Young Researcher Vision Camp

An international Career building Symposium

2017

Castle Wildenstein
Leibertingen
Germany

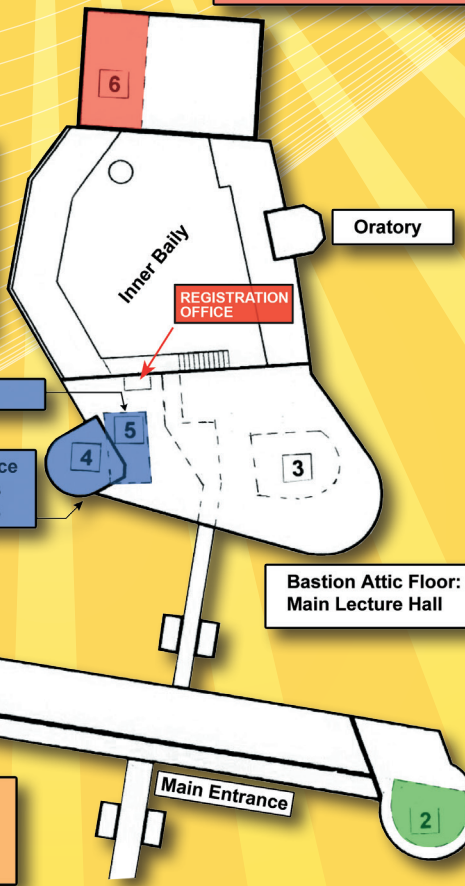
www.vision-camp.eu

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 Vision Camp 2017,

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

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

ENJOY THE
YOUNG RESEARCHER VISION CAMP

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

FRIDAY, JUNE 30TH, 2017

until 16:00	Arrival (for details see 'How to get there')
16:25 - 16:30	Welcome
16:30 - 17:00	**KEYNOTE LECTURE I** **'PRIMARY CILIA IN THE VISUAL SYSTEM'** Helen May-Simera
17:00 - 18:00	**SCIENTIFIC SESSION I: 'RETINAL DEVELOPMENT AND HOMEOSTASIS'** Chair: Jérôme Roger • Elena Braginskaja: "Glycogen Synthase Kinases 3 are Critical for Retinal Development and Homeostasis" • Giuliana Gagliardi: "Using human induced pluripotent stem cells to generate photoreceptors for cell therapy" • Annaig Hamon: "Role of Yap in retinal homeostasis" • Vithiyanjali Sothilingam: "New insights into the mechanisms of retinal degeneration due to Phosphodiesterase 6 (PDE6) deficiency"
18:00 - 19:00	**SCIENTIFIC SESSION II: 'A VIEW ON VISION THROUGH INVERTEBRATE EYES'** Chair: Gavin Taylor • Mikael Ljungholm: "Simulating spatial vision with raytracing" • Emelie Brodrick: "Visual adaptation to light and dark in crustaceans" • Alexis Buatois: "Behavioral assays on visual discrimination learning in tethered bee" • Andrea Adden: "Navigation in the Bogong moth"
19:00 - 19:30	**KEYNOTE LECTURE II** **„OUT OF BREATH – HYPOXIC SIGNALLING IN THE RETINA"** Christian Grimm
19:45 - open end	Open-air Barbecue
21:00 - 22:00	**[Optional]** Evening Tour with Wild Life Ranger in the Valley Danube (due to extra charges; 5€/person) **[Optional]** Evening Tour through the Medival Castle and its hidden Secrets (due to extra charges; 5€/person)

SATURDAY, JULY 1ST, 2017

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

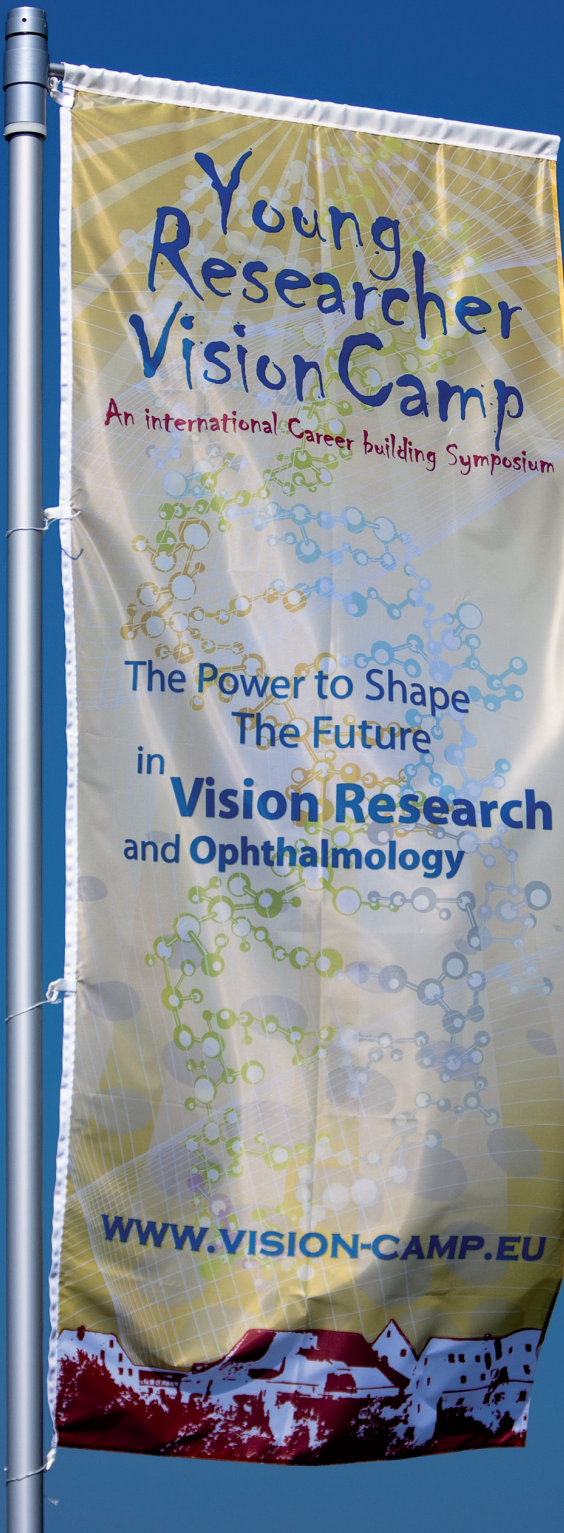
06:00 - 07:00	Early morning exercises
07:00 - 08:00	Breakfast
08:00 - 09:00	**SCIENTIFIC SESSION III: 'CELL DEATH MECHANISM'** Chair: Francois Paquet-Durand • Soumyaparna Das: "Targeting CNG-Ca2+ channels for the treatment of hereditary photoreceptor degeneration" • Michael Power: "Imaging of Ca2+ and calpain activity in dying photoreceptors" • Stephen Carter: "RAB-28, linked to retinal degeneration, associates with the BBSome and IFT in C. elegans" • Merve Sen: "Effects of Sustained Delivery of VCP Inhibitors in Animal Models of Retinitis Pigmentosa"

