cells-Microglia interplay." • Cardillia-Joe Simon: Institut de la Vision (Paris) "Gene therapy to reactivate the cones during retinal degeneration ."
12: 30 - 14:00	Lunch
	EDUCATIONAL SESSIONS (25 min input; 20 min discussion)
14:00 - 14:45	Educational Sessions 1 - **"On the importance of clinical trials"** Lecturer: **Prof. Heiko von der Leyen,** Hannover Clinical Trial Centre, Hannover, Germany
14:45 - 15:30	Educational Sessions 2 - **"Artificial Intelligence in Ophthalmology"** Lecturer: **Dr. Abouzar Eslami** Head of Translational Research Lab – Carl Zeiss Meditec AG
15:30-16:00	Coffee Break
16:00 - 16:45	Educational Sessions 3 - **"How to build your own company?"** Lecturer: **Prof. Nils Högsdahl** Dean of the HdM Stuttgart
16:45 - 17:45	**ZEISS SESSION** **SESSION 7: "APPLICATION OF VIRTUAL REALITY FOR OPHTHALMOLOGY AND VISION SCIENCE"** Chair: Alexander Leube • Sophia Tatiyosyan, Institute for Ophthalmic Research – Eberhard Karls University Tübingen "Virtual diagnosis for low vision patients: An optokinetic nystagmus based contrast sensitivity test in virtual reality" • Efe Bozkir, Perception Engineering - Eberhard Karls University Tübingen "Safer Driving Experience and Cognitive Load Assessment in Risky Situations via Virtual Reality" • Niklas Stein, Institute for Psychology – University of Münster "Distorted VR - Simulating progressive lenses in virtual reality" • Alexander Leube, Practical show case: "Simulation of cataract and central scotoma using virtual reality for educational purpose"
17:45 - 18:30	**KEYNOTE LECTURE III:** **"CATCHING SCIENCE IN LANGUAGE: A SHORT INTRODUCTION TO PATENT LAW"** Dr. Stijn van Dongen, NLO, Amsterdam, The Netherlands
18:35 - 18:45	Group Photo
19:00 - open end	Poster Session
19:30 - open end	Buffet in the inner bailey

SUNDAY, JULY 14TH, 2019

Each session consists of 4 talks a 10min; total 60 min per session; including introduction (up to 3 min) and discussion

7:00 - 8:00	Early morning exercises
7:30 - 9:00	Breakfast
9:00 - 10:00	**SESSION 8 – "POST-TRANSLATIONAL MODIFICATION AND EPIGENETIC SIGNALING IN THE RETINA"** Chair: Martial Mbefo • Atta Ur Rehman, University of Lausanne (Switzerland) "Homozygosity mapping and mutation identification in consanguineous Pakistani families with inherited retinal degenerations" • Rebecca Ward, University College Dublin (Ireland) "Genetic and Pharmacological analysis of Inherited Retinal Degeneration Genes in Zebrafish" • Daria Fresia, Jules-Gonin Eye Hospital, University of Lausanne (Lausanne) "Role of metabolic memory and epigenetic modifications in the pathogenesis of diabetic retinopathy" • Jiaming Zhou, Ophthalmology, Lund University (Sweden) "Proteins controlled by cGMP-dependent protein kinase (PKG) in normal and degenerating retinas"
10:00 - 11:00	**SESSION 9 – "COMPUTATIONAL TOOLS TO UNDERSTAND THE RETINA AND ITS PROCESSES"** Chair: Daniele Dell'Orco • Charlotte Beelen, University of Oldenburg (Germany) – "Simulating single photon responses in rods: a comprehensive modeling approach" • Daniel Zeymer, University of Tübingen (Germany) – "Quantitative analysis of the retinal vascular structure" • Valerio Marino, University of Verona (Italy) - "Molecular dynamics simulations to unveil physiological and pathological mechanisms in vision" • Akane Yamashita, Aichi Prefectural University, Nagakute (Japan) "A simulation analysis of the photoresponse of rod photoreceptors
11:00 - 11:30	Coffee Break
11:30 - 12:30	**SESSION 10 – "YOUNG DOG @ VISION CAMP"** Chair: Sven Schnichels • Yanhong Hou, Department of Ophthalmology, University of Cologne, Germany "Corneal UV-light crosslinking promotes high-risk corneal graft survival by regressing mature pathologic corneal lymphatic and blood vessels". • Bettina Hohberger, Department of Ophthalmology, University of Erlangen-Nuernberg "Agonistic β2-adrenergic receptor autoantibodies influence microcirculation in glaucoma patients". • Chantal Dysli, Department of Ophthalmology, Inselspital, University Hospital Bern "Fluorescence lifetime imaging ophthalmoscopy (FLIO): from molecular dimensions to macular diagnosis". • Carsten Balser, Laboratory for Experimental Immunology of the Eye, Department of Ophthalmology, University of Cologne "Co-inhibition of PGF and VEGF blocks their expression in mononuclear phagocytes and limits neovascularization and leakage in the murine retina".
11:00 - 12:00	ZEISS Poster Awards Poster Awards & Talks of the Awardees
12:00 - 13:30	Farewell Lunch (optional)
From 13:00	Shuttle Bus to the train station in Beuron (optional)


Young Researcher Vision Camp
An international Career building Symposium
The Power to Shape The Future in Vision Research and Ophthalmology
WWW.VISION-CAMP.EU

Content

Aït-Ali, Najate 14
The RdCVF metabolic signaling and its origin

Avesani, Anna 15
Biochemical and biophysical characterization of human GCAP2

Beelen, Charlotte J. 16
Mechanisms underlying light adaptation in rods investigated with comprehensive modeling

Beelen, Charlotte J. 17
Simulating single photon responses in rods: a comprehensive modeling approach

Belhadj, Soumaya 18
Towards the development of new biomarkers for retinal neurodegeneration

Bozkir, Efe 19
Safer Driving Experience and Cognitive Load Assessment in Risky Situations via Virtual Reality

Breher, Katharina 20
A metrological approach to the analysis of choroidal thickness by OCT in the context of myopia research

Carrillo Aleman, Andrea 21
Why might reading make myopic?

Chen, Yiyi 22
Studies into the energy metabolism of the retina

Christensen, Gustav 23
Cationic and anionic PEGylated liposomes for delivery of cGMP-based drugs to photoreceptor cells

Dal Cortivo, Giuditta 24
Effects of missense mutations in the GUCA1A gene associated with cone-rod dystrophy and perturbation of second messenger homeostasis in photoreceptors

Das, Soumyaparna 25
The effect of Ca2+ channel blockers on Ca2+ level and calpain activity

De Angeli, Pietro 26
Cas9-based strategies to rescue of deep-intronic mutations in ABCA4 gene

Domdei, Niklas 27
Photoreceptor-targeted psychophysics with adaptive optics scanning laser ophthalmoscopy

Duda, Sabrina 28
Alpha ganglion cells of the guinea pig retina

Gantes Nuñez, Francisco Javier 29
Personalized eye models to predict quality of vision in Age-Related Macular Disease (AMD)

García García, Miguel 30
Peripheral 2D image quality metrics

Garcia-Garcia, Diana 31
Retinal regeneration and inflammation: role of YAP in Müller cells-Microglia interplay

Gehlen, Jana 32
Pharmacological manipulation of oscillations in the retina of the retinitis pigmentosa mouse model rd10 improves efficiency of electrical stimulation

Giamundo, Giuliana 33
Retinal degeneration in MPSIII-A knockout (-/-) mice.

Hölzel, Maj-Britt 34
A retinal origin of nystagmus in nyx-/- mice

Ingensiep, Claudia 35
Establishment of a hypoxia model for the MEA-based analysis of the electrical activity in murine retinae

Intartaglia, Daniela 36
The light-responsive miR-211 regulates retinal cell clearance by modulating lysosomal biogenesis via Ezrin targeting

Jenisch, Peter 37
Inosine-5'-monophosphate dehydrogenase 1 (IMPDH1) as a novel target for the treatment of Retinitis Pigmentosa

Karpińska, Anna 38
Optimisation and up-scaling of nanoparticles for drug delivery system.

Kieninger, Sinja 39
Gene-editing based elimination of intronic mutations as a therapeutic approach for OPA1-linked optic neuropathies

Korympidou, Maria Magdalini 40
Chromatic processing at the mouse outer retina

Kutluer, Meltem 41
INVESTIGATION OF A NEW IN VITRO SYSTEM TO STUDY ON ROD PHOTORECEPTOR CELLS

Li, Huang 42
Phosphodiesterase 6 inhibition induces cell death of rod photoreceptor-like cells differentiated from 661W

Lorenzo-Soler, Laura 43
Angiotensin Receptor Blockers in Cyclodextrin Nanoparticle Eye Drops: Ocular Pharmacokinetics and Pharmacologic Effect on Intraocular Pressure

Lovas, Sándor 44
Quantification of Cell Death in Long-Term Organotypic Culture of the Adult Human Retina

Magda, Daniel 45
Temporal Changes of Microglial Phenotype in long-term Organotypic Culture of the Human Retina

Marino, Valerio 46
Molecular dynamics simulations to unveil physiological and pathological mechanisms in vision

Mihaylova, Veselina 47
Effects of blockade of ionotropic GABA receptors on the oscillatory potentials in the ON- and OFF-response of the frog electroretinogram

Mompeán, Juan 48
High Performance Computing for OCT images of cataractous eyes

Pawliczek, Daniel 49
Retinal dysplasia and cortical cataracts in neonatal mice exposed to ionizing radiation reduce visual acuity

Pensieri, Pasquale 50
Role of Otx2 transcription factor in the adult retina photoreceptors

Pfäffle, Clara 51
Optophysiology in the living human eye

Pourreza Ghoushchi, Vahid 52
Is myopia affecting accommodation?

Rasmussen, Michel 53
Potential novel cGMP binding partners in mouse retina may be relevant in photoreceptor degeneration

Rodríguez Bocanegra, Eduardo 54
Potential innate immune responses to adeno-associated virus mediated gene therapy

Roy, Akanksha 55
Towards assessment of phospho signaling in retinal tissues in vitro and in vivo by kinase activity profiling

Sanchez-Cruz, Alonso 56
PRESERVATION OF RETINAL SYNAPSIS AND VISUAL FUNCTION WITH HUMAN PROINSULIN IN THE rd10 MOUSE MODEL OF RETINITIS PIGMENTOSA.

Sanz Diez, Pablo 57
Accommodative response following contrast adaptation.

Scheel, Constanze 58
The Role of Tet3 in the Retina

Schneider, Sandra 59
Ablation of primary cilia exclusively in the RPE leads to a pathogenic phenotype and consequent retinal degeneration

Schneider, Sandra 60
Possible role of epithelial-to-mesenchymal transition underlying retinal pigment epithelium phenotype in cilia mutant mice

SIMON, Cardillia 61
Gene therapy to increase and maintain light sensitivity in

Solaki, Maria 62
Functional assessment of CNGA3 variants using an aequorin-based bioassay

Stein, Niklas 63
Distorted VR - Simulating progressive lenses in virtual reality

Szatko, Klaudia 64
Chromatic processing in mouse retinal ganglion cells

Tolone, Arianna 65
New treatments for hereditary photoreceptor degeneration: Testing cyclic nucleotide analogues on organotypic retinal explant cultures

von der Leyen, Heiko 66
On the importance of clinical trials

Wagner, Johanna 67
Preclinical evaluation of rAAV.CNGB1 in the Cngb1 knockout mouse model of retinitis pigmentosa.

Wagner, Sandra 68
Does close work affect the ciliary muscle morphology?

Wolfram, Lasse 69
The impact of substrate stiffness on the expression of miRNAs in retinal pigment epithelial cells

Xiao, Ting 70
Exploration of AON-based uORF Blockage to Counteract OPA1 Haploinsufficiency

Yamashita, Akane 71
A simulation analysis of the photoresponses of rod photoreceptors.

Yiyi, Chen 72
Studies into the energy metabolism of the retina

Zamboni, Davide 73
Rhodopsin Kinase (GRK1) affects the affinity for Ca2+ of Recoverin

Zhou, Jiaming 74
Proteins controlled by cGMP-dependent protein kinase G (PKG) in normal and degenerating retinas

Zobel, Lena 75
In vivo characterization of AAV capsids for gene therapeutic usage.


Young Researcher
Vision Camp
WWW.VISION-RESEARCH.EU
YOUTH HOSTELLING
INTERNATIONAL
DJH
JUGENDHERBERGE

The RdCVF metabolic signaling and its origin

Najate Aït-Ali, Frédéric Blond, Ying Yang, Laurence KLIPFEL, Géraldine Millet-Puel, Emmanuelle Clerin, Thierry Léveillard.

Sorbonne Université, INSERM, CNRS, Institut de la Vision, 17 rue Moreau, F-75012 Paris, France.

Purpose
Retinitis pigmentosa (RP) is characterized by a progression from night blindness due to rod photoreceptor death to the dysfunction of cone photoreceptors that leads to complete blindness. Transplantation of a wild-type photoreceptor layer in the subretinal space of the rd1 mouse decrease the secondary degeneration of cones suggesting that in RP patient the rods death could result in the loss of a secreted factors necessary for cone survival. The protein, rod-derived cone viability factor (RdCVF)

Methods
was identified. RdCVF is a splicing variant of the nucleoredoxin-like-1(NXNL1) gene encoded when the unique intron was retained, whereas the splicing of that intron produces an active thioredoxin enzyme, RdCVFL. In an evolutionary perspective, RdCVFL precedes the truncated thioredoxin RdCVF. 580 million years ago (mya), additionally to RdCVFL, the ancestral NXNL genes that are ubiquitously expressed, produced RdCVF with no function. 400 mya, rods emerged from cones, the expression of NXNL1 became

Results
restricted to the retina and 70 mya mammals emerged with rod dominated retina. Those cells express RdCVF and RdCVFL contrarily to cones that express only RdCVFL. RdCVF secreted by rods interacts with a complex made of BSG1 and the glucose transporter GLUT1 stimulating glucose uptake and aerobic glycolysis. The accidental splicing leading to a truncated thioredoxin as RdCVF would not work without BSG1. In addition, in cone RdCVFL play a redox homeostatic role relying on NADPH produced through the pentose phosphate pathway. So, the activity of RdCVFL depends on the uptake of glucose stimulated by RdCVF thereby, both products of NXNL1 has

Conclusions
complementary activities for protecting the cones. The ancestral NXNL1 gene originally encodes for a thioredoxin and then after for another protein RdCVF boosting the activity of the first one. Both proteins define a superthioredoxin system. 40 mya, cones became concentrated in the fovea, requiring the protection by rods to maintain their visual acuity in primate. Today, RP patients lose central vision resulting from the interruption of the NXNL1 metabolic and redox signaling between rods and cones, so the delivery of a recombinant adeno-associated virus (AAV) vector encoding for RdCVF and RdCVFL will delay the onset of the central blindness.

Statement on proprietary interests

Acknowledgement

Avesani, Anna

Biochemical and biophysical characterization of human GCAP2

Anna Avesani 1, Valerio Marino1,2, Daniele Dell'Orco1

1 Department of Neurosciences, Biomedicine and Movement Sciences, Section of Biological Chemistry, University of Verona, Verona, Italy 2 Department of Translational Research and New Technologies in Medicine and Surgery, University of Pisa, Pisa, Italy

Purpose
Phototransduction is a cascade of biochemical events tuned by [cGMP] and [Ca2+]. Variations in [Ca2+] are sensed by Guanylate Cyclase Activating Proteins (GCAPs), responsible for the activation or the inhibition of the Guanylate Cyclases (GCs) upon Mg2+ or Ca2+ binding, respectively. Therefore, the dysregulation of the Ca2+ and cGMP levels leads to retinal degeneration. To date, 20 point mutations in GCAP1 were found in GCAP1, while one GCAP2 variant seems to be involved in Retinitis Pigmentosa.

Methods
We heterologously expressed GCAP2 in E. coli in and assessed myristoylation efficiency by Matrix-Assisted Laser Desorption/Ionization) mass spectrometry. We investigated hydrodynamic size and aggregation propensity by a combination of Size Exclusion Chromatography and Dynamic Light scattering. We evaluated ion-dependent conformational changes and thermal stability by Circular Dichroism spectroscopy and electrophoretic mobility. Finally, we checked GCAP2 functionality by enzymatic assay.

Results
MALDI analysis confirmed a GCAP2 myristoylation efficiency close to 100%. SEC profiles of mGCAP2 suggested a monomer-dimer equilibrium, at odds with nmGCAP2 whose Mg2+ form is a monomer, while the Ca2+ form is a dimer. DLS showed that the hydrodynamic diameter of mGCAP2 was slightly smaller than nmGCAP2, a Ca2+-dependent size increase and no aggregation propensity. CD spectra suggest that mGCAP2 structure is sensitive to both ions, while nmGCAP2 seems to be sensitive only to Ca2+. GCAP2 showed the typical Ca2+-dependent gel mobility shift upon Ca2+-binding. GCAP2 showed a very low capability to regulate the putative target GC1.

Conclusions
SEC profiles of mGCAP2 highlighted a monomer-dimer equilibrium regardless of the presence of ions. Differently, nmGCAP2 activating form eluted like as monomer, while the inhibiting form is a dimer, at odds with DLS results which suggest that the hydrodynamic diameter is always compatible with a dimer whose size increases upon Ca2+ binding with no time-dependent aggregation. CD experiments showed a Ca2+-dependent conformational change for m/nmGCAP2, which occurred also upon Mg2+-binding for mGCAP2, in line with gel mobility shift assays. Finally, we observed that GCAP2 were not able to regulate their putative target GC1.

Statement on proprietary interests

Acknowledgement

Beelen, Charlotte J.

