Young Researcher Vision Camp

An international Career building Symposium

2015

Castle Wildenstein
Leibertingen
Germany

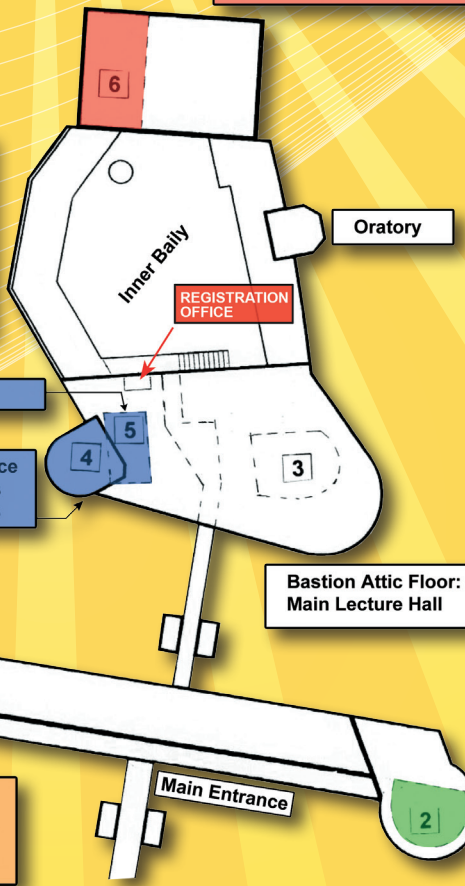
www.vision-camp.eu

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

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Preamble

Dear Colleagues,
Dear Participants of the Young Researcher's Vision Camp,

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

The Power to Shape Your Future in Vision Research and Ophthalmology in particular targeting young people from the field

and its main mission to develop innovative possibilities for young academics (clinicians, natural scientists) from ophthalmology and vision research for their future careers in public and private organisations.

A medieval castle solely dedicated for the future of vision research and ophthalmology. A completely different approach than all existing 'traditional conferences' by combining the scientific demand (talks by doctoral students, sessions chaired by young post-docs with networking opportunities (e.g. morning exercises; barbecue), keynote lectures & round table discussions.

Thomas Wheeler-Schilling

on behalf of the organising committee (in alphabetical order)

Michaela Bitzer
Sigrid Diether
Philipp Hunger
Norbert Kinkl
Francois Paquet-Durand
Vera Schmid
Timm Schubert

FRIDAY, JUNE 12TH, 2015

until 16:30	Arrival (for details see 'How to get there')
18:00 – 18:05	Welcome
18:05 - 19:05	**KEYNOTE LECTURE I** **„PARALLEL PROCESSING IN THE MAMMALIAN RETINA"** Heinz Wässle Max-Planck-Institute for Brain Research, Frankfurt, Germany
19:30 - open end	Open-air Barbecue

SATURDAY, JUNE 13TH, 2015

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

06:30 - 07:15	Early morning exercises
07:30 – 8:30	Breakfast
08:30 - 09:30	**SCIENTIFIC SESSION I: ‚PHOTORECEPTOR CELL DEATH: MECHANISMS AND TREATMENTS'** Chair: Francois Paquet-Durand, Department for Ophthalmology, Tuebingen, Germany • „Increased calcium influx via the synaptic terminal may contribute to cone photoreceptor cell death" Manoj Kulkarni (Institute for Ophthalmic Research, Tübingen) • „ROS-related aggrephagy in Retinal Pigment Epithelial cells" Luis Bonet-Ponce (Catholic University of Valencia) • „Pharmacological restoration of visual function in zebrafish following histone deacetylase inhibitor treatment" Conor Daly (School of Biomolecular and Biomedical Science, Dublin) • „PARP inhibition as a strategy for the treatment of hereditary retinal degeneration" Melanie Barth (Institute for Ophthalmic Research, Tübingen)
09:30 - 10:30	**SCIENTIFIC SESSION II: ‚THERAPEUTIC STRATEGIES FOR RETINAL DEGENERATIVE DISEASES'** Chair: Dominik Fischer, Nuffield Laboratory of Ophthalmology, Oxford, UK • „Serous retinopathy associated with MEK inhibition (binimetinib)" Elon H.C. van Dijk (Leiden, NL) • „Translational read-through of retinal dystrophy causing nonsense mutations" Fabian Möller (Mainz, DE) • „Pre-treatment characteristics in Choroideremia patients" Immanuel P. Seitz (Tuebingen, DE) • „Cone rescue through gene therapy in Retinitis Pigmentosa" Daniyar L. Dauletbekov (Oxford, UK)
10:30 - 11:00	Coffee Break

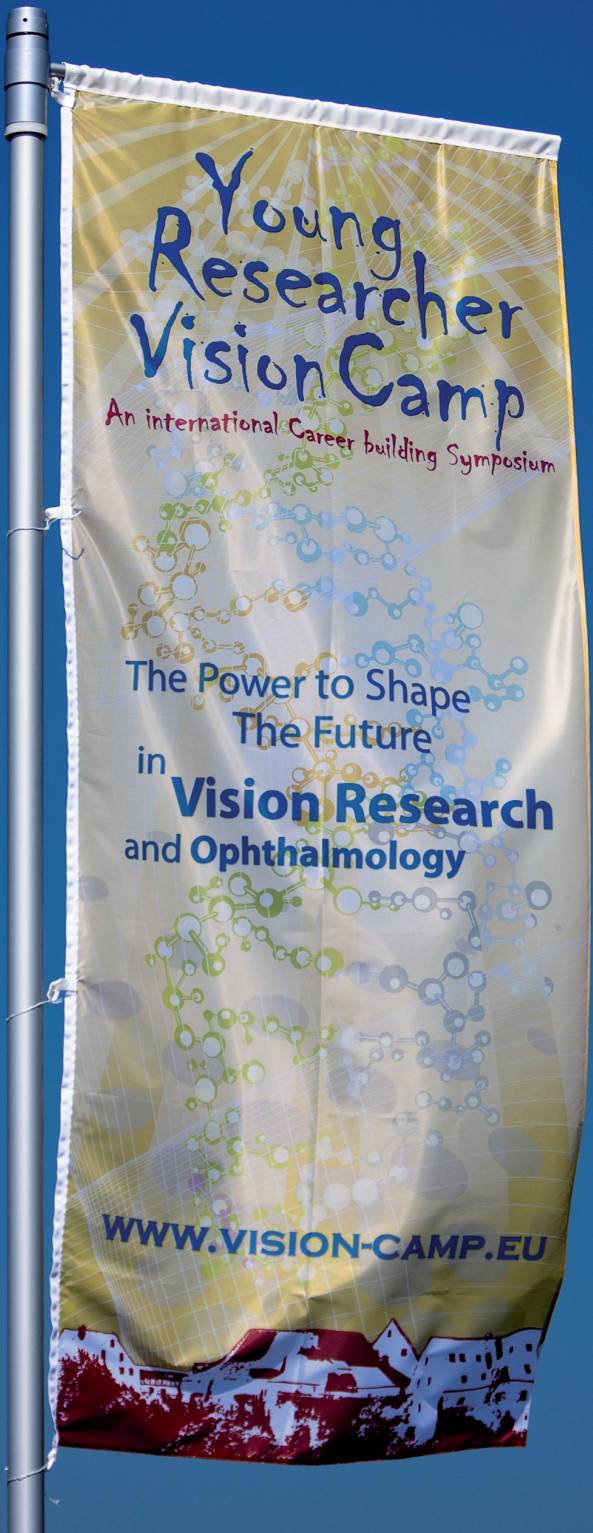
11:00 - 12:00	**Scientific Session III: ‚Spineless eyes: How invertebrates see the world‘** Chair: Emily Baird, Lund Vision Group, Lund University, Sweden • „A Twisted View of the World: Why Mantis Shrimp Rotate Their Eyes“ Ilse Daly (University of Bristol, UK) • „Seeing with primitive eyes: Vision in marine worms“ John Kirwan (Lund University, Sweden) • „Looking through the Schwarzwald: Spatial vision in bees“ Aravin Chakravarthi (Lund University, Sweden) • „Hoverfly visual responses depend upon the statistics of natural scenes“ Olga Dyakova (Uppsala University, Sweden)
12:00 - 13:00	**Scientific Session IV: ‚Neuronal wiring of the retina‘** Chair: Timm Schubert, Centre for Integrative Neuroscience, Tuebingen, Germany • „Impaired electroretinogram responses in ceramide synthase-deficient mice“ Bianca Brüggen (University of Oldenburg) • „Receptive field properties of alpha-like ganglion cells from retinas with GluA4-deficient horizontal cells“ Sebastian Ströh (University of Oldenburg) • „Retinal processing around saccades“ Saad Idrees (Centre for Integrative Neuroscience, Tübingen) • „Functional characterization of the signal processing chain in the mouse early visual system“ Miroslav Roman Roson (Centre for Integrative Neuroscience, Tübingen)
13: 00 - 14:00	Lunch
14:00 - 15:00	**Scientific Session V: ‚Immunology of the Eye‘** Chair: Marcus Karlstetter, Experimental Immunology of the Eye, Department of Ophthalmology, University of Cologne, Germany • „Interplay between complement system, inflammasome activation, and RPE atrophy in AMD“ Carolina Brandstetter (Department of Ophthalmology, University of Bonn) • „Recombinant and Endogenous Expression of Age-Related Maculopathy Susceptibility Protein 2 (ARMS2)“ Yuchen Lin (Leibniz Institute for Natural Product Research and Infection Biology, Jena) • „IFNß-treatment as a therapy targeting microglia in a murine model of retinal degeneration“ Anika Lückoff (Department of Ophthalmology, University of Cologne) • „Translocator protein (18 kDa) (TSPO) and microglia as a therapeutic target in retinal degeneration“ Rebecca Scholz (Department of Ophthalmology, University of Cologne)
15:00 - 16:00	**Keynote Lecture II** **‘On preventing degenerative retinopathies: the reality of extreme genetic heterogeneity’** Peter Humphries Trinity College Dublin, Ireland
16:00 - 16:30	Coffee Break
16:30 - 17:10	**Educational Session I** ‘Acceleration of the innovation process in research’ Siegfried Wahl, ZEISS Vision Science Lab, Germany
17:10 - 17:50	**Educational Session II** ‘How to develop a career outside of academia?’ Pieter J. Gaillard

17:50 - 18:30	**EDUCATIONAL SESSION III** 'From the idea to the invention – Intellectual Property in the industry' Adam Muschielok – Rodenstock GmbH, Germany
18:30 - 18:45	Group Photo
19:00 - open end	Poster Session
20:30 - open end	Buffet in the inner bailey

SUNDAY, JUNE 14TH, 2015

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

7:00 - 7:45	Early morning exercises
8:00 – 9:00	Breakfast
9:00 - 10:00	**SCIENTIFIC SESSION VI: ‚VERTEBRATE ANIMAL AND CELL MODELS FOR FUNCTIONAL AND THERAPEUTIC RESEARCH ON IRDS'** Chair: Erwin van Wyk, Department of Otorhinolaryngology, Radboudumc, Nijmegen, The Netherlands • „Using zebrafish as a model to develop a therapeutic intervention for USH2A-associated retinitis pigmentosa" Ralph Slijkerman (Radboudumc Nijmegen, The Netherlands) • „Generation of a zebrafish eys knock-out model for inherited retinal degeneration" Muriel Messchaert (Radboudumc Nijmegen, The Netherlands) • „Analyzing retinal degeneration in a canine model of RPE65 deficiency" Fei Song (Justus-Liebig University, Giessen, Germany) • „Towards an in vitro model for type II Leber congenital amaurosis" Florian Udry (Hôpital Ophtalmique Jules-Gonin, Lausanne, Switzerland)
10:00-11:00	**SCIENTIFIC SESSION VII: ‚VISUAL ADAPTATION TO THE NATURAL AND OPTICAL ENVIRONMENT'** Chair: Arne Ohlendorf, Zeiss Vision • „Monocular adaptation to spatially varying distortions" Selam Habtegiorgis (ZEISS Vision Science Lab, Tübingen, Germany) • „Visual perception and adaptation to bifocal patterns" Aiswaryah Radhakrishnan (Visual Optics and Biophotonics Lab, Madrid, Spain) • „Development of fine-tuned aberration" Tomasz Kozlowski (Lund Vision Group, Lund, Sweden) • „Lack of oblique astigmatism in the chicken eye" Felix Maier (Section of Neurobiology of the eye, Tübingen, Germany)
11:00 – 12:00	**POSTER AWARDS** • Carl Zeiss Award Winners Short presentations (each 5 minutes) • Charles River Award Winners Short presentations (each 5 minutes)
12:00 - 13:30	FAREWELL Lunch (optional)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Increased calcium influx via the synaptic terminal may contribute to cone photoreceptor cell death

Manoj Kulkarni (Institute for Ophthalmic Research, Tübingen)

Purpose
n/a

ROS-related aggrephagy in Retinal Pigment Epithelial cells

Luis Bonet-Ponce
Catholic University of Valencia, C/Quevedo nº1, 46001, Valencia, Spain

Retinal pigment epithelial (RPE) cells play a central role in retinal physiology, due to its localization and function. In fact, RPE pathological degeneration leads to photoreceptor damage, and subsequent blindness. Especially relevant is the one named age-related macular degeneration (AMD) because it represents the main cause of blindness worldwide. AMD is a brutal neurodegenerative disease, highly idiopathic, which makes it really difficult to address. Indeed, there is no possible treatment right now.

At a cellular level, it is well established that an imbalance of the redox status gives rise to cellular damage, and as a consequence massive RPE cell death. Unfortunately, the cellular pathways involved in the pathology are currently unknown. Thus it is crucial to study the cellular and molecular mechanisms underlying ROS-induced damage in RPE cells. Autophagy is a catabolic process that engulfs cytosolic material and transports it to the lysosome for its final degradation. Autophagy has been demonstrated to play a crucial role as a cytoprotective mechanism in RPE cells under oxidative conditions. Recent evidences have evidenced mitochondrial complex I damage as a possible actor in ROS-mediated damage in RPE cells during degeneration.

Thus we treated ARPE-19 cells with the selective mitochondrial complex I inhibitor, rotenone. We denoted a massive increase in mitochondrial ROS and 4-HNE adducts (a lipid peroxidation product). Oxidative stress produced by mitochondria leads to an increase in tubulin acetylation, which impairs autophagosome to lysosome fusion blocking the autophagy process. As a consequence, ARPE-19 cells are unable to degrade 4-HNE adducted proteins that accumulate in the cytosol.

Hence, we herein demonstrated the cellular pathway in the basis of ROS-induced damage upon mitochondrial complex I inhibition. Our work suggests a correct tubulin acetylation as a central event to assure the autophagy function, which is crucial to protect cells against oxidative stress.

Pharmacological restoration of visual function in zebrafish following histone deacetylase inhibitor treatment

Conor Daly, Eugene Dillon, Lisa Shine, Theresa Heffernan, David Duffy, Gerard Cagney, Breandán N. Kennedy
(School of Biomolecular and Biomedical Science, Dublin)

Purpose
Controversially, pharmacological inhibition of Histone Deacetylase (HDAC) proteins is in clinical trial for the treatment of inherited retinal degenerations. Previous studies report that patients suffering from the inherited retinal degeneration Retinitis Pigmentosa (RP) may show improved visual field and acuity following treatment with the HDAC inhibitor valproic acid (VPA). Utilising zebrafish models of retinal degeneration we rescued retinal morphology and visual function of a blind zebrafish mutant (dye mutant) by treatment with the HDACi Trichostatin A (TSA).

Methods
Visual function was assessed by Optokinetic Response (OKR) and Visual Motor Response (VMR) assays. Cone photoreceptor outer segment (OS) morphology, cilliary marginal zone (CMZ) apoptosis and cone photoreceptor outer segment (OS) length were assessed by light microscopy or transmission electron microscopy. Larvae were drug treated with HDACi (1 µM TSA, 10 µM MC1568 and 10 µM MS275) with or without 100-500 nM ANA-12 from 3-5 dpf at 28.5 C. Expression levels of candidate genes mediating dye rescue were analysed by quantitative real-time PCR. An unbiased shotgun proteomic analysis of TSA-treated dye eyes was carried out by LC-MS/MS and the resulting dataset analysed using Ingenuity Pathway Analysis (IPA).

Results
The dye mutant has reduced visual behaviour and several defects in retinal morphology compared to unaffected sibling larvae. HDACi treatment of dye results in improved OKR and VMR, rescue of gross morphological defects, an 80% decrease in the number of dead cells in the CMZ and an increase in cone photoreceptor OS length. Proteomic analysis identified significantly differentially expressed proteins in response to treatment, including phototransduction proteins Gnat2, Pde6c, and Gc3. qRT-PCR analysis showed increased bdnf transcription. ANA-12 treatment blocks Bdnf/Trkb signaling and HDACi meditaed rescue in dye.

Conclusions
HDAC inhibition is effective in restoring visual function and rescuing morphological defects in a zebrafish model of retinal degeneration.

Acknowledgement
Health Research Board, Fighting Blindness

PARP inhibition as a strategy for the treatment of hereditary retinal degeneration

Melanie Barth[1,2], Ayse Sahaboglu[1] and Francois Paquet-Durand[1]

[1]Institute for Ophthalmic Research, [2]Graduate School of Cellular and Molecular Neuroscience

Purpose
Inhibition of the enzyme poly(ADP-ribose) polymerase (PARP) can partially rescue photoreceptor degeneration in rd1 mice, a mouse model for inherited retinal degeneration (RD). Recently, several PARP inhibitors have been successfully tested in late phase clinical trials for cancer therapy. Here, I investigated whether some of these clinically tested inhibitors are able to rescue photoreceptor cell death in rd1 mice.

Methods
Retinas of rd1 mice were explanted at P5 and cultured in vitro under serum-free, entirely controlled conditions until P11 (i.e. 6 days in vitro; DIV). From DIV2 on, they were treated with PARP inhibitor concentrations ranging from 10 nM to 50 µM or corresponding concentrations of DMSO as solvent control. On cryosections, cell death was assessed using the TUNEL assay, while PARP activity was determined by immunohistochemical DAB staining against PAR-residues. Further immunohistochemical fluorescent stainings were performed to gain an insight into cell death related pathways, like cGMP level alterations and epigenetic changes.

Results
The outer nuclear layer (ONL) of treated retinal explant cultures displayed significantly reduced PAR positive and TUNEL positive cells. This effect was dependent on the inhibitor concentration used, with some inhibitors showing protective effects at submicromolar concentrations. While no changes in cGMP levels due to PARP inhibition were apparent, epigenetic changes could be observed in treated cultures.

Conclusion
These results confirm the importance of PARP activity for rd1 retinal degeneration and point to the ambiguity of PARP actions for cell death and survival in neurodegeneration and cancer. The efficacy of clinically tested PARP inhibitors highlights their potential for a rapid translation into a therapy for RD.

Serous retinopathy associated with MEK inhibition (binimetinib) for metastatic cutaneous and uveal melanoma

Elon H.C. van Dijk (1), Carla M.L. van Herpen (2), Marina Marinkovic (1,3), John B.A.G. Haanen (4), Drake Amundson (5), Gré P.M. Luyten (1), Martine J. Jager (1), Ellen H.W. Kapiteijn (6), Jan E.E. Keunen (7), Grazyna Adamus (5), Camiel J.F. Boon (1)

1 Department of Ophthalmology, Leiden University Medical Center, Leiden, the Netherlands

2 Department of Medical Oncology, Radboud University Medical Center, Nijmegen, the Netherlands

3 Department of Ophthalmology, Onze Lieve Vrouwe Gasthuis, Amsterdam, the Netherlands

4 Division of Medical Oncology, The Netherlands Cancer Institute Antoni van Leeuwenhoek, Amsterdam, the Netherlands

5 Department of Ophthalmology, Oregon Health and Science University, Portland, United States of America

6 Department of Medical Oncology, Leiden University Medical Center, Leiden, the Netherlands

7 Department of Ophthalmology, Radboud University Medical Center, Nijmegen, the Netherlands

Purpose
To analyse the clinical characteristics of a serous retinopathy associated with MEK inhibition with binimetinib treatment for metastatic cutaneous melanoma (CM) and uveal melanoma (UM), and to determine possible pathogenetic mechanisms that may lead to this retinopathy.