09:00 - 10:00	**SWITCHBOARD SESSION** **SCIENTIFIC SESSION IV: 'RETINAL PROCESSING'** Chair: Timm Schubert • Meng-Jung Lee: "Electrical imaging bipolar cell activity using high-density micro-electrode arrays" • Prerna Srivstava: "Alterations in bipolar cell function due to degeneration of the outer retina" • Maxime Zimmermann: "Chromatic processing in the zebrafish inner retina" • Janina Leyk: "HDAC6 – a novel target for retinal degeneration?"
10:00 - 10:30	Coffee Break
10:30 - 11:30	**EYERISK SESSION I** **SCIENTIFIC SESSION V: 'RISK FACTORS AND DISEASE PATHWAYS IN AGE-RELATED MACULAR DEGENERATION (AMD)''** Chair: Anneke den Hollander • Anneke den Hollander: "The EYE-RISK project" • Annemarie Colijn: "Risk profiling for late AMD" • Eveline Kersten: "Identification of systemic biomarkers in AMD" • Soufiane Ajana: "Prediction models in high dimensional settings: Application to AMD"
11:30 - 12:30	**EYERISK SESSION II** **SCIENTIFIC SESSION VI: 'AMD DISEASE MODELLING'** Chair: Berta de la Cerda Haynes • Sofia Calado: "Patient-specific iPSC-derived retinal pigment epithelial cells (RPE) for modelling AMD" • Fran Pool: "Mathematical Modelling of Biological Systems" • Eduardo Rodriguez: "A Swine Model of Selective Geographic Atrophy Mimicking Atrophic AMD" • Ana Belén García Delgado: Animal models for the study of AMD
12:30 - 13:00	Pickup of Packed Lunches
13:00 - 16:00	**BUS EXCURSION TO THE "CAMPUS GALLI"** More information on www.campus-galli.de (due to extra charges; 10€/person)
16:00 - 16:30	Coffee Break
16:30 - 17:30	**SCIENTIFIC SESSION VII: 'NOVEL DEVELOPMENTS IN VISUAL REHABILITAION'** Chair: Arne Ohlendorf • Maria Barraza: "Novel strategies to enhance visual performance using simulation of central vision loss for the visually impaired" • Alex Ochakovski: "Gene therapy - enabling cone vision in complete achromatopsia" • Stefan Küster, "Effect of explorative saccade training to search-tasks in children with suprachiasmal lesions" • Sunetra Basavaraju, "Strategies for vision rescue: The electrical chip - a sub retinal implant."
17:45 - 18:30	**KEYNOTE LECTURE III** **'HOW TO SHAPE YOUR CAREER IN INDUSTRY?'** Christer Säfholm
18:35 - 18:45	Group Photo
19:00 - open end	Poster Session
19:30 - open end	Buffet in the inner bailey

SUNDAY, JULY 2ND, 2017

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

7:00 - 8:00	Early morning exercises
8:00 – 9:00	Breakfast
9:00 - 10:00	**MYFUN SESSION** **SCIENTIFIC SESSION VII: ‚UNDERSTANDING IN MYOPIA‘** Chair: Arne Ohlendorf • Barbara Swiatczak: „In vivo markers of myopia" • Sandra Gisbert: "Inter-individual variability of myopia" • Pablo Sanz Diez: "Adaptation to contrast and its influence on accommodation" • Petros Papadogiannis: "Effect of chromatic aberration on detecting the sign of defocus"
10:00-11:00	**SCIENTIFIC SESSION VIII: ‚GENETICS AND THERAPEUTICS OF RETINAL DEGENERATIONS‘** Chair: Alex Garanto • Franziska Wagner: „The status of DNA repair mechanisms in the healthy and degenerating retina" • Thilo Buck: „Human iPSCs-derived retinas as tool for AAV-vector selection in retinal gene replacement therapy" • Sarah Naessens: „Antisense oligonucleotide-based splice correction of two neighboring deep-intronic ABCA4 mutations causing Stargardt disease" • Matthijs de Boer: „Studying visual perception in inherited retinal dystrophies with optogenetics: using Lca5-/- mice as a model"
11:00 – 12:00	**ZEISS POSTER AWARDS** Poster Awards & Talks of the Awardee
12:00 - 13:30	Farewell Lunch (optional)


Young Researcher Vision Camp
An international Career building Symposium
The Power to Shape The Future in Vision Research and Ophthalmology
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Adden, Andrea

Unravelling the visual magnetic compass in moths

Andrea Adden, David Dreyer, Kristina Brauburger, Stanley Heinze, Eric Warrant

Vision Group, Lund University, Lund, Sweden

Purpose
Animals that migrate towards a specific location need to know the direction of their goal – essentially, they need a compass. Long-distance migrating birds and sea turtles can use the Earth's magnetic field as a compass. Although we do not yet understand the mechanisms that underlie the magnetic sense in these animals, recent work has focussed on a light-dependent chemical reaction involving cryptochromes: the radical-pair mechanism. After absorbing a photon, the cryptochrome's FAD domain forms a radical-pair with adjacent amino acids. The ratio of radical-pair reaction products depends on the ambient magnetic field. Since the mechanism is light-dependent, it has been suggested that this reaction takes place in the photoreceptors. Here, we introduce a new animal model for testing the radical-pair hypothesis from multiple angles: the Bogong moth (Agrotis infusa), a nocturnal moth that performs long-distance migration.

Methods

Results
Preliminary results from behavioural experiments show that the Bogong moth can detect magnetic fields and use them for selecting a flight direction as long as a visual landmark is provided. In a cue conflict situation, the moths lose the ability to orient.

Conclusions
We conclude that a light-dependent magnetic compass indeed exists in the Bogong moth, and outline further steps to unravel the visual magnetic compass in moths on a cellular and molecular level.

Ajana, Soufiane

Prediction models in high dimensional settings: Application to AMD

Soufiane Ajana

University Bordeaux, INSERM, Bordeaux Population Health Research Center, UMR, Bordeaux, France

Purpose
High dimensional data contain often many irrelevant variables for predicting an accurate group assignment or an outcome of interest. Even if the contributions of such variables to the model (regression or classification) are small, they can sum up to significant (non-)information and result in performance loss. Traditional methods cannot be applied in high dimensional settings because of the complexity (multicollinearity) of the data. However, sparse methods like lasso, sparse partial least squares (spls) or elastic net are able to address this issue by shrinking the contributions of potential noise variables towards zero. The objective of the present work is to give an exhaustive overview of these sparse methods and to compare their properties.

AMORIM, ANDRÉ

Changes in activity of different retinal cells when processing different degrees of defocus: pilot study

Amorim A., Fernandes PRBF, Amorim-de-Sousa A, González-Méijome JM.

CEORLab, Center of Physics – University of Minho, Braga, Portugal.

Purpose

Use of functional models of the human retina might be relevant to understand if the retinal information processing of optically degraded images may be relevant in several practical aspects from the perception of dysphotopsia with modern implants to correct presbyopia to the ocular development and myopia progression. The purpose of the present communication is to analyze the changes in the processing activity at different levels of the retinal tissue.

Methods

The standard condition (SC) of stimulation was a white circle (r = 15 pixels) centered on a black background. The optically degraded stimuli were produced by applying Gaussian filters (G'X') with increasing degree of standard deviation to the standard condition (X = 1, 3 and 5). The model contemplated a total of 35x35 pixels, representing about 7 degrees of visual field with constant cell density. The COREM software [Martínez Cañada et al., 2016] was used including five cellular modules, representing retinal layers of photoreceptors, horizontal cells (sending feedback on photoreceptors), center-ON bipolar cells, amacrine cells (whose bipolar cell conductance depends on), and center-ON ganglion cells. Each module consisted on a Gaussian filter (except for photoreceptors) followed by a temporal low-pass filter (replaced by a single compartment model for bipolar cells) and by a static-non linearity. The output of each module is the change of voltage relatively to the resting potential. Due to the lack of experimental data, chosen parameters were based on available COREM models. The output of each module was compared between all conditions (SC, G1, G3, G5). The changes relatively to the SC condition were used as metrics for each module, mainly the area under the spatial curve (sAUC) of the data along a horizontal line running the entire simulated visual field and crossing its center. We assumed this area to be related with the overall activity (or response magnitude) of each retinal layer, being an increased sAUC associated with higher voltage changes on the cell membranes.