Mechanisms underlying light adaptation in rods investigated with comprehensive modeling

Charlotte J. Beelen (1), Sabrina Asteriti (2,3), Lorenzo Cangiano (2), Karl-Wilhelm Koch (1), and Daniele Dell'Orco (3)

(1) Biochemistry Group, Department of Neuroscience, Faculty VI, University of Oldenburg (2) Department of Translational Research and New Technologies in Medicine and Surgery, University of Pisa (3) Section of Biological Chemistry, Department of Neurosciences, Biomedicine, and Movement Sciences, University of Verona

Purpose
Rod phototransduction is a well-studied system that displays a vast range of kinetic behavior. When rods are subjected to a background light, the flash response becomes faster. We investigate the underlying mechanism by computationally studying rod phototransduction in a model of phototransduction.

Methods
We use a comprehensive model of the mammalian phototransduction cascade, which explicitly models the relevant molecular species and their interactions, mainly using mass-action kinetics. This allows us to study a vast array of stimuli and predict the photoresponse of genetically altered photoreceptors. We compare the results from simulations of two different light stimulation paradigms to electrophysiological recordings from single rods and investigate possible mechanisms responsible for light adaptation.

Results
When investigating the response of rods to a flash superimposed on light backgrounds, we find that light adaptation leads to a faster response depending on the intensity of the background. The model is able to reproduce this measured effect very well. When we remove the calcium-dependent regulation of Recoverin and its binding to the Rhodopsin kinase in the model, this light adaptation effect disappears.

Conclusions
We conclude that the calcium-dependent regulation of the phosphorylation of Rhodopsin via its kinase being released by Recoverin is essential for light adaptation in the discussed case. When calcium feedback on this mechanism is removed, we see no remaining effect of light adaptation. In our model, the effect of light adaptation in the presence of background light is therefore completely due to this regulation.

Statement on proprietary interests
-

Acknowledgement
Charlotte Beelen wishes to acknowledge funding from the DAAD as well as the DFG-GRK 1885.

Beelen, Charlotte J.

Simulating single photon responses in rods: a comprehensive modeling approach

Charlotte J. Beelen (1), Sabrina Asteriti (2,3), Lorenzo Cangiano (2), Karl-Wilhelm Koch (1), and Daniele Dell'Orco (3)

(1) Biochemistry Group, Department of Neuroscience, Faculty VI, University of Oldenburg (2) Department of Translational Research and New Technologies in Medicine and Surgery, University of Pisa (3) Section of Biological Chemistry, Department of Neurosciences, Biomedicine, and Movement Sciences, University of Verona

Purpose
Rod cells have reproducible single photon responses and therefore operate at the physical measurement limit. It has been shown that single photon responses show a rather small coefficient of variation. Using a computational model of the phototransduction cascade, we investigate the statistical properties of single photon responses and study which mechanisms lead to the observed high reproducibility. Furthermore, we study the effect of mutations on single photon response kinetics and statistics.

Methods
We apply a systems biology approach to simulate single photon responses in different conditions. We use a comprehensive model of the mammalian phototransduction cascade, which explicitly models the molecular species and their interactions. The model has been extensively tested over a vast range of stimulus conditions and mutations. The results from the stochastic simulations are compared to electrophysiological measurements of dim flash responses in mouse rod cells.

Results
When splitting up the model into a stochastic frontend and a deterministic backend, it is possible to simulate single photon responses that are comparable to measurements. Using a scaled-down version of the model, we were able to include new results such as the novel activation stoichiometry of PDE by transducin. The model reproduces known results for single photon responses in different knockout mutations. We are also able to shed light on the relevance of multiple phosphorylation sites of Rhodopsin for the reproducibility of single photon responses.

Conclusions
Using the bottom-up modelling approach has the advantage that many different experimental settings can be evaluated without retuning of any parameters. Thus, we are able to investigate various genetic modifications or stimulus paradigms with robust simulation results. We can investigate the stochastic properties of single photon responses by performing stochastic simulations.

Statement on proprietary interests
-

Acknowledgement
Charlotte Beelen wishes to acknowledge funding from the DAAD as well as the DFG-GRK 1885.

Belhadj, Soumaya

Towards the development of new biomarkers for retinal neurodegeneration

Soumaya Belhadj1,2, Frank Schwede3, Norman Rieger1, François Paquet-Durand1

1 Cell Death Mechanism Laboratory, University of Tübingen, Tübingen, Germany 2 Graduate Training Center of Neuroscience, University of Tübingen, Germany 3 Biolog Life Science Institute, Bremen, Germany

Purpose
Inherited retinal degeneration is a group of diseases characterized by progressive photoreceptor death leading to blindness. These diseases are still untreatable. A key problem for therapy development is the lack of in vivo biomarkers. The aim of the present study is to promote the development of novel biomarkers that can be used both for an early diagnosis of RD and for the rapid assessment of the in vivo efficacy of neuroprotective treatments.

Methods
Molecular probes for the detection of cell death related processes were tested initially on unfixed retinal tissue sections (ex vivo) and afterwards on live organotypic retinal explant cultures (in vitro). The probe development focused on the detection of the activity of poly-ADP-ribose-polymerase (PARP) using a variety of different NAD+ analogues (6-Biotin-17-NAD+, 6-Fluo-10-NAD+, ε-NAD+). Retinal sections were incubated in a PARP reaction mixture with/wo the NAD+ analogues and PARP-activity-dependent fluorescence increases were visualized under the deconvolution microscope.

Results
The NAD+ analogues 6-Biotin-17-NAD+ and 6-Fluo-10-NAD+ revealed large numbers of PARP activity positive cells in sections obtained from the rd1 retinal degeneration model, while in sections derived from wild-type animals the number of PARP activity positive cells was relatively low. The result suggested especially 6-Fluo-10-NAD+ as a candidate probe for the assessment of cell death in vivo. However, when tested on live organotypic retinal explant cultures no PARP activity could be detected with 6-Fluo-10-NAD+.

Conclusions
The compound 6-Fluo-10-NAD+ may be used as a new molecular probe to assess PARP activity in ex vivo retinal tissue sections. However, this probe does not appear to be suitable for in vivo testing since it may not be able to penetrate the membrane of live cells. The further development towards an in vivo probe may require the use of a pro-drug approach or, alternatively, a drug delivery system to enable the NAD+ analogue to reach into photoreceptor cells.

Statement on proprietary interests
The authors declare no conflict of interest

Acknowledgement
This research was funded by a grant from the European Union (transMed; H2020-MSCA-765441).

Bozkir, Efe

Safer Driving Experience and Cognitive Load Assessment in Risky Situations via Virtual Reality

Efe Bozkir, David Geisler, Enkelejda Kasneci

Perception Engineering, University of Tuebingen

Purpose

Traffic accidents which involve pedestrians on urban roads can be fatal. In order to prevent these accidents, gaze-aware warning systems can help to increase driver attention and detect risky pedestrians. However, it is difficult to test such systems or train drivers using these systems in safety critical situations in the wild. Firstly, this work investigates whether a low-cost virtual reality (VR) setup can be used to increase driver attention properly in a safety critical pedestrian crossing in VR. Our analyses show significant differences in the distance to pedestrians, pupil diameters and driver accelerator inputs when the gaze-aware pedestrian cues were provided. Secondly, we used pupillary information along with accelerator and brake inputs of drivers to classify driver cognitive load using multiple classifiers in a person-independent, privacy-preserving and real-time fashion. Results show that our VR setup allows to predict cognitive load at a high accuracy above 80%. Overall, beyond the specific setup and scenario, there is a strong indication that VR setups can be used to study safety critical situations in driving.

Methods

Results

Conclusions

Statement on proprietary interests

Acknowledgement

Breher, Katharina

A metrological approach to the analysis of choroidal thickness by OCT in the context of myopia research

Katharina Breher (1), Arne Ohlendorf (1,2), Siegfried Wahl (1,2)

(1) Institute for Ophthalmic Research, Tübingen, Germany (2) Carl Zeiss Vision International GmbH, Aalen, Germany

Purpose
Studies found the choroid to be able to change its thickness in response to defocus in a bi-directional fashion. However, measuring choroidal thickness from optical coherence tomography (OCT) images can cause difficulties due to a limited visibility of the choroidal-scleral interface. The study evaluated the agreement of a freely available software for automated choroidal segmentation in two spectral-domain OCTs compared to manual segmentation.

Methods
Three consecutive OCT volume scans were performed in 23 healthy subjects using two different spectral-domain OCT (Cirrus HD-OCT 5000, Carl Zeiss Meditec AG, Dublin, CA, USA and HRA+OCT Spectralis, Heidelberg Engineering, Heidelberg, Germany). Subfoveal choroidal thickness was obtained by manual measurements of one naïve (Examiner 1) and one experienced examiner (Examiner 2), additionally to an automated segmentation software developed by Mazzaferri et al. (2017). Volume scans were chosen as scan pattern, as the automated software is able to analyze choroidal thickness in OCT volume scans particularly. Subjects with a difference of ≥100µm in choroidal thickness between segmentation methods were excluded. ICC, 95% Limits of Agreement (LoA) and coefficients of repeatability were calculated.

Results
The repeatability of three measurements was between 34µm and 47µm for different segmentation methods and examiners, whereas automated segmentation yields less repeatability compared to manual segmentation. The mean difference between methods range from 1µm to 11µm, indicating higher thickness values with the Cirrus OCT. 95% LoA were between ±44µm and ±86µm, thus, nearly twice as high for the automated segmentation method compared to manual segmentation.

Conclusions
Repeatability and agreement values exceed previously reported effect sizes of choroidal thickness changes in response to defocus. The obtained metrological results should be considered for the future evaluation and interpretation of changes in choroidal thickness in myopia research.

Statement on proprietary interests
Katharina Breher: None. Arne Ohlendorf and Siegfried Wahl are employed by Carl Zeiss Vision International GmbH, Aalen, Germany and are scientists at the University of Tübingen.

Acknowledgement
Funding was received from Eberhard-Karls-University Tuebingen (ZUK 63) as part of the German Excellence initiative from the Federal Ministry of Education and Research (BMBF).

Carrillo Aleman, Andrea

Why might reading make myopic?

Andrea C Aleman, Min Wang, Frank Schaeffel

Payerstrasse 26

Purpose
Experiments by Crewther and Crewther in chicks (Neuroreport 2002, 2003) as well as studies in knock-out mice with deficient ON or OFF channels have shown strong evidence of a role of ON and OFF pathways in refractive error and myopia development.

Methods
Software was written in Visual C++ for realtime analysis of the ON and OFF contributions in video frames (640x480 pixels). At 285,000 regularly spaced positions, the gray levels of surrounding pixels were subtracted from the gray level of the center pixel. If positive, the position was considered ON stimulating, if negative, OFF stimulating. The analysis was done for different receptive field sizes, making it possible to read out the strength of ON and OFF stimulations in different scenes at different spatial frequencies. Seven young subjects (age 23-29) were tested with stimuli that preferentially stimulated ON or OFF in a larger monitor (65") positioned at 3.2 m distance and subfoveal choroidal thickness was measured with OCT Spectralis (Heidelberg Instruments Inc, Massachusetts, USA) before and after exposure for 30 and 60 minutes. Screen brightness was matched to 35 cd/m^2

Results
(1) Natural scenes were generally balanced; however, black text of white represented a strong overstimulation of OFF pathways while white text on black overstimulated the ON pathways. This was valid for both arabic and chinese letters. (2) After reading black text on white for 60 minutes, the choroid thinned in all subjects (on average of both eyes: -16.1±4.6 µm, $p<0.001$). After reading white text on black background, the opposite happened: the choroid thickened significantly (on average of both eyes: +10.0±6.5 µm, $p=0.01$). (3) After reading for 30 minutes, similar trend was achieved for both conditions of reading, but the effect in choroidal thickness was smaller

Conclusions
It is possible to change choroidal thickness bi-directionally by overstimulating either ON or OFF pathways. Reading black text on white overstimulates OFF, causes choroidal thinning and may induce myopia without defocus. On the contrary, reading white text on black background stimulates ON, causes choroidal thickenning, and may have an inhibitory effect on myopia

Statement on proprietary interests
The authors report no conflicts of interest

Acknowledgement
This work was supported by the European Union's Horizon 2020 research and innovation programme under the Marie Sklodowska-Curie Research training Network MyFun Grant MSCA-ITN-2015-675137

Chen, Yiyi

Studies into the energy metabolism of the retina

Yiyi Chen

Institute for Ophthalmic Research - Tuebingen, Germany | Universitätsklinikum Tübingen - Universität Tübingen

Abstract:
The retina is the most metabolically active tissue in our body and alterations in energy metabolism are often associated with retinal disease. However, the biochemical pathways of retinal energy supply and consumption are still not fully understood. Even further, surprisingly little is known about the retinal expression patterns of the enzymatic systems relevant for neuronal energy metabolism.

In this study, we first analysed the expression pattern of the lactate transporter monocarboxylate transporter 1 (MCT1) and the glucose transporter 1 (GLUT1) in the retina, using immunofluorescence staining. MCT1 and GLUT1 were expressed in retinal pigment epithelium and photoreceptor outer segments, respectively. To confirm the expression of MCT1 functionally, we then used organotypic retinal explant cultures treated with the MCT1 inhibitor AZD3965. These studies showed an increased number of dying cells in the outer nuclear layer (ONL), suggesting that photoreceptors energy metabolism critically depends on the import of lactate.

Future studies will be performed to identify metabolites changes and the metabolic pathways that could be affected both under normal and diseased conditions in mice retinas.

CHRISTENSEN, GUSTAV

Cationic and anionic PEGylated liposomes for delivery of cGMP-based drugs to photoreceptor cells

Gustav Christensen

Cell Death Mechanism Group Institute for Ophthalmic Research University of Tuebingen Elfriede Aulhorn Straße 5-7, 72076 Tuebingen, Germany

Purpose
Retinal degeneration has been linked to elevated levels of cGMP in photoreceptor cells (PRCs). Certain analogues of cGMP have previously been shown to slow down the degeneration. To enhance delivery of analogues to PRCs, they can be encapsulated in liposomes, followed by intravitreal injection. This can reduce clearance of drugs from the vitreous. Biodistribution of liposomes depends on surface properties like charge and hydrophobicity, which can be altered to find the optimal formulation.

Methods
Cationic and anionic liposomes, with or without polyethylene glycol (PEG), were prepared. Liposomes were loaded with cGMP and cGMP analogues. Fluorescently labelled cationic and anionic PEGylated liposomes were incubated with retinal explant cultures from wild-type mice. Biodistribution of anionic PEGylated liposomes was tested in ex vivo porcine eyes. Encapsulated fluorescein was injected into the eye, and the mobility monitored using a Fluorotron Master Ocular Fluorophotometer. The mobility of free fluorescein was used as comparison.

Results
No difference was observed for neutral and positively charged liposomes in the encapsulation efficiency (EE) of cGMP (~25%). For anionic PEGylated liposomes, EE of cGMP reached 80 %, and EE of the cGMP analogue DF004 were ~30 %. Both cationic and anionic PEGylated liposome formulations reached the photoreceptor cell layer in explant cultures, although possible toxicities were observed for the cationic formulation at 100 µg/mL. Biodistribution studies in porcine eyes showed that the liposome encapsulated fluorescein remained in the eye for at least 24h while free fluorescein was almost completely gone after 24h.

Conclusions
The anionic PEGylated liposome formulations investigated showed potential as drug delivery carriers for cGMP based drugs, as these could remain in the vitreous for at least 24h, reach the photoreceptor cell layer in explant cultures, and encapsulate both cGMP and its DF004 analogue with great efficiency. The cationic PEGylated formulation may be less useful as potential toxicities in retinal explant cultures were observed.

Statement on proprietary interests
No conflict of interest

Acknowledgement
The study was supported by the Deutsche Ophthalmologische Gesellschaft and the Baden-Württemberg Stiftung

DAL CORTIVO, GIUDITTA

Effects of missense mutations in the GUCA1A gene associated with cone-rod dystrophy and perturbation of second messenger homeostasis in photoreceptors

Giuditta Dal Cortivo, Valerio Marino, Daniele Dell'Orco

Dep. of Neuroscience Biomedicine and Movement Sciences, University of Verona, Verona, Italy Dep. of Translational Research and of New Surgical and Medical Technologies, University of Pisa, Pisa, Italy.

Purpose
GCAP1 is a Ca2+-sensor protein involved in phototransduction, specifically in the Guanylate Cyclase (GC) regulation. To date, 20 point mutations in GCAP1 encoding gene are known to be associated with Cone and Cone/Rod Dystrophies (COD and CORD), autosomic dominant diseases characterized by progressive central and/or peripheral vision loss. Here we present a biochemical and functional characterization of four mutants directly involved in Ca2+ coordination, namely D100G, E111V and E155A/G.

Methods
A combination of techniques was used to investigate the mutations effects: circular dichroism (CD) provides structural information, analytical SEC allows the investigation of the oligomeric state and dynamic light scattering (DLS) can evaluate aggregation propensity. Ca2+ affinity was assessed combining SDS-PAGE (gel-shift) and a competition assay with chromophoric chelator Br2-BAPTA. GC activity was measured at different Ca2+ concentrations by functional assays.

Results
All the mutants analyzed in this study are correctly folded and change conformation upon ion binding. All the variants are dimers in all tested conditions and do not show time-dependent aggregation, exception made for Mg2+-E111V. Both gel-shift and Ca2+-binding assays highlight a reduced affinity for Ca2+ which reflects into an inability to regulate the target under physiological Ca2+ fluctuations. Finally, the presence of increasing concentrations of WT GCAP1 partially mitigates the effects of the mutations on the Ca2+-dependent regulation of the target GC.

Conclusions
COD/CORD-associated point mutations in GCAP1 affecting residues directly involved in Ca2+-coordination are less sensitive to physiological Ca2+ fluctuations, leading to severe dysregulation of the GC activity and consequently of the downstream processes. However, preliminary biochemical results suggest that high concentration of WT GCAP1 may mitigate the dominant effect of the COD/CORD mutants, thus opening new scenarios for protein-therapies.