Methods
Thirty patients with metastatic CM and 5 patients with metastatic UM, treated with the MEK inhibitor binimetinib (CM) as single agent or a combination of binimetinib and the PKC inhibitor sotrastaurin (UM) were included in this study. An extensive ophthalmic examination was performed in all patients, including Early Treatment of Diabetic Retinopathy Study best-corrected visual acuity, applanation tonometry, slit-lamp examination, indirect ophthalmoscopy, digital color fundus photography, and optical coherence tomography (OCT). In selected cases, additional examinations were performed, including visual field testing with a Humphrey Field Analyser and electro-oculography (EOG). Blood samples were obtained from 3 CM and 3 UM patients to analyse the presence of autoantibodies against retinal and retinal pigment epithelium (RPE) proteins.

Results
Six CM patients (20%) and 2 UM patients (40%) reported visual complaints during the period of administration of the study medication. The median time until onset of visual complaints, which were mild and transient in all patients, was 3.5 days (range, <1 hour-3 weeks). On OCT, subretinal fluid (SRF) was detected in 77% of CM patients and 60% of UM patients. In the 26 patients with SRF, the fovea was affected in 85%, while SRF was detected extrafoveally in 85% of patients. In 19 eyes of 11 patients, an EOG was performed after the start of the binimetinib medication: 16 of these eyes (84%) developed SRF on OCT. Fifteen (94%) of these eyes showed an abnormal Arden ratio (<1.65), and 1 eye (6%) showed a subnormal Arden ratio (1.65-1.8). After the start of binimetinib treatment, a broad pattern of anti-retinal antibodies was found in 3 CM and 2 UM patients tested, while anti-RPE-antibodies were detected in all 6 patients. Anti-bestrophin antibodies were detected in 3 of these cases.

Conclusions
A time-dependent and reversible serous retinopathy can develop in metastatic CM and UM patients treated with binimetinib or a combination of binimetinib and sotrastaurin. A minority of patients develop visual complaints, which are generally mild and transient. A possible cause of binimetinib-associated serous retinopathy may be toxicity of study medication, but autoantibodies may also be involved.

Statement on proprietary interests
The authors have no proprietary or commercial interest in any materials discussed in this article.

Translational read-through of retinal dystrophy causing nonsense mutations

Fabian, Ananya, Inessa Uwe, Kerstin,
Möller F.[1], Penner I.[1], Samanta A.[1], Baasov T.[2], Wolfrum U.[1] and Nagel-Wolfrum K.[1]

[1] Cell and Matrix Biology, Inst. of Zoology, Johannes Gutenberg University of Mainz, Germany;

[2] Edith and Joseph Fischer Enzyme Inhibitors Laboratory, Schulich Faculty of Chemistry, Technion-Israel Institute of Technology, Haifa, Israel

Inherited retinal dystrophies are a genetically heterogeneous group of diseases, caused by a variety of different mutations with no effective cure available so far. Approximately 12% of pathologic mutations found in patient screenings are nonsense mutations. A therapeutic strategy targeting these mutations promises to benefit a large cohort of patients.

Nonsense mutations result in premature stop codons (PTC) in corresponding mRNAs and thereby terminate translation, resulting in truncated protein fragments. Since the termination of translation at PTCs has a lower fidelity compared to native stop codons, the read-through of PTCs using pharmacologic compounds has become a promising approach. This translational read-through allows the recovery of functional protein without interfering with gene expression and gene splicing.

We analyzed the biocompatibility and read-through efficacies of different translational read-through inducing drugs (TRIDs). These studies show lower toxicity profiles for designer aminoglycosides, such as NB84 and the structurally unrelated compound Ataluren, in comparison to canonical aminoglycosides like gentamicin. We show translational read-through of several syndromic nonsense mutations in cell culture, focusing on mutations causing human Usher syndrome (USH), Bardet Biedl syndrome and Senior Loken syndrome, all of which exhibit retinal degeneration. In addition we have demonstrated read-through of nonsense mutations in organotypic retina cultures and in vivo in mice.

Taken together our data demonstrates excellent biocompatibility, along with substantial read-through efficacies of the TRIDs analyzed. These results emphasize the feasibility of designer aminoglycosides and Ataluren for therapeutic strategies tackling retinal dystrophies caused by nonsense mutations.

Supports
German Ministry of Education and Research (E-Rare-2, the ERA-Net for Research on Rare Diseases) "EUR-USH", FAUN-Stiftung, Nuremberg, Foundation Fighting Blindness (FFB), EU FP7 "SYSCILIA", Tistou and Charlotte Kerstan Foundation,

Multimodal assessment of choroideremia patients defines pre-treatment characteristics

Immanuel P. Seitz[124], Ahmad Zhour[1], Susanne Kohl[2], Pablo Llavona[2], Tobias Peters[3], Barbara Wilhelm[3], Eberhart Zrenner[12], Marius Ueffing[2], Karl Ulrich Bartz-Schmidt[1], M. Dominik Fischer[1456]

1 University Eye Hospital, University of Tübingen, Tübingen, Germany, 2 Institute for Ophthalmic Research, University of Tübingen, Tübingen, Germany, 3 STZ Eyetrial, Centre for Ophthalmology, University of Tübingen, Tübingen, Germany, 4 Nuffield Laboratory of Ophthalmology, University of Oxford, Oxford, UK, 5 Oxford Eye Hospital Oxford, University Hospitals NHS Trust, Oxford, UK, 6 Merton College, University of Oxford, Oxford, OX1 4JD, UK

Purpose
Choroideremia (CHM) is a X-chromosomal disorder leading to blindness by progressive degeneration of choroid, retinal pigment epithelium (RPE), and retinal neurons. A current clinical gene therapy trial (NCT01461213) showed promising safety and efficacy data in a carefully selected patient population. The present study was performed to shed light on pre-treatment characteristics of a larger cohort of CHM patients using a high resolution multi-modal approach.

Methods
In a retrospective cross-sectional study, data from 58 eyes of 29 patients with clinically confirmed CHM were analysed including best-corrected visual acuity (BCVA), refractive error, spectral-domain optical coherence tomography (SD-OCT), fundus autofluorescence (FAF), perimetry, and tonometry. Residual retinal volume, area of residual RPE, and foveal thickness were quantified to further define natural disease progression and assess symmetry.

Results
We evaluated 98 data points of BCVA [0.34 ± 0.06 (logMAR); mean ± 95 % confidence interval], 80 of IOP (14.6 ± 0.6 mmHg), and 98 of refraction (−2.16 ± 1.08 spherical equivalent). Visual fields (n = 76) demonstrated variable degrees of concentric constriction (54 % 30°). Mean residual RPE area on FAF (n = 64) measured 8.47 ± 1.91 mm^2 (range 0.30–38.5 mm^2), while mean neuroretinal volume (n = 42) was found to be 1.76 ± 0.12 mm^3. Age at examination was exponentially associated with BCVA, while logarithmic functions best described progressive loss of retinal area and volume. A high degree of left to right symmetry was found in all modalities with structural markers showing the best correlation (r^2area = 0.83; r^2volume = 0.75).

Conclusions
Analysis of these widely available clinical data defines the natural disease characteristics of a relevant patient population eligible for gene therapeutic intervention. In the wake of preliminary reports on safety and efficacy of CHM gene therapy (NCT01461213), this multi-modal assessment of a cohort of CHM patients provides important evidence of the natural rate of disease progression and degree of symmetry between eyes.

Acknowledgement
Tistou & Charlotte Kerstan Stiftung

Cone rescue through gene therapy in Retinitis Pigmentosa

Daniyar L. Dauletbekov (Oxford, UK)

n/a

A Twisted View of the World: Why Mantis Shrimp Rotate Their Eyes

Ilse Daly
University of Bristol, UK

Purpose
Gaze stabilization is an almost ubiquitous animal behaviour, one that is required in order to see the world clearly and without blur. Stomatopods, however, rarely fix their eyes, choosing instead to wander over the visual field in a series of rapid pitch, yaw and torsional (roll) rotations. In this work, we demonstrate that the lack of gaze stabilization is counterweighted by an enhancement to the linear polarization vision system.

Methods
We demonstrate that the lack of gaze-stabilization induced by torsional rotation is counterweighted by an enhancement to the linear polarization vision system. Torsional rotations may be employed to preferentially align particular photoreceptors with the e-vector of a linearly polarized stimulus against an unpolarized background, or to enhance the contrast between a polarized object and a polarized background.

Conclusions
This is the first documented example in any animal of dynamic linear polarization vision, whereby the polarization information is actively optimised through eye movements.

Acknowledgement
BBSRC, AFOSR

Seeing with primitive eyes: Vision in marine worms

John Kirwan
Lund University, Sweden

Although photoreceptive organs are widespread among the Bilateria, investigation of visual systems has concentrated on a few taxa with high-perfomance image-resolving vision. Consequently, there remains a gap in our understanding of the structure and function of many visual systems, the visual information they use and (crucially) which behaviours vision facilitates. We are testing the basic visual capabilities of representatives of several invertebrate groups, including errant polychaetes, echinoderms, myriapods and onychophorans. We are chiefly concerned with the simplest forms of true 'vision': perceiving an image, however simple, from light stimuli (as opposed to simply detecting changes in intensity) and with the ecological circumstances which necessitate this.

To understand the physical limitations of simple eyes, we use microscopic imaging (from which a geometric model of structure can be developed) as well as interferometry and electrophysiological recording. Thereby, we can understand the optical properties of eyes and the parameters of light being perceived. In tandem with this, we carry out behavioural assays of the animals' visual capabilities to assess the characteristics and limitations of the animals vision in vivo and their sensory ecology. By investigating form and function of simple eyes, in their evolutionary and developmental context, we can further speculate as to their evolutionary history and to the circumstances of their origin.

Looking through the Schwarzwald: Spatial vision in bees

Aravin Chakravarthi (Lund University, Sweden)

n/a

Hoverfly visual responses depend upon the statistics of natural scenes

Dyakova, O. and Nordström, K.
Department of Neuroscience, Uppsala University, Box 593, 75124 Uppsala, Sweden

Purpose
Hoverfly habitats are very cluttered, while their visual systems allow them to perform their daily routines very well and amazingly fast. In particular, we have noticed that hoverflies seem to favor certain sites over others. We hypothesize that among other factors image statistics may play an essential role in making some sites more attractive. Here, we aim to investigate whether there is a link between visual features of the surround and the optomotor response in hoverflies.

Methods
We used image analysis to quantify image statistics that could be essential for hoverflies' visual system. We used one of these statistics, the slope of the amplitude spectrum of an image (α), which can quantifies an image's blurriness. Images with a high slope, appears to us as a very blurry. Next, we manipulated the slopes of artificial (random noise) and natural images to create panoramas with different alphas. We used these manipulated images for trackball experiments, where a tethered fly was walking on a ball and two sensors provided information about the ball's movement.

Results
We analysed the total yaw in response to the different images. We show that the optomotor response in hoverflies is influenced by the slope of the amplitude spectrum, and that the total yaw is largest when the stimuli have an alpha close to 1.2, which is close to that of a typical natural scene.

Conclusions
The slope of the amplitude spectrum of an image is in natural scenes is distributed between 0.6 and 1.7, with a peak just over 1. Thus, our data suggest that the optomotor response in hoverflies is tuned to the slopes that are most prevalent in scenes typically encountered by hoverflies.

Acknowledgement of funding, if applicable
US Air Force Research Laboratory (AFRL, FA9550-11-1- 0349)

Impaired electroretinogram responses in ceramide synthase-deficient mice

Bianca Brüggen (1), Christiane Kremser (2), Reto Weiler (1, 3), Klaus Willecke (2), Karin Dedek (1, 3)

(1) Neurobiology, University of Oldenburg, 26111 Oldenburg, Germany(2) Life and Medical Sciences Institute, University of Bonn, 53115 Bonn, Germany (3) Research Center Neurosensory Science, University of Oldenburg, 26111 Oldenburg, Germany

Purpose
Ceramides form the backbone of all complex sphingolipids. They also act as signaling molecules on a variety of intracellular cascades and are involved in autophagy and apoptosis. The de novo synthesis of ceramides in the endoplasmic reticulum is catalyzed by six ceramide synthases: CerS1-6. CerS deficiencies cause abnormalities in lipid composition and signaling cascades (such as apoptosis) in a variety of tissues, including the brain. CerS1 has been shown to be neuronally expressed in the brain, CerS2 occurs in oligodendrocytes and CerS4 is mainly expressed in the skin. However, expression of CerS – in retinal neurons and glia cells or the cornea - and the effects of CerS deficiencies on the retina have not been studied so far.We investigated retinal function and morphology in CerS1-, CerS2- and CerS4-deficient mice to find out if CerS deficiency-induced defects in the brain such as cell loss and tissue shrinkage also occur in the retina.

Methods
CerS expression in the cornea and retina was investigated using western blots. Retinal function was measured electrophysiologically by electroretinograms (ERGs) under scotopic and photopic light conditions. Retinal morphology was investigated immunohistologically, using antibodies against a variety of cell markers, receptors and synaptic proteins. Labeled photoreceptors, glutamate receptors and synaptic markers were quantified in retinal slices. Membrane composition was investigated by lipid analyses using mass spectrometry.

Results
CerS1, CerS2 and CerS4 are expressed in the cornea and retina. All tested CerS-deficient mice showed reduced responses in the ERG compared to wild-type littermates. Reductions were seen in a-wave and b-wave amplitudes and in oscillatory potentials. Cell marker stainings revealed no loss of retinal neurons, but preliminary quantification of glutamate receptors and synaptic proteins suggest differences between genotypes. Moreover, CerS1 KO and CerS4 KO mice displayed significant reduction in C18 and C20 ceramides, respectively.

Conclusions
The reduced a- and b-wave amplitudes in ERGs suggested defects at the photoreceptor synapse. These would lead to impaired signaling between photoreceptors and bipolar cells, possibly caused by changes in the size and number of receptors or synaptic proteins. ERG effects may be influenced by altered lipid and membrane compositions, potentially leading to altered electrical properties of the cornea or retinal neurons and thus altered extracellular field potentials. Mice deficient for CerS show reduced ERG signals. The underlying cause may be the altered lipid composition in cornea cells, retinal neurons and/or glia cells, potentially leading to 1) altered electrical properties of cornea and retina, 2) changes in corneal light transmission and/or 3) changes in vesicular signal transmission.

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Receptive field properties of alpha-like ganglion cells from retinas with GluA4-deficient horizontal cells

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Purpose
Center-surround antagonism is a key feature of receptive fields of retinal bipolar and ganglion cells and has been scrutinized for decades (Kuffler, 1953; Barlow, 1953; Werblin and Dowling, 1969). However, whether horizontal cells or amacrine cells or both contribute to surround antagonism in bipolar and ganglion cells remains uncertain (Masland, 2012; Thoreson and Mangel, 2012).Investigating the contribution of horizontal cells to ganglion cell surrounds proves to be difficult because (1) pharmacological approaches tend to influence other retinal cell populations and (2) simultaneous electrophysiological recordings from horizontal cells and ganglion cells are difficult to establish.To overcome this, we used a mouse line in which the ionotropic glutamate receptor subunit 4 (GluA4) is exclusively lacking in horizontal cells. As glutamate-induced currents are reduced by ~75% in GluA4-deficient horizontal cells (Ströh et al., 2013), horizontal cell impact on ganglion cell responses should also be strongly reduced. To investigate this, we compared light responses from ganglion cells in retinae with GluA4-expressing and GluA4-deficient horizontal cells.

Methods
Mice (8-12 weeks) were dark-adapted for 1 hour prior to dissection and retinae were dissected under dim red light. Whole-mounted retinae were placed onto the preparation chamber with the photoreceptor side facing downwards. For patch-clamp recordings, alpha-like ganglion cells were chosen, light-adapted (0.3 µW/cm²) for 10 min prior to seal formation and again for 10 min after establishing the seal.OFF alpha ganglion cells were stimulated with white spots of increasing diameter on a black background. For whole-cell recordings, inhibitory currents were measured clamping the cell at the reversal potential of cations (0 mV); excitatory currents were measured at the reversal potential of chloride (-73 mV). Area-response-functions were created and fitted with a difference of two Gaussians (DOG) model. Additionally, excitatory and inhibitory conductances were analyzed.For cell-attached recordings, OFF alpha ganglion cells were stimulated with increasing white spots on a dark background, and additionally with increasing white and black spots on a 50% grey background. Cells were recorded in voltage-clamp mode. Peristimulus time histograms were plotted and area-response-functions were created and fitted with a DOG-model.

Results
Preliminary results showed no differences for whole-cell recordings and conductance analyses between OFF alpha ganglion cells of retinae with GluA4-deficient horizontal cells compared with retinae with GluA4-expressing horizontal cells. Cell-attached recordings and spike analyses also showed no differences in receptive field organization of OFF alpha ganglion cells of retinae with GluA4-deficient horizontal cells. In line with Pang et al. (2003), two different OFF alpha ganglion cell populations were identified, but neither of which showed differences in center-surround antagonism between genotypes.

Conclusions
Under the current experimental conditions, no difference between genotypes was found, suggesting only a minor role for horizontal cells in generating center-surround antagonism in OFF alpha ganglion cells. As Van Leeuwen et al. (2009) suggested that the major role of horizontal cells is gain control in the outer retina, additional ganglion cell recordings may be necessary to reveal effects on light sensitivity and adaptation in ganglion cells of GluA4 transgenic mice.

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Retinal processing around saccades

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Purpose
Active visual exploration involves frequent transitions between rapid eye movements (saccades) and periods of fixation. The influences of such eye movements on visual processing have received increasing interest recently, for example to understand how perceptual stability is possible in the face of constant image displacement across the retina. Most studies rely solely on cortical or sub-cortical neuronal recordings and/or on behavioral measures of perceptual state. The retinal contribution to the observed phenomena (i.e., the visual feed-forward component) is not well understood, often resulting in diametrically opposite models of retinal involvement. This problem is further exacerbated by the fact that there is little knowledge about how retinal responses themselves are altered by eye movements. We are therefore studying the retinal contribution to active vision by characterizing the responses of retinal ganglion cells and underlying circuit mechanisms of inter-saccadic visual processing. Our central hypothesis is that retinal ganglion cells (of both mice and humans) exhibit stimulus-dependent modulations of their responses to stimuli flanked by saccade-like image displacements, and that such modulations may act to bridge sensory processing across saccades.

Methods
We record activity of ganglion cells from isolated mouse retina using multi-electrode array (MEA) while showing a visual stimulus paradigm mimicking saccades. The paradigm consists of rapid saccade-like image shifts across the retina followed by a test stimulus (in this case full field flash) at different intervals after the saccade.

Results
Our results suggest that response to the test stimulus get modulated as a function of time after the saccade-like image shifts. Furthermore, different cells show different modulation of test stimulus response. For example, some cells show suppression of activity after a saccade and then recover to baseline activity while others also show enhancement of activity before return to baseline. Moreover, different cells follow different time courses for recovering to baseline activity.

Conclusions
These results confirm our initial hypothesis that the activity of retinal ganglion cells in mice gets modulated by saccade-like image displacements and that there are different pathways leading to this modulation within the retina itself. Future work will focus on identifying the circuitry and mechanisms behind such modulations and how they relate to neural modulations observed in retina recipient areas of the brain.

Functional characterization of the signal processing chain in the mouse early visual system

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Purpose
More than 20 types of retinal ganglion cell (RGC) represent parallel channels transmitting different aspects of visual information from the retina to various parts in the brain. Retinal output is most directly conveyed to the cortex via the retino-geniculo-cortical pathway, comprised of RGCs, relay cells in the dorsolateral geniculate nucleus (dLGN) and the primary visual cortex (V1). While it has long been known that this pathway is not homogenous but consists of parallel channels each carrying specific information, it is still debated which RGC types project to the dLGN and how their output is transformed at the level of the dLGN. Here, we started to characterize, in the mouse model, the functional properties of dLGN-projecting RGCs and to compare responses of RGCs and dLGN neurons to the same set of visual stimuli.

Methods
We explored two approaches for selective labeling and physiological characterization of dLGN-projecting RGCs. First, we injected a retrograde tracer ("mini-Ruby", Molecular Probes) into the mouse dLGN. After 7 days, the retina was removed and electroporated with a synthetic calcium indicator (Briggman & Euler, J Neurophysiol 2011). Using two-photon in- vitro imaging, we recorded light-evoked calcium activity from the population of RGCs that had been labelled by the retrograde tracer. Visual stimuli included frequency/contrast modulated full-field flicker, dense noise, moving bar, and chromatic stimuli. Second, we injected an adeno-associated virus (AAV) encoding the calcium biosensor GCaMP6 into the dLGN. Through transfection of RGC terminals this leads to retinal biosensor expression, which enabled us to selectively record light-evoked calcium responses in dLGN-projecting RGCs. In a separate set of experiments, we characterized the responses of dLGN neurons to the same visual stimuli using in-vivo extracellular multi-electrode recordings in the dLGN of awake, head-fixed mice.