Results

The photoreceptors layer's response directly follows the intensity of the stimulus. The horizontal cells' is roughly a difference of gaussian (DoG) filtered version of the photoreceptors' signal, which feedbacks back on the photoreceptors layer to compose the receptive field. Temporally, the horizontal cells' response is a delayed and smothered version of the photoreceptors'. The response magnitude of the bipolar cells layer follows the contrast of the image features. A consequence is that it changes spatially in a smoother way when the blur increases. Temporally, there is a peak right after the stimulus onset followed be a decay to a steady level which increases with contrast. The amacrine cells layer's response has a similar shape compared to the bipolar cells. It slightly smoothens the bipolar cell's response before forwarding it to the ganglion cells, whose response represents the post-synaptic potentials at their dendrites. The sAUC of each module was compared relatively to the standard condition SC. The sAUC showed a progressively steeper decline (between 7-10% for G5) with increased blur for the photoreceptors and horizontal cells. The amacrine and ganglion cells showed a steep decline at first (~7.5% for G1, ~13% for G3), but this decline decreased for the G5 condition. For bipolar cells, the decline was even steeper for G1, but never surpassed 10% and almost recovered completely for G5, meaning that its overall activity was of the same intensity as in SC. The shapes of the spatial curves of these last 3 layers were significantly different for each condition, varying from perfectly concave (SC) to a convex shape (G5), and a closer sAUC between them may suggest that their overall response magnitude may be similar for different contrast conditions.

Conclusions

Increasing the degradation of the image induces changes in the contrast of the stimuli and spreads the stimulation area with a decaying level of intensity from the center to the periphery. This condition induces more changes in the bipolar, amacrine and ganglion cell layers, but the overall response magnitude may not be so different. This theoretical paradigm of study might have implications in several practical conditions and the results must be confirmed experimentally using electrophysiology techniques.

Statement on proprietary interests

No author has interests in the methods or devices mentioned in this communication.

Acknowledgement

Present study has been partially funded by Fundação para a Ciência e Tecnologia (Portugal) through project PTDC/FIS-OPT/0677/2014 granted to CEORLab and Strategic program UID/FIS/04650/2013 granted to Center of Physics.

Amorim-de-Sousa, Ana

Topographic distribution of the human retinal cells: a review

Amorim-de-Sousa A, Fernandes PRBF, Amorim A, Queirós A, González-Méijome JM

CEORLab, Center of Physics – University of Minho, Braga, Portugal.

Purpose

The human retina is a complex tissue spread over an area of 1100 mm2 where the light is transformed on an electrical sign and the visual information is firstly treated and modulated before gets to the brain. This review aims to describe the variety of retinal cell types and when possible their distribution along the retinal tissue from published data.

Methods

We performed a PubMed database search for studies about the different retinal cell types and the cellular retinal distribution using "retinal cell types", "retinal topography" and "retinal cell density" as key-words.

Results

From the literature, there are 3 types of photoreceptors (L-M-S); 11 different types of bipolar cells (Bc), 3 types of horizontal cells (Hc), 18-25 types of amacrine cells (Ac) and ~23 types of ganglion cells (Gc) in the mammalian retina, including the human species. Photoreceptors: from the approximately 130 million photoreceptors in the human retina, 120 million are rods and start to appear at 5° from the center of the fovea reaching its peak density of ~160 000 rods/mm2 at 18-20° eccentricity from the fovea. The center of the fovea is composed mainly by L- and M-cones, with S-cones representing less than 12% of all cones and then decreasing towards periphery. L-and M- cones have their highest density at fovea (~21 000 and 40 000 cells/degree, respectively), while for S-cones at the center of fovea there are ~2500 cells/degree, reaching it maximum density of ~7500 S-cones/degree at approximately 3° from central fovea. Three different types of HC has been described (HI, HII and HIII) and despite the supposed connectivity function described (HI with cones and rods; HII only with cones; HIII L-M cones and alleged with rods), no information about the distribution of the Hc was found in the literature search. Eleven types of Bc have been described; 1 directly connected with rods (RB), 7 concerned with information convergence from cones (6 types of diffuse (DB) and 1 type of a giant bistratified (GBB)), and 3 concerned with single-cones contacts in a 1:1 relationship called invaginanting (iMBc) and flat (fMBc) midget Bc and the blue-cone Bc (BB).At the center of the fovea there are only iMBc and fMBc connected with L- and M-cones (1iMBc+1fMBc:1cone) till 40° eccentricity. The BB is connected to a single S-cone at fovea but as it moves away start to receive information from 2, 3 or 4 S-cones with a maximum distribution at 3° with ~70 000 BBc/degree. The 6 types of diffuse Bc (DBc) are similarly distributed along the retina with a density of ~2500 cells/mm2 (DB3 and DB6 ~930 cells/mm2). The GBB is related with S-cones OFF-pathway and is usually connected to ~20 cones pedicles. Its distribution have a peak density of ~140 000 per degree at 1-3°eccentricity, decreasing to ~37 500 per degree at 20-30° eccentricity. Regarding Ac, there is a lack of information with respect the density and distribution of Ac, however is has been reported from 18 up to 25 different types of Ac along the retina. The literature reports about 23 different types of Gc, however there are not much information about all of them. Like the Bc, there are invaginating and flat midget/parvocellular Gc (iPGc and fPGc, respectively) responsible for red/green color opponency with a distribution of ~2800 cells/mm2 at 1mm of eccentricity, with a decreasing density up to 280 cells/mm2 at 15mm from the foveal area. The S-cone Gc (blue/yellow) is a specialized Gc that represents only 3% of all the retinal Gc with a spatial density distribution comparable to the S-cones, with ~400 cells/mm2 at fovea and ~20 cells/mm2 in more peripheral areas. Another well studied GC is the parasol Gc (MGc), also classified as invaginating (iMGc) and flat (fMGc), and represents ~10% of Gc population, with only 2% of them at fovea, increasing its density to peripheral areas (peak density at ~7mm from the nasal fovea with ~3400 cells/mm2 in the macaque retina) with responsibility in detection of movement, depth perception and small changes on brightness. Some authors reported no significant differences in cell density between the four retinal quadrants (nasal, temporal, superior and inferior), while others observed significant differences along the horizontal and vertical meridians, reporting a higher cells density (up to ~1000 cells/mm2) in the nasal and superior peripheral retina.

Conclusions

The retina is constituted by multiple different cells with specific functions, rearranged by receptive fields. Each cell class and type has its own function. The diversity of cells and they specific connections, as well as the hypothetical asymmetries along the horizontal and vertical retinal meridians, is what makes the retina a complex tissue of light transduction. We think that the retinal cells distribution should be considered when forming hypothesis about the electric retinal activity.

Statement on proprietary interests

No author has interests in the methods or devices mentioned in this communication.

Acknowledgement

The present study has been partially funded by Fundação para a Ciência e Tecnologia (Portugal) through project PTDC/FIS-OPT/0677/2014 granted to CEORLab and Strategic program UID/FIS/04650/2013 granted to Center of Physics.

ASCARI, GIULIA

Missense mutation in CEP78 in a family with cone-rod dystrophy, sensorineural hearing loss, obesity and subfertility

Ascari, Giulia1; Van De Sompele, Stijn1; Derycke, Lara2; Gabriële, Holtappels2; Krysko, Olga2; Van Dorpe, Jo3; Creytens, David3; Van Laethem, Thalia1; Balikova, Irina4, 5; Gerris, Jan6; Bachert, Claus2; Leroy, Bart P.1,4; De Baere, Elfride1; Coppieters, F

1. Center for Medical Genetics Ghent, Ghent University, Ghent, Belgium. 2. Upper Airways Research Laboratory, Ghent University Hospital, Ghent, Belgium. 3. Department of Pathology, Ghent University Hospital, Ghent, Belgium. 4. Department of Ophthalmology, Ghent University Hospital, Ghent, Belgium. 5. Free University of Brussels, Brussels, Belgium. 6. Gynecology Department, Ghent University Hospital, Ghent, Belgium.