Statement on proprietary interests

Acknowledgement
Telethon Foundation

Das, Soumyaparna

The effect of Ca2+ channel blockers on Ca2+ level and calpain activity

Soumyaparna Das (1,3), Michael Power (1-3), Luke Rogerson (2,3), Thomas Euler (2), Francois Paquet-Durand (1)

(1) Institute for Ophthalmic Research, University of Tübingen, Germany (2) Werner Reichardt Centre for Integrative Neuroscience (CIN), University of Tübingen, Germany (3) Graduate Training Centre of Neuroscience, University of Tübingen, Germany

Purpose
Primary degeneration of rods followed by cones occurs in retinitis pigmentosa. In this, high accumulation of cGMP over-activates CNGCs leading to high influx of Ca2+ in the cells. It is believed to be the cause of photoreceptor degeneration by activation of Ca2+-dependent calpain-type proteases. Cyclic nucleotide gated channels (CNGC) are the main source of Ca2+ in the photoreceptor outer segment (OS) and voltage gated calcium channels (VGCC) are a major Ca2+ source in cell body and synapse.

Methods
To explore further, we inhibited Ca2+ channels namely CNGC and VCGG using pharmacological blockers L-cis and D-cis-diltiazem respectively. The effects were studied on organotypic retinal explant cultures using different concentration of the compounds and were analysed using TUNEL assay (25, 50 and 100 µM) and calpain activity assay (100 µM). Furthermore, the acute effects of the compounds on Ca2+ dynamics in cones were tested using two-photon imaging.

Results
We found a significant decrease of calpain positive cell count in rd1 ONL, by 30% (0.91 ± 0.07, n=3) on treatment with L-cis-diltiazem, and a decrease by 34% (0.88 ± 0.07, n=2) on treatment with D-cis-diltiazem. In untreated condition, the calpain positive cells (per 1000 µm2) in the rd1 ONL is around 330% higher (1.18 ± 0.08, n=4), as compared to wt (0.36 ± 0.03, n=4). Ca2+-imaging showed that D-cis-diltiazem did not affect the light induced Ca2+ response in cones, while L-cis-diltiazem suppressed this response in concentration dependent manner.

Conclusions
Our preliminary data suggests that pharmacological block of both VGCC and CNGC can decrease photoreceptor Ca2+ levels and the activity of calpains. Activity of calpains is controlled by cytoplasmic Ca2+ concentration. The differential effect of L-cis- and D-cis diltiazem on light-induced responses indicates that CNGC inhibition reduces Ca2+-levels in photoreceptor OS, while VGCC inhibitor may reduce Ca2+ in the cytoplasm.

Statement on proprietary interests

Acknowledgement
ProRetina foundation, Germany German Research Foundation (DFG)

De Angeli, Pietro

Cas9-based strategies to rescue of deep-intronic mutations in ABCA4 gene

Pietro De Angeli

Institute for Ophthalmic Research, Centre for Ophthalmology, University of Tübingen

Summary

Stargardt disease (STGD) is an autosomal recessive inherited retinal disorder caused by biallelic mutations in the ABCA4 gene. It is one of the most common autosomal recessive retinal dystrophies with an estimated incidence of one in 10,000. To date, more than 1000 different mutations have been classified as pathogenic. Nevertheless, in a considerable number of STGD cases only monoallelic or no mutations have been identified. In order to unravel the missing heritability, intronic sequences have been studied allowing the identification of several pathogenic deep-intronic variants which have been shown to cause defects in mRNA splicing (either by pseudoexon formation or exon skipping). In this project, we want to apply, asses and compare several CRISPR-Cas9-based approaches to rescue such splicing defects. Furthermore, in order to easily assess the different strategies, we will also establish fluorescence-reporter cell lines in which enable to sense the rescue of the deep-intronic mutations back to correct splicing. Efficacy of these approaches will be tested in photoreceptor precursor cells (PPCs) derived from patient fibroblasts.

Acknowledgement

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 813490

Domdei, Niklas

Photoreceptor-targeted psychophysics with adaptive optics scanning laser ophthalmoscopy

Niklas Domdei, Jenny Reiniger, Frank Holz, Wolf Harmening

Department of Ophthalmology, University of Bonn, Germany

Purpose
Adaptive optics scanning laser ophthalmoscopy (AOSLO) allows imaging of single photoreceptors in vivo. Combined with real-time eye tracking and correction for chromatic aberrations, the AOSLO can be used as a microscopy platform to make single cells optically accessible for functional testing. We here tested the impact of individual cones on human vision and their relationship with retinal features such as preferred retinal locus of fixation (PRL) and point of peak cone density.

Methods
For imaging and wavefront correction we use 840 nm light. Online eye-tracking enables the presentation of retina stabilized stimuli and therefore repeated stimulation of the same cone. Small spot sensitivity (543 nm; 26.1 µsec; 1.4 µm full width at half maximum) was tested in 5 eyes of 5 participants with an adaptive staircase method (QUEST) over 12 trials per test site. The 9 test sites were arranged in a cross-shaped pattern with 0.1 arcmin spacing and centered manually close to the PRL.

Results
Across all examinations, no significant correlation between the distance to the PRL and sensitivity was found ($p=0.07$). Furthermore, there was no significant correlation between the normalized cone density of the test site and sensitivity ($p=0.25$). Individual differences in peak cone density [cones/deg^2] between participants (~50%) were also not correlated with differences of highest sensitivity values (~7%).

Conclusions
The average detection thresholds were similar for all tested participants, ~3.5 log10 photons at the cornea. Differences in foveal sensitivity to cone-sized light stimuli did not correlate with the variation of cone density at test locations. While cone density decreased rapidly up to 30% with increasing eccentricity (0.2 degree), sensitivity was stable across those test sites. Even though we observed a tendency for higher visual sensitivity closer to the PRL, trends were not significant. Therefore, the principles of PRL formation remain unknown.

Statement on proprietary interests

Acknowledgement
DFG grants HA5323/5-1, Carl Zeiss Förderfonds

Duda, Sabrina

Alpha ganglion cells of the guinea pig retina

Sabrina Duda*, Christian Puller*, Yousef Arzhangnia, Martin Greschner

Visual Neuroscience, Department of Neuroscience, Carl von Ossietzky Universität Oldenburg, Germany

Purpose
α-ganglion cells (αGCs) of the mouse retina have been shown to consist of four types with distinct anatomical and physiological features. This is inconsistent with the classification of αGCs in other mammals, where only two types were termed αGCs. Here, we have combined multi-electrode array (MEA) recordings with immunolabeling and single-cell dye injections to investigate molecular identities, morphological features and light response properties of αGCs of the guinea pig retina.

Methods
Light responses to a random noise stimulus were recorded by a large scale MEA from retinas of adult, pigmented guinea pigs and used to classify GCs into different types. Subsequently, the recorded retinas were labeled with immunomarkers, such as RBPMS, SMI-32 and ChAT. The individual positions of labeled cells were then matched to the functional mosaic of the corresponding type from MEA recordings. GCs were injected with neurobiotin to reveal morphological features of individual cells.

Results
Cell bodies and dendrites of two types of ganglion cells were most intensely labeled with SMI-32. Their dendrites stratified below the ON ChAT band or below the OFF ChAT band. Dye-injections revealed a typical α-like morphology. Matching of labeled cells with their prior recorded receptive fields showed that they corresponded to two functional types, ON sustained and OFF transient. In addition, we mapped their functional counterparts showing mirror-symmetric light responses, OFF sustained and ON transient, onto two further populations of SMI-32 positive GCs with corresponding dendritic stratification patterns.

Conclusions
The matching procedure allows a characterization of molecular identities and light response properties of the same ganglion cell. Our data suggests that four GC types with distinct physiological and morphological features could be classified as αGCs in guinea pig. Two cell types had been named αGC in previous studies of the guinea pig, which correspond to two of the four α-like GC types presented here, the ON sustained and OFF transient αGCs. Contrary to implications of the classical naming, these two types do not form a functionally symmetrical cell pair.

Statement on proprietary interests
No proprietary interests

Acknowledgement
This work was supported by VolkswagenStiftung (M.G.)

Gantes Nuñez, Francisco Javier

Personalized eye models to predict quality of vision in Age-Related Macular Disease (AMD)

a) Francisco Javier Gantes Nuñez b) Daniel Malacara Hernández c) Juan Tabernero

a),b) Centro de Investigaciones en Óptica, C.P. 37150, León, Gto., Mexico c) Departamento de Electromagnetismo y Electrónica, Universidad de Murcia, 30100 Murcia, Spain

Purpose
The purpose of this study is to link the size/volume/magnitude of macular edemas or drusen, caused by Age-Related Macular Disease (AMD) to expected visual distortions obtained from Amsler grids.

Methods
Retinal images from subjects with AMD were acquired using an OCT, (Spectralis, HRT). The characteristics of drusen were obtained from the retinal images using the scale information showed in the retinal files. With this information and the localisation of drusen, a personalized matrix that models the retinal was generated using MATLAB. Then, computational eye models with AMD were done using an exact ray tracing software (Zemax) with lens parameters from the Escudero-Navarro eye model. From each model eye, and using a ray-tracing calculation, the shape of an Amsler grid was simulated in accordance to the drusen.

Results
A series of customized eye models with AMD were obtained using Zemax. The simulated Amsler grids images presented the main affectations caused by AMD and were well in agreement with features described in the classical literature: Metamorphopsia, micropsia and macropsia. Also, the shapes and size of drusen were reproduced in a high feature compared with the retinal images.

Conclusions
The drusen models reproduced in the eye models showed reliable and accurate results compared with the retinal images. Furthermore, the virtual Amsler grids obtained from the eye models simulated in Zemax, presented promising results for the development of a simple method to evaluate the visual effects of AMD from objective data. This method can be useful to quantitatively measure the visual progression of drusen in AMD patients.

Statement on proprietary interests
None of the authors have any conflict of interest

Acknowledgement

García García, Miguel

Peripheral 2D image quality metrics

Miguel García García[1], Dibyendu Pusti[2], Pablo Artal[2], Siegfried Wahl[1] and Arne Ohlendorf[1]

[1]^Carl Zeiss Vision International GmbH, Aalen, Germany. [2]^Laboratorio de Óptica, Universidad de Murcia, Murcia, Spain.

Purpose
To evaluate the impact of different designs of multifocal contact lenses on the peripheral image quality metrics.

Methods
Series of Hartmann-Shack images were recorded by using an open-view high-resolution peripheral wavefront sensor (VPR, Voptica SL, Murcia, Spain) in 13 young myopic subjects (mean spherical equivalent error: -3.25D; range: -0.75 to -6.50D). For each eye, horizontal scans covering 40° of visual field were obtained at five different vertical fixations (0°, +10°, +20°, -10°, -20°). Measurements were taken while subjects were wearing: 1) no lens 2) a multifocal center-distance (MFCD) or 3) a multifocal center-near (MFCN) soft contact lens (Xtensa by mark'ennovy; distance Rx: -0.25DS). At each peripheral angle, the point spread function (PSF) was calculated and used to convolve the "Lena" image. After this, the convolved images passed a low pass filter to remove the spatial frequencies that where above the cutoff spatial frequency for the corresponding eccentricity. Subsequently, the filtered images were cropped and compared with the original image, to obtain 2-D cross-correlation values as an image quality metrics. This was used to determine 2D significance maps, based on the crosscorrelation values for all the subjects and conditions. Additionally, one significance map was defined by using the theoretical spatial frequency limit. The differences (root mean square errors: RMSE) between the 2-D significance maps and the cut-off map were used to quantify the overall differences produced by each condition for each subject.

Results
While analyzing the full visual field, the naked eye condition (1) obtained the highest similarity to the ideal image quality (RMSE=0.2315). In contrast, the MFCN design significantly degraded the overall quality metric (RMSE= 0.2897; p=0.0166), while the MFCD did not (RMSE=0.2720, p=0.3552).

Conclusions
The amount of blur on the retina can be modified by optical treatments such as contact lenses. A better understanding of the image quality with different optical treatments can shed some light on the efficacy of different solutions for myopia management.

Statement on proprietary interests
Miguel Garcia Garcia: Commercial Relationship(s);European Union:Code F (Financial Support);Carl Zeiss Vision International:Code E (Employment);Carl Zeiss Vision International:Code F (Financial Support) | Siegfried Wahl: Commercial Relationship(s);Carl Zei

Acknowledgement
MYFUN receives funding from the European Union's Horizon 2020 research and innovation programme under the Marie Sklodowska- Curie grant agreement N° 675137.

GARCIA-GARCIA, DIANA

Retinal regeneration and inflammation: role of YAP in Müller cells-Microglia interplay

Garcia-Garcia, Diana

Paris-Saclay Institute of Neuroscience, CNRS, Univ Paris Sud, Université Paris-Saclay, Orsay, France

Purpose
Contrasting with fish or amphibians like Xenopus, retinal regeneration from Müller glia is largely limited in mammals. In our quest towards the identification of molecular cues that may boost their stemness potential, we investigated the involvement of the Hippo pathway effector YAP, which is upregulated specifically in mouse Müller cells following retinal injury.

Methods
YAP function was assessed specifically in mouse Müller cells by conditionally knockout YAP (Yapfl/fl;Rax-CreERT2 mice) and by overexpression of a constitutive active form of YAP (YAP5SA) using an adeno-associated viral (AAV) variant ShH10 as vector. Retinal degeneration was induced in vivo via MNU intraperitoneal injection or ex vivo in the retinal explant model. Inflammatory experiments of microglial modulation were performed by LPS stimulation both on in vivo and ex vivo models.

Results
Conditional Yap deletion in mouse Müller cells prevents cell cycle gene upregulation that normally accompanies their reactivation upon degeneration. On the contrary, overactivation of YAP enhances Müller cell proliferative response up to 25% in retinal explants. Besides, the upregulation of inflammatory genes that accompanies Müller cells reactivation upon injury was also prevented in the absence of YAP suggesting a role of YAP on the inflammatory response. In addition, preliminary data from microglial modulation in mice also suggest a relationship between Müller cells reactivation and microglia.

Conclusions
These findings reveal a role for YAP in Müller cells exit of their quiescent state in a degenerating retina and signal inflammation as one of the factors capable of influencing regenerative capacity. Supporting our mice results, the laboratory has also shown that YAP is required for Xenopus Müller cells proliferation in response to injury and that this proliferation can be modulated by LPS. Moreover, YAP has recently been shown by others to be involved in the control of the inflammatory response. Further investigations will be done to determine the role of YAP in the coupling between Müller cells regeneration and the inflammatory response.

Statement on proprietary interests

Acknowledgement

Gehlen, Jana

Pharmacological manipulation of oscillations in the retina of the retinitis pigmentosa mouse model rd10 improves efficiency of electrical stimulation

Jana Gehlen1, Stefan Esser1, Kim Schaffrath2, Sandra Johnen2, Peter Walter2, Frank Müller1

1: Institute of Complex Systems, Cellular Biophysics, ICS-4, Forschungszentrum Jülich, Germany 2: Department of Ophthalmology, RWTH Aachen University, Germany

Purpose
To eliminate or suppress pathological electrical activity in the retina that occurs upon photoreceptor degeneration and that may compromise the efficiency of retinal ganglion cell (RGC) stimulation by an electrical prosthesis.

Methods
Electrophysiological recordings were obtained in vitro from wild type (WT) retinae and from rd10 retinae using multi electrode arrays (MEA). Retinae were obtained from animals at the age of 3-4 months. Local field potentials (LFP) and spike activity of RGCs were recorded and RGCs were stimulated electrically. The effects of different agonists at inhibitory neurotransmitter receptors on oscillatory activity were investigated.

Results
As described earlier, in rd10 retinae but not in WT retinae, we observed oscillations in the LFP at a frequency of 3-6 Hz. Glycine and GABA abolished oscillations in a reversible way. Moreover, diazepam, flunitrazepam and lorazepam, all allosteric modulators of the benzodiazepine family acting at the GABAA receptors, also eliminated oscillations. We found that the efficiency of electrical stimulation–measured as ratio of spike rate after and before the stimulation pulse– was lower in rd10 than in WT retina. Most importantly, treatment of the rd10 retina with GABA or the different types of benzodiazepines increased the stimulation efficiency.

Conclusions
In rd10 retina, pathological oscillatory activity seems to reduce the efficiency of electrical stimulation. Abolishing oscillations improved stimulation efficiency to values similar to WT retina. This study may open the way to a therapy that supports electrical stimulation by retinal prostheses with pharmacological treatment.

Statement on proprietary interests
none

Acknowledgement
The study was supported by the DFG grants MU-3036/3-3, WA-1472/6-3, JO-1263/1-3 and Pro-Re/Projekt/Johnen-Diarra.1-2015.

GIAMUNDO, GIULIANA

Retinal degeneration in MPSIII-A knockout (-/-) mice.

Giuliana Giamundo,Daniela Intartaglia,Veronica Maffia, Elena Marrocco, Francesco Giuseppe Salierno, Edoardo Nusco,Nicolina Cristina Sorrentino, Ivan Conte.

Telethon Institute of Genetics and Medicine, Via Campi Flegrei 34, Pozzuoli (Naples), 80078, Italy

Purpose
Characterization of rods retinal degeneration in MPSIII-A knockout (-/-) mice.

Methods
Immunofluorescence analysis and longitudinal acquisition of electroretinograms to investigate retinal morfologhy and function during disease progression.

Results
MPSIII-A inactivation in mouse leads to a progressive rod dysfunction followed by rods loss, without the apparent involvement of cones. Among the evidence supporting the latter conclusion, a special emphasis should be placed on the lack of a decrease in ONL thickness, even in very old mice.

Conclusions
MPSIII-A knockout (-/-) mice exhibited a progressive rod dystrophy accompanied by significant alteration in visual function.