Results
Combining the data sets from the retina and the dLGN, we seek to build computational models that will test if and how dLGN responses can be described as specific combinations of RGC output channels and the influence of local inhibitory circuits. Specifically, we will ask if response features are simply inherited from the RGC input or if they are modified within the LGN.

Conclusions
In conclusion, this study promises to yield a functional characterization of the population of dLGN-projecting RGCs, and to provide fundamental insights into how the representation of visual information changes along the first stages of the retino-geniculo-cortical pathway.

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Interplay between complement system, inflammasome activation, and RPE atrophy in AMD

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Purpose
Photooxidative damage of the retinal pigment epithelium (RPE) is associated with the pathogenesis of age-related macular degeneration (AMD). In addition, involvement of a chronic immune response in the sub-RPE space including activation of the complement system has been demonstrated. To identify a molecular link between these mechanisms we investigated the capability of activated complement components to prime RPE cells for activation of the NLRP3 inflammasome by lipofuscin phototoxicity

Methods
Lipofuscinogenesis was induced in primary human RPE cells and ARPE-19 cells by incubation with isolated photoreceptor outer segments following modification with lipid peroxidation products. For inflammasome priming, lipofuscin-loaded cells were incubated in serum-free media or media supplemented with full human serum, C5-deficient serum, or isolated C5a. Specific C5a receptor (CD88) antibodies were used to block C5a binding. Control cells were primed with IL-1α. Following priming, cells were irradiated with blue light for up to 6 hours. NLRP3 inflammasome activation was assessed by measuring IL-1β and IL-18 secretion. Pyroptotic cell death was analyzed using LDH release assay, TUNEL staining, and DNA/histonespecific ELISA

Results
Priming of RPE cells with full human serum or isolated complement component C5a resulted in a lipofuscin load- and light dose-dependent activation of the NLRP3 inflammasome with secretion of IL-1β and IL-18. Complement heat-inactivation, C5 depletion, or C5a receptor inhibition suppressed the priming effect of human serum. Specific inhibition of caspase-1 or cathepsin B, L, or D likewise prevented NLRP3 activation. Inflammasome activation was followed by RPE cell death by pyroptosis as identified by morphological and molecular characteristics

Conclusions
Complement component C5a is capable of providing the priming signal for subsequent activation of the NLRP3 inflammasome by phototoxic effects of lipofuscin. This molecular pathway may represent a functional link between hallmark features of AMD such as lipofuscin accumulation, photooxidative damage, chronic immune response, and progressive degeneration of the RPE and may provide a novel target for therapeutic intervention in AMD

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Recombinant and Endogenous Expression of Age-Related Maculopathy Susceptibility Protein 2 (ARMS2)

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Purpose
The formation of drusen at the macula and the degeneration of RPE cells is characteristic for AMD and represents the most common cause of blindness in developed countries. The ARMS2 variant at 10q26 (A69S, rs10490924) is strongly associated with AMD. This polymorphism is linked to a mutation in the 3' untranslated region in the ARMS2 gene, which introduces an instability motif into the transcribed mRNA. So far the endogenous protein expression of ARMS2 is still unclear and whether the ARMS2 risk variant leads to protein deficiency in certain cells.

Methods
56 patients with neovascular AMD were sequenced and the polymorphisms rs2736911, rs10490924 or del443ins54 in ARMS2 were evaluated. Monocytes isolated from whole blood of patients with defined genotypes, were stained with ARMS2 antiserum and ARMS2 expression was followed by laser scanning microscopy. Furthermore ARMS2 protein expression and location was evaluated in human retinal sections from ARMS2 genotyped patients.

Results
We identified ARMS2 expression in human blood derived monocytes by gene expression and laser scanning microscopy using ARMS2 specific antiserum. Expression of ARMS2 in monocytes, as well as microglia cells was confirmed by siRNA, laser scanning microscopy of human monocytes as well as co-staining of retinal sections with ARMS2 antiserum and CD68, a specific marker for monocytic cells. Interestingly ARMS2 was absent in monocytes derived from AMD patients homozygous for the ARMS2 risk variant (A69S, rs10490924) and also in microglia cells of retinal sections from individuals homozygous of the ARMS2 risk variant. Thus, the risk variant of ARMS2 leads to ARMS2 protein deficiency in monocytes and microglia cells.

IFNß-treatment as a therapy targeting microglia in a murine model of retinal degeneration

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Purpose

Age-related macular degeneration (AMD) is a leading cause of vision loss in the elderly. Typical hallmarks of AMD are chronic activation of the innate immune system and reactive microglial cells in the retina. Here, we analyzed the role of interferon beta (IFNß) signaling and the effect of IFNß therapy on microglial activation and choroidal neovascularization in a murine model of AMD-like retinal damage.

Methods

Laser-rupture of Bruch's membrane was used as a murine model for AMD. Retinal inflammation and choroidal neovascularization (CNV) were analyzed in IFN-alpha/beta receptor knockout (IFNAR-/-) mice, IFNß-treated C57BL6/J mice and C57BL6/J wild type controls using fundus fluorescein angiography (FFA), lectin staining and optical coherence tomography (OCT). Microglial morphology in laser-induced lesions was analyzed by Iba1 and Tspo staining of flatmounted retinas and retinal pigment epithelia (RPE).

Results

Laser-induced lesions in IFNAR-/- animals showed increased vessel leakage as well as CNV compared to control animals, indicating that IFNAR-/- deficiency enhanced inflammation. In contrast, IFNß-treated animals showed reduced vessel leakage and CNV compared to untreated controls. OCT-analysis of IFNß-treated and untreated wild type mice 7 and 14 days after induction of the retinal damage revealed diminished edema formation in IFNß-treated animals. Immunohistological analysis of flat-mounted laser-damaged retinas displayed both, a higher number and a longer presence of activated microglial cells at the sites of damage in IFNAR-/- mice compared to controls. The amount of activated microglia cells in IFNß-treated animals was lower than in respective control groups.

Conclusions

Knockout of IFNAR leads to enhanced retinal inflammation and microglial reactivity. In contrast, IFNß therapy significantly prevented vessel leakage, CNV and microglial activation. We conclude that IFNß signaling dampens microglial reactivity and is a protective mechanism in retinal degeneration.

Translocator protein (18 kDa) (TSPO) and microglia as a therapeutic target in retinal degeneration

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Purpose
Microglia activation is a common hallmark of several retinal degenerative diseases. Our previous work showed that TSPO is as marker for reactive retinal microglia and that the selective TSPO ligand XBD173 exerts strong anti-inflammatory effects on microglia in vitro. In the present study, we investigated whether XBD173 has the capacity to modulate retinal microglia in vivo and thereby protects from light induced retinal degeneration.

Methods
BALB/C mice were treated with vehicle or 10 mg/kg XBD173 by intraperitoneal injection prior to exposure to 15.000 lux white light for one hour. Daily XBD173 treatment was continued for four consecutive days. After this time period, retinal flat-mounts and sections were prepared to analyze microglia morphology, localization and reactivity using Iba1 and TSPO protein expression. Optical coherence tomography, morphometric measurements of retinal thickness and TUNEL stainings were used to to determine the extent of retinal degeneration and photoreceptor apoptosis.

Results
In control eyes that were not exposed to light, Iba1 staining revealed that microglia were located in the inner and outer plexiform layers and showed a ramified morphology. Light exposed animals that were sham treated displayed a severe thinning of the photoreceptor layer and prominent photoreceptor apoptosis which was accompanied by the migration of amoeboid microglial cells into the outer nuclear layer and the subretinal space. TSPO staining revealed a strong TSPO expression in these microglia, indicating a highly reactive status. In contrast, light exposed mice that received XBD173 injections showed a well preserved photoreceptor layer and strongly reduced apoptosis. Significantly fewer numbers of amoeboid microglial cells were present in the ONL and subretinal space and nearly all of them displayed a ramified cell shape. Furthermore, these microglia showed much less staining for the activation marker TSPO.

Conclusions
TSPO-specific XBD173 treatment of mice challenged with intense withe light reduced the number of reactive microglia and protected retinal photoreceptors from light induced apoptosis. We conclude that TSPO and its ligands represent promising targets for neuroprotective and anti-inflammatory therapy of retinal degenerative diseases.

Exon-skipping as a promising therapeutic approach for treatment of retina degeneration in USH2A pseudoexon 40 patients

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Purpose
Usher syndrome (USH) is the most common cause of combined deaf-blindness in man. The hearing loss can be partly compensated by providing patients with hearing aids or cochlear implants, but for the loss of vision currently no treatment is available. Recently we reported the identification of the first deep intronic mutation in the USH2A gene (c.7595-2144A>G) leading to the inclusion of a pseudoexon (PE40), thereby giving rise to a premature stopcodon and thus a truncated protein. In this study we explore the therapeutic potential of antisense oligonucleotides (AONs) to restore the native USH2A transcript. Our strategy is to induce skipping of PE40 by interfering with the splicing machinery in the PE40 region.

Methods
The PE40 region together with 500 bp flanking sequence was cloned into a minigene splice assay to model PE40 splicing. Next we used engineered AONs with complementary chemical backbones (2'OMe-phosphorothioate (2'OMe-PT) and Morpholino) directed against intron-exon boundaries and exonic splice enhancer (ESE) regions of PE40 to induce its exclution and thereby lead to wildtype USH2A mRNA transcript.

Results
All tested AONs, both 2'OMe-PT and Morpholinos, were capable of redirect splicing towards the exclusion of PE40 in our minigene splice assay, albeit with different efficiencies.

Conclusion
By using AONs we were able to specifically induce the skipping of PE40 from the mature USH2A mRNA transcript, predicting translation of fully functional wildtype USH2A protein. Following this approach we expect to be able to stop the progression of this devastating blinding disorder by AON therapy and provide USH2A PE40 patients a prospect on vision in the future.

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Generation of a zebrafish eys knock-out model for inherited retinal degeneration

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Purpose
Retinitis pigmentosa (RP) is a clinically diverse and genetically heterogeneous group of inherited retinal disorders with a prevalence of approximately 1 in 4,000 individuals. Mutations in eyes shut homolog (EYS), a gene expressed uniquely in the photoreceptor cells of the retina, are among the most frequent causes of autosomal recessive (ar)RP, accounting for approximately 5-10% of all arRP cases. In order to develop a treatment strategy for EYS-associated RP, a suitable model system is needed. Therefore, our goal is to develop a stable Eys knock-out in zebrafish using CRISPR/Cas9 genome editing.

Methods
We cloned and characterized Eys in zebrafish, via RT-PCR analysis on zebrafish eyes cDNA and sequencing. Furthermore, we are generating a stable Eys knock-out in zebrafish via CRISPR/Cas9 technology. The effect of EYS knock-out on retinal structure and function will be studied using immunohistochemistry and behavioral assays. Subsequently, this model will be used to assess the effect of therapeutic rescue constructs that are being developed.

Results
RT-PCR on zebrafish eyes cDNA resulted in the identification of a 8598 bp cDNA consisting of 45 exons. The transcript is predicted to encode a 2866-aa protein containing 38 EGF-like domains and five laminin A G-like domains, which is highly similar compared to the human EYS gene. On protein level, zebrafish and human show a sequence identity of 32%.

Conclusions
We characterized Eys in zebrafish, which shows high similarity with human EYS. Our work will serve as the basis for understanding the function of EYS, and to initiate pre-clinical therapeutic intervention studies.

Analyzing retinal degeneration in a canine model of RPE65 deficiency

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Purpose
Mutations in the RPE65 gene are associated with early onset severe retinal dystrophy, a rare form of blindness in humans that is characterized by severe visual impairment within the first years of life. The Swedish Briard dog is a naturally occurring animal model for this disease. Adeno-associated virus (AAV) mediated gene therapy in these dogs resulted in tremendous treatment benefits in terms of restoration of function in both, rods and cones. However, treatment benefit in humans is less pronounced. The aim of the study was to characterize mophology of the canine RPE65-/- retina before and after successful AAV mediated gene therapy in order to obtain a better understanding of the disease.

Methods
Retinae from unaffected and affected dogs were used in this study. The affected dogs were treated unilaterally with AAV vector carrying the human RPE65 gene. Dogs were euthanized at various ages, between three and 96 months. Different markers were used as primary antibodies to mark diverse cells in the retina. In vivo OCT imaging was performed to study retinal layer thickness over time.

Results
At two years of age, in the affected dogs L/M-cone opsin expression was uniformly reduced over the whole retina. However, S-cone opsin expression loss was limited to superior central part of the retina. Opsin delocalization was observed in L/M and S cones. Delocalization of ribbon synapses to the outer nuclear layer was founded in the peripheral retina. In the further progression of the disease, these changes were determined in the entire retina. After successful gene therapy relocalization of cone opsins were discovered in both L/M and S cones. Reduction of both S cone loss and delocalization of ribbon synapses was observed in the treated area. Likewise, OCT analysis showed preserved ONL thickness in the treated area.

Conclusions
There are remarkable changes in the retina at early stage of the disease. The changes extend with age and cannot all delayed by gene therapy. While S cones have a strong treatment effect, L/M cones seem to benefit less efficiently from gene therapy. In humans the central visual acuity is L/M cone dependent. The limited effect on L/M cones might be an explanation of the discrepancy between the canine and human gene therapy studies.

Towards an in vitro model for type II Leber congenital amaurosis

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Purpose
Leber congenital amauroses (LCA) are a group of hereditary retinal diseases characterized by a severe and early visual loss often resulting in blindness. Mutation in the RPE65 gene has been identified as the cause of type II LCA, impairing completion of the visual cycle taking place in the retinal pigmented epithelium (RPE) and leading to cones and rods degeneration. Gene therapy has emerged as the only available treatment for LCA, however to date, no human gene therapy was able to prevent the photoreceptors degeneration.

Methods
Using a lentiviral-mediated gene therapy, we were able to rescue the phenotype of the RPE65-/- as well as the Rpe65RW91/RW91 adult mice and prevent photoreceptors degeneration. In order to further assess the efficiency of this lentiviral-mediated gene therapy, an in vitro model for type II LCA is currently being developed. After the derivation of induced-pluripotent stem cells (iPSCs) from type II LCA affected patients, iPSCs are differentiated into retinal pigmented epithelium (RPE).

Results
The in vitro model will permit the analysis of various parameters of the gene therapy such as the efficiency of RPE65 protein rescue, RPE65 correct localization or its impact on the RPE transcriptome.

Conclusions
In conclusion, we hope that this model will provide an in vitro proof-of-concept of our lentiviral-mediated gene therapy for future clinical perspectives.

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Monocular adaptation to spatially varying distortions

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Purpose
Progressive lenses distort the visual image in a spatially varying and gaze-direction-dependent way, and after some time, the visual system adapts to it. Mechanism of visual adaptation to optically induced spatially varying distortions (SVDs) were investigated.

Methods
Adaptation to spatially varied distortions was investigated by measuring an adaptation after effect of two oppositely oriented skew distortions in parallel. Skewed video images were shown to 23 subjects as an adapting stimuli. The adaptation aftereffect of each skew distortion was evaluated by measuring the perceived undistorted angle (PUA) of a skewed checkerboard image in an adjustment task.

Results
Adaptation was obtained to both distortions. The PUA shifted from the baseline in the direction of the adapting skew direction after each adaptation step.

Conclusions
The visual system induces enhanced adaptation abilities to different distortions in parallel with which it can recalibrate at fast rate for several distortion profiles. Thus, a parallel memory of two different distortions was demonstrated.

Visual perception and adaptation to bifocal patterns

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Purpose
Presbyopia is the physiological inability to focus objects at near distance. Conventional treatment for presbyopia include bifocal or multifocal correction in the form of alternative vision or simultaneous vision. In a simultaneous bifocal correction, a sharp image is superimposed with a blurred image at any distance resulting in a complex image degradation. We previously measured changes in adaptation to such simultaneous vision corrections optically introduced with custom developed vision simulator. The adaptation to a simultaneous vision corresponded to the amount of blur in the simultaneous correction and its energy distribution. We also showed that subjects show systematic preferences to different bifocal patterns (angular vs radial), that were partly influenced by the amount of blur present in the eye. An alternative, more common method of presbyopia correction is monovision. These intentionally introduce long term differences in interocular blur and has consequences in binocular vision. We explored potential differences in the natural blur adaptation in both eyes of subjects inherent differences in interocular blur, defined by the ocular higher order aberrations. Calibrated images were presented through an adaptive optics system and changes in the perceived-best-focus (image blur producing neutral percept) were measured. We found that both eyes of the subjects had similar perceptual blur, even when the retinal image quality were different. Also, no after-effects (to adaptation) were observed when the eyes were adapted to the retinal image quality of the eye with less aberrations and a greater effect of adaptation was seen when adapted to the retinal image quality of eye with more aberrations. Our results indicate that in eyes with interocular optical blur differences, long term adaptation to blur is driven by the eye with better optical quality. The visual calibrations we report, are important for understanding the consequences of interocular differences in optical errors present in refractive corrections such as monovision or even anisometropia.

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Development of fine-tuned aberration

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n/a

Lack of oblique astigmatism in the chicken eye

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Purpose
Primate eyes display considerable oblique off-axis astigmatism which could provide information on the sign of defocus that is needed for emmetropization. The pattern of peripheral astigmatism is not known in the chicken eye, a common model of myopia.

Methods
Peripheral astigmatism was mapped out over the horizontal visual field in three chickens, 43 days old, and in three near emmetropic human subjects, average age 34.7 years, using infrared photoretinoscopy.

Results
There were no differences in astigmatism between humans and chickens in the central visual field (chicks: 0.35D, humans: 0.65D, n.s.) but large differences in the periphery (i.e. astigmatism at 40° in the temporal visual field: humans: 4.21D, chicks: 0.63D, p< 0.001, unpaired t-test). The lack of peripheral astigmatism in chicks was not due to differences in corneal shape. Perhaps related to their superior peripheral optics, we found that chickenshad excellent visual performance also in the far periphery. Using an automated optokinetic nystagmusparadigm, no difference was observed in spatial visual performance with vision restricted to either thecentral 67° of the visual field or to the periphery beyond 67°. Accommodation was elicited by stimuli presented far out in the visual field. Transscleral images of single infrared LEDs showed no sign of peripheralastigmatism.

Conclusions
The chick may be the first terrestrial vertebrate described to lack oblique astigmatism. Since corneal shape cannot account for the difference in astigmatism in humans and chicks, it must trace back to the design of the crystalline lens. The lack of peripheral astigmatism in chicks also excludes a role in emmetropization. None of the authors has any proprietary interests

Acknowledgement
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Investigation of induced pluripotent stem cell derived optic vesicles from Retinitis pigmentosa patients carrying CRB1 mutations

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Purpose
Retinitis Pigmentosa (RP) and Leber's contigenital amaurosis (LCA) are two forms of retinal degeneration taking place either before/right after birth (LCA) or late in life (RP). For both diseases there are several dozen known causative mutations affecting in some way the development or the homeostasis of retinal cells most prominently photoreceptor cells. Amongst them is the CRB1 gene, which is believed to be involved in the stability of the outer segment of photoreceptor cells. Since the loss of eyesight is a severe impairment of life quality the thoroughly investigation of the pathogenetic background is crucial.

Methods
In 2012, Nakano and his group developed a protocol which allows the differentiation of human induced pluripotent stem cells (hiPSC) to three dimensional optic vesicle/cup structures matching to the corresponding embryonic structures.

Results
Making use of optic vesicle differentiation protocol we like to recapitulate human retinogenesis and development of photoreceptors in vitro especially focused on the role of CRB1. One of our approaches is to use a fluorophore under a CRB1- specific promotor to track CRB1 expressing cells during development. Further, we obtained hair keratinocytes from two siblings suffering from Retinitis Pigmentosa carrying a C948Y mutation in the CRB1 gene. From those we generated iPS cells which can be used for retinal differentiation.

Downregulation of the canonical Wnt signalling pathway by TGF-β1 inhibits photoreceptor differentiation of adult human Müller stem cells

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Purpose
Müller glia regenerate the adult zebrafish retina after injury. Müller glia with stem cell characteristics (hMSC) can be isolated from the adult human retina and induced to differentiate into photoreceptors in vitro. Activation of Wnt signalling promotes proliferation and differentiation of neural progenitors, whilst transforming growth factor-β (TGF-β), highly upregulated during retinal gliosis, is known to promote regeneration of the zebrafish retina. We investigated whether TGF-β may control the Wnt signalling pathway and whether it may influence the ability of human Müller glia to differentiate into photoreceptors in vitro.