Purpose
Bi-allelic truncating mutations in CEP78 were recently found to cause cone-rod dystrophy (CRD) with sensorineural hearing loss. Here, we aimed to identify the causal mutation in a family with CRD and sensorineural hearing loss, and to explore other ciliary phenotypic features.

Methods
Whole exome sequencing was performed in 2 affected siblings and segregation analysis was done in 5 additional family members. Skin biopsies and nasal brush samples were obtained from both affected individuals and their parents to study ciliary structure and length.

Results
We identified a novel missense mutation in CEP78 in a homozygous state in both affected individuals: c.449T>C, p.(Leu150Ser). This mutation segregates with disease in 7 individuals. The affected amino acid is highly conserved, and the change is predicted to be damaging by several in silico prediction tools. Phenotyping revealed the presence of additional features reminiscent to a ciliopathy such as recurrent airway infections, obesity and subfertility. Morphology of primary cilia in fibroblasts was evaluated and induced cilia in patient's fibroblasts were significantly longer in comparison to control cells. Functional studies are ongoing on nasal epithelial cells and semen samples to investigate if this CEP78 mutation affects ciliary structure, and if it is associated with the ciliary phenotypic features observed.

Conclusions
In conclusion, we identified the first missense mutation in CEP78 causing CRD and sensorineural hearing loss, a recently identified syndrome distinct from Usher syndrome. The family studied here displayed additional features, suggesting a potential involvement of CEP78 in more complex ciliopathies.

Acknowledgement
FRO, FWO

Barraza Bernal, Maria Jose

Novel strategies to enhance visual performance using simulation of central vision loss for the visually impaired.

Maria Barraza Bernal, Katharina Rifai & Siegfried Wahl.

ZEISS Vision Science Lab, Institute for Ophthalmic Research, University of Tübingen, Germany.

Purpose
Apply eye tracking technology to investigate the changes in PRL when subjects undergo a simulation of progressive central scotoma.

Methods
Five normally sighted subjects participated in the study. A foveally centered mask was presented gaze-contingently to simulate the scotoma. Initially subjects developed a PRL under simulation of a six degree scotoma, which was used as a baseline. The simulation consisted of a gradual increase, and an abrupt increase of scotoma size in separate conditions. In the incremental progression, the diameter of the scotoma increased by a fixed amount of either one or two degrees of visual angle, thus scotomas of eight, ten and eleven degrees of visual angle were simulated. In the abrupt progression, the diameter was adjusted individually to span the area of the visual field used by the current PRL.

Results
Subjects located the PRL along the same meridian under simulation of scotoma progression. Furthermore, no differences between the fixation stability of the baseline PRL and the gradual progression PRLs were found, whereas in abrupt progression, fixation stability dropped significantly.

Conclusions
These results provide first insight into fixation behavior in a progressive scotoma and might contribute to the development of training tools for patients with progressive central maculopathies.

Statement on proprietary interests
None

Acknowledgement
NA

Basavaraju, Sunetra

Strategies for vision rescue: The electrical chip – a subretinal implant.

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Purpose
Various research techniques are being developed in order to restore vision in blind patients, whose blindness is caused by progressive loss of vision due to photoreceptor degeneration in Retinitis pigmentosa (RP) [Zrenner E et al., 2011. Zrenner E, 2013]. One amongst these research approaches is to use microelectronics. Thus, in this direction of research, the Tübingen subretinal implant proves to be moving towards faster and reliable results [Stingl K et al., 2015]. The Implant is a micro-electronic chip (alpha IMS subretinal Implant), operating as a light to voltage converter. It is composed of an active area of 1500 independent pixels, each consisting of a photodiode, an amplifier and a stimulating electrode that partially replace the degenerated photoreceptor function [Stingl K et al., 2013].

Methods
Considering the fact that photodiodes convert light to electrical pulses, the amplified output pulses that are 1ms long are presented in a defined frequency depending on the amount of light projected onto it. The strength and working range of these pulses can be determined by the sensitivity and gain controls respectively. These parameters can be controlled by implant patients through an external hand held device depending on the intensity of light in the room they are in. The power supply and the control signals are transferred to the chip through the skin with the help of a coil placed behind the ear which is connected to the external control unit. The implant chip is surgically introduced into the subretinal space and placed below the foveal region in RP patients, in order to stimulate the bipolar cells [Zrenner E, 2013]. Several psychophysical tests were conducted during clinical trials to assess the performance of the patients with the retina implant chip. [Stingl K et al., 2016]

Results
It was observed during the clinical tests, that the visual acuity of the patients was improved. The visual functions such as, light perception, light localization, motion detection, grating acuity were restored to a great extent [Stingl K et al., 2013, Stingl K et al., 2015]. The patients could use the implants during their daily tasks; some of them claimed that they could carry out their daily life activities and also recognize letters and combine them to form words [Zrenner E et al., 2011, Stingl K et al., 2013]. Patients also reported that they could recognise facial expressions, such as: a smile, contour of people, clothing patterns, localizing objects like a telephone, cutlery and different parts of meal. All of these objects were recognized within the visual field of 10° [Stingl K et al., 2013].

Conclusions
The patients who were implanted with the retinal chip were at an advanced stage of retinal degeneration. That is, the time between implantation and the onset of the disease were a few decades. During the course of degeneration, the retina undergoes both morphological and structural changes. In our lab (AG Zrenner / Haq), we aim to target the disease at an early stage by investigating if an earlier implantation time proves to be advantageous. Thus, we perform functional tests on mouse retinal models by using methods like Calcium imaging and multielectrode array recordings [Haq et al., 2017 in preparation].

Brodrick, Emelie

Adaptation to extreme changes of light in crustaceans

Emelie Brodrick

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Purpose
A variety of mechanisms have been described for adjusting the sensitivity of the arthropod compound eye to changes in environmental light. The fiddler crab has large vertically elongated compound eyes on stalks, with a panoramic field of view. As well as spending time in the darkness of a burrow, fiddler crabs are active during diurnal low tides on tropical marine mudflats. Sunlight levels can be intensely bright here and the sea and mud surfaces reflect strong horizontally-polarized glare. How do the visual systems of these animals cope with rapid and extreme changes in light across the different regions of the eye?

Methods
Fiddler crabs collected on a Spanish mudflat were either light or dark adapted before the eyes were dissected and prepared for transmission electron microscopy (TEM). From the TEM images, observations and measurements were taken of the microvillus banding pattern of the rhabdom in different regions of the eye. The distribution of screening pigment granules within the photoreceptor cells was also compared between light and dark adapted animals.

Results
Fiddler crabs use the polarization properties of light to add contrast to their visual world by having bands of orthogonally-arranged microvilli on the photoreceptor cells, orientated to detect either horizontal or vertically polarized light. My research explores the idea that a differential banding pattern exists in the microvilli along the rhabdom length, which results in a greater sensitivity to vertically polarized light than horizontal, acting like "polaroid sunglasses" to filter out reflected glare. The TEM images also suggest migrations of pigment granules along microtubules within the photoreceptors in response to light and dark, which provide screening to parts cells during high light intensities.