Statement on proprietary interests
none

Acknowledgement
Sanfilippo Children's Foundation

Hölzel, Maj-Britt

A retinal origin of nystagmus in nyx-/- mice

Maj-Britt Hölzel 1, Beerend H.J. Winkelman 1,2, Marcus H. Howlett 1, Coen Joling 1, Gobinda Pangeni 3, Sander Kamermans 4, Huib J. Simonsz 5, Maureen A. McCall 3, Chris de Zeeuw 1,2, Maarten Kamermans 1,6

1 Netherlands Institute for Neuroscience, Amsterdam, the Netherlands 2 Erasmus University, Rotterdam, the Netherlands 3 University of Louisville, Louisville, USA 4 Polder Animation, Utrecht, the Netherlands 5 Department of Ophthalmology, Erasmus MC, Rotterdam, the Netherlands 6 Department of Biomedical Physics, Academic Medical Center, University of Amsterdam, the Netherlands

Purpose
The origin of infantile nystagmus is commonly thought to be located in the brainstem. However, Simonsz et al (2009) found that a specific group of young nystagmus patients had mutations in nyctalopin (nyx), a protein implicated in the cone to ON-bipolar cell synapse. The location of the mutation indicates a retinal origin. To test this hypothesis we investigated whether nyx-/- mice had a similar form of nystagmus and aimed at resolving the underlying mechanism.

Methods
Eye movement and optic nerve recordings, whole cell patch clamp recordings of ONDS-GCs and MEA recordings. For targeted patch clamp recordings we crossbreed the nyx-/- mice with a SPIG1+ mouse line in which all ON-dsGCs coding for upward motion express GFP.

Results
Nyx-/- mice have a disturbed optokinetic response and show small amplitude oscillating horizontal eye-movements with a frequency of about 5 Hz. Interestingly, the oscillating eye-movements disappear in the dark. Since Nyx-/- mice have no functional ON-bipolar cells we asked the question whether the retinal output of these mice was affected using optic nerve recordings. We found that the ganglion cells in these mice oscillate with a frequency of about 5 Hz. The ganglion cell oscillations synchronized after light stimulation. A specific type of GCs, the ON-directional selective ganglion cells (ON-DSGCs) detect global motion and send this information to the accessory optic system (AOS) where it is used to control the eye-position, such that we can stabilize images on our retina. We found that nyx-/- mouse ON-DSGCs are not responsive to light, but oscillate with a frequency of about 4.5 Hz. Blocking the ganglion cell oscillations pharmacologically with a cocktail of DNQX and D-AP5, which block AMPA and NMDA receptors, stopped the oscillation eye-movements. Furthermore, shifting the ganglion cell oscillation frequency by application of strychnine also shifts the eye movement oscillations. This data shows that there is a causal relation between the ganglion cell oscillations and the oscillatory eye movements.

Conclusions
We propose that the synchronized oscillating output of those ON-DSGCS to the AOS is interpreted as oscillating global image motion inducing compensatory eye-movements.

Statement on proprietary interests

Acknowledgement
switchBoard - In the Eye of the Observer: Visual Processing at the Heart of the Retina SwitchBoard receives funding from the European Union's Horizon 2020 research and innovation programme under the Marie Sklodowska-Curie grant agreement No 674901

Ingensiep, Claudia

Establishment of a hypoxia model for the MEA-based analysis of the electrical activity in murine retinae

Ingensiep, C., Schaffrath, K., Walter, P., Johnen, S.

Department of Ophthalmology, University Hospital RWTH Aachen, 52074 Aachen, Germany

Purpose
Several eye diseases, e.g., diabetic retinopathy or glaucoma, are associated with retinal hypoxia. Especially the lack of oxygen in retinal ganglion cells (RGCs) causes cell damage up to cell degeneration leading to blindness. Thus, to examine the activity of RGCs and to analyze the effect of neuroprotective substances under hypoxic conditions is of great ophthalmologic interest.

Methods
Using multielectrode array (MEA) recordings, two ex vivo models were established to analyze the electrical activity of murine wild-type(wt)-retina under hypoxic stress conditions. Hypoxic conditions were initiated by stopping the perfusion with oxygen saturated medium (model 1) or by exchanging the perfusion with oxygen saturated medium by nitrogen saturated medium (model 2). To analyze the influence of neuroprotective agents on the firing behavior of RGCs under hypoxia-induced conditions, 2-aminoethanesulfonic acid (taurine, 1 mM) was added during a hypoxia time of 30 min.

Results
The electrical activity vanished during hypoxia. However, it conditionally returned after reestablishment of the conventional test conditions. With increasing duration of hypoxia, the number of recording channels decreased, on which activity could be redetected. After a hypoxic period of 30 min and a subsequent recovery time of 30 min, 52.14 ± 26.69% (model 1) and 59.43 ± 11.35% (model 2) of the initially active channels showed a restored activity. The application of taurine had a positive influence on the excitability of RGCs but not on the number of recording channels and the firing frequency of the spontaneous activity after hypoxia.

Conclusions
The hypoxia models established here allow for the analysis of electrical RGC activity before, during and after hypoxic conditions and the effects of any added protective substance. Additionally, a microarray-based transcriptome-wide gene expression analysis of the retina has been performed using different culture conditions.

Statement on proprietary interests
none

Acknowledgement
none

Intartaglia, Daniela

The light-responsive miR-211 regulates retinal cell clearance by modulating lysosomal biogenesis via Ezrin targeting

Federica Naso1#, Daniela Intartaglia1#, Danila Falanga1, Chiara Soldati1, Elena Polishchuk1, Paola Tiberi1, Elena Marrocco1, Paolo Scudieri1, Ivana Trapani1, Edoardo Nusco1, Francesco Giuseppe Salierno1, Enrico Maria Surace1,2, Luis J. V. Galietta1,2, San

1. Telethon Institute of Genetics and Medicine, Via Campi Flegrei 34, Pozzuoli (Naples), 80078, Italy. 2. Department of Translational Medicine, University of Naples Federico II, 80131, Naples, Italy. 3. Medical Genetics, Department of Biochemistry, Biophysics and General Pathology, University of Campania "L. Vanvitelli", via Luigi De Crecchio 7, 80138, Naples, Italy.

Purpose
Efficacy of induction of lysosomal biogenesis and activity, via pharmacological repression of Ezrin, for the clearance of lipofuscin accumulation in the miR-211-/- mice, which could represent an important therapeutic strategy to treat the most common cause of blindness worldwide such as age-related macular degeneration.

Methods
This study used a variety of in-vivo and in-vitro approaches to investigate how miR-211 regulates lysosomal biogenesis.

Results
We found that miR-211 is a daily crucial regulator of lysosomal biogenesis and function in the RPE/retina crosstalk by targeting Ezrin gene. MiR-211-mediated inhibition of Ezrin leads to a Ca2+-mediated activation of TFEB, a master regulator of lysosomal biogenesis.The dysregulation of the light/dark expression pattern of Ezrin caused impairment of both daily lysosomal biogenesis and degradation of POS in RPE from miR-211-/- mice, which show an age-dependent accumulation of both phagolysosomes containing poorly processed POS and lipofuscin granules, accompanied by a compromised vision.

Conclusions
Pharmacological activation of lysosomal biogenesis and function, through Ezrin inhibition, rescued the miR-211-/- phenotype in vivo, pointing to a new lysosomal-based therapeutic intervention to treat retinal degeneration.

Statement on proprietary interests
None

Acknowledgement
BrightFocus Foundation

JENISCH, PETER

Inosine-5'-monophosphate dehydrogenase 1 (IMPDH1) as a novel target for the treatment of Retinitis Pigmentosa

Peter K. Jenisch

Cell Death Mechanism Group Institute for Ophthalmic Research University of Tuebingen Elfriede Aulhorn Straße 5-7, 72076 Tuebingen, Germany

Purpose
Retinitis pigmentosa is a degenerative genetic disorder in which cell death can be connected to high cGMP levels in photoreceptors. In the rd1 mouse model this is due to a phosphodiesterase(6b) mutation lacking cGMP hydrolysis. The enzyme inosine-5'-monophosphate dehydrogenase-1 is the rate-limiting step in the biosynthesis leading to cGMP. IMPDH1 may be a target for the reduction of rod cGMP levels / cell death by selectively inhibiting via the registered immunosuppressive drug mycophenolate.

Methods
To characterize the retinal expression pattern of IMPDH1, immunofluorescence staining and the anti-IMPDH1 antibody (ab33039; Abcam) were used. Stainings were performed in C3H rd1 mouse retina as well as in its congenic C3H Pde6b+/+ wild-type counterpart. In control staining the primary IMPDH1 antibody was omitted.

Results
In both rd1 and wild-type mice the IMPDH1 signal was seen in inner segments, outer plexiform layer, and in processes spanning the whole width of the retina. The latter likely indicates Müller glia cell labelling. Control staining (negative control) delivered no or only weak background labelling of all retinal layers.

Conclusions
The positive detection of the IMPDH1 enzyme, notably in photoreceptor inner segments, indicates that IMPDH1 can indeed be targeted by specific drugs such as mycophenolate. Future studies, initially in retinal explant cultures in vitro, may reveal whether such drug treatment will decrease photoreceptor cGMP levels and cell death.

Statement on proprietary interests

Acknowledgement
Kleinprojektförderung PRO RETINA-Stiftung

Karpińska, Anna

Optimisation and up-scaling of nanoparticles for drug delivery system.

Anna Karpińska and Matej Buzgo

InoCure s.r.o.

Purpose
Our main aim is to develop and optimize the particles/fibers production technology by design the electrospraying/electrospinning device, theoretical analysis of the features of the process and parameters affecting the formation of the elements.

Methods
To achieve our goal, the simulations have been done in COMSOL Multiphysics software. Then, our model will be constructed and verified in practical use to finally characterize the particles/fibers features.

Results
We have examined the impact of the geometry of the needleless emitter/spinneret and its features on particles/fibers formation.

Conclusions
Our study can greatly enhance the efficiency of the electrospraying/electrospinning,that is promising for drug delivery technology.

Statement on proprietary interests

Acknowledgement
The authors would like to thank the European Union's Horizon 2020 research and innovation program under the Marie Skłodowska Curie grant agreement No 765441 for financial support.

Kieninger, Sinja

Gene-editing based elimination of intronic mutations as a therapeutic approach for OPA1-linked optic neuropathies

Sinja Kieninger1, Jasmin Haderspeck2, Tobias Bonifert1, Stefan Hauser3, Ludger Schöls3, Stefan Liebau2, Bernd Wissinger1

1Centre for Ophthalmology, Institute for Ophthalmic Research, Molecular Genetics Laboratory, University of Tuebingen, Germany 2Institute for Neuroanatomy, University of Tuebingen, Germany 3Hertie-Institute for Clinical Brain Research, University of Tuebingen, Germany

Purpose
Deep intronic mutations (DIMs) in OPA1 have been recurrently described in patients with autosomal dominant optic atrophy or Behr syndrome. DIMs activate cryptic splice sites, which lead to an inclusion of a pseudoexon bearing a premature stop codon into mutant OPA1 transcripts. In this project, we intend to further develop and validate a therapeutic approach for OPA1-linked optic neuropathies using the CRISPR/Cas9 technology to excise the pseudoexon and thereby restore normal OPA1 splicing.

Methods
Patient-derived fibroblasts were used to establish iPSCs with the c.610+364G>A DIM in OPA1. IPSCs are transfected with plasmids expressing dCas9 nickase and a sgRNA pair. This approach induces single-strand breaks in the vicinity of the DIM which are repaired by NHEJ. Splicing rescue was quantified by Pyrosequencing. The spectrum of edited OPA1 alleles was determined by Sanger sequencing of individual clones. Minigene assays are used to analyze the splicing rescue of the CRISPR-edited alleles.

Results
We observed a 50 % rescue of normal splicing of the DIM allele upon CRISPR/dCas9-based editing in iPSCs. Sequencing of the CRISPR-treated cell clones revealed a large variety of insertions and deletions at the DIM site. A total of 28 alleles containing the DIM were analyzed, of which 21 were edited by CRISPR/dCas9. Of 31 wildtype alleles, only 4 were edited, which demonstrates specificity for the DIM allele. The CRISPR-mediated editing events were also checked with different splice site prediction tools. Those editings which seem to eliminate the cryptic splice acceptor site are currently individually analyzed using minigene assays.

Conclusions
We have demonstrated proof-of-concept of successful CRISPR/dCas9-based elimination of a recurrent DIM in OPA1 in patient-derived iPSCs. Moreover, we could show that the approach is fairly specific for the mutant allele although wildtype and mutant sequence differ by just a single base.

Statement on proprietary interests

Acknowledgement
Funded by a grant of the Deutsche Forschungsgemeinschaft [WI1189/11-1] within the framework of the ERA-NET E-Rare 3 project “TreatOPON”.

Korympidou, Maria Magdalini

Chromatic processing at the mouse outer retina

Maria Magdalini Korympidou 1-3; Klaudia Szatko 1,3,4; Philipp Berens 1,4; Deniz Dalkara 5; Thomas Euler 1,2,4; Timm Schubert 1-2; Katrin Franke 1,4

1 Institute for Ophthalmic Research, 2 Centre for Integrative Neuroscience, 3 Graduate Training Centre of Neuroscience, 4 Bernstein Centre for Computational Neuroscience, all: University of Tübingen, Germany, 5 Institut de la Vision, Sorbonne Université INSERM, CNRS, Paris, France

Purpose
Chromatic signals from different photoreceptor types are locally compared by downstream retinal circuits – an important prerequisite for color vision. Despite the uneven opsin distribution in their retina (Applebury et al. Neuron 2000), mice are able to discriminate colors (Jacobs et al. Vis Res 2004) – at least in the upper visual field (Denman et al. eLife 2018), which corresponds to the ventral retina. Here we investigate where color-opponency arises within the retinal circuit.

Methods
We studied cone responses to chromatic stimuli by two-photon imaging of glutamate release from their axon terminals in the whole-mounted mouse retina.

Results
We found that color-opponency is already present in the cone output, but varies along the dorso-ventral axis: Ventrally, where the S-opsin is predominantly expressed, cones possessed an antagonistic green-ON surround. Dorsally, however, the chromatic tuning of the surround was not as clear cut; instead, many cones possessed surrounds that elicited ON responses both to green and UV stimuli. Blocking horizontal cell function pharmacologically eliminated the surround responses suggesting that lateral inhibition provided by horizontal cells is involved in the generation of cone color-opponent signals.

Conclusions
Our findings support the existence of rod-cone color-opponency in the ventral mouse retina (as proposed by Joesch and Meister Nature 2016), suggesting that the green-sensitive rods create the antagonistic surround of UV-sensitive cones via horizontal cells.

Statement on proprietary interests
The authors declare no competing financial interests.

Acknowledgement
BMBF, FKZ (01GQ1002 to KF); MPG (M.FE.A.KYBE0004 to KF); DFG (CIN-EXC307 to TE/TS)

Kutluer, Meltem

INVESTIGATION OF A NEW IN VITRO SYSTEM TO STUDY ON ROD PHOTORECEPTOR CELLS

Meltem Kutluer, Antonella Comitato, Valeria Marigo

Universtiy of Modena and Reggio Emilia

Purpose
Our aim is to develop a new in vitro system to mimic retinal degeneration and to enable molecular studies on rod photoreceptor cells.

Methods
661W cells were genetically modified with Nrl gene by a retrovirus. Retroviral integration was defined by PCR on genomic DNA. Rod photoreceptor specific gene expression was determined with RT-PCR analysis. Different differentiation media were tested on 661W cells and genetically modified 661W cells. Differentiation was assessed by Immunofluorescence analysis to determine expression of rod photoreceptor proteins (markers) such as Rhodopsin and PDE6B.

Results
Nrl genetically modified 661W cells increased expression of rod-specific genes when compared to unmodified cells. Differentiation media caused changes in cell shape such as cell elongation. One of these media could significantly increase differentiation of cells into rod-like cells with an elongated shape. Immunofluorescence analysis showed expression of rod specific proteins such as Rhodopsin, PDE6B, RET-P1, Peripherin.

Conclusions
Transduction of 661W cells with a retrovirus for the expression of the Nrl gene demonstrated to be intrumental for differentiating 661W cells into rod-like photoreceptor expressing rod specific proteins. This cell line will be used for in vitro studies to mimic retinal degeneration.

Statement on proprietary interests

Acknowledgement
The transMed project has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No 765441

Phosphodiesterase 6 inhibition induces cell death of rod photoreceptor-like cells differentiated from 661W

Huang Li, Antonella Comitato, Valeria Marigo

Department of Life Sciences, University of Modena and Reggio Emilia, Modena, Italy

Purpose
Rod-specific cyclic guanosine monophosphate (cGMP) phosphodiesterase 6 (PDE6) accounts for a sizable fraction of cases of autosomal recessive retinitis pigmentosa. To gain insight into the precise mechanisms eventually leading to cell death under the impacts of PDE6 mutation in this disease, here, we characterize a cell model mimicking rod photoreceptor cell death in vitro by pharmacological PDE6 inhibition on 661W cells, a photoreceptor-like cell type.

Methods
661W cells were genetically modified by expression of neural retina leucine zipper (Nrl). Differentiation was triggered by treatment with differentiation medium. PDE6 inhibition was induced by zaprinast for 72 hours. Cell death was evaluated by TUNEL assay and ethidium bromide staining. Calpain activity, cGMP and calcium contents were visualized by immunofluorescence.

Results
PDE6 inhibition induced cGMP accumulation and triggered cell death, as determined by immunofluorescence and TUNEL assay. Immunostaining indicated that cell death was accompanied by calcium and calpain activation in cells treated with zaprinast. Our results indicated that PDE6 inhibition could increase intracellular cGMP level, which would cause photoreceptor cell death via opening of the cGMP- gated channels in photoreceptor plasma membrane and influx of Ca2+ ions.