Methods
hMSC were differentiated into photoreceptors by culture with FGF-2, Taurine, Retinoic acid and IGF (FTRI). Cells were also cultured with or without TGFβ1, TGFβ inhibitors and the Wnt inhibitor XAV-939. mRNA and protein isolated from these cells were examined for expression of molecules of the Wnt signalling pathways and the photoreceptor markers Nr2e3 and recoverin using RT-PCR, western-blot analysis and confocal microscopy of immuno-stained cells

Results
Culture of hMSC with FTRI increased gene and protein expression of WNT2B and β-catenin, components of the Wnt canonical signalling pathway. Whilst TGFβ1 decreased gene and protein expression of WNT2b and β-catenin, it also inhibited differentiation of hMSC into photoreceptors as judged by decreased expression of NR2E3 and recoverin. The TGF-β type I receptor inhibitor SB431542 and the Smad3 inhibitor SIS3 blocked the TGF-β inhibition of WNT2B. In addition, cells undergoing photoreceptor differentiation showed a decrease in the expression of DKK1, an antagonist of the Wnt signalling pathway. Addition of the β-catenin inhibitor XAV-939 to hMSC undergoing differentiation prevented upregulation in gene and protein expression of NR2E3.

Conclusions
The results suggest that down-regulation of the canonical Wnt signalling pathway by TGFβ1 inhibits photoreceptor differentiation of hMSC. This could be due to the inhibition of the expression of Wnt ligands in these cells. There is no evidence that the adult human retina regenerates despite harbouring Müller glia with stem cell characteristics. Since TGFβ is highly upregulated during gliosis, it may be possible that this cytokine may inhibit the potential regenerative ability of these cells. It would be therefore important to investigate how these pathways can be modulated in order to induce endogenous regeneration of the adult human retina.

Canine exome sequencing identifies a nonsense mutation in EYS in Chihuahuas with progressive retinal atrophy

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Purpose
To identify the molecular genetic defects underlying progressive retinal atrophy (PRA) in dogs using exome sequencing.

Methods
Exome sequencing was performed in one affected and one unaffected dog from twelve breeds (Airedale Terrier, Berger de Pyrenée, Boxer, Chihuahua, Coton de Tulear, Finnish Lapphund, Husky, Löwchen, Miniature Schnautzer, Old German Shepherd, Rauhaar Teckel, and Saarloos Wolfshond). An automated bioinformatics pipeline was employed to map and annotate large scale genomic variations in canines. Variants were prioritized by their occurrence in other dogs and/or SNP databases, and in silico pathogenicity prediction. Special consideration was given to genes that are known to be associated with inherited retinal dystrophy (IRD) in humans.

Results
Interestingly, exome sequencing yielded compound heterozygous variants in EYS in the PRA-affected Chihuahua, i.e. a nonsense mutation (c.1566C>T; p.R556*), and a missense variant (c.1522G>A; p.A508T) with unknown pathogenicity. In addition, a homozygous causative variant in PDC (c.244C>G; p.R82G) was identified in the Miniature Schnautzer dogs. Screening of large cohorts following the prioritization of candidate variants has excluded several other potentially pathogenic variants, for instance in SNRNP27 and THSD7B in the Airedale Terrier, in CABP4 in the Berger de Pyrenées and in RP1L1 in the Saarloos Wolfshond. No clear-cut pathogenic mutations likely contributing to PRA were found in the other dogs.

Conclusions
Although the causality of the p.A508T change is unknown, the identification of the EYS p.R556* nonsense mutation in the PRA-affected Chihuahua allows selective breeding to generate a homozygous mutant animal model for this specific subtype of IRD, in order to understand the disease mechanisms and to perform preclinical therapeutic studies. Moreover, the PDC finding demonstrates the robustness of exome sequencing and the reliability of our bioinformatic analysis. Further improvement of our pipeline, supported by an increase of publically available canine sequencing data, will likely facilitate the elucidation of additional molecular genetic defects causing PRA.

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Cataract surgery affects the pupil response to both blue- and red light

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Purpose
The aim of this study was to evaluate the effect of unilateral cataract surgery on the pupillary light response to sustained chromatic light stimuli.

Methods
In a prospective study involving 11 otherwise healthy subjects, consensual pupil responses to monochromatic red (633 nm, 2 loglux) and blue (463 nm, 2 loglux) light were recorded before, 1 day and 3-weeks after cataract surgery using a binocular chromatic pupillometer (DP-2000, NeurOptics). The unoperated eye was illuminated and the pupil response was recorded in the surgery eye. During one measurement, pupillary diameters of both eyes were recorded continuously before (10 s), during (20 s) and after (60 s) illumination. Main outcomes were late post-illumination pupillary response (PIPR10-30s), early post-illumination pupillary response (PIPR0-10s) and maximal pupillary constriction amplitude (CAmax) to blue- and red light stimulations.

Results
The late post-illumination pupillary response (PIPR10-30s) to either blue- or red light did not change significantly after the cataract surgery. The blue light elicited early post-illumination pupillary response (PIPR0-10s) at baseline was 0.30 ±0.23 decreasing by 20% at day-1 and 13% at 3-weeks after unilateral cataract surgery. Similarly, PIPR0-10s to red light reduced by 17% and 13% at 1-day and 3-weeks, respectively. The maximal contraction amplitude (CAmax) to blue light was 0.59 ±0.02 prior to surgery, decreasing by 9% and 7% at 1-day and 3-weeks, respectively. CAmax to red light was reduced significantly by 7.3% at 1-day, but did not change significantly at 3-weeks.

Conclusions
Cataract surgery decreases the pupil response to both red and blue light. This effect is presumably due to mechanical damage to the iris sphincter, since the pupil constriction decreased. Reduced early light-off parameter can be explained by the carry-over effect of the decreased pupil constriction during the light stimulation. Conversely, no effect of cataract surgery was found on the late post-illumination pupillary response.

Visual behavior at the presence of simulated central vision loss and forced Preferred Retinal Loci

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Purpose
People who suffer from central vision loss has to fixate an object eccentrically at a preferred retinal locus of fixation (PRL) to acquire visual information. The efficiency of the PRL positions depend on the central vision loss and the visual task features. It can happen that a person develops a PRL at an inefficient location. The study targets whether a preferred retinal locus of fixation can be forced to be at a specific location.

Methods
A central scotoma is simulated in 10 healthy subjects, separated into 2 groups; the control group and induced group. The PRL training was carried out in one hour training sessions where different visual task had to be performed. The performance is tracked along the training with a reading task. At the induced group, to force the location of the PRL, the target was shifted to the left half of the visual field every time a target is placed in the right half of the visual field.

Results
The training showed that healthy subjects under central vision loss simulation developed a PRL and in addition, subjects from the induced group located the targets on the left half of the visual field.

Conclusions
It demonstrates that PRL position can be induced using the previous training paradigm.

Recessive mutations in the polyglutamylase TTLL5, present in photoreceptor cells and spermatozoa, cause cone-rod degeneration and incompletely penetrant male infertility

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The effect of red light treatment on dendropathy in a mouse model of mitochondrial optic neuropathy.

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Purpose
To test the effectiveness of mitochondrial augmenting 670 nm light in preventing retinal ganglion cell (RGC) dendritic pruning, which occurs in the wild-type (WT) mouse and in a mouse model of autosomal dominant optic atrophy due to the heterozygous mutation B6;C3-Opa1(Q285STOP) (Het). This mutation leads to a 50% reduction in the transcript of the mitochondrial fusion protein Opa1.

Methods
4 J/cm2 of 670 nm light was delivered in vivo over 88 seconds, to the eyes of 13-15 month old WT and Het mice for five consecutive days prior to axotomy of the retinal ganglion cells, upon which the retinas were flat mounted. Retinal ganglion cells from WT and Het mice, and sham treated aged matched controls, were diolistically labelled with lipophilic dyes after 0, 8 and 16 hours in culture. The cells were imaged by confocal microscopy and analysed using Fiji software to monitor changes in their dendritic architecture.

Results
Sholl analysis of retinal ganglion cells showed a statistically significant loss in the number of dendrite intersections from 20 to 160 µm from the neuronal soma at 16 hours post axotomy in both WT and Het cells. The loss of dendrites was partly prevented in both light treated WT and Het cells. After 16 hours the areas under the Sholl curves were decreased by 47% in sham treated WTs and Hets, whereas in light treated retinas the decreases were 7% and 8% for Wts and Hets, respectively. A reduction in the total dendritic length was observed after 16 hours in sham treated WT (from 2448 ± 159 to 1300 ± 209 µm) and het (from 2291 ± 243 to 1278 ± 183 µm) cells. After light treatment the total dendritic lengths were 2305 ± 328 and 2181 ± 514 µm for WTs and Hets, respectively.

Conclusions
Exposure of the retinal ganglion cells in WT and Het mice to 670 nm light prior to axotomy provides resistance against degeneration in terms of dendrite loss. Our results demonstrate a potential of 670 nm light treatment for therapeutic applications for optic neuropathies/retinal ganglion cell degeneration.

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The role of neuroinflammation in the regenerating adult zebrafish retina

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Purpose
Neuroinflammation was shown to have a positive impact on stem cell activity in the zebrafish central nervous system after traumatic injury. Since little is known about the inflammatory response of the zebrafish retina upon lesion, we aim to identify general contribution of inflammation to regeneration.

Methods
To identify novel marker genes that are differentially expressed after lesion, we studied transcriptome data from injury activated Müller glia. Potential candidates were further analysed by performing In situ hybridization in combination with immunohistochemistry. Microglia are among the first cells to react to injury in an inflammatory context, and are labelled by mpeg1:mCherry. We analyzed microglia for morphological changes as well as cell migration towards the lesion site. Finally, to study the general role of the immune system for regeneration, we performed drug-mediated suppression of the immune system and analysed microglia activity, cell proliferation and the expression of markers for inflammation.

Results
We identified matrix metalloproteinase 9 (mmp9) as a novel marker gene for inflammatory processes in Müller glia cells after photoreceptor ablation. In addition, microglia undergo the same morphological changes as described in murine retina and zebrafish telencephalon, by changing from a ramified to a more amoeboid morphology, by contracting phylopodia, and by accumulating at the site of lesion. Under immune suppression, Müller glia proliferation was significantly decreased in comparison to controls. Furthermore, the number of microglia strongly decreased under these conditions, and mmp9 expression was almost not detectable via ISH.

Conclusions
MMP9 is a marker for an early inflammatory response of Müller glia cells. The dynamics of microglia suggests their involvement in the regeneration response of zebrafish retina. Furthermore, the decreased regenerative response after inhibition of the immune system suggests that inflammation might have a positive role in stimulating retinal regeneration, although more details on this process remain to be elucidated.

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Differential splicing of PRPH2 in rods and cones affects the expression of disease associated point mutations on transcript level

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Purpose
Mutations in peripherin-2 (PRPH2) are associated with retinal disorders characterized by defects of rod or cone photoreceptors. So far the molecular mechanisms addressing the differential role of peripherin-2 in rods and cones were analyzed exclusively on protein level and remained largely elusive. However, point mutations can also affect the corresponding gene on transcript level by influencing mRNA splicing. Hitherto, there is no experimental approach that allows for systematic analysis of impacts of point mutations on splicing in photoreceptors. In this study, we designed PRPH2 minigenes containing the full coding sequence and shortened intronic regions of human PRPH2 wild type (WT) as well as 11 PRPH2 point mutants. The mutations are localized in exon2 which represents a mutation hotspot within PRPH2. For splicing analysis six of the PRPH2 mutants associated with autosomal dominant retinitis pigmentosa (adRP) were expressed in rods, the remaining five which are linked to different types of cone diseases were expressed in cones.

Methods
We took advantage of the recombinant adeno-associated virus (rAAV)-mediated gene transfer to deliver the PRPH2 minigenes to murine rods and cones. Titer-matched rAAV particles containing WT or mutant PRPH2 minigenes were subretinally injected to WT mice on postnatal day 14. Six PRPH2 minigenes containing mutants associated with adRP were expressed in rods and another five mutants linked to cone diseases were expressed in cones using the human rhodopsin (hRHO) and mouse short wavelength opsin (mSWS) promoter, respectively. The specificity of the promoters was tested on retinal slices from mice injected with WT PRPH2 minigenes. The splicing products were identified by reverse transcriptase PCR (RT-PCR) analysis using minigene specific primers followed by direct sequencing. Quantification of the band intensities of the splicing products was performed from five independent RT-PCR experiments.

Results
We show that the single constructs were specifically targeted to rods and cones, respectively. Splicing of the single PRPH2 minigenes lead to three splice variants: i) the unspliced product ii) the intron1 retention iii) the correctly spliced PRPH2. Surprisingly, splicing of WT PRPH2 in cones resulted almost exclusively in the unspliced variant whereas rods predominantly expressed the correctly spliced WT PRPH2. Quantification of the relative intensities revealed that three of five mutants linked to cone diseases expressed remarkably high levels of the correctly spliced PRPH2 variant compared to the WT in cones. By contrast, in rods for five of six PRPH2 mutants associated with adRP the relative expression levels of the correctly spliced PRPH2 transcript were unchanged and in one case slightly reduced. The reduction was caused by one mutation which leads to a generation of a novel donor site in exon2.

Conclusions
Taken together, using a new experimental approach we provide evidence for differential splicing in rods and cones. Furthermore, we unveil novel pathomechanisms and a novel genotype phenotype correlation of PRPH2 mutants associated with rod or cone diseases which might explain the differential penetrance of these mutations in rods and cones.

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Impaired electroretinogram responses in ceramide synthase-deficient mice

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Purpose
Ceramides form the backbone of all complex sphingolipids. They also act as signaling molecules on a variety of intracellular cascades and are involved in autophagy and apoptosis. The de novo synthesis of ceramides in the endoplasmic reticulum is catalyzed by six ceramide synthases: CerS1-6. CerS deficiencies cause abnormalities in lipid composition and signaling cascades (such as apoptosis) in a variety of tissues, including the brain. CerS1 has been shown to be neuronally expressed in the brain, CerS2 occurs in oligodendrocytes and CerS4 is mainly expressed in the skin. However, expression of CerS – in retinal neurons and glia cells or the cornea - and the effects of CerS deficiencies on the retina have not been studied so far.We investigated retinal function and morphology in CerS1-, CerS2- and CerS4-deficient mice to find out if CerS deficiency-induced defects in the brain such as cell loss and tissue shrinkage also occur in the retina.

Methods
CerS expression in the cornea and retina was investigated using western blots. Retinal function was measured electrophysiologically by electroretinograms (ERGs) under scotopic and photopic light conditions. Retinal morphology was investigated immunohistologically, using antibodies against a variety of cell markers, receptors and synaptic proteins. Labeled photoreceptors, glutamate receptors and synaptic markers were quantified in retinal slices. Membrane composition was investigated by lipid analyses using mass spectrometry.

Results
CerS1, CerS2 and CerS4 are expressed in the cornea and retina. All tested CerS-deficient mice showed reduced responses in the ERG compared to wild-type littermates. Reductions were seen in a-wave and b-wave amplitudes and in oscillatory potentials. Cell marker stainings revealed no loss of retinal neurons, but preliminary quantification of glutamate receptors and synaptic proteins suggest differences between genotypes. Moreover, CerS1 KO and CerS4 KO mice displayed significant reduction in C18 and C20 ceramides, respectively.

Conclusions
The reduced a- and b-wave amplitudes in ERGs suggested defects at the photoreceptor synapse. These would lead to impaired signaling between photoreceptors and bipolar cells, possibly caused by changes in the size and number of receptors or synaptic proteins. ERG effects may be influenced by altered lipid and membrane compositions, potentially leading to altered electrical properties of the cornea or retinal neurons and thus altered extracellular field potentials. Mice deficient for CerS show reduced ERG signals. The underlying cause may be the altered lipid composition in cornea cells, retinal neurons and/or glia cells, potentially leading to 1) altered electrical properties of cornea and retina, 2) changes in corneal light transmission and/or 3) changes in vesicular signal transmission.

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Generation of hESC/hIPSC- derived photoreceptors: Modelling retinal degenerative diseases in vitro

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Purpose
Human Induced Pluripotent Stem cells (hiPSCs) are a promising tool to model retinal diseases in vitro, and to generate photoreceptors for cell therapy. Usher syndrome is an autosomal recessive disease (affecting 1:25000 children) that causes retinitis pigmentosa and hearing loss due to defects of the neurosensory cells in the cochlea. Although animal models exist for this disease, these do not show retinal degeneration, making the disease pathways leading to retinal cell loss difficult to elucidate. A systemic characterisation of the expression of Usher proteins Myosin 7a and Usherin during human retinal development is also lacking to our knowledge, mainly due to the lack of human tissues for study. Here we generated hiPSCs from patients with Usher syndrome and established 3D retinal tissue differentiation cultures to provide new models to shed light on the disease pathophysiology.

Methods
Two iPSC lines carrying the following mutations were generated: NCUS:35 (compound heterozygous MYO7A p.Arg2024X and p.Asp75His) and NCUS:24 (compound heterozygous USH2A p.Glu767Serfs*21 and p.Lys2532Thrfs*56) representing Usher type 1 and type 2, respectively. Mutant hIPSCs and control lines (from individuals without a retinal phenotype, and embryonic stem cell line WA09) were maintained on a feeder-dependent culture system and expanded weekly via enzymatic passaging. To closely mimic the in vivo retinal microenvironment and the specific tissue organisation we established protocols for generation of 3D retinal tissue according to Meyer et al, 2009 (Proc Natl Acad Sci,106(39): 16698-703), with some modifications.

Results
We show generation of optic vesicles and retinal neuroepithelium from two Usher patient iPSCs, as well as control iPSC and ESC lines. Photoreceptors were generated by 5 weeks of differentiation determined by co-labelling of Crx and Recoverin. Down regulation of markers of pluripotency and up regulation of retinal markers was evident by RT-PCR analysis. USH2A (Usherin 2A) and MYO7A (Myosin 7A) transcripts were detected in undifferentiated pluripotent stem cells, and expression increased by 2 weeks of differentiation in control cultures. The respective Usher proteins, Usherin and Myosin 7a were also detected at later time points. Percentage yields of optic vesicles and photoreceptors at early time points (week 2-5 of differentiation) were not significantly different between lines. Analyses of long-term cultures (12-14 weeks of differentiation) will allow comparison of photoreceptor maturation and survival across different lines.

Conclusions
3D retinal differentiation cultures of Usher patient-derived iPSCs provide a model system to study the consequences of disease-causing mutations. We successfully differentiated hiPSCs and ESCs into optic vesicles and laminated retinal neuroepithelium and showed that photoreceptor genesis followed in vivo developmental stages, validating the in vitro system to model disease. Future comparative analysis of the patient-derived and control cell lines may highlight differences in signalling pathways needed for maintenance and/or survival of patient-derived photoreceptors.

Acknowledgement
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Retinal Image Simulation

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Purpose
The retinal image, that is the image that reaches the retina, depends on the optics of the eye and it represents the optical information that the visual system processes. Schematic eye models are a theoretical representation of an idealised eye, in which the refractive structures of the eye are described as lenses. In this work, we simulate the retinal image of a 3D scene, shaped by schematic eye models, on a spherical retina.

Methods
We use PBRT, a physically based rendering tool, that relies on a 3D representation of a virtual scene and on ray tracing. We developed a plug-in to render the retinal image of a scene, shaped by schematic eye models. The plug-in is capable of representing any eye model that consists of rotationally symmetric surfaces with conicoid shape. Moreover, flat and ellipsoidal retinal shapes can be considered.

Results
We compare the irradiance of the retinal image produced by different eye models. Contrary to what is commonly expected, there is no major deterioration of the retinal image on the periphery, when a hemispherical retina is used. This occurs because, in this case, the retinal image suffers no significant peripheral decay of irradiance.

Conclusions
The developed tool gives a new insight into the properties of the retinal image generated by schematic eye models, both foveally and peripherally.The simulation of the retinal image can be used as a more precise input than photographs for image-based vision perception models, because the latter suffer artificial distortions due to the camera lens system and do not reproduce the correct irradiance distribution on the retina.

Determining the minimal cohort of cells required for visual coding.

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Purpose
The characterization of visual sensory neurons in a laboratory setting is canonically determined using simple stimuli. Thus stimuli however, are not representative of the real world which is interpreted in complex visual stimuli and in motion. Discerning how this information is decoded at the level of the retina can be problematic due to the complexity in the many variables that modulate the electrical responses by the different types of cells that define this tissue.In order to understand how the retina encodes natural images in motion we carried out extracellular recordings from ganglion cell evoked responses using a MEA and custom software designed to reconstruct these complex visual stimuli. Using this setup we demonstrate that with recorded activity of only a small cohort of cells we are able to decode and accurately reconstruct the actual image displayed onto the retina.