Conclusions

Statement on proprietary interests
N/A

Acknowledgement
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Buatois, Alexis

Associative visual learning by tethered bees in a controlled visual environment

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Purpose
Efficient foraging in bees is ensured by flower constancy: the bees tend to visit flowers from the same species as long as profitable. This behavior demonstrates the bees' ability to recognize and memorize the visual and olfactory flowers characteristics. The pioneer works of Karl von Frisch a century ago showed that bees are indeed able to associate a reward with an odor, a color or a shape. More recently, higher cognitive abilities (Avarguès-Weber et al., 2011) such as visual categorization (e.g. symmetric vs. asymmetric objects or Human face vs. non-face) or relational concept learning (e.g. 'same' or 'above') were evidenced in bees. Despite these progresses in our knowledge on the visual cognitive abilities of honeybees and some advances in the anatomical description of the visual brain circuitry, neural correlates of simple associative visual learning are still largely unknown in bees by contrast with the neuronal mechanisms of olfactory learning (Giurfa and Sandoz, 2012). This absence of in vivo functional brain analysis is mainly due to the difficulty to couple invasive neurobiological methods with classical free flight learning protocols. It is therefore both timely and necessary to develop efficient visual learning paradigms on harnessed bees. Avarguès-Weber, A., Deisig, N., Giurfa, M., 2011. Visual cognition in social insects. Annu. Rev. Entomol. 56, 423–443. doi:10.1146/annurev-ento-120709-144855 Giurfa, M., Sandoz, J.-C., 2012. Invertebrate learning and memory: Fifty years of olfactory conditioning of the proboscis extension response in honeybees. Learn. Mem. 19, 54–66. doi:10.1101/lm.024711.111

Methods
We developed a new setup, in which tethered bees are placed on a locomotion compensator system (air-suspended ball) facing a hemi-cylindrical screen associated with a high frequency video-projector. The bees' choices between projected visual stimuli are recorded by monitoring the ball movements due to the bee's walking behavior. The bees could then be subjected to a differential conditioning procedure in which one stimulus (e.g. a green square) was associated with a reward while an other (e.g. a blue disc) was associated with a distasteful substance (quinine).

Results
After this conditioning protocol, the bees manage to learn to discriminate the two virtual stimuli.

Conclusions
These positive results obtained in our setup open new doors toward functional studies of visual learning in bees as well as offering control conditions for cognitive studies in this pollinator.

Acknowledgement
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Human iPSCs-derived retinas as tool for AAV-vector selection in retinal gene replacement therapy

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Purpose
Based on our previous Adeno-associated virus (AAV) Crumbs rescue study in Leber's congenital amaurosis and retinitis pigmentosa mouse models, the success of a clinical trial depends on the efficacy of expressing Crumbs preferably in Müller Glia Cells (MGCs) and photoreceptor cells (PRCs) in the retina. Here, Crumbs proteins support the cell adhesion between PRCs-PRCs, MGCs-MGCs and PRCs-MGCs (at the subapical region adjacent to adherens junctions at the outer limiting membrane). So far, information on cell tropism and efficacy of AAVs in humans has been a hurdle because AAV infection and promoter strength can differ between species. Here, we set up reliable human retina potency assays that can be used to select appropriate promoters and AAV serotypes for Crumbs gene therapies.

Methods
1. Set up AAV screening assays for human tissue in: a) cultured explant retinas and b) iPSCs-derived retinas. 2. Screen different AAV serotypes and promoters on PRCs and MGCs infectivity General: We infected human iPSCs-derived retinas and adult human donor retina explants with different AAV capsids (AAV9, AAV5, ShH10Y), titres, promoters (CMV, hRLBP1) and collected the retinas at 14 or 28 days post-infection. We quantified GFP expressing cell types in the human retina by immunohistochemistry using confocal microscopy. The potency assay includes three components: (1) cell-specific expression, (2) promoter activity, and (3) cross-validation in two models. Human donor retina culture: Eyes were enucleated within 24 h post-mortem. The retina was dissected and punched into 6 mm pieces. Retinas were cultured on membranes (0.4 µm inserts). Retinas were infected in a 50 µL AAV/medium mix and cultured in Neurobasal® A-based serum free medium. Human iPSCs-derived retina culture: We followed mainly the Zhong et al Canto-Soler (2014) protocol for the differentiation. We infected series of two organoids in 50 µL AAV/medium mix per group. AAV: AAV concentrations were determined by qPCR in genomic copies (gc) / mL.

Results
Human donor retinas: All AAV.CMV.GFP vectors showed GFP expression in PRCs, MGCs and the GCL. AAV9 infected many more inner retina cells (Bipolar cells, Amacrine cells) compared to AAV5 and ShH10Y. AAV5 and ShH10Y were 10-fold more efficacious in infecting human retinal cells than AAV9 at 1010 genome copies. iPSCs-derived retinas: All AAVs infected and expressed GFP in the human RPE. AAV9 transduced poorly the immature iPSCs-derived retina at 1010 AAV particles. AAV5 and ShH10Y showed expression in inner nuclear cells indicative of early MGCs. A similar expression pattern was found with a MG-specific promoter (RLBP1).

Conclusions
Human MGCs in explant retinas or iPSCs-derived retinas can be efficiently infected by AAV5 and ShH10Y. The AAV5 shows great potential in infecting PRC and MGCs cells at 10x lower dose than AAV9. Our results suggest that instead of AAV9—previously used in a proof-of-concept study in Crb2 and Crb1Crb2 KO mouse retinas (3)—AAV5 and ShH10Y might be preferentially used to rescue Crumbs expression in human MGCs as well as photoreceptors. Furthermore, the developed assays will be helpful in testing novel AAV capsids and cell type specific promoters for gene rescue studies.

Statement on proprietary interests
Patent holder (LUMC) and inventor J. Wijnholds on Crumbs-AAV gene therapy vectors for LCA-8 and progressive RP. None for TM. Buck, PM. Quinn, CH. Alves, C. Ohonin, EHC. van Dijk, and CJF. Boon.

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CALADO, SOFIA M.

Patient-specific iPSC-derived retinal pigment epithelial cells (RPE) for modeling AMD

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Purpose
Age-related macular degeneration (AMD) is a multifactorial neurodegenerative disease that affects the central part of the retina. It is characterized by the formation of lipid-rich extracellular deposits, localized inflammation, and degeneration of photoreceptors due to retinal pigment epithelium (RPE), Bruch's membrane, and choroidal vasculature dysfunction and atrophy. Currently, no ideal animal models are available to study the pathophysiology of AMD. In this work we have used patient-specific induced pluripotent stem cells (iPSC)-derived RPE cells as an in vitro model of AMD.

Methods
Peripheral blood monocyte cells (PBMC's) were isolated from AMD patients showing reticular drusen and cultured for 7 days before reprogramming with CytoTune®-iPS 2.0 Sendai Reprogramming Kit. The generated iPSC were characterized for there their stemness fate and ability to generate the three germ layers. The iPSC were then differentiated towards RPE and the iPSC-derived RPE cells were validated by immunocytochemistry, PCR and Western blot, as well as by its phagocytic capacity and basal/apical polarity.

Results
The iPS-derived RPE cells display the RPE-specific markers RPE65, CRALBP and BEST1, as well as are able to generate tight junctions and phagocyte photoreceptor outer segments (POS).

Conclusions
We were able to generate iPSC-derived RPE cells from patients with AMD. These cells display several features of RPE and for this reason are suitable models to study AMD pathophysiology and test new therapeutic strategies.

Acknowledgement
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Carrillo Aleman, Andrea

Are focussing errors during accommodation also error signals for emmetropization?

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Purpose
Emmetropization (the process of fine-tuning axial eye growth to minimize refractive errors) has been shown to be controlled by the retina. The retina detects image defocus and fine-tunes the growth rates of the sclera. However, accommodation continuously modifies the retinal image defocus and it is not known how this input is taken into account. We have measured accommodation errors and eye growth in high detail in chickens wearing negative spectacle lenses, to find out whether larger accommodation errors lead to larger changes in eye growth.

Methods
Chickens (n=6) aged 10 to 12 days wore lenses (-7D) monocularly for 4-5 days. Fellow eyes remained untreated and served as controls. Vitreous chamber depth (distance from the backside of the lens to the retina) and refraction were measured during the lens treatment period, on average 5 times a day to obtain detailed growth traces. After some training, it was possible to measure vitreous chamber depth (VCD) in alert chickens with extreme precision (standard deviations about 15µm), using a low coherence interferometer (Lenstar LS 900, Haag-Streit, Koeniz, Switzerland). Refractive states were measured by infrared photoretinoscopy with and without lenses in place. Three readings were taken for each eye and at each time point.