Conclusions
Our study suggests that this cell model could serve as an alternative in vitro model for rod photoreceptor cell biology to study the mechanisms of rod photoreceptor death in retinitis pigmentosa as well as the therapeutic research to test novel neuroprotective drugs.

Statement on proprietary interests

Acknowledgement
TransMed receives funding from the European Union's Horizon 2020 research and innovation programme under the Marie Sklodowska-Curie grant agreement No 765441

Lorenzo-Soler, Laura

Angiotensin Receptor Blockers in Cyclodextrin Nanoparticle Eye Drops: Ocular Pharmacokinetics and Pharmacologic Effect on Intraocular Pressure

Laura Lorenzo-Soler, Gerhard Garhöfer, Ólöf Birna Ólafsdóttir, Phatsawee Jansook, Íris Mýrdal Kristinsdóttir, Thorsteinn Loftsson, Einar Stefánsson

University of Iceland, Medical University of Vienna, University Hospital of Iceland, Oculis ehf.

Purpose
Orally administered angiotensin II receptor blockers (ARBs) reduce intraocular pressure (IOP). Topical administration may reduce potential side-effects. The main purposes of this study are (1) to determine the pharmacokinetics of irbesartan and candesartan in γ-cyclodextrin nanoparticle eye drops in the anterior segment of the rabbit eye and (2) to test the hypothesis that irbesartan and candesartan eye drops lower IOP in rabbits.

Methods
1.5% irbesartan and 0.15% candesartan eye drops were applied to rabbits. The pharmacokinetics in cornea and aqueous humor of single eye drop application were studied in 25 rabbits. The effect of the eye drops on IOP was studied in 10 rabbits using an iCare tonometer and compared with 0.5% Timolol eye drops.

Results
Candesartan lowered the IOP from 24.6 ± 5.1 mmHg to 19.0 ± 2.9 mmHg (p=0.030, n=10). Irbesartan lowered IOP from 24.2 ± 1.7 mmHg to 20.2 ± 0.9 mmHg (p=0.14, n=10). Timolol decreased the IOP from 24.9 ± 4.2 mmHg (mean ± SD) to 20.4 ± 4.8 mmHg (p=0.036, n=10). The pharmacokinetics data show that both formulations are able to deliver effective amounts of drug into the intraocular tissues, reaching a concentration of 121.4 ± 68.8 ng/g (mean ± SD) in the aqueous humor after a single dose administration.

Conclusions
Topical application of irbesartan and candesartan eye drops delivers effective drug concentrations to the anterior segment of the eye in rabbits. They reduce IOP in normotensive rabbits comparable to timolol. ARB eye drops have potential as a new class of glaucoma drugs.

Statement on proprietary interests
Ólafsdóttir and Íris M. Kristinsdóttir (Employees, Oculis), Thorsteinn Loftsson and Einar Stefánsson (Personal Financial Interest, Oculis)

Acknowledgement
This study has received partial funding from the European Union's programme Eurostar under the project No PREVIN E11008 and the Horizon 2020 research and innovation programme under the Marie Sklodowska-Curie grant agreement No 765441.

Lovas, Sándor

Quantification of Cell Death in Long-Term Organotypic Culture of the Adult Human Retina

Sándor Lovas[1], Dániel Magda[1], Ferenc Kilin[1], Ákos Kusnyerik[2], Anita Csorba[2], Zoltán Zsolt Nagy[2], Arnold Szabó[1]

1, Department of Anatomy, Histology and Embryology, Semmelweis University, Budapest 2, Department of Ophthalmology, Semmelweis University, Budapest

Introduction:
We developed a culture technique that maintains human adult post mortem retinas in excellent condition for more than three months, enabling research on human retinal tissue that was previously impossible. In this study, we analyzed spatial and temporal characteristics of apoptosis occurring in culture.

Methods:
Retinal cultures from multiple donors were cultured for up to 10 weeks. The cultures were fixed at different time points and were subjected to TUNEL analysis and immunohistochemistry.

Results:
In general, every major cell types survived and all retinal layers were maintained. The rate of apoptosis was low at all ages and remained low during the whole experiment. The initial average apoptosis of 7,662687 ± 5,21597 cells / 100 µm at DIV7 decreased to 2,912 ± 2,229 by the third week and remained similar to that until the end of the 10th week. At all ages studied, the most intense cell death occurred in the outer nuclear layer. The overwhelming majority of TUNEL-positive cells were calbindin-negative rods. Cones didn't undergo severe apoptosis and a mean density of 4500- 5000 cones/mm2 were measured even in long-term cultures. In the inner retina apoptosis affected all cell types uniformly, none of them showed disproportionately high levels of cell death.

Conclusion:
Our human retinal explant culture system can maintain human retinas in culture for over 3 months while retaining a high level of morphological preservation. The low level of apoptosis and small deviation makes our explant culture system an excellent in vitro model for safety and efficacy testing of drug candidates and retinal regeneration studies.

Magda, Daniel

Temporal Changes of Microglial Phenotype in long-term Organotypic Culture of the Human Retina

Daniel Magda1, Sandor Lovas1, Ferenc Kilin1, Annamária Hesz1, Akos Kusnyerik2, Zoltan Zsolt Nagy2, Arnold Szabo1

1, Department of Anatomy, Histology and Embryology, Semmelweis University, Budapest 2, Department of Ophthalmology, Semmelweis University, Budapest

Purpose
Specific roles of microglia in healthy and diseased retina is an emerging topic. Recently, we developed an organotypic culture system that allows the survival of the post mortem human retina over 12 weeks. The method provides a suitable tool to study gliotic activation in the full-thickness three-dimensional retinal tissue. In this study, we examined changes in microglial phenotype in detail.

Methods
Human eyes were acquired through organ donations. Small pieces of the freshly isolated retina were placed onto a polycarbonate membrane and cultured for up to 10 weeks. The cultures were supplied with chemically defined serum-free medium and were fixed at different time points. The samples were analyzed by immunohistochemistry using glia-specific markers.

Results
All retinal layers were well-maintained and every major cell types survived. Both Müller cells and astroglia became hypertrophic and showed increased GFAP expression. In ex vivo controls microglia cells were distributed along retinal vessels and populated the inner retina. In cultures a rapid change in phenotype was observed. Microglia cells with mainly ameboid morphology appeared in the outer retinal layers. Parallel to the morphological changes, expression levels of CD68 became highly elevated, while the expression of Iba-1 largely decreased. The expression of LAMP1 lysosomal marker indicated maintained phagocytic function.

Conclusions
The adult human retina can be maintained in an appropriate culture system for at least three months. Our model provides a reliable tool for the investigation of interactions between neurons, macroglia and microglia cells. By long-term culturing, both acute and chronic effects of pharmacological compounds could be tested directly on human tissue in a cost- and time-effective manner. Further, by using post mortem human tissue we can reduce the use of animals both in academic and industrial research.

Statement on proprietary interests

Acknowledgement

Marino, Valerio

Molecular dynamics simulations to unveil physiological and pathological mechanisms in vision

Valerio Marino1,2, Daniele Dell'Orco1

1 Department of Neurosciences, Biomedicine and Movement Sciences, Section of Biological Chemistry, University of Verona, Verona, Italy 2 Department of Translational Research and New Technologies in Medicine and Surgery, University of Pisa, Pisa, Italy

Purpose
Neuronal Calcium Sensors (NCS) are a highly conserved protein family expressed in neurons and involved in vision. Calcium (Ca^{2+}) binding to their EF-hand motifs triggers a conformational change required for the regulation of a specific target. Here, we investigated the structural effects of ion or membrane binding to Recoverin (Rec) and GCAP1 to describe experimental results at the atomistic scale. In addition, we studied the role of key residues of GCAP1 associated with cone dystrophies (COD).

Methods
Different Rec and GCAP1 signaling states were subjected to exhaustive 1 μs all-atom Molecular Dynamics simulations. The dynamic information from each trajectory was encoded in a Protein Structure Network (PSN) accounting for persistent non-covalent interactions. We then analyzed the topology of each PSN to identify residues involved in a high number of interactions (hubs).

Results
MD simulations were able to reproduce the physiological Ca^{2+}-dependent binding of Rec to the photoreceptor membranes. The comparison between the simulations of WT and GCAP1 COD variants suggested the enhanced thermal stability exhibited by L84F was due to the tighter hydrophobic packing of residue 84. Moreover, the distortion of the Ca^{2+}-binding loop of GCAP1 variant E111V was found to be responsible for the measured decrease in Ca^{2+} affinity. Finally, PSN analysis identified key conserved residues involved in many interactions (hubs), whose mutations are associated with retinal dystrophies.

Conclusions
All-atom MD simulations are a reliable tool for the investigation of the atomistic mechanisms underlying complex protein behaviors such as membrane binding, thermal stability and Ca^{2+}-coordination. Indeed, we were able to explain experimental observables through the structural analysis of MD simulations. Moreover, PSN analysis of MD simulations allows to identify high-degree residues target of retinal dystrophy mutations, some of which are conserved in the NCS family. Such analysis is virtually extendable to any protein/target complex for which structural information is available.

Statement on proprietary interests

Acknowledgement

Mihaylova, Veselina

Effects of blockade of ionotropic GABA receptors on the oscillatory potentials in the ON- and OFF-response of the frog electroretinogram

Mihaylova V, Popova E, Kupenova P

Dept. Physiology, Medical University of Sofia, Bulgaria

Purpose
The involvement of ionotropic (GABA-A and GABA-C) receptors in generation of oscillatory potentials (OPs) in the electroretinogram (ERG) is well documented. However, the specific contribution of these receptors to the OPs generated at stimulus onset (ON-OPs) and offset (OFF-OPs) is not clarified. The goal of our study was to fully separate the ERG ON- and OFF-responses and to characterize the GABA-A and GABA-C receptor-mediated effects on the ON- and OFF-OPs.

Methods
The experiments were carried out on perfused eyecup preparations of frog (Rana ridibunda). To separate the ON- and OFF-responses, long-lasting (3s) light stimuli were presented in the dark at 30s intervals. The OPs were extracted from the ERG by digital filtering (20-300 Hz bandpass). GABA receptor-mediated contribution to ON- and OFF-OPs was evaluated by following the effects of selective GABA-A receptor blockade with 50 uM bicuculline and GABA-C receptor blockade with 100 uM TPMPA.

Results
The GABA-A receptor blockade with bicuculline produced an amplitude increase and shortening of peak latency of the ERG b- and d-waves. The amplitude of the early (O1, O2) OPs in both ON- and OFF-responses was increased. In contrast, the late (O4-O8) OPs were suppressed and eventually abolished. The GABA-C blockade with TPMPA resulted in a b- and d-wave amplitude increase and peak latency delay. TPMPA, like bicuculline, produced an increase in the amplitude of the early ON- and OFF-OPs. However, the late OPs were changed in a different manner: the amplitude of the ON-OPs was strongly increased, while that of the OFF-OPs was not changed.

Conclusions
In the present study we demonstrated that GABA-A and GABA-C receptors contribute in a similar manner to early oscillatory potentials in both ON- and OFF-responses of the frog ERG. However, the two types of ionotropic receptors are differently involved in generation of late OPs. There is clear ON/OFF asymmetry in GABA-A and GABA-C receptor contribution to late OPs. Our results imply that GABA-A and GABA-C receptors may participate in a specific way in multiple generators of OPs in the retinal ON and OFF channels.

Statement on proprietary interests

Acknowledgement
This study was supported by Grant 58/03.05.2018 from the Council for Medical Science, Medical University of Sofia.

Mompeán, Juan

High Performance Computing for OCT images of cataractous eyes

Juan Mompeán, Juan L. Aragón, Pablo Artal

Laboratorio deÓptica, IUiOyN, Universidad de Murcia, Spain Dept. Ingeniería y Tecnología de Computadores, Universidad de Murcia, Spain

Purpose
Our OCT system has been optimized to obtain the best results from both the anterior chamber and the lens of the eye. Furthermore, it has been optimized to image cataracts. However, it generates over 1GB of data per measurement (more than 2GB while processing). As a result, the processing time for each measurement is very high. Our main goal in this work is to use a GPU to speed-up the high computing time required by the processing of each OCT volumes to enable a real-time processing system.

Methods
Our initial implementation was developed using Labview, but its performance was poor. Several computing platforms have been evaluated to provide a broad overview of what fits better our problem and computing demands. Those platforms include several CPUs and GPUs, moreover, several programming languages have been tested: Labview, MATLAB, C++, OpenMP, CUDA and OpenCL. Furthermore, several libraries have been tested to perform the FFT (FFTW, cuFFT and clFFT) obtaining very good results.

Results
The initial MATLAB and Labview implementations took more than 100 seconds to process one OCT volume. While the C++ sequential version takes 40 seconds on a modern CPU, the OpenMP implementation obtains very good results reducing the processing time to 8.4 seconds running on an 8-core/16-thread CPU, and 11.8 seconds on a 4-core/4-thread- CPU. A low-end but modern GPU (NVIDIA GTX 1050) has been measured to take 2.9 seconds to process a volume, while an older AMD R7 250 takes 7.2 seconds. Finally, a high-end and modern NVIDIA GTX 1080 Ti requires only 1.35 seconds to process the whole volume.

Conclusions
A GPU parallel implementation enables either real-time processing of the OCT volumes or very quick post-processing. Therefore, improving the ease of use of the system for a practitioner or optician and the quality of the obtained results due to more the chances to repeat any measurement for the same patient if necessary.

Statement on proprietary interests

Acknowledgement
This research has been supported by “Fundación Sóneca”, Murcia, Spain (grant 19897/GERM/15), and the Spanish SEIDI under grants FIS2013-41237-R, and TIN2015-66972-C5-3-R, as well as European Commission FEDER funds.

PAWLICZEK, DANIEL

Retinal dysplasia and cortical cataracts in neonatal mice exposed to ionizing radiation reduce visual acuity

Daniel Pawliczek, Claudia Dalke, Jochen Graw

Helmholtz-Zentrum München, Institut of Developmental Genetics, Eye Diseases

Purpose
The retina is known to be radiation-sensible only in early stages of development, while the lens is susceptible to irradiation without restrictions. Despite this fact, a clear assessment of the vision of a model organism with these impairments is lacking.

Methods
Wild-type and heterozygous Ercc2+/- mice were whole-body irradiated by 2 Gy of γ-radiation (Dose rate=0.3 Gy/min) just 2 days after birth. All mice were investigated by optical coherence tomography (OCT) and virtual drum and eyes were collected for histology and immunostaining after killing.

Results
Retinae of all irradiated mice investigated in vivo with OCT revealed a decrease of thickness by at least 40%, 9 months after irradiation. Counting of outer nuclear cells revealed a reduction of up to 90% compared with the controls. These changes were accompanied by an increase of GFAP expression of the retinal glial cells. Every irradiation group displayed mild or strongly affected eye lenses with lesions in the inner cortex at the interface to the nucleus. Up to 80% of the irradiated lenses were alternated compared to none in the control group. Visual acuity of the irradiated mice was reduced by at least 45% compared to control mice.

Conclusions
Altogether, postnatal irradiation with a high dose of 2 Gy affected the eye of postnatal mice permanently, but damage in the retinae and the lenses was not enough to impair totally mice's vision or at least the possibility to differ contrasts. That helps to assess the radiation consequences in catastrophic cases of exposure.

Statement on proprietary interests
-

Acknowledgement
This project has received funding from the Euratom research and training programme 2014-2018 under grant agreement No 662287.

PENSIERI, PASQUALE

Role of Otx2 transcription factor in the adult retina photoreceptors

Pasquale Pensieri, Annabelle Mantilleri, Thomas Lamonerie

Institut Biologie Valrose

Purpose

Photoreceptors (PRs) are the cells responsible, in the retina, to convert light in electric signals, that are sent to the brain after a sophisticate elaboration. Their development and specification are under control of a complex gene network in which a key role is played by the transcription factor Otx2. Even if PRs keep Otx2 expression in adult stage, together with the neighbour Retinal Pigmented Epithelium (RPE) and bipolar cells (BPs), the role of this protein at this stage is not yet fully characterized. Recently our laboratory showed that, depleting Otx2 expression in these three retinal compartments, induces PRs specific degeneration, a phenotype that is interestingly recapitulated when the protein is abolished only from the RPE. On the other side, in the same full retina KO context, Otx2 re-expression just in the RPE, is sufficient to maintain alive Otx2-null PRs. Those observations open two main questions, actually under investigation during this PhD fellowship: - What is the role of Otx2 in the PRs? - How Otx2 expression in RPE is sufficient for Otx2-depleted PRs?

Methods

To answer the first question, we induce Otx2 ablation specifically in the adult PRs, in a particular transgenic mice line, analysing later at different biological levels (RNA-seq, cell-viability and identity, PRs functionality). For the second question we speculated, according with some recent publications, a direct transfer of Otx2 from RPE to PRs. To test this interesting hypothesis, we inject in the retina AAVs, driving tagged-Otx2 expression specifically in the RPE and then we check if the tagged protein is up-taken by PRs.

Results

Otx2-depleted PRs are still alive 90 days after KO-induction, confirming that endogenous Otx2 has a marginal role in PRs and that expression in Retinal Pigmented Epithelium is sufficient for their viability and, also, the cell identity. Anyway, different tests are ongoing in the lab to verify also the correct functionality of depleted-PRs. RNA-seq data from our conditional Otx2-KO showed, surprisingly, a downregulation of RPE genes, even if KO is specific for PRs. Moreover, by immunostaining on retina is evident a reduction of Otx2 signal even in the RPE. Those data are coherent with a possible neuroprotective mechanism based on a possible Otx2 transfer from RPE to PRs. Unfortunately subretinal injection of the AAVs driving tagged-Otx2 expression in the RPE still did not give a clear answer about Otx2 transfer, even if the feeling is really that one for different reasons.