Methods
Fresh retinas were isolated and maintained in darkness with temperature controlled oxygenated physiological solution. We used a 100 array with a inter electrode distance of 400 µm (Utah Electrode Array) that enables extracellular recordings from the ganglion cell layer. The photoreceptor layer was stimulated with light at different intensities, several space/temporal patterns and natural scenes in motion. The waveforms and the time responses were stored together with the state of the visual stimulus for later offline analysis. Once each ganglion cell was characterized (type, location and size of their receptive fields…) the data provide by each different ganglion cell in response to the actual scenes was normalized as follow: the maximum response corresponds to the brightest color of the part of actual scene that each cell decodes. In addition, as some receptive field areas expand to more than a single line, a weighted sum of the lines adjacency was performed.

Results
Recordings were taken from wild type retinas displaying activity from at least 40 ganglion cells. Their spatial position and receptive fields were then characterized using light stimulation with bars. The ganglion cells responses were then recorded during the presentation of a moving actual scene stimulus repeated 30 times within 3 minutes. After spike sorting the data was plotted into a PSTH and was normalized by a linear regression between the gray values range of the corresponding rows. Using this method actual visual stimulus was reconstructed. Our images were analyzed for their degree of similarity by comparing Bhattacharyya distances between the reconstructions and the processed actual images. Here, we obtained a value of 0.32 for the images with 1 assigned to the biggest difference and 0 to an equal distribution. We also demonstrate that reconstruction of high congruency was possible with the recorded activity from as little 11 ganglion cell responses.

Conclusions
Our results demonstrate that with responses from just 11 ganglion cells we are able to reconstruct accurately a complicated natural moving image. This opens a window for further investigation using images in color to improve our understanding of the visual coding that takes place at the level of the retina. Our ultimate goal is to apply the data acquired from this procedure and begin to compare ganglion cell visual responses in healthy retinas to those suffering from injury or neurodegenerative visual diseases. This could provide valuable information to the processes and development underlying the functional degradation of ganglion cells in visual impairments.

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In vivo imaging and stimulation of human cone photoreceptors with adaptive optics scanning laser ophthalmoscopy

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Purpose
Adaptive optics scanning laser ophthalmoscopy (AOSLO) can image 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. Due to its scanning nature, visual stimuli can be encoded into the imaging beam with high-speed acousto-optic modulation (AOM), thereby creating an acutely focused visual display directly on the retina. We here characterize the limits and possibilities of such a micro display for the studies of visual function on the level of individual receptor cells in the living eye.

Methods
Measurements were performed with a multi-wavelength AOSLO, with 840 nm light for imaging and 543 nm for stimulation. Since space and time are interlinked in a scanning system, spatial characteristics can be inferred by recording temporal beam intensity modulation. Modulation accuracy for benchmark stimuli (gratings, lines, dots, Gabor patches, complex gray scale images) was measured using a high-speed Si analog photodetector sampled at 1.25 - 5 Gigahertz. A simple light capture model was used to calculate nominal light delivery under experimental conditions. Stimulus fidelity and visible contrast was validated psychophysically under foveal inspection when higher order aberrations were compensated for.

Results
The smallest full contrast stimuli presentable were on the order of 3 pixels across in raster scanning coordinates. This corresponds to about 2 µm on the retina or ~120ns as the beam continuously sweeps over the retina with a typical raster scanning excursion of 1.2 deg of visual angle. Optical modelling confirms that this size would place almost all light within the dimensions of a single cone inner segment diameter. Maximum light intensity contrast for extended stimuli achieved in our setup was ~0.99 (Michelson, or about 355:1), a level limited by the extinction ratio of the acousto-optic device used for optical switching. Residual light leak (~4.3 cd/m2 at 543 nm, around 4100 isomerizations per second) through these switches likely saturates any rod photoreceptor contribution, thus AOSLO-based visual psychophysics is currently limited to cone photoreceptor responses.

Conclusions
AOSLO-based micro-stimulation has enough spatial resolution to drive individual cone photoreceptors in the living eye, allowing investigators to probe the relationship between retinal structure and visual function on single cell level. This technique promises to be useful for a host of fundamental and clinical vision research applications.

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NINL and DZANK1 co-function in vesicle transport and are essential for photoreceptor development in zebrafish.

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Purpose
The cytoplasmic dynein 1 motor complex is known to be essential for photoreceptor outer segment formation and function. NINL, an important interaction partner of three ciliopathyassociated proteins (lebercilin, USH2A and CC2D2A), was previously shown to associate with this motor complex. In this work, we scrutinize the role of NINL using a combination of affinity proteomics and in vivo zebrafish studies, in order to gain insight into the pathogenic mechanisms underlying these three associated hereditary disorders.

Results
We identify Double Zinc Ribbon and Ankyrin Repeat domains 1 (DZANK1) as a novel interaction partner of NINL and show that loss of Ninl, Dzank1 or both synergistically leads to dysmorphic photoreceptor outer segments, accumulation of trans-Golgi-derived vesicles and mislocalization of Rhodopsin and Ush2a in zebrafish. In addition, retrograde melanosome transport is severely impaired in zebrafish lacking Ninl or Dzank1. We further demonstrate that NINL and DZANK1 are essential for intracellular dynein-based transport by associating with complementary subunits of the cytoplasmic dynein 1 motor complex, thus shedding light on the structure and stoichiometry of this important motor complex.

Conclusions
Altogether, our results support a model in which the NINL-DZANK1 protein module is involved in the proper assembly and folding of the cytoplasmic dynein 1 motor complex in photoreceptor cells, a process essential for outer segment formation and function.

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Müller glia-derived cytokines are predominantly increased in the gliotic human retina.

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Purpose
Although it is well documented that cytokines and growth factors play a key role in retinal gliosis, the effects of cytokine activation on the potential modulation of retinal neurogenesis by resident human Müller stem cells (hMSC) has not yet been addressed. Before exploring the effect of cytokines on the promotion or inhibition of hMSC proliferation and differentiation in the degenerated retina, we have investigated whether the pattern of cytokine expression by Müller glia mimics that of the gliotic retina.

Methods
Normal cadaveric retina was obtained from Moorfields Eye Bank. Retinectomy specimens were obtained upon written consent from patients undergoing retinal surgery at Moorfields Eye Hospital and used according to guidelines from the Local Ethics Committee. Protein lysates from tissue and MIO-M1 Müller glial cells were examined for cytokine expression using a dot blot proteome profiler antibody array. Quantification analysis was also conducted using a multiplex immunoassay for 27 different cytokines.

Results
Qualitative cytokine arrays of gliotic retinae showed that 24 cytokines exhibited 2-fold increase, as compared with the normal retina. 76 factors out of 102 analysed were found in the Muller glia lysate. Quantitative cytokine arrays revealed a significant increase in the levels of 19 cytokines in the gliotic retina compared to the normal retina. Interestingly, cytokines predominantly produced by Müller glia were found to be highly upregulated in the gliotic retina as compared with the normal retina. These included G-CSF, MCP-1, PDGF-bb, RANTES, VEGF and TGFβ2.

Conclusions
This study has identified a significant upregulation of a wide spectrum of inflammatory and regulatory cytokines in the gliotic retina which are predominantly expressed by Müller glia. Interestingly, the pattern of cytokine expression by Müller glia mimics that of cytokines found to be highly upregulated in gliotic retina, suggesting that Müller glia may be the principal source of cytokines present in the gliotic retina. It is hoped that results of this study will lead to investigations of the effects of selective cytokines on the inhibition of the regenerative ability of Müller glia in the human retina.

Potential roles of ON and OFF channels in spatial- and contrast vision, and perhaps myopia

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Purpose
In animal studies it was found that the retinal ON channel mediates an inhibitory effect on myopia (Crewther and Crewther 2003). The ON channel is also more sensitive to contrast, while the OFF channel is more involved in spatial vision (Zaghloul, Boahen et al. 2003, Schiller 2010). The purpose of this study was to find out whether selective adaptation to ON or OFF stimulation affects visual acuity and suprathreshold contrast sensitivity.

Methods
Eleven subjects, aged 28.6 ±7.2 (range 22 to 47 years) years old participated the study. Two subjects were male, 9 were female. Their vision was corrected and ensured that a minimum visual acuity of 0.0 log MAR was reached (equivalent to a visual acuity of 1.0). All subsequent experiments were done with a defined defocus of 2 D because it is known that fully corrected visual acuity is scarcely affected by adaptation. Visual acuity was measured with the FrACT ("Freiburg Visual acuity and Contrast Test") and the supra threshold contrast sensitivity was measured with a custom-developed program involving a binocular match of supra-threshold contrast of sine wave gratings embedded in a Gabor patch (Ohlendorf and Schaeffel 2009). Each eye was differently stimulated using a divider and a divided screen. One eye was stimulated with a dynamic ON or OFF stimulus, the other was exposed to a stationary pattern. ON and OFF stimuli consisted of several hundreds of small fields (visual angles 1.87 cyc/deg) with saw-tooth shaped temporal luminance profiles - rapid ON for the ON stimulus and rapid OFF for the OFF version, repeated at 1.2 Hz. Stimulated eyes were randomly alternated. Stimuli were shown either in a dark room with adaptation periods of 7/14/21/28 minutes, or in bright room with 28 min adaptation time.

Results
Visual acuity increased in both eyes after 28 minutes of ON or OFF stimulation. However, this effect can be considered as adaptation to low pass filtering induced by the defocus. The ON or OFF stimulated eyes displayed a larger increase in visual acuity than the eyes exposed to stationary stimuli. Comparing the increase in visual acuity after ON and OFF stimulation after 28 minutes, they also differed from each other, both in darkness ($p=0.03$) and brightness ($p= 0.018$). Visual acuity increased maximally in the case of ON stimulation in a bright room ($p=0.01$), and after the OFF stimulation in darkness (p

Conclusions
Temporally modulated saw-tooth shaped luminance profiles increase visual acuity under defocus more than temporally stable patterns. The differences in visual acuity after 28 minutes of ON and OFF stimuli suggests that the different pathways were successfully stimulated. To increase visual acuity under defocus, the ON pathway should be stimulated in brightness and OFF pathway in darkness. Most likely, inhibition of myopia with ON or OFF stimulation is luminance-dependent.

Comparison of different DNA-based nanoparticles for the treatment of anterior segment diseases

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Purpose
An improvement of drug delivery to the eye is highly demanded to enhance efficacy of eye medication. Therefore, it is desired to explore delivery systems, which prolong the effectiveness of the applied drugs. We present a new class of DNA-based nanoparticles (NPs) for the treatment of anterior segment diseases. This whole variety of DNA-based NPs exhibit different affinity to the eye surface, allowing for tailored adhesion times and several loading possibilities. By tailoring the duration, time of the medication a lower regime can be attained, resulting in an improved compliance. In addition, it allows for a lower drug concentration, what leads to fewer side effects.

Methods
To evaluate the NPs, they were conjugated to a fluorescent marker and dropped on pig (from the slaughterhouse) and rat eyes (conscious animals). The incubation time was 5, 15 & 30 min for the pig eyes and 30 min and 2h for the rat eyes. Additional time points for some selected NP were performed and analyzed via histology and fluorophotometry. To exclude toxicity of the NPs, three ocular cell lines (661W, ARPE-19 & RGC-5) were incubated with the NP for 24h and cell viability, cell amount and caspase activity was measured. In addition, local in-vivo toxicity was evaluated via TUNEL-staining.

Results
In this study 13 different DNA-based NPs were tested in pig eyes. Seven of them showed a very good or excellent adhesion to the cornea. These seven NPs were further tested on conscious rats in which six showed good binding to the rat cornea epithelium for at least 30 min after application. The best NP showed an adherence to the corneal epithelium of at least four hours. Fluorophotometry studies confirmed the restriction of the NP to the cornea and the adherence time. Our NP did not show any toxicity at the used concentration.

Conclusions
In this study we selected the best DNA-NP out of 13 different types of DNA-NPs for further studies. We found that by changing the properties of the NP, i.e. length and number of lipid-modified bases, we are able to influence the adhesion time of the NP to the cornea. This fact is an important bonus, as the medication can be patient tailored. A first functionalization of the NP is shown on the poster of Strudel et al.

Statement on proprietary interests
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IZKF Promotionskolleg

GPU accelerated light propagation through retinal volumes mapped by multiphoton microscopy

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Purpose
The architecture of photoreceptor cell (PRC) nuclei differs considerably between nocturnal and diurnal mammals: whereas diurnal mammals possess conventional PRC nuclei, nocturnal mammals show a uniquely inverted PCR architecture characterized by the compaction of dense heterochromatin in the nuclear center instead of the usual euchromatin [1]. As the refractive index increases with molecular density, this nuclear inversion was suggested to reduce light scattering as inferred by 2D simulation, and direct measurement on individual isolated nuclei [2,3].The aim of this project is to create a realistic model of light propagation in the retina, initially focused on the optical characteristics of the outer nuclear layer.

Methods
First, we obtained a realistic model of the retina by multi-photon microscopy, taking advantage of its superior penetration depth and a reduction of artifacts related to scattering. Second, we implemented a beam propagation method on GPU accelerators that considerably speeds up the calculations and allows for simulating the propagation of light through the realistically large 3D retinal volume.

Results
By studying the evolution of the angular spectrum of light on its way through the 3D model of the outer nuclear layer we find that the near field coupling between cell nuclei of inverted architecture indicating an overall reduction in scattering, as opposed to a linear addition of each cells scattering contributions.

Conclusions
These results strongly support the initial hypothesis of reduced light scattering in the ONL due to an architectural inversion as previously inferred from the 2D simulations [1] by considering a much more detailed anatomical model. It further demonstrates that the arrangement and inversion of the outer nuclear layer nuclei in a 3D model creates a favourable light propagation environment for nocturnal mammals.References: [1] I. Solovei et al, Cell 137:2 (2009)[2] M. Kreysing et al, Optic Letters 35:15 (2010)[3] Z. Błaszczak et al, Optics Express 22:9 (2014)

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Rare variants associated with age-related macular degeneration result in a lower age at onset and higher familial occurrence

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Purpose
Recently, rare variants in the CFH (Arg1210Cys), CFI (Gly119Arg), C3 (Lys155Gln) and C9 (Pro167Ser) genes were found to be highly associated with age-related macular degeneration (AMD). The aim of the current study was to determine the contribution of these rare variants in the development of AMD in 22 multiplex families. In addition, we aimed to describe differences in clinical characteristics in carriers versus non-carriers of rare genetic variants, in these multiplex families and in a retrospective case-control cohort.

Methods
We included 707 AMD patients and 518 control individuals (>50 years) from the European Genetic Database (EUGENDA) database, including 114 affected and 60 unaffected members of 22 multiplex AMD families. All individuals underwent an ophthalmic examination including grading according to the standard protocol of the Cologne Image Reading Center and Laboratory, and completed a questionnaire on non-genetic risk factors, family history for AMD and age at onset of first symptoms. Venous blood was obtained for genetic analysis and measurement of complement activation levels.

Results
Rare variants CFI Gly119Arg, C9 Pro167Ser and C3 Lys155Gln were identified in five out of 22 multiplex families, but did not completely segregate with the disease phenotype. AMD patients who carried rare variant CFI Gly119Arg, C9 Pro167Ser or C3 Lys155Gln had a significantly lower age at first symptoms (65.7 vs 71.8 years; p = 0.011), and more often had a positive family history for AMD (52.5% vs 19.8%; p < 0.001) than patients who did not carry these rare variants. Advanced AMD patients with geographic atrophy carried these rare variants more frequently than patients with neovascular AMD (p = 0.03).

Conclusions
Rare genetic variants CFI Gly119Arg, C9 Pro167Ser and C3 Lys155Gln are more prevalent in patients with a positive family history for AMD, but do not completely segregate within families. Patients who carry one of these rare variants differ clinically from patients without this rare variant, as they have a lower age at first symptoms and more often progress to geographic atrophy.

Statement on proprietary interests
The authors have no proprietary or commercial interest in any materials discussed in this article.

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Hyperhydroxymethylation in rod photoreceptor cell death

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Purpose
DNA hydroxymethylation by Tet enzymes, oxidizing 5-methylcytosine (5mC) to 5-hydroxymethylcytosine (5hmC), is an important epigenetic process proposed to regulate gene expression, cell death and survival. 5hmC is found at high levels in the brain but the exact functions in neurons remains elusive. In this study we use mouse retina to examine the role of 5hmC in neurodegeneration.

Methods
5hmC and TdT-mediated dUTP-biotin nick end labeling (TUNEL) positive rod photoreceptors in rd1 mice were labelled using immunohistochemistry. To reveal differences in the retinal epigenome between the rd1 mouse model for retinitis pigmentosa and wild type (wt) mice hydroxymethylated DNA immunoprecipitation (hMeDIP) followed by next generation sequencing (NGS) was performed.

Results
Elevated levels of 5hmC were detected in rod photoreceptors at their peak time point of degeneration in rd1 mice. Furthermore 5hmC accumulation correlated with TUNEL in rod photoreceptors. hMeDIP data showed differences in the hydroxymethylation patterns of rd1 and wt mice with a higher amount of hydroxymethylation in rd1 than in wt mice. After mapping the peak locations to genes, 1797 genes were identified to be hyperhydroxymethylated in rd1 mice, whereas only 445 genes were hypohydroxymethylated compared to wt mice. Gene ontology (GO) analysis of the hyperhydroxymethylated genes in rd1 retina revealed regulation of phosphorylation as the most significant GO term.

Conclusions
The present study reveals an enrichment of 5hmC in degenerating rod photoreceptors suggesting a contribution to an epigenetically regulated cell death program. In summary, these findings provide novel insights into the role of 5hmC in neurodegeneration.

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Adaptation to spatially varying distortions

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Purpose
Progressive lenses distort the visual image in a spatially varying and gaze-direction-dependent way, and after some time, the visual system adapts to it. Mechanism of visual adaptation to optically induced spatially varying distortions (SVDs) were investigated.

Methods
Adaptation to spatially varied distortions was investigated by measuring an adaptation after effect of two oppositely oriented skew distortions in parallel. Skewed video images were shown to 23 subjects as an adapting stimuli. The adaptation aftereffect of each skew distortion was evaluated by measuring the perceived undistorted angle (PUA) of a skewed checkerboard image in an adjustment task.

Results
Adaptation was obtained to both distortions. The PUA shifted from the baseline in the direction of the adapting skew direction after each adaptation step.

Conclusions
The visual system induces enhanced adaptation abilities to different distortions in parallel with which it can recalibrate at fast rate for several distortion profiles. Thus, a parallel memory of two different distortions was demonstrated.

Diabetic retinopathy and visual acuity in Funen, Denmark: the value of big datasets in Type 2 diabetes mellitus

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Purpose
To determine if the level of diabetic retinopathy (DR) affect best corrected visual acuity (VA) amongst patients with Type 2 diabetes mellitus on the Funen Diabetes Database (FDDB), Denmark.

Methods
The FDDB was established in 2003; since when patients living with diabetes mellitus (DM) and their health-care providers have access to latest relevant results including those from GPs, diabetic consultants and ophthalmologists. All results of body mass index, blood pressure, blood tests and DR screening are stored in this single point of entry database. FDDB contained 22,098 Funen patients at the point of data extract on the 28/11/2014. On this date, anonymized results for each patient at the time of their latest DR screening were extracted from FDDB. The patients' visual acuity (VA) was measured on a decimal scale, with 1.33 being the best. For the current statistical analysis, data for patients with Type 2 diabetes and their DR grade, visual acuity and HbA1c were used.

Results
Of the 22,098 patients', 14,230 had type 2 DM. Due to data distribution, the non-parametric Kruskal-Wallis test was performed. It showed that there was a statistically significant difference in VA between the different DR levels, Chi^2=502.3, p below 0.05, with a mean rank score of 7,383.6 for no DR; 6,611.2 for mild DR; 6,121.0 for moderate DR; 5,540.1 for severe DR and 3,545.4 for proliferative DR (PDR). It equates to median VA of 0.9 (range 0-1.33) in the no DR group and 0.6 (range 0-1) in the PDR group, a difference that can only be appreciated in such large sample. HbA1c is statistically different between the no DR, mean 52.5 mmol/mol (95% CI, 52.2-52.7) and PDR patients, mean 60.9 mmol/mol (95% CI, 59.4-62.5).

Conclusions
We have shown a statistically significant decrease in visual acuity with increasing levels of DR severity and also for patients with worse DM control, but our data argues the need for large samples as such a differences would have been impossible to appreciate in a smaller study.

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Neuroprotective effect of hypothermia and cyclosporine A in an in vitro retinal ischemia model

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Purpose
Retinal ganglion cell death induced by ischemia can lead to irreversible loss of vision as found in central retinal artery occlusion (CRAO). Currently there is no therapy available to treat this ophthalmic infarct. However studies indicated that reperfusion within four hours could improve the clinical outcome. Here we investigate the neuroprotective effect of hypothermia and cyclosporine A (CsA) as possible candidates to extend the therapeutic window until reperfusion of the retina.