Results
After the negative lenses were attached, the refractions of the chickens did not shift into the hyperopic direction by 7D, as one would expect from the power of the lens (average refractions before lens was attached +2.48±0.63 D, refractions with the lens in place +5.17±0.76 D), indicating that the chickens tried to accommodate to at least partially refocus their eyes. We quantified accommodation errors as the difference between expected refraction versus measured shift in refractions in the different animals. Accommodation errors varied among animals from +3.44±1,05 to +5.73±0.59D. VCD became significantly deeper in eyes with negative lenses in 3 chickens (paired t-test). In two of those animals, VCD was already increased already after 2h of lens treatment (+0.07±0.11mm, $p<0.05$; +0.07±0.01 mm, $p<0.01$) and in one chicken, the difference was significant after 20h (+0.05±0.01mm, $p<0.01$). However, accommodation errors did not predict the subsequent changes in eye growth. No correlation was found between the magnitude of the accommodation errors in the individual animals and the subsequent changes in VCD.

Conclusions
Retinal focus error signals generated by insufficient accommodation did not predict subsequent changes in eye growth. Possible conclusions are that (1) short transient changes in focus by accommodation are not sufficient to elicit changes in eye growth, (2) the short intermittent periods where the lenses were removed to measure whether they were already myopic were sufficient for partial recovery, (3) the growth response of the eye is a all or none response- if there is a consistent focus error signal, it grows as fast as possible to achieve correction of the hyperopic refractive error, no matter how large the error is, (4) focus error signals from accommodation do not contribute to emmetropization in the chicken, perhaps because there is an efference copy from the accommodation feedback loop, (5) other unknown factors. Studies on these topics are important because they could explain why undercorrection or no correction does not consistently block myopia development in children.

Acknowledgement
MyFun: Myopia: Fundamental understanding needed.H2020-MSCA-ITN-2015.

Finite Element Method Based Modeling Of A New Electrode Design And Stimulation Strategy For Retinal Stimulation

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Purpose
Spatial resolution of the visual prostheses needs to be increased to the the desired level. There are different aspects to handle this problem, but one of the main problems encountered is that the number of electrodes cannot be increased because of electrode interaction. This study presents Central-Weighted Time Shifted Stimulation Strategy developed using Finite Element Method that aimed both to reduce the effect of electrode interaction, power dissipation and temperature rise generated as a result of stimulation on the tissue. So, each electrode could be used as an individual pixel.

Methods
The proposed method includes (1) an electrode design that both provides localized stimulation and is formed by denser electrodes with hexagonal layout in the center compared to periphery, (2) a stimulation strategy that the electrodes are fired in sequence. The electrical stimulation of the retina was modeled by the Finite Element Method while solutions were made using Maxwell's equations and its derivatives. A stimulation current was applied to the pre-selectod electrodes placed on the retinal tissue. The resulting electric field distribution over the tissue was calculated and examined. Various parametric analyzes were been carried out by taking into consideration that the electric field intensity between 1000-3000 V/m could stimulate nerve cells. The temperature rise in the tissue was examined using the Bio Pennes equation. During simulations it was assumed that the retinal tissue was homogeneous and isotropic under the conditions that capacitive factors were ignored.

Results
The advantages of the proposed method were shown by comparing the results of standard planar electrode array with a standard stimulation strategy. When the electric field distribution was examined, localized stimulation could be achieved with the proposed approach by significantly decreasing electrode interaction. Besides, nerve cells could be stimulated with lower stimulation current amplitudes. It was observed that electrode interaction increased only when the distance between the electrodes decreased to below 5 µm and the stimulation current exceeded about 10 µA. The proposed sequential stimulation strategy provided lower power consumption, 0.239 mW/mm3 against 4.19 mW/mm3, and a significant decrease in tissue temperature rise, 0.4 °C against 1.38 °C, was observed.

Conclusions
It was shown that proposed approach was an in silico method that could be used to reduce power consumption, induced temperature rise in the tissue and to minimise electrode interaction which was an important constraint for increasing the resolution of visual prostheses.

Statement on proprietary interests
This work presents limited version of another study which is accepted by Journal of the Faculty of Engineering and Architecture of Gazi University with the ID 5000188600.

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COLIJN, J. M.

Burden of Age-Related Macular Degeneration now and in the future

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Purpose:
Age-related macular degeneration (AMD) is one of the major causes of blindness in the Western world. Exact figures on the prevalence of AMD are lacking which hampers proper clinical and public health planning. One of the aims of the European H2020 EYE-RISK project is to estimate the burden of AMD in Europe.

Methods:
Data from the European Eye Epidemiology (E3) consortium were used to estimate the prevalence of Early and Late AMD per age category in Europe. Projection models were developed to predict the number of AMD cases by 2040.

Results:
Prevalence of Early AMD increased with age from 3.5% (95% confidence interval [CI] 2.1-5.0) in those aged 55-59 years to 17.6% [95% CI 13.6-21.5] in those aged 85+ years. For Late AMD, these figures were 0.1% [95% CI 0.04 - 0.3] in the youngest age category and 9.8% [95% CI 6.3-13.3] in the oldest category, respectively. A decreasing prevalence of Late AMD was observed in the older age categories after 2006. By the year 2040 the number of AMD patients almost doubled, mostly due to the increased proportion of elderly in the population. The number of individuals with Early AMD is estimated to be 14.9-21.5 million, the number of individuals with Late AMD 3.9-4.8 million.

Conclusion:
Although the prevalence of Late AMD seems to be declining, the absolute number of patients is expected to almost double by 2040. This underlines the need for prediction models to differentiate between patients progressing to Late AMD and those who do not . First steps are taken to provide such a tool.

Das, Soumyaparna

Inhibition of CNG channel activity for the treatment of retinitis pigmentosa

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Purpose
Retinitis pigmentosa (RP) is a disease caused by mutations in rod photoreceptors which leads to blindness and for which no effective treatment is currently available. High levels of Ca2+ concentration in the photoreceptors are thought to contribute to the neurodegenerative process. Cyclic nucleotide gated channels (CNGCs) are one of the major sources of Ca2+. A knockout study of CNGC showed an improvement of rod viability in the rd1 mouse model for RP. Here, we target CNGCs using pharmacological blockers and antisense oligonucleotides (AON) mediated knockdown to try and prevent rod photoreceptor degeneration.

Methods
We used C3H rd1 and C3H wild-type mice to generate organotypic retinal explant cultures. These were treated with the CNGC blocker L-cis-diltiazem and its enantiomer D-cis-diltiazem from postnatal day (P) 7 to P11. Dose response data was collected using the TUNEL assay for the quantification of cell death. In addition, C3H wild-type explants were treated with D-cis-diltiazem and L-cis-diltiazem. As an alternative to pharmacological CNGC inhibition, we are developing an anti-sense-oligonucleotide (AON) approach, based on exon skipping or RNase H mechanism of the CNGB1 gene, targeting genomic sequences conserved between mice and man. To allow for a rapid analysis of efficacy, human retinoblastoma cell lines WERI-RB1 and Y-79 were tested for the expression of CNGB1 by PCR of cDNA derived from their total RNA. The expression of Cngb1 was also checked in different mouse tissues.

Results
L-cis-diltiazem was found to reduce photoreceptor degeneration on retinal explants derived from rd1 animals as compared to untreated [Average percent TUNEL positive cells – Treated 7.3008; n=10, Untreated 9.0847; n=15, p=0.0233] while D-cis-diltiazem had no significant effect. The analysis of the mouse and human Cngb1/CNGB1 gene sequences identified 6 exons and 9 potential candidate AON sequences with conserved sequences. The selected regions are expected to selectively knockdown the gene, allowing for direct translation from mouse to man. Human retinoblastoma cell lines WERI and Y-79 were found to express CNGB1 and may help rapid evaluating AON knockdown efficacy.