Conclusions

We clearly confirmed a marginal role of endogenous Otx2 in the adult photoreceptors, but we are still testing their functionality when Otx2 is removed. Different datas are strongly suggesting a possibility of Otx2 transfer from RPE to PRs, but we still miss a clear proof.

Statement on proprietary interests

Acknowledgement

PFÄFFLE, CLARA

Optophysiology in the living human eye

Clara Pfäffle(1), Dierck Hillmann(1,3), Hendrik Spahr(1,2), Sazan Burhan(1), Lisa Kutzner(1), Felix Hilge(1), Yoko Miura (1,2), Gereon Hüttmann (1,2)

(1) Institute of Biomedical Optics, University zu Lübeck, Peter-Monnik-Weg 4, 23563 Lübeck (2) Medical Laser Centre Lübeck (MLL), Peter-Monnik-Weg 4, 23562 Lübeck (3) Thorlabs GmbH, Maria-Goeppert-Straße 9, 23562 Lübeck

Purpose
OCT has been established as a standard imaging technique in ophthalmology. Morphological changes in the µm-range give us the possibility to diagnose retinal diseases and track their progression. However in most cases an irreversible damage is already done when first morphological changes occur. Functional imaging of the retina could detect diseases in an earlier stage. However, functional changes are much smaller than the resolution of common OCT systems and are therefore hard to detect.

Methods
Coherence imaging methods are able to detect the phase and the amplitude of the back scattered light. The phase is much more sensitive to changes than the amplitude. However, due to scanning artifacts and eye motion the phases is rendered useless in most OCT techniques. Full-Field swept-source OCT parallelizes the acquisition of the whole field of view, thereby avoiding any disturbances of the wavefront. The resulting phase stable measurement can than be used to detect small morphological changes in the nm-range.

Results
Those phase sensitive measurements allowed us to measure small changes of the optical path length of different retinal layers. During illumination with a white light stimulus we could detect an elongation of the optical path length of the photoreceptor outer segments. The elongation were sharply limited to the area of stimulation. At the same time we observed an elongation of the optical path length between the ganglion cell and inner plexiform layer at a slightly different lateral position. We were thereby able to measure the functional connections of those two layer in dependence of its retinal position.

Conclusions
In a first attempts we showed that phase sensitive measurements are able to detect robust and reproducible intrinsic optical signals in the living human eye. Due to these measurements we were able to map the functional connection between different neuronal layers.

Statement on proprietary interests
DH is working for Thorlabs GmbH. DH and GH are listed on related patents.

Acknowledgement
Funded by the DFG (Holo-OCT HU 629/6-1)

Is myopia affecting accommodation?

Vahid Pourreza Ghoushchi, Pedro M. Pierto, Pablo Artal

Laboratorio de Óptica, Universidad de Murcia, Murcia, Spain

Purpose
It has been speculated for a long time whether altered accommodation dynamics may be an effect, cause or predictor of myopia development. Our aim is to establish whether there is a difference in the accommodation dynamics between myopic and emmetropic subjects.

Methods
A group of 11 young emmetropes and myopes (spherical refraction within 0 D to –4.75 D) with low astigmatism (less than 1 D) and normal best corrected visual acuity were studied. Accommodation dynamics were measured with an open-view binocular Hartmann-Shack sensor operating in real time. The instrument provides the subject's relative defocus and aberrations for both eyes in real time (25 Hz). Each subject underwent 3 runs of 3 cycles of accommodation and deaccommodation between a far target presented on a flat screen at 2.8 m and a near target at 0.35 m. Accommodation response, accommodation speed, convergence, convergence speed, pupil miosis, pupil miosis speed, spherical aberration change, and HOA-RMS change were measured 9 times for each subject, in 3 runs of 3 presentations each, were estimated.

Results
The average speed of accommodation ranged among subjects from 2.2 to 8.1 D/sec (mean value: 5.3 D/ sec). For a 2.5 D demand, the average lag of accommodation was 0.50 D, with wide variability in individual values from 1.3 D to -0.5 D (only one case of lead). Correlation coefficients between velocity of accommodation and other variables were: R = 0.15 with refractive error; -R = 0.078 with accommodation response; R = 0.080 with subject's age; R = 0.017 with pupil miosis speed; R = -0.254 with spherical aberration; R = -0.354 with HOA-RMS; R = 0.0.5327 convergence amplitude; R=0.168 with pupil miosis amplitude.

Conclusions
We did not find a correlation between accommodation dynamics and myopia in the studied population. Only accommodation speed was (mildly) correlated with convergence amplitude (R^2=0.28) and HOA-RMS change (R^2=0.13). Correlation analysis of the subject's refraction (SE) with accommodation dynamics parameters suggest that myopia could affect convergence speed (R^2=0.29) and accommodation response (R^2=0.24).

Statement on proprietary interests
There is no commercial interest.

Acknowledgement of funding, if applicable
This study was supported by the following grants: European Research Council Advanced Grant (SEECAT, #ERC-2013-AdG-339228); MYFUN, European Union's Horizon 2020 research and innovation programme under the Marie Sklodowska-Curie grant agreement No 675137, Secretaría de Estado de Investigación, Desarrollo e Innovación (SEIDI; #FIS2016-76163-R); Fundación Séneca - Agencia de Ciencia y Tecnología de la Región de Murcia (#19897/GERM/15); European Regional Development Fund (#EU-FEDER).

Rasmussen, Michel

Potential novel cGMP binding partners in mouse retina may be relevant in photoreceptor degeneration

Michel Rasmussen (1), Charlotte Welinder (2), Frank Schwede (3), Per Ekström (1)

1 Lund University, Faculty of Medicine, Department of Clinical Science Lund, Ophthalmology, Lund, Sweden. 2 Division of Oncology and Pathology, Dept. of Clinical Sciences Lund, Lund University, Lund, Sweden. 3 BIOLOG Life Science Institute GmbH & Co. KG, Bremen, Germany

Purpose
Within the field of inherited retinal degenerations, there is a great interest in identifying unknown cGMP-binding proteins; 1) The second messenger cGMP accumulates in degenerating photoreceptors. 2) Known cGMP-binding proteins appear over-activated in dying photoreceptors and treatments that inhibit these proteins lead to reduced cell death. 3) There may however be yet other cGMP-binding proteins that contribute to the degeneration. The present project aims to find such.

Methods
Retinas from enucleated rd2 eyes were dissected followed by homogenization. Affinity chromatography using beads with immobilized cGMP, chemically linked at different positions, were used to enrich for cGMP-binding proteins, which were eluted with an excess ligand, i.e. cGMP. rd2 samples were subjected to mass spectrometry, where the enriched cGMP-binding partners were identified using Proteome Discovery 2.3. Final selection of the identified proteins was conducted using BLAST (Basic Local Alignment Search Tool) to search for possible conserved ligand binding domains similar to cGMP-dependent protein kinase I.

Results
The identified proteins from the rd2 samples included the presence of cGMP-binding partners like PKGI, PKAI/II, PDE6, PDE5, which supported the validity of the approach. However, also multiple indirect proteins like anchor proteins, which are known to interact with cGMP-binding partners, were identified. The BLAST search selected 20 proteins out from the identified proteins - 10 of which are known cGMP-binding partners and 10 potential novel cGMP-binding partners.

Conclusions
This emphasizes that the developed protocol for enriching for cGMP-binding partners in RP-retinas works. Multiple proteins that may bind indirectly are likewise identified, making the identification of direct cGMP-binding partners more difficult, although it at the same time may improve our understanding of the intracellular disease mechanism.

Statement on proprietary interests

Acknowledgement
This work was performed within the transMed program, which receives funding from the European Union's Horizon 2020 research and innovation program under the Marie Sklodowska-Curie grant agreement No 765441

Rodríguez Bocanegra, Eduardo

Potential innate immune responses to adeno-associated virus mediated gene therapy

E. Rodríguez-Bocanegra 1-2, K. Bucher 1-2, D. L. Dauletbekov 1-2, M. Bonillo 1-2, J. Pfromm 1-2, M. D. Fischer 1-2

1. University Eye Hospital and 2. Institute for Ophthalmic Research, Centre for Ophthalmology, University Hospital Tübingen, Tübingen, Germany.

Purpose
Adeno-associated virus (AAV) vectors have been established as a gold standard in terms of safety and efficacy in retinal gene therapy. However, previous studies revealed that AAV could induce innate and adaptive immune responses potentially compromising the therapeutic success. Here we aim to evaluate the pattern recognition pathways which are activated in an innate immune response to AAV serotype 8 using human differentiated THP-1 cells as a model for macrophages.

Methods
THP-1 cells were differentiated into macrophages by incubation with 100nM phorbol 12-myristate 13-acetate (PMA) and subsequently incubated with AAV8-CMV-eGFP at different multiplicities of infection (MOI; 1:10⁴, 1:10⁵, 1:5x10⁵ and 1:10⁶). Bacterial lipopolysaccharide (LPS) served as positive control. Supernatant was collected at various time points after stimulation and analysed for the presence of inflammatory cytokines and chemokines (IL-1β, TNF-α, IL-8, MIP-1α, MIP-1β) and type I IFNs (IFN-β) release using ELISA.

Results
AAV8-CMV-eGFP induced the release of TNF-α, IL-1β, IL-8, MIP-1α and MIP-1β but did not stimulate the production of IFN-β. The time when this release peaked varied between the different cytokines/chemokines. For all cytokines/chemokines the response was dose-dependent: highest at the two higher MOIs (1:5x10⁵ and 1:10⁶) and low or absent with the two lower MOIs (1:10⁴ and 1:10⁵). All cytokines were detected after stimulation with LPS.

Conclusions
AAV2/8 can trigger a dose-dependent inflammatory innate immune response in differentiated THP-1 cells which served as a model for human macrophages. In a next step, this model will allow us to dissect the dynamics of the relevant pattern recognition pathways involved in the innate immune response to AAV.

Statement on proprietary interests

Acknowledgement
Deutsche Forschungsgemeinschaft (DFG, German Research Foundation). Project number: D.27.15236

Roy, Akanksha

Towards assessment of phospho signaling in retinal tissues in vitro and in vivo by kinase activity profiling

Akanksha Roy 1,2, Riet Hilhorst 1, Wesseling S 2, Ivonne MCM Rietjens 2, John P Groten 1

1 PamGene International BV, 's-Hertogenbosch, The Netherlands 2 WUR Department of Toxicology, Wageningen, The Netherlands

Purpose
Cyclic guanosine-3',5'-monophosphate(cGMP) is a key molecule for signal transduction.It activates cGMP-dependent kinase(PKG).RD is characterized by very high cGMP levels in the photoreceptors which leads to (over)activation of PKG thus making cGMP analogues promising candidates for treatment of RD.The aim of this project is to develop clear-cut readouts (biomarkers) of cellular protein phosphorylation signatures induced by novel cGMP analogues that have major effect on cell signaling processes.

Methods
Substrates for PKG1&2 were identified on PamChip®microarray comprising of142Ser/Thr containing peptides.Each peptide represents a13amino acid sequence derived from phosphorylationsites in humanproteins.Phosphorylation is detected with fluorescently labeledSer/Thr antibodies.The effect ofATP,cGMP&analogues on kinase activity was investigated for recombPKG1&2,661Wcell lysates&retina tissue from rd1mice model.Samples were calibrated by proteincontent/array&signals analyzed by BioNavigator®software.

Results
Relevant peptides were identified based on 1. phosphorylation status and dependency on the ATP, cGMP & cAMP concentration 2. by the effect of selective PKG activators and inhibitors. Among those bona fide peptide substrates for PKG1 and PKG2, novel substrates were identified and the Ka for cGMP and cAMP was determined. Furthermore, kinase activity in the retinal cell lysates was shown to be modulated by cAMP, cGMP and cGMP analogues.

Conclusions
PKG substrate preference and modulation of PKG activity by cGMP analogues as measured on peptide microarrays provides a useful tool to study the effect of cGMP analogues and novel PKG inhibitors to assess retinal degeneration in an isolated and more complex environment like photo receptor cell lysates or the whole retina.

Statement on proprietary interests

Acknowledgement

PRESERVATION OF RETINAL SYNAPSIS AND VISUAL FUNCTION WITH HUMAN PROINSULIN IN THE rd10 MOUSE MODEL OF RETINITIS PIGMENTOSA.

Alonso Sánchez-Cruz, Alberto Hernández-Pinto, Concepción Lillo, Carolina Isiegas, Miguel Marchena, Fátima Boch, Ignacio Lizasoain, Pedro de la Villa, Enrique J. de la Rosa and Catalina Hernández-Sánchez

Lab 3D Centro de Investigaciones Biologicas (CIB-CSIC) Facultad de Medicina (Universidad Complutense de Madrid) Universidad de Alcala de Henares Universidad Autonoma de Barcelona

Purpose
Retinitis pigmentosa is a group of inherited retinal dystrophies. Retinitis pigmentosa courses with the dysfunction and death of photoreceptors, leading to blindness. Our group is focused in the development of neuroprotective strategies aimed to extend the visual function. One promising molecule is proinsulin (Pi), the insulin precursor. Our goal is to determine the mechanism of proinsulin visual function protection and to establish a gene therapy strategy to deliver proinsulin.

Methods
Insulin receptor expression was analyzed by PCR and immunostaining in wild-type (WT) and rd10 retinas. Analysis of retinal synapsis was carried out by immunostaining and electron microscopy. Adeno-associated viral vectors carrying the human Pi cDNA (AAV-hPi) were administrated to rd10 mice at P12 by intramuscular injection. hPi levels were determined by ELISA. The effects of Pi were examined at P30. Retinal function was assessed by electroretinography (ERG) and optomotor test.

Results
The predominant isoform of the Insulin Receptor (IR) present in the retina was the IRA. IR was widely expressed in the retina, but enriched in axons of horizontal and ganglion cells. The IR levels were decreased in the axons of horizontal cells of the rd10 retina. The analysis of the OPL synaptic connectivity unraveled that the rd10 retinas display a decreased proportion of triad synapses and an increased rate of rod terminals lacking post synaptic partners. AAV-hPi administration in rd10 mouse resulted in preservation of both rod-bipolar and rod-horizontal synapses. Moreover, treated rd10 mice showed a delay in visual function loss.

Conclusions
IRA, that drives proinsulin signaling, is the predominant isoform in the retina. IR is highly enriched in horizontal cell axons and this expression is downregulated in the rd10 mouse with the progression of retinal degeneration. Parallel to that, we observed aberrant synapses in the OPL of the rd10 retinas. Sustained production of hPi preserved photoreceptor cells and synaptic connectivity, as well as vision in the rd10 mouse. No metabolic alterations were observed upon hPi treatment. All together, these results suggest that proinsulin is a multi- facet neuroprotective factor and a potential therapy for Retinitis pigmentosa.

Statement on proprietary interests
None

Acknowledgement
This work was supported by grant SAF2016-75681-R

Sanz Diez, Pablo

Accommodative response following contrast adaptation.

Pablo Sanz Diez 1,2, Siegfried Wahl 1,2, Frank Schaeffel 2, Arne Ohlendorf 1,2.

1) ZEISS Vision Science Lab, Institute for Ophthalmic Research, University of Tuebingen, Elfriede-Aulhorn-Strasse 7, Germany 2) Institute for Ophthalmic Research, University of Tuebingen, Elfriede-Aulhorn-Strasse 7, Germany

Purpose
To analyze the effect of adaptation to contrast on the accommodative response (AR).

Methods
10 myopic subjects (mean age 25.20±2.89, mean spherical refraction: -2.91±1.36D) and 10 emmetropic subjects (mean age 25.70±2.33, mean spherical refraction: -0.19±0.14D) participated in the study. Subjects were exposed to natural grayscale videos whose blur and contrast were manipulated digitally in the Fourier domain. A 2-stage procedure was used: (1) to determine the minimum spatial frequency content necessary to produce a proper AR on an individual level; and (2) measuring the accommodation response, before and after adaptation to low-pass (s=-0.5), control (s=0.0) and high-pass (s=+0.5) filtered videos. The adaptation period lasted 60 seconds and was preceded earlier and later by a video with the spatial frequency content calculated in the first stage. The videos subtended 8° of visual angle and were displayed on a monitor positioned at a fixed distance of 50cm. Subjects were corrected with their distance correction and the AR was continuously monitored under monocular viewing conditions using eccentric photorefraction with a sampling rate of 80Hz.

Results
In stage (1), myopes required an average of 10.00±4.05cpd, while emmetropes required 4.80±1.60cpd ($p<0.01$). (2) After adaptation to low-pass the AR increased by 0.41±0.33D in the myopic group, however emmetropes showed an inverse trend with a reduction of 0.31±0.25D in AR ($p<0.01$ for both). After adaptation to high-pass manipulated videos, both groups showed similar results with an increase in the AR of 0.41±0.40D and 0.46±0.29D for myopes and emmetropes, respectively ($p<0.01$ for both). The control paradigm showed a stable AR without any increase of the AR, for both refractive groups ($p=0.82$, for myopes and $p=0.47$, for emmetropes).

Conclusions
The accommodative response is affected by the target spatial frequency information. Our measurements suggest that contrast adaptation can have a significant short-term effect on the accommodative response.

Statement on proprietary interests
The authors declare that they have no conflict of interest.

Acknowledgement
European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement (grant number 675137).