Methods
Retinal ischemia was mimicked in vitro using a custom-made ischemic chamber. In this chamber retinal explants were kept under oxygen free conditions for 75 minutes. Treatment with 1mM glutamate was used as a comparative model for ischemic cell death. The potential agents hypothermia and CsA were tested at different temperatures/concentrations (20°C or 30°C and 6µg/ml or 9µg/ml respectively). The neuroprotective effect on retinal ganglion cell survival was analyzed on mRNA and protein level (THY1, NEFH, TUBB3, BAX, BCL2, GFAP, CD11B, CASP3 and HSP70) and additionally on a functional level via multi-electrode array recordings. Furthermore retinal thickness was measured using optical coherence tomography.

Results
Ischemia induced by the in vitro model caused a reduction in retinal thickness, accompanied by a decrease of neuronal markers. Furthermore, microglial and astrocytic activation, an increase in apoptotic markers as well as a reduced spontaneous retinal ganglion cell activity were observed. Hypothermia and CsA counteracted the loss in retinal thickness and expression of neuronal markers of retinal ischemia. In addition, both hypothermia and CsA rescued the spontaneous retinal ganglion cell activity and less glial activation was observed.

Conclusions
Both hypothermia and CsA exert neuroprotective effects on the survival of retinal ganglion cells after ischemia. In the occurrence of a central retinal artery occlusion a cooled irrigation solution or a solution containing CsA could be used to extend the therapeutic window until reperfusion of the retina.

Unraveling mechanisms of photoreceptor degeneration in Achromatopsia

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Purpose
Gene mutations interfering with the 3'-5'-cyclic guanosine monophosphate (cGMP) signalling pathway in the retina are known to cause severe and currently untreatable retinal degeneration, such as achromatopsia. In the outer segment of photoreceptors the level of this crucial second messenger is controlled by light. Downstream, cGMP regulates the activity of the cyclic nucleotide-gated (CNG) cation channel. We found that degenerating photoreceptors in mouse models of achromatopsia lacking CNG channels accumulate high amounts of cGMP presumably via sustained activation of retinal guanylyl cyclase (retGC). It is still unclear whether this cGMP accumulation is directly linked to cell death or results as a side effect of the degenerative disease.

Methods
To evaluate the mechanisms of cGMP accumulation we interfered with the cGMP signalling pathway by subretinal delivery of specific AAVs. With the aid of shRNA we knocked down (KD) retGC in our achromatopsia mouse model (CNGA3 ko) to monitor cone degeneration. In order to understand mechanisms downstream of cGMP we analysed the effect of high cGMP dependent kinase (cGK) activity by delivering the active protein in the retina of wildtype mice. To finally understand the contribution of cGKs to cone degeneration and cell death we examined photoreceptor cell survival in CNGA3 ko mice cross bred with cGK ko mice.

Results
The progression of cone degeneration is severely delayed in the absence of cGMP accumulation. We show that a downregulation of the cGMP producing retGC prolongs cone survival in CNGA3 ko mice after AAV application. Further on overexpressing a kinase downstream to cGMP leads to a progressive photoreceptor degeneration in wildtype mice while knocking out cGK delays cone cell death.

Conclusions
We show a strong cGMP accumulation during photoreceptor degeneration in CNGA3 ko mice and involvement of the second messenger in the degeneration process. Clearence of cGMP accumulation prolongs cone photoreceptor survival. In the absence of CNG channels cGMP-dependent protein kinases (cGKs) are the most promising candidates as mediators of cGMP-dependent cytotoxicity of photoreceptors. Photoreceptor degeneration can be induced by high activity of cGK. Most importantly the degeneration proceeds slower in absence of cGK. These results show that retinal degeneration is associated to cGMP signaling but further experiments are crucial to fully unravel the role of cGMP in retinal degeneration and to identify potential targets for the neuroprotection of cones in retinal diseases.

Statement on proprietary interests
The authors have no proprietary interests in this research project.

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The role of Snf2h gene during retinal development and differentiation.

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Purpose
Snf2h gene encodes an ATP-dependent chromatin remodeling protein whose task is associated with regulation of number of nuclear processes. The current issue is the role of Snf2h in development of individual tissues and organs. Mice with inactivated Snf2h gene die at the blastocyst stage, due to the lack of trophoectoderm and inner cell mass. However, its role during the later embryonic stages remains unknown. A recent study have shown an effect of Snf2h on development of the nervous system. Because of the neural origin of the retina, we are interested whether the function of Snf2h is also necessary for proper eye morphogenesis and differentiation. The retina is organized into three major layers, which consist of seven cell types. We are talking about the ganglion cells in the ganglion cell layer; amacrine, horizontal, bipolar and Müller glial cells in the inner nuclear layer; and two types of photoreceptors, rods and cones, in the outer nuclear layer. The aim of this study is to determine how Snf2h is involved in the development and differentiation of individual cells of the retina.

Methods
The most common way to identify the function of a particular gene is its deletion. We know from the available literature that the whole-body deletion of Snf2h gene is lethal. Therefore we use the Cre/ LoxP system, which permits the tissue specific gene deletion (conditional deletion). We use Snf2h flox/flox mice in combination with mRx_Cre mice. The expression of Cre recombinase is controlled by regulatory sequences of the mouse Rx gene, one of the earliest determinants of retinal development. A detail histological and expression analysis of wild-type and Snf2h mutant eye will be performed. We use both the cryo- and paraffin-sections and we perform the immunofluorescence analysis.

Conclusions
Our present data show, that the thickness of Snf2h mutant retina is in comparison with wild-type individuals significantly reduced. Some layers of the retina are completely lost and the main damage is visible in photoreceptors. Therefore it is likely that the mutant mice do not see.

Relationship between the subjectively and objectively determined depth of focus of the human eye using defocus curves

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Purpose
The study compared the depth of focus (DoF) of the human eye, calculated from objective image quality metrics (IQM) and subjectively measured defocus curves.

Methods
40 subjects with a mean age of 24.9±3.2 years and a mean spherical equivalent refractive error of M=-0.54D±2.15D participated and mydrias was assessed using three drops of 1% cyclopentolat (assessed three times with 10 min between applications). Monocular subjective defocus curves (range: ±1.5D in 0.5D steps) were measured in a distance of 5m in the fully corrected dominate eye using a 4mm artificial pupil. The DoF was calculated as the dioptric range under the defocus curve at the threshold "maximum visual acuity [logMAR] + 0.1". A commercial aberrometer (i.Profilerplus, ZEISS, Germany) was used to assess the ocular wavefront. The point spread function (PSF) and the optical transfer function (OTF) were analyzed of the single wavefront aberrations for a 4mm pupil, using Matlab (MathWorks, Natick, USA). The DoF was calculated using the augmented visual Strehl-Ratio of the OTF (VSOTFa) at the thresholds 80% and 50% of the maximum value as well as the visual Strehl-Ratio of the PSF (VSPSF) at a threshold of 80% and 50% of the maximum value. A two-tailed Student's t-test was used for statistical analysis.

Results
Using the VSOTFa, the DoF was 0.41±0.14D for the 80% and 0.76±0.19D for the 50% threshold, while the DoF was 0.69±0.15D for the VSPSF at the 50% and 0.35±0.10D at the 80% threshold. Subjective assessment of the DoF gave a mean value of 0.81±0.26D. DoF was significantly different for 80% VSOTFa ($p<0.001$) as well as for 80% and 50% VSPSF ($p<0.001$, $p=0.007$) compared to the subjective DoF, while the DoF at 50% VSOTFa ($p=0.349$) was not. Significant correlations were found between the RMS of higher order aberrations and the matching thresholds for VSOTFa ($y=324.2x+13.1$, $r=0.409$) and for VSPSF ($y=334.8x+8.6$, $r=0.505$). Nevertheless, there was no significant relationship between the metrics and the subjective measurements of the DoF (50% VSOTFa $r=0.20$; 50% VSPSF $r= 0.24$) neither with fixed or individual threshold levels, based on the HOA-RMS.

Conclusions
The estimation of the DoF using the VSOTFa at a 50% threshold from the maximum showed no significant difference to the subjectively measured DoF, but lacked a significant correlation. There was no relationship between the subjectively measured and objectively determined depth of focus of the human eye.

HDAC6 is present in retinal cells and is involved in protein aggregate formation

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Purpose
Retinitis pigmentosa (RP) and age-related macular degeneration (AMD) are retinal diseases characterized by the loss of photoreceptor cells. Recent studies suggest the involvement of histone deacetylases (HDACs) in neurodegeneration and aging, and that inhibition of HDACs plays a protective role. Using a pan-inhibitor of HDACs, it was demonstrated that in a mouse model for RP photoreceptor cell death could be prevented. Furthermore, HDAC activity was shown to be increased as an early effect following retinal ischemic injury in a rat model. HDACs comprise a family of 18 members, among these HDAC6 does not deacetylate histones, but has α-tubulin, HSP90 and cortactin as main substrates. HDAC6 is involved in protein aggregate formation, heat shock responses, macroautophagy and may play a critical role in cellular responses to stress situations. The present study was undertaken to investigate whether HDAC6 is present in retinal cells and whether its inhibition modifies protein aggregate formation which occurs after proteasomal inhibition.

Methods
To analyze the presence of HDAC6 retinae derived from C57BL/6 mice and the photoreceptor cell line 661W were used. Cell extracts were prepared and immunoblot analysis was carried out. Furthermore, indirect immunofluorescence and immunohistochemistry using antibodies against HDAC6, α-tubulin and acetylated tubulin was performed. To inhibit HDAC6 enzymatic activity tubastatin A (TST), a specific inhibitor of the second catalytic domain responsible for deacetylation activity, was used.

Results
As demonstrated by immunohistochemistry, HDAC6 is expressed in the mouse retina and prominent in photoreceptor inner segments as well as in the outer plexiform layer. It is also a constituent of 661W cells. During retinal development (1 – 28 days) a slight decrease in HDAC6 abundance was observed. The amount of acetylated tubulin is not altered. Inhibition of HDAC6 by TST (1 – 10 µM, 24 h) leads to the induction of heat shock proteins (HSPs) and to an increase in acetylated tubulin in 661W cells. Indirect immunofluorescence shows that microtubules appear more bundled when HDAC6 is inhibited. Treatment with MG-132, a proteasomal inhibitor, causes protein aggregate formation. Preincubation with TST impairs the formation of aggregates and the proteins are diffusely distributed within the cytoplasm.

Conclusions
HDAC6 is present in the mouse retina and its expression is decreased during development. Inhibition of HDAC6 by TST leads to hyperacetylated microtubules and to an increase in heat shock protein levels. Furthermore, protein aggregation, which is observed after proteasomal inhibition, is modified by TST. Our data indicate that HDAC6 is constitutively expressed in retinal cells and may play a major role in stress responses.

Acknowledgement
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Recombinant and Endogenous Expression of Age-related Maculopathy Susceptibility Protein 2 (ARMS2)

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Purpose
The formation of drusen at the macula and the degeneration of RPE cells is characteristic for AMD and represents the most common cause of blindness in developed countries. The ARMS2 variant at 10q26 (A69S, rs10490924) is strongly associated with AMD. This polymorphism is linked to a mutation in the 3' untranslated region in the ARMS2 gene, which introduces an instability motif into the transcribed mRNA. So far the endogenous protein expression of ARMS2 is still unclear and whether the ARMS2 risk variant leads to protein deficiency in certain cells.

Methods
56 patients with neovascular AMD were sequenced and the polymorphisms rs2736911, rs10490924 or del443ins54 in ARMS2 were evaluated. Monocytes isolated from whole blood of patients with defined genotypes, were stained with ARMS2 antiserum and ARMS2 expression was followed by laser scanning microscopy. Furthermore ARMS2 protein expression and location was evaluated in human retinal sections from ARMS2 genotyped patients.

Results
We identified ARMS2 expression in human blood derived monocytes by gene expression and laser scanning microscopy using ARMS2 specific antiserum. Expression of ARMS2 in monocytes, as well as microglia cells was confirmed by siRNA, laser scanning microscopy of human monocytes as well as co-staining of retinal sections with ARMS2 antiserum and CD68, a specific marker for monocytic cells. Interestingly ARMS2 was absent in monocytes derived from AMD patients homozygous for the ARMS2 risk variant (A69S, rs10490924) and also in microglia cells of retinal sections from individuals homozygous of the ARMS2 risk variant. Thus, the risk variant of ARMS2 leads to ARMS2 protein deficiency in monocytes and microglia cells.

IFNß-treatment as a therapy targeting microglia in a murine model of retinal degeneration

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Purpose
Age-related macular degeneration (AMD) is a leading cause of vision loss in the elderly. Typical hallmarks of AMD are chronic activation of the innate immune system and reactive microglial cells in the retina. Here, we analyzed the role of interferon beta (IFNß) signaling and the effect of IFNß therapy on microglial activation and choroidal neovascularization in a murine model of AMD-like retinal damage.

Methods
Laser-rupture of Bruch's membrane was used as a murine model for AMD. Retinal inflammation and choroidal neovascularization (CNV) were analyzed in IFN-alpha/beta receptor knockout (IFNAR-/-) mice, IFNß-treated C57BL6/J mice and C57BL6/J wild type controls using fundus fluorescein angiography (FFA), lectin staining and optical coherence tomography (OCT). Microglial morphology in laser-induced lesions was analyzed by Iba1 and Tspo staining of flatmounted retinas and retinal pigment epithelia (RPE).

Results
Laser-induced lesions in IFNAR-/- animals showed increased vessel leakage as well as CNV compared to control animals, indicating that IFNAR-/- deficiency enhanced inflammation. In contrast, IFNß-treated animals showed reduced vessel leakage and CNV compared to untreated controls. OCT-analysis of IFNß-treated and untreated wild type mice 7 and 14 days after induction of the retinal damage revealed diminished edema formation in IFNß-treated animals. Immunohistological analysis of flat-mounted laser-damaged retinas displayed both, a higher number and a longer presence of activated microglial cells at the sites of damage in IFNAR-/- mice compared to controls. The amount of activated microglia cells in IFNß-treated animals was lower than in respective control groups.

Conclusions
Knockout of IFNAR leads to enhanced retinal inflammation and microglial reactivity. In contrast, IFNß therapy significantly prevented vessel leakage, CNV and microglial activation. We conclude that IFNß signaling dampens microglial reactivity and is a protective mechanism in retinal degeneration.

Peripapillary retinal nerve fiber layer thickness corresponds to drusen location and extent of visual field defects in superficial and buried optic disc drusen

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Purpose
Optic disc drusen (ODD) are hyaline deposits located within the optic nerve head. Peripapillary retinal nerve fiber layer (RNFL) thinning is presumably associated with the high prevalence of visual field defects seen in ODD patients. The goal of this study was to investigate the basic characteristics of patients with ODD, to compare the peripapillary RNFL thickness to the anatomic location (superficial or buried) of ODD, to classify the type and extent of visual field defects and correlate these to peripapillary RNFL thinning, and to assess the risk of drusen-associated complications.

Methods
Retrospective, cross sectional study.

Results
149 eyes of 84 ODD patients were evaluated. 65% were female and 76% had bilateral ODD. 109 had superficial ODD and 40 had buried ODD. Peripapillary RNFL thinning was seen in 83.6% of eyes where OCT was performed (n =61). Eyes with superficial ODD had greater mean peripapillary RNFL thinning (P

Conclusions
Present basic characteristics for ODD patients correspond well with previous reports. Peripapillary RNFL thickness correlates with anatomic drusen location and the extent of visual field defects. Anterior ischemic optic neuropathy is the most frequent severe complication of ODD.

Statement on proprietary interests
There are no conflicts of interest

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Who let the dogs out?: Detrimental role of Galectin-3 in hypoperfusion-induced retinal degeneration

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Purpose
Retinal ischemia results in a progressive degeneration of neurons and a pathological activation of glial cells, resulting in vision loss. In the brain, progressive damage asfter ischemic insult has been correlated to neuroinflammatory processes involving microglia. Galectin-3 has been shown to mediate microglial responses to ischemic injury in the brain. Therefore, we wanted to explore the contribution of Galectin-3 (Gal-3) to hypoperfusion-induced retinal degeneration in mice.

Methods
Gal-3 knockout (Gal-3 KO) and WT C57BL/6 mice were subjected to chronic cerebral hypoperfusion by bilateral narrowing of the common carotid arteries using metal coils. Sham operated mice served as controls. After 17 weeks, the mice were sacrificed and the eyes were analyzed for retinal architecture, neuronal cell survival and glial reactivity using morphological staining and immunohistochemistry.

Results
Hypoperfusion caused a strong increase in Gal-3 expression and microglial activation in WT mice, coupled with severe degenerative damage to all retinal neuronal subtypes, remodeling of the retinal lamination and Müller cell gliosis. In contrast, hypoperfused Gal-3 KO mice displayed a retained laminar architecture, a significant preservation of photoreceptors and ganglion cells neurons and an attenuation of microglial and Müller cell activation.

Conclusions
Gal-3 expression after ischemic insult mediates Müller cell and microglial activation, and results in significant retinal degenerative damage. Gal-3 is thereby a potential target for treatment and prevention of hypoperfusion-induced retinal degeneration, and a strong candidate for further research as a factor behind retinal degenerative disease.

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Hypothermia promotes survival of ischemic retinal ganglion cells

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Purpose
Ischemic stroke in the retinal arteries leads to death of neural tissue and ultimately to blindness. The retina is known to die within four hours after onset of ischemia. It is debated whether hypothermia might increase the time window for medical treatment and thereby the chance of recovery of sight. In order to characterize the time course of cell death during ischemia and potentially beneficial effects of hypothermia in more detail, we investigated the survival of ganglion cells in ischemic pig and human retina as a function of time and temperature.

Methods
Eyes were obtained from minipigs and from post mortem human donors. Enucleated pig eyes were stored for defined durations at three different temperatures (37°C, 21°C, and 4°C). In order to assess the viability of the tissue, we measured ganglion cell activity (spiking) with a multi electrode array.

Results
Our results confirmed that pig retinal ganglion cell function was severely compromised already after 2 hours of ischemia at body temperature. After 4 hours under ischemic conditions at 37°C, ganglion cells did not fire action potentials anymore. However, ganglion cell activity in pig retina was maintained under ischemic conditions for up to 12 hours at 21°C and at least 40 hours at 4°C. Moreover, in post mortem human retina, we recorded spontaneous ganglion cell activity in retina harvested up to 27 hours after death.

Conclusions
Our results demonstrate that hypothermia greatly increases survival of retinal ganglion cells exposed to ischemia. These results might be relevant for the future treatment of retinal ischemia.

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Peripherin-2 Interacts with Cone Opsins

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Purpose
Mutations in peripherin-2 are one of the most common causes for autosomal dominant hereditary retinal diseases. Interestingly, some of these mutations lead to degeneration of rods whereas others primarily affect cones. Recently, we have shown that peripherin-2 interacts via its fourth transmembrane domain (TM4) with the rod-specific light sensor rhodopsin. Furthermore, we demonstrated that a mutation within TM4 (p.G266D), which is linked to rod disorders, selectively disrupts this interaction. In this study, we set out to investigate and quantify the interaction of peripherin-2 with cone opsins in HEK293 cells. In addition, we analyzed if another mutation in TM4 of peripherin-2 (p.V268I), which is linked to cone degeneration, affects the interaction of peripherin-2 with cone opsins.

Methods
We combined co-immunoprecipitation and fluorescence resonance energy transfer (FRET) to investigate the binding properties of wild type and p.V268I peripherin-2 with cone opsins in transfected HEK293 cells. Immunoelectron microscopy was used to analyze the co-localization of peripherin-2 and cone opsins in murine cone photoreceptors. To address the expression of the p.V268I mutation in cones, wild type mice were subretinally injected using recombinant adeno-associated virus (rAAV)-mediated gene transfer.

Results
We showed that peripherin-2 interacts with the short-wavelength (S-) and the medium-wavelength (M-) opsin. However, compared to rhodopsin, the binding affinity of peripherin-2 to cone opsins was significantly lower. Quantitatively, the p.V268I mutation leads to a selective increase of binding to M-opsin whereas binding to S-opsin and rhodopsin remains unaffected. The low binding affinities were confirmed by immunoelectron microscopy showing only a partial co-localization of peripherin-2 and cone opsins. Immunohistochemical staining of transduced murine retinae excluded any effects of the p.V268I mutation on protein expression and localization, respectively.

Conclusions
We unveil a novel role of peripherin-2 in cone photoreceptors and provide first insights into the disease mechanism of the p.V268I mutation. These results might contribute to explain the differential role of peripherin-2 in rods and cones.