Conclusions
We found that the known CNGC blocker L-cis-diltiazem can reduce photoreceptor cell death in rd1 retina in vitro, confirming the importance of CNGC for photoreceptor degeneration. Further CNGC targeting compounds and AONs will be tried in vitro, and the most successful of these will then be tested in vivo. Since several CNGC targeting drugs are already in clinical use (e.g. L-cis-diltiazem), the establishment of CNGC as a therapeutic target could facilitate a rapid translation into new treatments for RP patients.

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de Boer, Matthijs

Studying visual perception in inherited retinal dystrophies with optogenetics: using Lca5-/- mice as a model

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Purpose
The purpose of this study is to investigate the potential of optogenetics-based treatment to restore responses in the visual cortex of Lca5-/- mice. Lca5-/- mice show retinal degeneration starting at the second week of life and thus are an excellent model for studying early-onset visual impairment. In humans, Leber Congenital Amaurosis (LCA) is characterized by an onset of visual impairment within the first year of life.

Methods
Proper expression of the light-sensitive protein Opto-mGluR6, a fusion product of melanopsin and mGluR6, was analyzed in hTERT-RPE1 and HEK293T cells via immunocytochemistry and Western blot analysis. In order to link visually-driven behavior to retinal and cortical function, we have developed a virtual reality set-up for mice. In this set-up, head-fixed mice are trained in a virtual environment while subjected to a reward-driven visual discrimination task. This allows us to measure visually-driven behavioral choices in a motivational context.

Results
In hTERT-RPE1 cells transfected with Opto-mGluR6 fused to GFP, Opto-mGluR6 mainly localized inside the cell, whereas cells transfected with Opto-mGluR6 and either eYFP or TurboFP635 as separate products showed a more membrane-targeted localization of Opto-mGluR6. Protein expression was confirmed with Western blot analysis on isolated protein lysates from transfected HEK293T cells. Mice subjected to the behavioral training show a high motivation to learn the task.

Conclusions
Opto-mGluR6 is properly expressed under control of a ubiquitous promoter in hTERT-RPE1 and HEK293T cells. The virtual reality set-up is a useful paradigm for measuring visually-driven behavioral choices in mice. Future work should indicate whether Lca5-/- mice show an expected decrease of performance in the visual discrimination task, as well as whether optogenetics-based treatment is able to restore this performance. In addition, electrophysiological recordings and two-photon measurements during the task will allow us to link behavioral choices to responses within the visual cortex.

Acknowledgement
Project: Light after Dark, Grant number: NWO 58-14-002

Depping, Anne

Localisation of the Putative Magnetoreceptive Protein Cryptochrome 1b in the Retinae of Migratory Birds and Homing Pigeons

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Purpose
Birds can detect the Earth's magnetic field and use the magnetic field information as compass clue. For magnetic orientation, light of specific wave-lengths must be available and Cluster N, a light-processing forebrain region which is part of a known visual pathway in the birds' brain, is active. Therefore, the birds' eyes must be involved in some way in magnetoreception. The suggested magnetoreceptive proteins are the cryptochromes which are also involved in the regulation of the circadian clock in many animals. Four types of cryptochromes have been identified in birds and three of them are suitable candidates: Cryptochrome (Cry) 1a, Cry1b and Cry4. Here we analyse in which cell types of the retina of migratory birds and homing pigeons Cry1b is expressed.

Methods
Immunocytochemistry, cell transfection, immunohistochemistry, Western blot analysis, confocal microscopy

Results
In all tested species (European robin, pigeon and Northern wheatear), Cry1b was observed in the cytosol of ganglion cells, displaced ganglion cells, and in photoreceptor inner segments. The antibodies used for immunolabeling were proven to be specific by immunocytochemistry and Western blot analysis.

Conclusions
In all three species, retinal Cry1b is localised in cell types which have been discussed as potentially well suited locations for magnetoreception: Cry1b is observed in the cytosol of ganglion cells, displaced ganglion cells, and in photoreceptor inner segments. The cytosolic rather than nucleic location of Cry1b in the retina reported here speaks against a circadian clock regulatory function of Cry1b and it allows for the possible involvement of Cry1b in a magnetoreception mechanism.

Statement on proprietary interests
N/A

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Ecker, Christina

VEGFR2 mediated signaling protects against light-induced photoreceptor degeneration

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Purpose
Vascular endothelial growth factor receptor 2 (VEGFR2) is broadly expressed in the eye. To learn about its function, we conditionally deleted VEGFR2 in the entire eye and used the light damage paradigm to induce photoreceptor degeneration.

Methods
Floxed VEGFR2 mice were crossed with CAG-Cre mice, expressing the Cre recombinase under control of a tamoxifen-responsive chicken actin promoter. Light damage was applied with white light (5000 Lux, 30 min) and apoptotic cell death in the retina was analyzed using TUNEL- labeling. Retinal structure and function were studied by light and electron microscopy, ERG analyses, immunohistochemistry, real time RT-PCR and western blot analyses.

Results
Western blot analyses, real time RT-PCR and immunohistochemistry confirmed the successful deletion of VEGFR2. Retinal structure and function of VEGFR2 deficient mice did not show obvious alterations. However, following light exposure, VEGFR2 deficient mice had a significant higher number of apoptotic TUNEL-positive cells, concomitant with an impaired phosphorylation of proteinkinase B (AKT) compared to control animals. This resulted in a significantly elevated mRNA expression of the pro-apoptotic factor Bad in VEGFR2 deficient animals compared to controls.

Conclusions
VEGFR2 deficiency enhances the vulnerability of photoreceptors following light induced apoptotic cell death pathway.

Statement on proprietary interests
This indicates a neuroprotective role of the VEGFR2 signaling pathway.

FAYZIYEVA, MUNIS

COMPARATIVE ANALYSIS OF THE SEVERITY OF DRY EYE SYNDROME IN PATIENTS WITH DIABETES MELLITUS AND GLAUCOMA

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Purpose
To compare the severity of dry eye syndrome in patients with diabetes mellitus and glaucoma.

Methods
This study was carried out in the department of ophthalmology of the 2nd clinic of TMA. The study involved three groups of people at the age of 50 to 82. In the first group there were 13 patients with diabetes mellitus type 2. The second group consisted of 13 patients who had glaucoma associated type 2 diabetes mellitus. And third group consisted of 14 patients with primary open angle glaucoma. All patients underwent ophthalmic examinations such as history taking, complete OSDI questionnaire (uzbek version by Bakhritdinova F., Makarova E.), Schirmer I tear test, visual acuity, tonometry, perimetry, laboratory analysis (blood sugar level).

Results
23 (57,5%) are women and 17 (42,5%) are men, the mean age was 64±3,4 years old. All patients at the moment of examination had compensated blood glucose level. However in all the patients DE symptoms of different severity were found. Subjective evaluation of the severity of dry eye syndrome in terms of a history taking and the OSDI questionnaire. The OSDI scoring range is divided into degrees of severity: mild (13-22), moderate (23-32), and severe (33-100). In the I group the mean score was 16.8 (range of 11.4 – 20.8). In the II group -48.8 (range of 34.1 – 62.5), and in the III group the OSDI score was equal to 38.2 (range of 31.3 – 46.9). From Schirmer I tear test obtained that, 6 diabetic patients (46,1%) have DES. In the I group Schirmer I test the mean value was 7,8 mm (range of 4-13 mm). 1 patient (7,7%) has severe DE, 2 patients (15,4%) have middle DE, 3 patients (23,1%) have mild DE. In the II group estimated the highest prevalence of DES – 10 patients (76,9%), which had both of diseases. The mean value of Schirmer I test in this group was 4,8 mm (range of 2-10 mm). The measurement showed that, 3 patients (23,1%) have severe DE, 5 patients (38,5%) have middle DE and 2 patients (15,4%) have mild DE. In 8 glaucomatous patients (57,1%) determined the DES. The mean value of Schirmer I tear test in III group was 5,7 mm (range of 3-10 mm). We estimated in this group 2 patients (14,3%) with severe DE, 4 patients (28,6%) with middle DE and 2 patients (14,3%) with mild DE. Similar results were obtained by further examinations. In biomicroscopy the decreased marginal tear meniscus was observed in 6 patients (15%). In this examination obtained 1 patient (2,5%) with erosion and 2 patients (5%) with mucin filaments.