Scheel, Constanze

The Role of Tet3 in the Retina

Constanze Scheel1, Victoria Splith1, Franziska Traube2, Florian Giesert3, Wolfgang Wurst3, Thomas Carell2, Martin Biel1, Stylianos Michalakis1

1 Munich Center for Integrated Protein Science CIPSM at the Department of Pharmacy – Center for Drug Research, Ludwig-Maximilians-Universität München, Germany 2 Center for Integrated Protein Science at the Department of Chemistry, Ludwig-Maximilians-Universität München, Germany 3 Institute of Developmental Genetics, Helmholtz Zentrum, München, Germany

Purpose
Epigenetic mechanisms include posttranslational histone modifications, regulatory non-coding RNAs and DNA methylation. In mammals, DNA methylation mainly occurs at cytosines and is usually associated with the repression of gene transcription. Importantly, the Tet (ten-eleven translocation) protein family of dioxygenases catalyse the oxidation of the epigenetic mark 5mC to 5hmC which is considered as the initial step of active DNA demethylation and generally results in gene activation.

Methods
The hypothesis is that Tet3 enzymatic activity is crucial for proper retinal development and function. The aim is to analyse the role of Tet3 in neuronal differentiation and CNS function using tissue and isoform specific Tet3 KO mice and different methods such as immunohistochemistry, qRT-PCR, mass spectrometry and RNA-seq analysis.

Results

Conclusions
Factors such as aging, inflammation and oxidative stress influence the epigenetic state of retinal neurons and play a key role in the development of retinal disorders. This approach should lead to a better understanding of the mechanisms of Tet3 action in healthy and diseased retinal and cerebral neurons.

Statement on proprietary interests

Acknowledgement

Schneider, Sandra

Ablation of primary cilia exclusively in the RPE leads to a pathogenic phenotype and consequent retinal degeneration

Sandra Schneider, Viola Kretschmer, Elisabeth Sehn, Helen Louise May-Simera

Institute of Molecular Physiology, Johannes-Gutenberg University, Mainz, Germany

Purpose
Primary cilia dysfunction promotes retinal degeneration. So far, most research has focused on the highly specialized primary cilium, the photoreceptor outer segments (POS). However, our previous work showed that primary cilia in the retinal pigment epithelium (RPE) are essential for its development and function. Considering this, we aim to examine the importance of primary cilia in the RPE for RPE development, retinal degeneration and visual function via ablation of cilia exclusively in the RPE.

Methods
For knockout-validation, we used immunofluorescence microscopy to determine ciliation of the RPE. To characterize effects of Ift20 knockout in the RPE on the phenotype of RPE and development of the retina, we used immunofluorescence microscopy and transmission electron microscopy. Visual assessment was performed by electroretinogram (ERG), to check for visual function of aging mice.

Results
Using the Cre/LoxP-System, we were able to dramatically reduce ciliation in the mutant RPE. Since our previous data showed that primary cilia are essential for regulation of WNT signaling during RPE maturation, we first examined the expression of β-catenin in RPE flatmounts at P0. In Ift20 cKO β-catenin expression was higher and increased expression could be detected in the cytoplasm. Subsequent phenotypic analysis revealed severe changes, including pigmentation, cellular morphology, subretinal holes and accumulation of subretinal deposits. ERG of the Ift20 cKO mice revealed a reduction in scotopic and photopic response over time.

Conclusions
Our study shows for the first time that ablation of primary cilia exclusively in the RPE leads to a wide range of defects that lead to vision impairments, even though the highly specialized primary cilia in the retina are not affected. Due to misregulated ciliary signaling, the RPE develops pathological defects leading to a non-fully functional tissue. Since the RPE is indispensable for vision, this research is necessary to fully understand which events lead to RPE dysfunction and consequently retinal degeneration and vision loss.

Statement on proprietary interests

Acknowledgement

Schneider, Sandra

Possible role of epithelial-to-mesenchymal transition underlying retinal pigment epithelium phenotype in cilia mutant mice

Sandra Schneider, Sonja Boxhorn, Helen Louise May-Simera

Institute of Molecular Physiology, Johannes-Gutenberg University, Mainz, Germany

Purpose
Primary cilia, microtubule-based organelles, are present on almost every eukaryotic cell type and play important roles in physiological and developmental processes via regulation of signaling pathways. Dysfunction leads to retinal degeneration. So far, most research has focused on the highly specialized primary cilium of the photore-ceptor outer segments. However, our previous work showed that primary cilia in the retinal pigment epithelium (RPE) are essential for its development and function.

Methods
Since Bardet-Biedl syndrome (Bbs) genes encode for ciliary trafficking proteins es-sential for ciliary maintenance and function, we used Bbs-deficient mice to model the effect of cilia dysfunction on RPE maturation and maintenance via gene/protein expression and morphological analysis of RPE flatmounts.

Results
Gene expression analysis of visual cycle genes, considered as markers for RPE maturity, revealed maturation defects in Bbs8-/- mice compared to controls. This might be due to misregulation of miRNA-211. Targets of this miRNA are among oth-ers, genes associated with epithelial-to-mesenchymal transition (EMT). We observed that Bbs8-/- mice showed a trend towards EMT-like signature gene expression. Im-munofluorescence microscopy revealed severe changes in RPE cell morphology and patterning and abnormal microvilli compared to controls. Furthermore, a re-emergence of primary cilia in mature tissue was observed in Bbs8-/- RPE.

Conclusions
A fully functional RPE, which is indispensable for vision, is critically dependent on its maintenance of an epithelial phenotype. EMT, accompanied by trans-differentiation of epithelial into mesenchymal cells, is initiated and controlled by the convergence of many signaling pathways. Since the main function of primary cilia is to co-ordinate numerous signaling pathways, it is highly likely that dysfunctional ciliary signaling underlies the possible EMT phenotype in ciliary mutants. These results highlight the important role of primary cilia in the RPE, which needs to be considered when designing treatment strategies for retinal degeneration.

Statement on proprietary interests

Acknowledgement

Gene therapy to increase and maintain light sensitivity in

C. SIMON, D. DALKARA

Institut de la Vision

Purpose
Rod-cone dystrophy (RCD) is a heterogeneous group of inherited retinal diseases. The majority of RCD mutated genes are expressed in the rod photoreceptors and in the retinal pigment epithelium. Yet, the phenotype is the same and is characterized by the degeneration of rods followed by degeneration of peripheral cones, which leave the patients with tunnel vision in mid stages and blindness in the latest stages of disease. A previous study showed that halorhodopsin, a microbial chloride pump, expressed in mice cones, restored these cells' activity albeit with high light intensities for activation due to the lack of intracellular signal amplification. In order to develop a light sensitive cone reactivation strategy, we first examined the expression of the phototransduction cascade elements in cones during degeneration in RCD mouse models and patients.

Methods
Subretinal injection of a membrane-hyperpolarizing target channel activated by G proteins recruited by cone opsin in degenerating cones

Results
We found that opsin and arrestin migrate to the cone cell bodies after outer segment loss. We thus hypothesized that cone reactivation based on cone opsin signalling may be feasible which in turn will allow us to recover high sensitivity vision. The ectopic expression of a membrane- hyperpolarizing target channel activated by G proteins recruited by cone opsin in degenerating cones improved visual function in two RCD mouse models.

Conclusions
In RCD patients, we found the same phenotype as in the mouse models ensuring a possible clinical translation. This new approach has the potential to restore, for the first time, high acuity and color vision requiring only low light intensities.

Statement on proprietary interests

Acknowledgement

Solaki, Maria

Functional assessment of CNGA3 variants using an aequorin-based bioassay

Maria Solaki, Peggy Reuter, Susanne Kohl, Bernd Wissinger

Centre for Ophthalmology, Institute for Ophthalmic Research, Molecular Genetics Laboratory, University of Tuebingen, Germany

Purpose
Achromatopsia (ACHM) is a rare autosomal recessively inherited disease impairing cone photoreceptor function. 33% of ACHM patients carry mutations in CNGA3, which encodes the CNGA3 subunit of cyclic nucleotide-gated (CNG) channels, an essential component of the cone phototransduction cascade. Over 50 missense variants in CNGA3 with uncertain pathogenicity are published. Thus, we aim to establish an expert-based assessment of CNGA3 variants combining in silico and functional analysis.

Methods
Wildtype (WT)/mutant CNGA3 channels and the calcium-sensitive photoprotein apoaequorin were expressed heterologously in HEK293 cells. The luminescence was recorded before and after application of the membrane permeable CNG channel agonist 8-Br-cGMP for 60 seconds. The overall luminescence signal and the timing of the signal maximum (tmax) were evaluated. In silico analysis of CNGA3 variants was conducted using the Alamut Genova software.

Results
To validate the bioassay, we functionally analyzed 9 previously studied and 3 uncharacterized CNGA3 variants. Two variants (P48L, V266M) showed moderately increased luminescence signals and tmax similar to CNGA3WT classifying them as probable polymorphisms. Mutant channels carrying the variants A469T, E590K, R563H, E228K, G557R or R427C exhibited altered overall luminescence intensities and a marked increase in tmax suggesting impaired channel function. Barely detectable luminescence signals, indicating severely impaired or absent channel function, was observed after expressing the CNGA3 variants R563C, F547L, P271T and A262P.

Conclusions
The CNGA3 variants A469T, E590K, E228K, G557R and R427C, which have been previously shown to impair CNG channel function, were correctly classified as pathogenic using the aequorin-based bioassay. CNGA3 channels carrying the polymorphism P48L or V266M showed – as expected – responses similar to CNGA3WT. The variants P271T and A262P, which have not been characterized previously and were classified as probably pathogenic using in silico prediction tools, could be confirmed as pathogenic variants using the bioassay. Thus, the aequorin-based bioassay is valid and suitable to functionally evaluate CNGA3 variants.

Statement on proprietary interests

Acknowledgement
This project was funded by the German Research Foundation as part of the SPP 2127: Gene and cell based therapies to counteract neuroretinal degeneration (project number: 398539671).

Stein, Niklas

Distorted VR - Simulating progressive lenses in virtual reality

Niklas Stein

Universität Münster

Purpose
Modern progressive lenses are widely used to allow distance and near-view within one lens. Some users experience motion sickness during the adaptation phase and have problems performing everyday tasks e.g. going down stairs when first using progressive lenses. To conduct the perceptual processes of different lens designs in psychophysical experiments it would be helpful, to simulate these in VR.

Methods
We created two methods to generate a progressive lens-like distortion program in real-time and set up an experiment to find possible effects of a specific lens design on the heading perception of 11 subjects.

Results
The results showed, that the heading angle on the horizontal axis was significantly underestimated in the distortion condition. A comparison with a computational model using the subspace algorithm and the same experimental parameters is still in progress.

Conclusions
VR could be used to simulate lens distortion and the first user study already led to interesting results. Future applications and more detailed simulations e.g. including distance specific blur might help to enhance the workflow of lens design in the future.

Statement on proprietary interests
-

Acknowledgement

Szatko, Klaudia

Chromatic processing in mouse retinal ganglion cells

Klaudia Szatko 1-3; Philipp Berens 1,2; Timm Schubert 2,4; Thomas Euler 1,2,4; Katrin Franke 1,2

1 Bernstein Centre for Computational Neuroscience, Tübingen, Germany; 2 Institute for Ophthalmic Research, Tübingen, Germany; 3 Graduate Training Center of Neuroscience | International Max Planck Research School, Tübingen, Germany; 4 Werner Reichardt Centre for Integrative Neuroscience, Tübingen, Germany

Purpose
Color vision is an important factor in guiding visual behavior in animals. Most mammal species are dichromatic, as are mice. They express short (S) and medium (M) wavelength sensitive opsins in cone photoreceptors (cones). While true S-cones exclusively expressing S-opsin are homogenously distributed across the mouse retina (Haverkamp et al. J Neurosci 2005), M-cones co-express S-opsin with increasing co-expression levels towards the ventral retina (Röhlich et al. Neuron 1994; Calderone and Jacobs Vis Neurosci 1995). Basically, this results in a green-sensitive dorsal and an UV-sensitive ventral retina (Baden et al. Neuron 2013; Wang et al. J Neurosci 2011). Despite this uneven opsin distribution, behavioral studies have demonstrated that mice can discriminate colors (Jacobs et al. Vis Res 2004) in the upper visual field (Denman et al. eLIFE 2018). Here, we investigate the retinal correlates of that behavior by analyzing chromatic processing in mouse retinal ganglion cells (RGCs).

Methods
To systematically examine how RGCs encode chromatic information, we used two-photon calcium imaging combined with visual stimulation and recorded light-evoked signals in RGCs at the population level. Specifically, we employed a UV and green center-surround flicker stimulus to analyze chromatic preference of RGC receptive fields.

Results
Our dataset of >8,000 recorded ganglion cell layer cells confirms that the dorso-ventral opsin expression gradient determines the spectral sensitivity of center responses of RGCs. Interestingly, we found that chromatic preferences of RGC surround responses did not strictly follow this distribution, resulting in color-opponent center-surround antagonism for ventral and, to a lesser extent, dorsal RGCs: Here, some dorsal cells showed UV-shifted surround responses, while most surround responses in the ventral retina were green-dominant. As green-sensitive M-opsin rarely occurs in the ventral retina, green surround responses likely originate from rod photoreceptors, which are green-sensitive. In our dataset, center-opponent RGCs as described in other dichromats were rare.

Conclusions
In conclusion, we identified mouse RGCs with color-opponent responses predominantly located in the ventral retina. Our results are in line with recent behavioral studies demonstrating color discrimination in the upper visual field of mice.

Statement on proprietary interests
The authors declare no competing financial interests.

Acknowledgement
This study is part of the research program of the Bernstein Center for Computational Neuroscience, Tübingen, funded by the German Federal Ministry of Education and Research and the Max Planck Society (BMBF, FKZ: 01GQ1002; MPG M.FE.A.KYBE0004).

Tolone, Arianna

New treatments for hereditary photoreceptor degeneration: Testing cyclic nucleotide analogues on organotypic retinal explant cultures

Arianna Tolone1,3, Frank Schwede2, Norman Rieger1, and François Paquet-Durand1

1Institute for Ophthalmic Research, University of Tübingen, Germany 2BIOLOG Life Science Institute Forschungslabor und Biochemica-Vertrieb GmbH, Bremen 3Graduate Training Centre of Neuroscience, University of Tübingen, Germany

Purpose
Inherited retinal degeneration (RD) is one of the major causes of blindness. Different forms of RD show an abnormal accumulation of cGMP in the photoreceptor, which increases the activity of two effectors: PKG and CNG channels. The manipulation of these targets could slow or prevent the course of the disease. Using organotypic retinal explant cultures derived from rd1 and rd10 mice, we evaluated the effects on photoreceptor cell death of second-generation cGMP analogues that inhibit PKG.

Methods
Eyes were extracted from P5 rd1 and P9 rd10 animals and the retina dissected aseptically. The retina was placed on a culture membrane and incubated in complete R16 medium at 37°C/5% CO_2. After two days of culture, retinas were treated with cGMP analogues inhibiting PKG (at 10µM and 50µM) while the remaining retinas were incubated in R16 as a control. The cultures were concluded at P11 for rd1 and P17 for rd10 and fixed with 4% PFA followed by cryoprotection in graded sucrose solutions, embedding, and cryosectioning. The protective capacities of the cGMP analogues were evaluated using TUNEL staining, an assay for cell death detection, on 12 µm-thick retinal cryosections.

Results
Our preliminary results showed that when compared to untreated rd1 and rd10 controls (100%), treatment with the compounds at a concentration of 10µM and 50µM caused a reduction of TUNEL positive, dying cells in the photoreceptor layer. One compound, DF004, showed a significant reduction of photoreceptor cell death in rd1 at a concentration of 50µM, when compared to untreated.

Conclusions
The PKG inhibitors tested thus far did in part show protective capacities in retinal explants cultures at concentrations of both 10µM and 50µM. Future studies may reveal further insights into the mechanisms of photoreceptor degeneration and the protection mediated by PKG inhibitors.

Statement on proprietary interests
FS is an employee of the company BIOLOG who generated some of the compounds tested. FPD is a shareholder of the company Mireca who retains rights to some of the compounds tested.

Acknowledgement
transMed receives funding from the European Union's Horizon 2020 research and innovation programme under the Marie Sklodowska-Curie grant agreement No 765441

On the importance of clinical trials

Heiko von der Leyen

Hannover Clinical Trial Centre, Hannover, Germany

Summary

In medical research human ingenuity continuously leads to new therapeutic approaches which are aiming at improving health and wellbeing. As a bridge to standard treatment the cornerstone of translational medicine is the conduct of clinical studies. I

n my talk I will briefly touch some historical aspects of experiments with humans and will give some examples on the challenges of fact finding in clinical studies.

Furthermore I will outline a stepwise approach to plan a bench to bedside transition of a new therapeutic principle either in form of a medicinal product or a medical device.

The basis of all clinical research, though, is the Declaration of Helsinki and the guidelines of Good Clinical Practice which ensure patient safety and data reliability.

Wagner, Johanna

Preclinical evaluation of rAAV.CNGB1 in the Cngb1 knockout mouse model of retinitis pigmentosa.

Johanna E. Wagner 1, Christian Schön 1, Catherine R O'Riordan 2, Abraham Scaria 2, Seng H Cheng 2, Martin Biel 1, Stylianos Michalakis 1

1 Center for Integrated Protein Science Munich CiPSM at the Department of Pharmacy – Center for Drug Research, Ludwig-Maximilians-Universität München, Munich, Germany 2 Gene Therapy, Rare Diseases, Sanofi, Framingham, MA

Purpose
Mutations in the cyclic nucleotide-gated channel beta 1 subunit (CNGB1) gene are known to cause retinitis pigmentosa type 45 (RP45) – an incurable blinding disease. Here, a novel recombinant adeno-associated virus vector for gene supplementation therapy of CNGB1-linked RP was developed and tested for efficacy in the preclinical Cngb1 knockout (KO) mouse model of RP45.