Acknowledgement
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The Evolution of vertebrate eyes: Insights from Cephalochordates

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Purpose

The evolution of vertebrate camera eye was always puzzling. Even Charles Darwin pointed out in his ground-breaking work On the Origin of Species to topic of eye evolution: „To suppose that the eye, ..., could have been formed by natural selection, seems, I freely confess, absurd in the highest possible degree. Yet reason tells me, that if numerous gradations from a perfect and complex eye to one very imperfect and simple, ..., can be shown to exist; ..., then the difficulty of believing that a perfect and complex eye could be formed by natural selection, though insuperable by our imagination, can hardly be considered real.“Amphioxus (Branchiostoma) and Asymmetron belonging to subphylum Cephalochordates, thanks to their key phylogenetic position, serve as excellent model organisms for studying vertebrate body plan features evolution. In our study we focus on comparison of Cephalochordate and vertebrate eye development. Our previous work, done on Branchiostoma floridae, showed, that highly similar genes are utilized for amphioxus frontal eye (comprising of ~10 photoreceptor and ~10 pigment cells) as well as for vertebrate eye development (comprising of millions of photoreceptor and pigment cells). Currently we are focussing on comparison of frontal eye development between two Cephalochordate genera Branchiostoma and Asymmetron separated in evolution by ~200 million years to get better insight into general rules of Cephalochordate and vertebrate eye development.

Methods

In our study we mainly use whole-mount immunofluorescent and mRNA in situ staining of Branchiostoma and Asymmetron larvae with subsequent confocal microscopy imaging. With this approach we can identife molecular fingerprint of Cephalochordate photoreceptor and pigment cells on a single cell resolution. We strongly benefit from „homemade“ antibodies raised against amphioxus specific antigens.

Conclusions

Our previous and present data show, that development of photoreceptor and pigment cells is highly similar in phylum Chordata from amphioxus to vertebrates. This can help for understanding the evolution of highly complex camera type vertebrate eyes from simple frontal eye of Cephalochordates.

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Attenuation of photoreceptor cell death by p75NTR antagonists in murine models of Retinitis Pigmentosa.

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Purpose
Retinitis Pigmentosa (RP) is a heterogeneous group of genetic retinal dystrophies. In most forms of RP, photoreceptor cell death is associated with disease progression. Reactive Müller cell gliosis and microglial activation are also found, often prior to photoreceptor death. Here, we studied the involvement of proNGF signaling in the retinal degeneration and its possible utility as a therapeutical target.

Methods
We analyzed the expression of pro-inflammatory cytokines and of markers of Müller cell gliosis by qRT-PCR, as well as microglial activation by immunohistochemistry in retinas from wild type and rd10 mice. Antagonists of p75NTR were tested in retinal explants ex vivo and by intravitreal injection in vivo.

Results
A marked increase in GFAP and TNFα transcription preceded photoreceptor cell death in rd10 retinas. Neurotrophin signaling elements were present at identical levels in both, WT and rd10 retinas. However, the unprocessed proNGF increased in the rd10 retina prior to photoreceptor cell death. Upon p75NTR antagonist treatment, reduced photoreceptor cell death was observed in retinal explants, as well as a better preservation of the outer nuclear layer in vivo, in both rd10 and RhoP mouse models.

Conclusions
Our results suggest the existence in degenerating retinas of a pro-inflammatory cascade mediated by the signaling of proNGF on p75NTR. Modulation of the p75NTR appears as a possible target to attenuate retinal dystrophy.

Hydrophilic vitreous substitute based on cross- linked hyaluronate – assessment of effects on retinal function and intraocular pressure using alternatives to animal testing

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Purpose
Vitreous substitutes are needed as tamponades after vitrectomy for retinal detachment. Hydrophilic biopolymers such as cross-linked hyaluronate are promising substances with the potential to overcome the drawbacks associated with current hydrophobic tamponade materials. Before a potential use in humans, biocompatibility of these hydrogels with respect to retinal function and intraocular pressure (IOP) needs to be demonstrated.

Methods
Sterilized cross-linked hyaluronate (Princess Volume, Croma Pharma, Austria) with a refractive index of 1.33 (identical to human vitreous) was injected through 23 or 20 G needles either into ex vivo vitrectomized pig eyes or on top of bovine retinal flat mounts.Using this non-animal testing strategy, hydrogel biocompatibility was evaluated by measuring the scotopic electroretinogram (ERG) of the isolated perfused bovine retina and continuously assessing the IOP in the anterior chamber of the whole pig eye.

Results
In the experimental setup of the isolated perfused bovine retina the B wave dropped by 22% 5 min after application of the hydrogel, which had been injected during a stop of perfusion for 120 s. At the same time the A wave showed a slight increase of 5.8%. At the end of the measurement, the B wave had decreased by 7.8% (p=0.099; n=5) while the A wave revealed an increase of 13.7 % (p=0.108; n=5). In the modified anterior chamber eye model the pressure the gel exerted on the eye was constant and comparable to BSS. The IOP value did not differ more than 1.8 mmHg from control in the eyes treated with the cross-linked hyaluronate hydrogel. In the first hour significant differences were found, that could be explained with adaption to the perfusion. In the next 23 h no significant differences were observed (p>0.05; n=4).

Conclusions
According to the ERG results no negative or toxic effects with respect to retina functionality were observed in the isolated perfused retina model. The swelling of the hyaluronate hydrogels did not result in significant increase of the IOP in the modified anterior chamber model, indicating their potential clinical applicability. Thus, the hyaluronate hydrogel met two important criteria required for vitreous replacements. The use of the described ex vivo models will allow to reduce the number of animal experiments.

Statement on proprietary interests
Kai Januschowski: Commercial Relationship(s);Croma Pharma GmbH (Financial Support) |Sven Schnichels: Commercial Relationship(s);Croma Pharma GmbH (Financial Support) | Martin Prinz: Commercial Relationship(s);CromaPharma GmbH (Personal Financial Interest) | Christine Hohenadl: Commercial Relationship(s);Croma PharmaGmbH (Employment) | Charlotte Reither: Commercial Relationship(s);Croma Pharma GmbH (Employment) | Martin Spitzer: Commercial Relationship(s);Croma Pharma GmbH (Financial Support)

Activation of the complement system in retina and optic nerve in an autoimmune glaucoma model

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Purpose
Glaucoma is a multifactorial disease and its pathomechanisms are still unclear. Studies showed that the immune system is involved in glaucoma. Here, we compared the activation of the complement system, as part of the innate immune system, in the retina and the optic nerve in an autoimmune glaucoma model.

Methods
Rats were immunized with optic nerve homogenate (ONA) or S100 protein. The control group (Co) received sodium chloride. After 3, 7, and 14 days cross-sections of the retina and the optic nerve were stained with antibodies against C3 and membrane attack complex (MAC, n=5-6/group). Cells were counted using ImageJ software. At 7 days, C3 was analyzed via Western Blot. Statistic was performed using t-test. Retinas were also used for quantitative rt-PCR of C3 and C5 (n=3-5/group) and this data were analyzed using REST software.

Results
In the retinas, no changes in C3 and MAC staining could be noted for both immunized groups at 3 days. At 7 days significant more C3+ and MAC+ cells could be observed in the ONA group (C3: p=0.04, MAC: p=0.03), while no changes were noted in the S100 group. Protein analysis via Western Blot revealed an increase of C3 in the ONA group (p=0.049), while no alterations were noted in the S100 group. At 14 days, an increase of C3+ cells was observed in the S100 group (p=0.04), but not in the ONA group. No alterations were noted in MAC staining for ONA and S100. PCR data revealed an increase of C3 mRNA in the S100 (1.6 fold, p

Conclusions
Immunization with ocular antigens leads to an activation of the complement system at an early point in time in glaucoma degeneration. We could show that C3 and MAC were not solely activated in the retina, but also in the optic nerve. We assume that the activation of the complement system triggers RGC death in the retinas, while the complement might play a role in optic nerve degeneration. The complement activation seems to occur simultaneously in both tissues.

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A tool for predicting disease genes associated with dominant retinal degenerations.

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Purpose
Variant filtering remains a bottleneck for the identification of candidate genes in any Next Generation Sequencing (NGS) project. This is even more critical in genetically heterogeneous diseases, such as retinal degenerations. The purpose of this work was to predict the likelihood for a gene to be involved in a dominant disease, considering the distribution of pathogenic and non pathogenic alleles in the general population.

Methods
Dominant properties of genes were computed by using information available on public databases of exonic variants. We selected 291 autosomal dominant (AD) and 446 autosomal recessive (AR) genes, which were assessed for specific characteristics, such as length, number and type of variants, conservation. The similarities and differences were scored with parametric and nonparametric tests and Monte Carlo simulations. We evaluated our algorithms by using a list of known genes associated with AD and AR retinitis pigmentosa (RP), as reported in the RetNet database.

Results
AR genes carried a higher number of nonsynonymous (NS) variants compared to AD genes (p=0.0035). This is probably because pathogenic recessive alleles are tolerated in a heterozygous state, while dominant ones are not. This effect was even more significant when the per gene ratio between NS and synonymous variants was considered (p inf 10-10). A predictive tool was constructed based on the distribution of these ratios, and tested on ADRP vs. ARRP. ADRP genes could be predicted with 30% sensitivity and 100% specificity.

Conclusions
By analyzing specific patterns of variant distribution, we could differentiate AD genes from AR ones. Although our model fails to detect many true positives, it does not provide false negative results, which represent the major obstacle in successful NGS filtering procedures. Analyses of additional features of AD vs. AR genes are currently ongoing, to ascertain whether other elements could improve our final prediction rate.

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Read-through strategies for therapeutic suppression of nonsense mutations in Usher syndrome

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Purpose
The Usher syndrome (USH) is the most frequent cause of inherited combined deaf-blindness, distinguished into three clinically and genetically subtypes. So far no effective treatment for the retinal degeneration of USH exists. Data base revealed that approximately 20% of all USH cases are caused by nonsense mutations leading to the expression of truncated non-functional protein. Translational read-through (TR) mediates over-read of nonsense mutations and thereby induces the expression of full length proteins. The read-through of nonsense mutations by translational read-through inducing drugs (TRIDs) has become a promising pharmacogenetic strategy for degenerative diseases. Here we aim to analyse the read-through efficacy of TRIDS on mutations from different USH causing nonsense mutations.

Methods
We generated a bidirectional reporter construct (PBI-CMV-HA) coding for dsRed to determine the transfection efficacy and the expression of the gene of interest with an HA-tag. Next we transfected selected nonsense mutations in the cDNAs of USH1C isoform harmonin a1 (p.R155X), harmonin b3 (p.R155X), USH3A (p.Y63X; p.Y176X) within the reporter constructs respectively and TRIDs i.e. PTC124, aminoglycosides and the designer aminoglycosides NB84 and NB124 were applied to the culture media.We compared the read-through efficiency of different TRIDs via quantitative immunofluorescence and Western blot analyses for all selected nonsense mutations.

Results
Our studies revealed the recovery of protein expression after TRIDs applications for all analyzed nonsense mutations in Usher Syndrome.

Conclusions
Conclusively, the read-though efficacy of TRIDs on the diverse USH causing nonsense mutations emphasizes the potential of TRIDs for the treatment of USH.

Statement on proprietary interests
Gene based therapies for the treatment of inherited disorders.

Newly generated antibodies indicate CFHR3 function in the human complement system

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Purpose
The CFH-related protein family (CFHR) comprises a group of five plasma proteins structurally and functionally related to the main negative complement regulator, factor H (CFH). Mutations within the CFH/CFHR gene cluster are associated with various human diseases such as age-related macular degeneration (AMD), the leading cause of irreversible vision loss in the elderly. Deletion of the CFHR3/1 genes goes along with a lower risk for AMD although the precise functions of the CFHR proteins still remain unclear. Based on our previous work, which resulted in CFHR3 monoclonal antibodies (mAbs), we aim to elucidate the role of CFHR3 in complement regulation in degenerative diseases.

Methods
Generated mAbs were tested for specificity and avidity against human CFHR3 in indirect enzyme-linked immunosorbent assay (ELISA) and Western blot. MAbs were used for immunoprecipitation from human serum and following mass spectrometry analysis. Complement modulation was analyzed in functional in vitro hemolysis assays and complement ELISA. Sandwich ELISA for detection of CFHR3 from human serum was established. Further, the interaction of CFH and CFHR3 with the oxidative stress marker CEP (ω-(2-carboxyethyl)pyrrole) and the complement component 3b (C3b) was tested.

Results
Newly generated mAbs were highly specific for human CFHR3. MAb 269-5 showed the highest avidity in indirect ELISA and detected CFHR3 in human serum. Furthermore, mAb 269-5 immunoprecipitated CFHR3 in complex with alternative and terminal complement components from human serum. Preliminary in vitro analyses showed an inhibitory effect of high anti-CFHR3 mAb concentrations on the alternative pathway. Promising data exposed a binding of CFH as well as CFHR3 to an oxidative stress epitope (CEP) and to C3b. Both proteins competed for binding to these molecules, and mAb 269-5 interfered with this competition by inhibiting CFHR3 interaction. MAbs 269-5 and 552-3 detected specifically CFHR3 from human serum in an optimized sandwich ELISA.

Conclusions
We generated and characterized highly specific monoclonal antibodies against CFHR3. These first results for a mAb-based in vitro modulation of the complement system encourage further investigation into the functional role of CFHR3 in complement and AMD. A competition of CFH and CFHR3 to oxidative stress epitopes and C3b impairs the local complement system homeostasis in the eye. We identified CFHR3 as a promising target for immune modulation in AMD patients.

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Targeting the translocator protein (18kDa) (TSPO) prevents microglia reactivity and protects from light induced retinal degeneration

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Purpose
Microglia activation is a common hallmark of several retinal degenerative diseases. Our previous work showed that TSPO is as marker for reactive retinal microglia and that the selective TSPO ligand XBD173 exerts strong anti-inflammatory effects on microglia in vitro. In the present study, we investigated whether XBD173 has the capacity to modulate retinal microglia in vivo and thereby protects from light induced retinal degeneration.

Methods
BALB/C mice were treated with vehicle or 10 mg/kg XBD173 by intraperitoneal injection prior to exposure to 15.000 lux white light for one hour. Daily XBD173 treatment was continued for four consecutive days. After this time period, retinal flat-mounts and sections were prepared to analyze microglia morphology, localization and reactivity using Iba1 and TSPO protein expression. Optical coherence tomography, morphometric measurements of retinal thickness and TUNEL stainings were used to to determine the extent of retinal degeneration and photoreceptor apoptosis.

Results
In control eyes that were not exposed to light, Iba1 staining revealed that microglia were located in the inner and outer plexiform layers and showed a ramified morphology. Light exposed animals that were sham treated displayed a severe thinning of the photoreceptor layer and prominent photoreceptor apoptosis which was accompanied by the migration of amoeboid microglial cells into the outer nuclear layer and the subretinal space. TSPO staining revealed a strong TSPO expression in these microglia, indicating a highly reactive status. In contrast, light exposed mice that received XBD173 injections showed a well preserved photoreceptor layer and strongly reduced apoptosis. Significantly fewer numbers of amoeboid microglial cells were present in the ONL and subretinal space and nearly all of them displayed a ramified cell shape. Furthermore, these microglia showed much less staining for the activation marker TSPO.

Conclusions
TSPO-specific XBD173 treatment of mice challenged with intense withe light reduced the number of reactive microglia and protected retinal photoreceptors from light induced apoptosis. We conclude that TSPO and its ligands represent promising targets for neuroprotective and anti-inflammatory therapy of retinal degenerative diseases.

Mechanisms underlying the modulation of horizontal cell gap junctions by all-trans retinoic acid

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Purpose

Horizontal cells (HC) are extensively coupled via gap junctions (GJ) composed of connexins. Recently different connexin isoforms have been identified in carp HC. One isoform is cpCx53.8 which is expressed in all four HC types. There is evidence that GJ function is highly modulated by the phosphorylation of the involved connexins. Here we investigated whether cpCx53.8 phosphorylation is modulated by all-trans retinoic acid (at-RA), a byproduct of the phototransduction cascade. Therefore we used an antibody detecting the phosphorylated isoforms of cpCx53.8 in western blot and immunhistological experiments. To identify the underlying signaling cascade, we additionally tested if at-RA effects could be inhibited by different protein kinase blockers. As a control light adapted and dopamine (DA)-treated retinas were used, as it is known from several previous studies that DA induces the cAMP-triggered phosphorylation of cpCx53.8 via PKA.

Western blot analyses revealed increased cpCx53.8 phosphorylation following light adaptation. Treatment of dark-adapted retinas with at-RA resulted in a comparable intense phosphorylation of cpCx53.8. Pre-incubation of retinas with specific PKA and PKC activators (cAMP and PDBu, respectively) and blockers (H89 and stau, respectively) showed that DA and at-RA mediate their effects by the activation of two intracellular signaling cascades, whereby at-RA acts via PKC. Immunhistological studies on retinas treated with at-RA and PDBu alone and together with stau showed changes in cpCx53.8 immunoreactivity (IR) pattern, indicating changes in the distribution of the connexin as a result of phosphorylation.

To unravel mechanisms on the cellular bases, N2A cells transiently transfected with PCS2+/cpCx53.8 were used. Transfected cells were treated with at-RA and PDBu, whereas cAMP was used as a positive control. In this case activation of PKA and PKC resulted in an increase in cpCx53.8 IR associated with the plasma membrane. These effects were blocked by pre-incubation with specific PKA and PKC inhibitors (H89 and stau, respectively), indicating that phosphorylation of cpCx53.8 is important for its incorporation into the plasma membrane. Western blot analyses confirmed the observed increase in cpCx53.8 IR in membrane fractions of the cells after treatment with cAMP and PDBu.

In contrast, treatment with at-RA had no effect on the distribution of cpCx53.8 in N2A cells. These results suggest that at-RA does not interact directly with PKC to support incorporation of cpCx53.8 into the plasma membrane as shown for PDBu. Furthermore, this result is in contrast to retinal studies, which showed an at-RA induced increased cpCx53.8 IR in the OPL. In summary these findings suggest that at-RA exerts its effect on cpCx53.8 phosphorylation via PKC, whereupon in vivo an additional protein like a receptor seems to be involved in the signaling cascade, as at-RA treatments in the artificial system had no effect.

Therefore future experiments need to clarify whether an at-RA receptor is expressed in HC and which PKC isoform (Ca^{2+}-dependent or independent) is involved in the signaling cascade.

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In situ detection of chromatin remodeling during postnatal retinal development

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Purpose
Ten-eleven translocation (TET) hydroxylases can oxidize the epigenetic mark 5-methylcytosine (5mC) to 5-hydroxymethylcytosine (5hmC) which is usually associated with an activation of gene transcription in neurons. It is hypothesized that neuronal TET3 is recruited to the DNA by transcriptional regulators. Screening for proteins that co-precipitate with TET3 from mouse retina identified the transcriptional repressor REST and the histone H3 lysine 36 (H3K36) methyltransferase NSD3 as highly enriched interactors of TET3. To unravel the relations between hydroxymethylation and induction of gene expression, we set out to further characterize this interaction in the developing mouse retina.

Methods
Proximity ligation assay (PLA) allows the in situ visualization of protein-protein interactions in fixed cells and tissues. The assay only gives a fluorescent signal when the interacting molecules are in close proximity (below 40 nm). Based on the identified interaction of TET3 with REST, we used PLA to analyze the subnuclear localization of 5mC/5hmC and REST in retinal cryosections. Since TET3 was also shown to interact with the histone H3 lysine 36 (H3K36) methyltransferase NSD3, we analyzed the co-localization of their enzymatic products 5hmC and trimethylated K36 of histone 3 (H3K36me3). To gain mechanistic insights, we manipulated the system by overexpressing TET3 and NSD3 in HEK293T cells followed by PLA analysis of 5hmC and H3K36me3.

Results
In the retina REST showed a positive PLA signal with both epigenetic DNA modifications, 5mC and 5hmC. However, the REST/5mC signal was exclusively localized to heterochromatin, whereas REST/5hmC signals could only be observed in euchromatin. While the REST/5hmC PLA signal correlated positively with the 5hmC levels, increasing from postnatal week 2 to week 3, the PLA signal for REST/5mC decreased over the same period of time. PLA also revealed a clear increase in 5hmC/H3K36me3 co-localization in nuclei of 3-week old retina, whereas at 2 weeks only a few or no PLA signals were observed. Moreover, overexpression of TET3 and NSD3 in HEK293T cells resulted in a significant increase of the 5hmC/H3K36me3 PLA signal compared to overexpression of either only TET3 or NSD3 suggesting a functional interaction of these two epigenetic writers.