Methods
A recombinant AAV vector optimized for efficient expression of full-length human CNGB1 under control of a short human rhodopsin promoter was designed and used for packaging of AAV5-pseudotyped vectors (rAAV.CNGB1). rAAV.CNGB1 was tested for efficacy after subretinal delivery of three different dosages in 4 week old Cngb1 KO mice representing an early disease stage with short-term, mid-term, and long-term follow-up analysis after 2, 4, and 8 months. Expression of human CNGB1 protein was evaluated by immunohistochemistry at 10 months of age using a CNGB1-specific antibody. Efficacy was assessed in vivo by electroretinography (ERG) at rod and cone specific light stimuli and by measuring morphological preservation using optical coherence tomography (OCT). Furthermore, the behavior was observed by performing a visual water maze task.

Results
We designed and generated rAAV.CNGB1, a novel AAV vector optimized for efficient CNGB1 gene expression in human rod photoreceptors. The transgene expression assay of rAAV.CNGB1 confirmed efficient and specific human CNGB1 protein expression in rod photoreceptors. Moreover, ERG demonstrated a significant beneficial effect of the treatment at rod specific as well as at cone specific light stimuli. Furthermore, we found a significant preservation of rod outer segment structure and prolonged survival of cone photoreceptors. Finally, visual water maze demonstrated a significantly improved spatial orientation.

Conclusions
The novel rAAV.CNGB1 vector supports efficient and specific transgene expression and biological activity in the preclinical Cngb1 KO mouse model of RP45 at early, mid and late stage of the disease.

Statement on proprietary interests
This work provides the basis for one of the first AAV therapies to treat hereditary degenerative blinding diseases.

Acknowledgement
This work was funded by Sanofi.

Wagner, Sandra

Does close work affect the ciliary muscle morphology?

Sandra Wagner (1), Frank Schaeffel (2), Eberhart Zrenner (1,3), Torsten Strasser (1)

(1) Institute for Ophthalmic Research, Eberhard Karls University Tuebingen, Elfriede-Aulhorn-Str. 7, 72076 Tuebingen, Germany (2) Section Neurobiology of the Eye, Institute for Ophthalmic Research, Eberhard Karls University Tuebingen, Elfriede-Aulhorn-Str. 7, 72076 Tuebingen, Germany (3) Werner Reichardt Centre for Integrative Neuroscience (CIN), Otfried-Mueller-Str. 25, 72076 Tuebingen, Germany

Purpose
There is ample evidence for a possible relationship between myopia development and near vision. We have studied whether sustained nearwork may have an effect on ciliary muscle (CM) morphology and accommodation and whether there are differences between emmetropes and myopes.

Methods
17 emmetropic and 18 myopic students (19-25 yr; spherical equivalent right eye 0.05±0.29D and -2.50±1.09D, respectively) were included. Accommodation and CM morphology were assessed at far (0.25D) and near vision (4D) before and after a 30-min reading task at 25 cm. Accommodation response was recorded to a step pulse of 0.25D–4D–0.25D with a target presentation of 15 sec using eccentric infrared photorefraction. The right eye's temporal CM was imaged via anterior-segment optical coherence tomography. A custom-developed semi-automatic algorithm was used to determine CM thickness (CMT) profiles and CM dimensions in the perpendicular axis. Accommodation dynamics were assessed using a 4-parameter logistic fit. Pre- to post-task changes were analyzed by means of a univariate ANOVA with relative values (Δpre-post).

Results
On average, CM was thinner after reading, predominantly at 0.0 to 1.4 mm posterior to the scleral spur in emmetropes, and in a narrower region of 1.0 to 1.9 mm in myopes. The perpendicular axis was significantly reduced after nearwork ($F_{1,66}=26.313$, $p<0.001$), however without a significant impact of refractive error ($F_{1,66}=1.887$, $p=0.174$) or target distance ($F_{1,66}=0.014$, $p=0.907$). Accommodation responses for targets at infinity differed significantly before and after nearwork ($F_{1,32}=7.775$, $p=0.009$). There was also a significant effect of refractive error ($F_{1,32}=11.310$, $p=0.002$): While myopes exhibited a mean myopic shift of 0.20±0.21D, emmetropes showed little change after nearwork (-0.02±0.16D). No changes were seen in the velocity of accommodation.

Conclusions
Sustained nearwork caused thinning of the CM in both emmetropic and myopic eyes. In myopes, CMT changes are associated with a significant sustained increase in lens power. Sharing features of striated muscles, CMT was expected to rather increase after prolonged contraction but the opposite was found here. Further studies are necessary to understand possible influences of sympathetic innervation which is activated during intense nearwork.

Statement on proprietary interests
None of the authors.

Acknowledgement
Sandra Wagner acknowledges support from the Hector Fellow Academy.

Wolfram, Lasse

The impact of substrate stiffness on the expression of miRNAs in retinal pigment epithelial cells

Wolfram L., Gimpel C., Böhringer D., Schlunck G.

Klinik für Augenheilkunde, Experimentelle Ophthalmologie, Freiburg im Breisgau, Germany

Purpose
Various diseases and age-dependent processes, such as AMD and glaucoma, are accompanied by alterations in stiffness of the surrounding tissue and concomitant changes in cell differentiation and function. The aim of the project was to characterize the impact of substrate stiffness on the transcriptome of retinal pigment epithelial cells (RPE), with a distinct focus on the expression of microRNAs (miRNAs) as part of the gene regulatory network.

Methods
ARPE-19 cells were plated on polyacrylamide gels of defined substrate stiffness (30-80kPa). Small RNAs and mRNAs were isolated (Qiagen miRNeasy, RNeasy) after three weeks of confluent cultivation. RNA sample quality was assessed by a lab-on-a-chip system (Agilent Bioanalyzer), total miRNA content was determined with a fluorescence-based assay (Quant-iT). In a pilot experiment, small RNAs were studied by Next-Generation Sequencing (NGS). Real-time PCR (RT-PCR) was performed to verify NGS data.

Results
736 different miRNAs were detected by RNA sequencing, 557 of them on all substrates. 292 showed significant stiffness-dependent expression level alterations (at least twofold). Of those, 79 were more abundant on soft and 213 on stiff substrates. Several candidate miRNAs were validated further, amongst them hsa-miR-204-5p and hsa-miR-155-5p as examples of miRNAs being more highly expressed on stiff and on soft substrates, respectively. In silico gene expression analyses suggest an enrichment of gene clusters associated with the GO terms of extracellular structure organization, regulation of vascular development and regulation of cell size.

Conclusions
Substrate stiffness-dependent mechanotransduction modulates the expression patterns of small non-coding RNAs in retinal pigment epithelial cells. The miRNA fraction of small non-coding RNAs is enhanced on stiff substrates and distinct miRNAs are differentially expressed in a substrate-dependent manner. Changes in tissue stiffness with age or disease may therefore alter RPE function.

Statement on proprietary interests
The authors declare no competing or financial interests.

Acknowledgement
Doktorandenstipendium der Freunde der Universitäts-Augenklinik Freiburg e. V.

Xiao, Ting

Exploration of AON-based uORF Blockage to Counteract OPA1 Haploinsufficiency

Ting Xiao, Peggy Reuter, Bernd Wissinger

Molecular Genetics Laboratory, Center for Ophthalmology, University of Tuebingen, Germany

Purpose
To explore the modulatory effect of upstream open reading frame (uORF) presence on OPA1 translation and subsequently design and apply antisense oligonucleotide (AON) targeting the uORF to elevate OPA1 protein expression in vitro and in vivo. Thereby providing a potential route to overcome haploinsufficiency in OPA1-linked optic neuropathies.

Methods
To validate the functionality of the uORF in the human and murine OPA1 transcripts, a series of dual luciferase reporter gene constructs have been generated and are used to transfected HEK293T, HeLa and SH-SY5Y cells. Luminometric measurements are used to determine the validity and the extent to which the uORF in OPA1 contribute to protein expression in vitro. A series of chemical modified AONs targeting uORF translation initiation sites will be used to transfect mammalian cells and test reporter expression by luminescence. Finally selected AONs will applied to treat patient-derived primary fibroblasts with known OPA1 mutation and tested in vivo in mouse eyes to check the efficacy of the AONs in increasing the OPA1 expression in retinal ganglion cells.

Results
The 5' untranslated region including the uORF is highly conserved in mammals. Moreover, we noted a ribosome occupancy profile indicative for the translation of the OPA1 uORF. We are currently validating DLR reagents and luminometer instruments in order to determine the linear range of the luminescence response for both Firefly and Renilla luciferases and to establish an optimized protocol for a sensitive and reliable reporter assay.

Conclusions
Not available.

Statement on proprietary interests
No.

Acknowledgement
Funded by a grant of the Deutsche Forschungsmeinschaft [WI1189/11-1] within the framework of the ERA-NET E-Rare 3 project 'TreatOPON'.

YAMASHITA, AKANE

A simulation analysis of the photoresponses of rod photoreceptors.

***Yamashita Akane[1], Kamiyama Yoshimi[1]**

[1] Aichi Prefectural University, Nagakute, Japan

Purpose
In order to understand the membrane electrical properties, which is responsible for sending messages to second-order neurons, the computational model of the rod outer and inner segments was developed.

Methods
The model is based on the physiological characteristics and the previous studies, i.e., the model of the outer segment (OS) (Dell'Orco et al. (2009)) and the model of the inner segment (IS) (Kamiyama et al. (2009)). To evaluate the model, the photocurrent in the OS and photovoltage in the IS were analyzed under various conditions, including the OS associated disorders.

Results
The model reproduced the photoresponses observed in experiments and previous studies.

We simulated light responses in the LCA (Leber's congenital amaurosis) condition by changing the model parameters in terms of a reaction for rhodopsin reconstitution. It was found that the change of photocurrents in LCA condition was much lower than that in control condition, which results in little changes of the membrane potential. The results well corresponded to the disappearance of ERG in LCA patients.

Conclusions
The present model is capable of accounting for many features of rod responses.

Statement on proprietary interests
None

Acknowledgement of funding, if applicable
None

Yiyi, Chen

Studies into the energy metabolism of the retina

Yiyi Chen

Institute for Ophthalmic Research, Raum 2112, Cell Death Mechanisms Group, Elfriede-Aulhorn-Strasse 7

Purpose
improve understanding of retinal energy supply and consumption, as well as dysregulation in diseases

Methods
Histology, immunostaining and TUNEL assay,Organotypic retinal explant cultures

Results
Monocarboxylate transporter 1 and the glucose transporter 1were expressed in retinal pigment epithelium and photoreceptor inner segments , respectively. Organotypic retinal explant cultures treated with the MCT1 inhibitor AZD3965, showed an increased number of dying cells in the outer nuclear layer (ONL).

Conclusions
Photoreceptors energy metabolism critically depends on the import of lactate.

Statement on proprietary interests
The authors declare that there is no duality of interest associated with this manuscript.

Acknowledgement
china scholarship council

Zamboni, Davide

Rhodopsin Kinase (GRK1) affects the affinity for Ca2+ of Recoverin

Davide Zamboni, Matteo Riva, Valerio Marino, Karl-W. Koch, Daniele Dell'Orco

Department of Neurosciences, Biomedicine and Movement Sciences, Section of Biological Chemistry, University of Verona, Verona, Italy. Department of Translational Research and of New Surgical and Medical Technologies, University of Pisa, Pisa, Italy. Department of Neurosciences, Biochemistry Group, University of Oldenburg, Carl-von-Ossietzky-Strasse 9-11, D-26129 Oldenburg, Germany

Purpose
Recoverin (Rec) is a Ca2+-sensor protein that regulates the activity of Rhodopsin Kinase (GRK1). One of the questions about the Ca2+-induced conformational change necessary for this regulation regards the binding of this ion to Rec. Previous studies revealed an apparent Rec affinity for Ca2+ around 17 μM, incompatible with physiological conditions. The aim of this study is to verify the hypothesis that GRK1 may exert an allosteric effect on Rec resulting in increased Rec affinity for Ca2+.

Methods
The effects of a GRK1 peptide either with GST tag (GST-GRK1) or a solubility tag (NRK1-25) on Rec apparent Ca2+-affinity were determined with different techniques. The variation in the microenvironment of aromatic residues upon Ca2+-binding was monitored by Circular Dichroism, FRET (Förster Resonance Energy Transfer) with hydrophobic probe ANS and intrinsic Trp fluorescence. The competition assay with chelator BAPTA-5N-OG allowed the estimation of the affinity of each Ca2+ binding site.

Results
All the techniques showed similar apparent affinity values for both Rec alone and in complex with NRK1-25. Results suggest a significant increase of the Rec affinity for Ca2+ in the presence of NRK1-25, reducing of nearly a half the apparent dissociation constant values of Rec from ~20 μM to ~10 μM. In addition, FRET experiments showed a slight increase of the Hill coefficient upon NRK1-25 binding, from ~0.87 to ~1.40. The discrepancy between our values and those reported in literature lie in substantially different techniques used to evaluate them.

Conclusions
The two peptides used showed a different behavior: NRK1-25 clearly indicate a positive allosteric effect exerted by GRK-1 on the apparent affinity of Rec for Ca2+, nevertheless it is not strong enough to explain completely how this interaction takes place under physiological conditions. On the other hand, GST-GRK1 showed only minor effects and mainly on the Hill coefficient. This could be explained by the different size of the two tags (6 residues for NRK1-25 vs 35 KDa for GST-GRK1) that could either sterically hinder the interaction, or could also interact unspecifically with Rec.

Statement on proprietary interests

Acknowledgement

Zhou, Jiaming

Proteins controlled by cGMP-dependent protein kinase G (PKG) in normal and degenerating retinas

Jiaming Zhou, Per Ekström

Faculty of Medicine, Lund University, 22184 Lund, Sweden

Purpose
High levels of photoreceptor cyclic guanosine-monophosphate (cGMP) induce over-phosphorylation via cGMP dependent protein kinase G (PKG) to affect the photoreceptors' wellbeing. The vasodilator-stimulated phosphoprotein (VASP) has been reported as a PKG substrate in the field of retinal degeneration. The aim of this study is to explore the cGMP-PKG downstream signaling via the evaluation of VASP activity after modulation of the cGMP-PKG system to gain more insights for further investigation.

Methods
PKG-activation/-inhibition experiments were performed in organotypic retinal explants from wild type and retinal degeneration mouse models (WT, rd1). Activation and inhibition of PKG was obtained via addition of suitable cGMP analogs during the explant culture with varied dose (10, 50 or 100 µM) and treatment time (1, 2 or 4 h). Immunostaining and image analysis were used to compare the phosphorylated (activated) VASP status in the treated retinas with the relevant no-drug control samples (NC).

Results
For WT, increased photoreceptor phosphorylated VASP (pVASP) was observed when retinas were treated with high dose PKG activator (100 µM) at all treatment times. For the medium dose treatment (50 µM), the pVASP levels increased after 1 and 2 h, and dropped to similar level as NC after 4 h. pVASP was elevated after 2 h by 10 µM treatment, while it was lower at other times. For rd1, where pVASP had been elevated by the disease, high and low concentration of inhibitor counteracted the VASP phosphorylation, with stronger and weaker effects, respectively. For the 50 µM treatment, pVASP was increased at 1 h compared to NC, but dropped at 2 and 4 h.

Conclusions
These results demonstrate that appropriate addition of cGMP analogs in ex vivo can be used to modulate the cGMP-PKG system of the photoreceptors and hence provide insights of PKG substrate activities in different situations, including under normal and degenerative conditions. The results will offer clues for further studies in the downstream signaling.

Statement on proprietary interests

Acknowledgement
This study is supported by Marie Sklodowska-Curie grant agreement No 765441, Stiftelsen för synskadade i f.d. Malmöhus län, and Ögonfonden.

Zobel, Lena

In vivo characterization of AAV capsids for gene therapeutic usage.

Lena Zobel (1), Johanna Wagner (1), Marina Pavlou (1), Martin Biel (1), Stylianos Michalakis (1)

(1) Center for Integrated Protein Science Munich CiPSM at the Department of Pharmacy – Center for Drug Research, Ludwig-Maximilians-Universität München, Munich, Germany

Purpose
Retinitis pigmentosa (RP) is an incurable blindness-causing disease characterized by a degeneration of rods and subsequently also of cones. Many genetic mutations are known to be involved in RP and therefore supplementation of therapeutic genes is a promising approach for preventing the disease progress. Here, new AAV capsid variants were tested for their efficiency as gene delivery vehicle in BL6/J wildtype mice.

Methods
Different AAV variants expressing either eGFP or mCherry under control of an optimized short human rhodopsin promoter were for lateral spreading and cross-sectional localization after subretinal delivery in BL6/J wildtype mice. Expression of EGFP and mCherry was evaluated by fundus fluorescence and scanning laser ophthalmoscopy (SLO) as well as immunohistochemistry and rRT-PCR.

Results
SLO and fundus fluorescence imaging demonstrated that eGFP and mCherry expression increased with time after injection and ultimately decreased again until sacrifice after 2 months. Moreover, all tested AAV capsids showed very characteristic spreading capacities. As already known, AAV8 was able to transduce retinal cells more efficiently than AAV5 and the gold-standard AAV2. We also found an increased transduction of retinal cells by Anc80, which is an ancestor of most AAV serotypes, and therefore might be a promising capsid option for AAV-based gene therapy.

Conclusions
Various AAV variants show different spreading capacities after subretinal injection and therefore offer advanced possibilities in gene therapeutic approaches for retinitis pigmentosa and other retinal diseases.

Statement on proprietary interests

Acknowledgement

Courtesy Sponsored By

Contact Details

European Vision Institute EEIG

Main Office
Rue du Trône 98
B-1050 Bruxelles
Belgium

Tel: +41 (0)79 298 31 42
Fax: +41 (0)44 252 04 12
e-mail: info@europeanvisioninstitute.org
Website: www. europeanvisioninstitute.org

The Liaison Office

European Vision Institute LO Tübingen | SWM
Elfriede-Aulhorn-Straße 7, D-72076 Tübingen,
Germany

Fon: +49 7071 29-87644
Fax: +49 7071 29-3774

final Version 06 - 2019-07-01