Conclusions
PLA on cryosections of the developing mouse retina and HEK293T cells provided novel mechanistic insights into transcriptional activation in neurons involving REST-guided targeting of TET3 to the DNA for directed 5hmC generation and NSD3-mediated H3K36 trimethylation. Importantly, PLA enabled the in situ detection of chromatin remodeling in retinal neurons at a resolution below 40 nm.

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Adherence, efficacy,and safety of antibiotic delivery through DNA based nanoparticles

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Purpose
Currently diseases of anterior segments, like corneal infections, can only be treated with multiple daily doses of highly concentrated antibiotics. Therefore it is desired to explore a delivery system, which binds to the surface tissue and thus prolongs the effect. We developed a novel class of DNA nanoparticles (NPs) which can be loaded with several drugs.

Methods
In this study kanamycin was conjugated to DNA-based NPs via a DNA aptamer. Kanamycin and kanamycin loaded NPs were dropped on human corneas and on eyes of conscious rats and the adherence time to the cornea was evaluated via histology. Furthermore, the in-vitro functionality of kanamycin was proved in a MIC-Test using E.coli. Additionally, we dropped kanamycin loaded NPs and pristine kanamycin on corneas. After 5 min the corneas were washed and transferred to petrifilms. Then E.coli colonies were applied onto the corneas and incubated at 37°C for 48h. Then the amounts of colonies were counted. Finally, in-vivo and in-vitro toxicity was evaluated.

Results
Kanamycin conjugated NPs showed a prolonged adherence time of up to two hours in-vivo and in-vitro. In contrast the pristine kanamycin was washed away after five minutes. For kanamycin the functionality of the antibiotic was still conserved, when loaded to the NP. Both kanamycin alone and the loaded kanamycin-NPs were able to decrease the amount of colonies compared to the untreated corneas. However, kanamycin-NP showed a ten times longer activity on pig corneas than pristine kanamycin. In addition, no toxicity was observed in-vivo or in-vitro.

Conclusions
We present the functionality and safety of our DNA-based NP carrier system in-vitro and in-vivo. This is an important step to prove that drugs bound to the NP are still functional or functionality can be restored. The functionality of the drug depends on the binding constant of the drug to the NP, which can be influenced by different binding methods. Further research needs to be performed in infected animal studies to further explore the potential of this carrier system.

Statement on proprietary interests
SS: Novartis: Financial Support, University Eye Hospital Tübingen: Patent; MSS: Alcon Recipient, Novartis: Financial Support, University Eye Hospital Tübingen: Patent; AH: University Groningen: Patent; JWdV: University Groningen: Patent.

Optical Coherence Tomography Value of Multiple Sclerosis

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Purpose
The main aim of this study was to investigate whether OCT is a valuable technique to measure multiple sclerosis in Lithuanian population.

Methods
We performed a prospective study in the Vilnius University Hospital Santariskiu Klinikos departments of Neurology and Ophthalmology. In this study over all 71 patients with MS were enrolled accord ing to patients epidemiological (gender, age), instrumental examination (visual evoked potentials) and laboratory examination data (oligoclonal bands and cerebralspinal fluid (CSF) index). All subjects were examined with OCT by measuring RNFL and papillomacular bundle (PMB) thickness. Each eye was considered separately. Statistical analysis was performed using SPSS 22.0 program. P

Results
In to tal 142 eyes in 71 patients with MS were evaluated. There were 22 men (31%) and 49 women (69%). The mean age is 40.7±10.7 years. Average overall RNFL thickness in MS eyes was 85.5±15.06 mm in right eye and 86.3±13.2 mm in the left eye. In addition, we compared the right and the left eye segments. We have received a statistically significant difference between the NS segments and their RNFL thickness averages (p

Conclusions
OCT is an important method in assessing RNFL and PMB thinning in patients with MS. It is known that OCT refers to irreversible progression of the retina and the brain atrophy. Temporal segment is the most vulnerable part of the retina. It has been observed that VEP latency was significantly prolonged in patients with MS in comparison to RNFL thinning. The CSF in dex is increased by immunologically more active multiple sclerosis. We think that more active form of MS may be associated with retinal segments violation. Hence we may conclude that RNFL thinning could be related with irreversible progression of the disease.

Tissue level optical benefits of nuclear inversion in mouse photoreceptors.

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Purpose
It has puzzled biologists for centuries that the vertebrate retina is inverted with respect to its optical function: photons need to traverse multiple layers of living neuronal tissue before detection by photoreceptor cells. Recent findings indicate that cells situated in this light path might circumvent this unfortunate situation by aligning with the light path or minimizing light scattering by adapting their nuclear architecture (Solovei 2009, Kreysing 2010, Blaszczak 2014). My research aims to further explore the effects of the nuclei that account for significant volume fraction of the entire retina (in rodents) and their architecture at a tissue level in enhancing the optical property of retina.

Methods
Using the concept of modulation transfer function we are looking to confirm benefits of nuclear inversion at tissue level. The mechanism of inversion has been recently established (Solovei 2013) where in, the down regulation of proteins associated with nuclear envelope during developmental stages of mice trigger the inversion. In this regard we compare wild type mice vs transgenic mice that express the Lamin B receptor and consequently have conventional nuclear architecture to specifically look at the fingerprint of the spatial frequencies in the transmission spectra of retina due to this inversion. The experiments are complemented by computer simulations of wave optical light propagation through outer nuclear layer as mapped by two-photon excitation microscopy.

Conclusions
We present first experimental and detailed computer simulated evidence for enhanced optical property of the mouse retina at a tissue level owing to its nuclear architecture, stemming from the developmental studies capturing the inversion of nuclear architecture.

Removal of lipofuscin from the RPE of Abca4-/- mice with THPE: quantitative and toxicity studies

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Purpose
The accumulation of lipofuscin (LF) in the retinal pigment epithelium (RPE) is a hallmark of aging and is supposed to play a key role in the development of age-related macular degeneration (AMD) and Stargardt disease. We previously reported that tetrahydropyridoethers (THPE) are able to remove LF from monkey RPE cells in vivo and aged human donor RPE cells in vitro. Here we present that THPE are also effective in a mouse model for Stargardt disease and show no toxic effects.

Methods
In total, 55 pigmented Abca4-/- mice were intravitreally injected once with THPE or vehicle for control. For the quantitative study, eyes were either fixed and examined by light and electron microscopy or RPE cells were isolated and analyzed by imaging flow cytometry after two to six weeks. The area occupied by LF granules was quantified in 10 micrographs per eye on average. For the toxicity study, full-field electroretinographic examination (ERG, using scotopic single flash and scotopic flicker and photopic single flash followed by flicker series) was performed in 16 animals directly before (baseline) and one, two and four weeks after intravitreal injection. After four weeks, animals were sacrificed and the eyes were examined by light and electron microscopy, including quantification of photoreceptor nuclei.

Results
A single treatment in 12-month-old animals resulted in a loss of ~50% of the area occupied by LF compared to control after 3 weeks. In 6-month-old treated animals, there was no difference to control after 2 weeks, but after 6 weeks LF was reduced to ~25%. Two weeks after a single injection of 9-month-old animals with THPE, the number of cells with high LF autofluorescence was lower in treated eyes compared to control as measured with imaging flow cytometry. THPE treatment showed no effect on the a- and b-wave amplitudes in ERG compared to control. Histologically, there were no aberrant changes in retinal morphology, in particular, there was no loss of photoreceptor nuclei after treatment.

Conclusions
THPE reduce the amount of LF not only in the healthy but also in the diseased RPE. Treatment with intravitreal THPE does not cause any toxic effects as shown by ERG and histological examination. This suggests that THPE compounds have an extraordinary potential as possible treatment options for LF-related diseases like dry AMD and Stargardt disease.

Statement on proprietary interests
TT, TP, SP, AT, MR, SS, KSL: None; MB, SJ, US: Katairo GmbH (Patent)

The influence of background image and other external and internal factors on hoverfly locomotor behavior

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Purpose
To investigate the effect of different background images on hoverfly activity, and to quantify what role other external and internal factors play. It is important to have information about hoverfly baseline activity when analyzing how novel visual stimuli affect behavior.

Methods
A locomotor activity monitor was used to measure walking activity of the hoverfly Eristalis tenax. The monitor measures locomotor activity as beam breaks of an infrared light in the middle of a tube. We quantified activity with a one minute resolution. The hoverflies were kept in a 12 h light:dark cycle at ca 22 degrees. The experiments were carried out for 54 hours, and the mean activity measured for 5 hours in the middle of the second day.

Results
We found that the background image affects the locomotor behavior of Eristalis tenax, as does the gender of a conspecific companion. Quite remarkable is also the resilience the walking activity of these hoverflies show towards diet, age, gender and even starvation. Notable is also the fact that diet seems to affect survival and that females survive starvation far better than males.

Conclusions
We have generated a baseline of what internal and external factors affect hoverfly locomotor behavior. Our finding that the background image affects the walking behavior of Eristalis tenax indicates that the local visual environment is involved in shaping the behavioral output of hoverflies.

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Effects of pretreatment with the radioprotector ortho-phospho-L-tyrosine (pTyr) on Rb+/- mice after radiation exposure – Implication for the treatment of retinoblastoma patients with radiotherapy

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Purpose
Retinoblastoma (Rb) is the most frequent ocular tumor in children and if let untreated, can cause death. Like the most head and neck tumors it is sensitive to radiotherapy (RT). However, the therapy has its risks like damage of healthy tissues recurrence and development of treatment-induced secondary tumors. The aim of this study is to investigate the ability of the radioprotector pTyr to prevent RT-induced secondary tumors and other side-effects observed after RT.

Methods
B6;129-Rb1tm3Tyj/J mice having a mutation in one of the Rb -gene allele were used. Although these mice do not develop a Rb, this model was chosen because Rb-patients having a similar mutation have a higher risk of developing secondary tumors induced by RT. One group of mice was treated with intraperitoneal injections of pTyr 16 hours before each irradiation (IR). Another group was only irradiated. Both groups were irradiated over a period of 3 weeks 3 times a week with a dosage of 5 Gy per exposure (Fig.1). All animals were investigated using SLO/OCT and histologically 1, 3, 6 and 9 months after IR . Radiation-induced tumor induction as well as normal tissue radiation toxicity were evaluated as function of pTyr-treatment. B6;129-Rb1tm3Tyj/J mice having a mutation in one of the Rb -gene allele were used. Although these mice do not develop a Rb, this model was chosen because Rb-patients having a similar mutation have a higher risk of developing secondary tumors induced by RT. One group of mice was treated with intraperitoneal injections of pTyr 16 hours before each irradiation (IR). Another group was only irradiated. Both groups were irradiated over a period of 3 weeks 3 times a week with a dosage of 5 Gy per exposure (Fig.1). All animals were investigated using SLO/OCT and histologically 1, 3, 6 and 9 months after IR . Radiation-induced tumor induction as well as normal tissue radiation toxicity were evaluated as function of pTyr-treatment.

Results
The first visible effect of the IR was the graying of the hair coat in the area of IR. A hair sample from the area of IR was plucked from each animal and analysed as described under (fig. 2a). The effect of IR induced graying of the hair coat was reduced in the pTyr treated mice (fig. 2b). The results of the OCT- analysis showed that 3, 6 and 9 months after IR the thickness of the retina of the mice was significantly lower in the untreated group (n=12) compared to the pTyr treated one (n=12) (p

Conclusions
Our results show that the application of pTyr before IR significantly reduces the negative effects of radiation on the hair coat and retina 3, 6 and 9 months after IR. The appearance of secondary tumors which is an often seen side effect of a RT in patients could not be found in our model at the investigated time points. The development of IR-induced tumors might take a longer period of time.

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A novel feeder-free culture system to derive Human retinal pigment epithelium from pluripotent stem cells

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Purpose
The retinal pigmented epithelium (RPE) is a monolayer of pigmented cell located between the neural retina and the blood supplier, choroid. Its main contributions to the visual process are the synthesis and the recycling of the chromophore required for phototransduction, the phagocytosis of shed photoreceptor outer segments, the regulation of fluid and nutrient flow between the retina and the choroid. The neural retina activity relies on RPE functions and its deficiency give rise to several diseases, of which most of them result in visual impairments or blindness. The ability to generate hRPE for disease modelling, drug screening or transplantation would be particularly worth to answer these important challenges.

Methods
Here we present an easy, reliable, xeno- and serum-free method to quickly generate hRPE from iPSCs in culture. Starting from feeder-free culture conditions we established a simple three-step protocol able to induce the typical RPE cobblestone appearance, and pigmented foci as early as 20 days after differentiation. After a first step consisting on the formation of embryonic body-like aggregates, the neuroephitelium induction follows and then the third phase commit the neural cells to RPE fate.

Results
The cells are characterized by their pigmentation, the expression of mRNAs of typical RPE markers associated with the retinoid cycle (RALBP and RPE65), chloride channels (BEST1), phagocytosis (MERTK) and specific coexpression of transcription factors (PAX6 and MITF). The presence of protein involved in the tight junction formation (ZO-1) was revealed by immunocytochemistry. To obtain pure populations of RPE, pigmented foci were manually transferred at day 25-35 on matrigel and cultured until confluence.

Conclusions
In conclusion, the presented protocol provides a quick and consistent method to generate hRPE from pluripotent stem cells which will be utilised to generate RPE-like tissues from hiPSCs of affected patients with the aim to perform in-depth study of diseases mechanisms and test new treatments.

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Establishment of a Photoreceptor Degeneration Model in Rabbits

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Purpose
In patients suffering from retinal degenerative diseases, e.g. retinitis pigmentosa, functionality can be regained by prostheses, where electrical stimulation of surviving retinal cells is induced by microelectrodes. In order to test newly developed retinal implants, a large-eye animal model that mimics the properties of this type of retinal degeneration is required. In particular, an unilateral induction of photoreceptor degeneration is desired using the contralateral eye as control.

Methods
Pigmented chinchilla bastard rabbits, weighing 3.0 to 3.5 kg, received an intravitreal injection of n-methyl-n-nitrosourea (MNU) into the left eye. The applied MNU concentration was adjusted to the respective vitreous volume, which was calculated using ultrasound biometry. After injection, a weekly follow-up with macroscopy, funduscopy, optical coherence tomography (OCT), and electroretinogram (ERG) was performed. After three weeks, animals were sacrificed and both eyes were prepared for immunohistochemistry.

Results
Immunohistochemical stainings of MNU-treated eyes revealed scattered areas of photoreceptor degeneration. Additionally, various side effects on the retina itself (retinal detachment, retinal tears), as well as on other ocular tissues (e.g. chemosis, lens lesions) were observed. However, the contralateral eye was not affected. Retinal detachments observed in funduscopy also showed in ERG recordings and OCT scans.

Conclusions
Intravitreal injection of MNU induced photoreceptor degeneration in rabbits. However, several difficulties impeded the experiments: due to MNU's teratogenicity and carcinogenicity, a high organizational effort is mandatory to perform these experiments. In order to achieve the desired MNU concentrations, only small volumes of solvents can be used, thus decreasing the solubility of MNU. Its poor solubility together with its short half-life (30 to 40 minutes) affected the MNU concentration to be injected in an uncontrollable manner. Additionally, it is not exactly known, how MNU interacts with components of the vitreous body, possibly interfering with the consistent dispersion of MNU.

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Genetic and environmental factors in age-related nuclear cataract

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Purpose
Nuclear cataract is the most common form of age related cataract, a leading cause of world blindness. This study aimed to determine the genetic factors underling nuclear cataract, to calculate the heritability of nuclear cataract progression, and to explore prospectively the effect of dietary micronutrients on cataract progression.

Methods
Nuclear cataract was measured from Scheimpflug lens images in 2556 elderly twins at baseline and in 324 twins at 10 years follow-up from the TwinsUK cohort using objective grading. Genotypes were obtained using Illumina 610K/317K platforms and in a subset of 940 individuals Illumina HighSeq sequencing was performed. Common variants were imputed to HapMapII panel. Baseline dietary data was available from EPIC food frequency questionnaires. GWAS was performed using a score-test-based analysis (MERLIN) and genes in close proximity to associated variants were included for gene-centred association analysis (SKAT), adjusting for age and sex. Heritability was calculated using maximum likelihood structural equation twin modelling. Association between nuclear cataract change and micronutrients was investigated using regression analysis.

Results
The most associated GWAS variant was rs9792446(p=1.0e-7) in an intron of the TRPM3 gene. TRPM3 encodes for a lens expressed calcium channel previously linked to congenital cataract. Rare variants "burden" in 9 genes was associated with nuclear cataract, the most significantly associated gene (CALHM1, p=0.001) also encoding for a calcium channel. The best fitting model estimated that the heritability of nuclear cataract progression was 35% (95% CI:13%-54%). Dietary vitamin C was found protective against both nuclear cataract at baseline and nuclear cataract progression (p=0.01 and p=0.034 respectively).

Conclusions
Our results identify suggestive association between common variants at TRPM3 and nuclear cataract. We also found some evidence for an effect of rare variants in the CALHM1 gene on nuclear cataract. Genetic factors explained 35% of the variation in progression of nuclear cataract over a 10 year period. Environmental factors accounted for the remaining variance, and in particular dietary vitamin C protected against cataract progression. Ongoing replication studies will clarify the role of TRPM3 in age-related nuclear cataract, and larger samples will provide more power to discover further genetic and environmental associations.

Statement on proprietary interests
no interests to declare

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Controlling Retinal Cell Fate Using Nanotopography and Neurotrophic Factors

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Purpose
Bioscaffolds, supporting survival and guiding axonal growth, holds great promise for the advancement of cell-based therapies for retinal neurodegeneration. Increased knowledge is required on the effect of nanotopographies, extracellular matrix (ECM) proteins and neurotrophic factors on retinal cell survival, cell fate and axonal guidance. Hence, we investigated the influence of nanotopography, laminin (ECM protein) and neurotrophic factors on the behavior of retinal neuronal and glial cells.

Methods
We cultured mouse post-natal retinal cells for 7 days in vitro, at either Poly-L-Lysine coated chamber slides (control) or electrospun poly caprolactone (PCL) fiber substrates with random or aligned orientation and substrates were laminin coated or non-coated. Either basic (DMEM-F12, B27 supplement) or the enriched Full-SATO (Neurobasal, CNTF, BDNF, Forskolin, Insulin) medium was used.

Results
Excellent overall cell survival was found up to 7 DIV on all three substrates utilizing either medium. A significant increase in numbers of retinal ganglion cells (RGC; markers: RBPMS, NeuN, βtubulin III), photoreceptors (PR; marker: rhodopsin) and glial cells (marker: GFAP) was found using Full-SATO medium. Nanotopography per se significantly affected neuronal morphological formation; with mainly uni and bipolar profiles at aligned fibers and more multipolar profiles at random fibers and the control surface. Addition of laminin and use of Full-SATO medium in all three substrates clearly promoted both RGC and PR maturation, demonstrated by complex neuronal morphologies and extensive neurite outgrowth. A remarkable 90° switch of neurite orientation was found after coating with laminin.

Conclusions
With a future cell-based retinal therapy in mind, we here provide further in-depth knowledge on control of retinal cells fate using electrospun PCL fibers, ECM-guiding proteins and a supportive enriched culture media. Controlled neural development and axonal guidance was improved by using laminin-coated aligned fibers and Full-SATO medium.

Statement on proprietary interests
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Hoverfly higher-order visual neuron is tuned to the same image statistics as the human visual system

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Purpose
Natural scenes, which are more commonly encountered by freely flying flies, can be described in a variety of ways. One analysis, which is common in psychophysics, starts with doing a Fourier transform. From this it is possible to extract a rotationally averaged amplitude spectrum of the image. Its slope (α) characterizes the image blurriness, which increases with alpha. It is known that alpha the distribution of alphas in natural scenes has a peak around 1.2 and that the vertebrate visual system is tuned to this slope. We here investigate how neural responses in hoverflies depend on the alpha value.

Methods
We manipulated the amplitude spectra (the alpha) of artificial (random noise) and natural images. We performed electrophysiology, where we recorded from the cSIFE neuron that is excited by flicker stimuli and inhibited by stationary patterns. We investigated how the cSIFE response depends on image alpha.

Results
The results of the experiment show that the strongest inhibition is found when the alpha of the stationary stimulus is close to 1 and that there is no inhibition below spontaneous rate for alphas not related to natural scenes (e.g. 0 and 2).

Conclusions
Our data suggest that natural scene statistics directly affect the response properties of neurons in the hoverfly visual system similar to the human one. Hence, further knowledge will be obtained in hoverfly research, which can even be used for a better understanding of the mechanisms that underlie visual scene perception.

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