Conclusions
The results obtained confirm the importance of such an experiment. The severity of dry eye syndrome is supposed to be related to hyperglycemia, hypoinsulinemia and microvasculopathy, presence of glaucoma association. From our data we concluded that, the high prevalence of DES in patients with glaucoma associated type 2 diabetes mellitus. However the most pronounced signs of DES develop in patients with both of these diseases. It is important to assess patients with glaucoma and diabetes mellitus on presence of DES and treat in time.

Acknowledgement
250 $ (US)

FERRARO, LUCIA LEE

Validation of an adaptive optics scanning laser ophthalmoscope prototype in patients with degenerative retinal diseases.The LITE study.

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Purpose
To report the clinical validation of a newly designed and implemented confocal adaptive optics scanning laser ophthalmoscope (AOSLO) device in the context of the LITE ("Development of advanced laser imaging techniques for the anterior and posterior eye") study.

Methods
A prospective,observational,1-year study was conducted at the Institut de la Màcula (Barcelona, Spain) between August 2015 and November 2016 to validate the instrument. After testing the device in healthy subjects, patients with Stargardt's disease(STG) or retinitis pigmentosa(RP) were enrolled and visited at baseline, 3, 6 and 12 months. At each visit complete ophthalmic exam and retinal imaging were performed, in addition to AOSLO in regions of interest.

Results
Twenty-two patients were screened and 16 met the eligibility criteria: 5 healthy, 6 with STG and 5 with RP. Patients with degenerative diseases had a median of 47 years old (range, 35 to 64), there were 45.5% (5/11) females and 81.8% (9/11) were right eyes. Median baseline best-corrected visual acuity (BCVA) was 78 letters (range, 54 to 91) and median macular sensitivity was 16.8 dB (range, 3.5 to 27.8). AOSLO imaged a regular mosaic of photoreceptors in healthy individuals (Figure 1). In STG and RP patients (Figure 2) AOSLO showed irregular mosaics even in normal-appearing areas, according to fundus autofluorescence(FAF) and/or spectral domain optical coherence tomography(SD-OCT). These findings highlight the sensitivity of AOSLO imaging tools for assessing retina mosaic structure; moreover, damage to the outer retinal layers identified on AOSLO precedes that seen with current state-of-the-art instruments.

Conclusions
The newly developed AOSLO provided high-quality images of the photoreceptors(or lack thereof) in STG and RP, and may provide insights into disease progression and pathogenesis.

Acknowledgement
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Gagliardi, Giuliana

Using human induced pluripotent stem cells to generate photoreceptors for cell therapy.

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Purpose
For retinal cell therapy based on human induced pluripotent stem (hiPSCs), one of the major challenges is to identify surface antigens as specific markers of hiPSC-derived photoreceptors that could be used for the separation of transplantation-competent cell population. Here, based on our retinal differentiation method using adherent hiPSCs, we focused our study on the cell surface antigen CD73.

Methods
Retinal organoids containing photoreceptor precursors were generated according to our protocol (Reichman et al. 2017). At different time points of differentiation, expression of CD73 in retinal organoids was assessed by RT-qPCR and immunostaining. For cell separation, retinal organoids were dissociated into single cells, immunostained with a CD73-PE antibody and Magnetic-Activated Cell Sorting (MACS) was performed by using anti-PE Microbeads. Cells from dissociated organoids (unsorted, CD73+ and CD73- fractions) were analyzed and characterized by flow cytometry, RT-qPCR and immunohistochemistry. In vitro functionality of CD73+ cells was assessed by two-photon calcium imaging. In order to evaluate the risk of tumor development, NUDE rats were subretinally injected with hiPSCs, unsorted retinal cells or sorted CD73+ photoreceptor precursors from 120 day-old organoids. Eyes were histologically analyzed two months post-transplantation with antibodies directed against specific human antigens (MTCO2, Stem121).

Results
Analysis of CD73 expression in hiPSC-derived retinal organoids indicated that CD73 is specific of cells committed into the photoreceptor lineage, as all of the CD73+ cells co-localized with well-established photoreceptor markers (RECOVERIN, CONE ARRESTIN and OPSIN). Flow cytometry analysis indicated that the percentage of CD73+ cells in dissociated retinal organoids increased with maturation, with CD73+ cells representing more than 60% of cells at day 180 of differentiation. Dissociated retinal cells expressing CD73 could be sorted by Magnetic-Activated Cell Sorting (MACS), leading to enrichment to 90% of CD73+ cells in the positive sorted fraction. RT-qPCR analysis on sorted CD73+ cells showed over-expression of the most significant photoreceptor-specific genes compared to dissociated retinal cells before CD73 MACS. Re-plating of the CD73-sorted cells demonstrated the cell viability, even several days after the separation process, and homogeneity (CRX and RECOVERIN expression). Functional analysis by Fura-2 calcium imaging showed cGMP-dependent calcium influx in CD73+ photoreceptors from 180 day-old retinal organoids. In vivo experiments in NUDE rats, suggested that both unsorted retinal cells and sorted CD73+ cells at D120 are safe as no malignant hyperproliferation of human cells was observed in the eye as far as two months after subretinal transplantation. As expected, injection of undifferentiated hiPSCs did result in teratoma development in half of the rat injected.

Conclusions
Our results support the use of CD73 as a marker of hiPSC-derived photoreceptors. MACS of CD73+ cells resulted in a significant enrichment of photoreceptor cells. Importantly, this cell population do not present any tumorigenic risk as far as two months after transplantation. Hence, we think that CD73+ photoreceptor precursors hold great promise for future clinical translation.

Acknowledgement
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Garcia, Miguel

Defocus map across the visual field at indoor environments

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Purpose
There is abundant evidence that growth of the posterior eye segment is locally controlled at each position in the visual field and that local image defocus serves as an error signal for the feedback loop of emmetropization. Peripheral defocus error signals are further altered by accommodation which is controlled by the fovea. Based on these signals, the retina controls growth of the underlying sclera over the entire visual field. Mapping out local defocus over time could predict how the eye will grow in the future, and at which time myopia may start to develop. However, there is no device available at present that can map out defocus across the visual field and over the day in freely ranging subjects.

Methods
The "Kinect" v.1 camera was used (Microsoft) which is a RGB-D sensor that provides depth maps out of the box, using an infrared spot pattern technology. Its field of view is 58.5° (H) x 46.6° (V). The "Kinect" was attached to a helmet, together with a portable Eye Tracker that logged the direction of gaze. The distance from the eye to the fixation point provided the assumed plane of focus of the subject. Dioptric differences could now be recorded over the visual field, relative to the fovea, dioptric depth maps could be generated. Different tasks were performed in different scenarios and analyzed with a custom-developed script in Matlab (Mathworks).

Results
Comparisons of measured and real distances in depth showed a high correlation (R=0.9998, n=30), similar to published values for this device. Recordings of different indoor environments provided detailed defocus maps of the scenes, relative to the fixation point, with 640 x 480 pixel lateral resolution. In this pilot study, defocus maps were generated and averaged over 5 minutes (sampling rate 3 frames/sec).

Conclusions
With the presented approach it will be possible to generate defocus maps over the central visual field over time. These maps can be convoluted with the individual peripheral refraction profiles in order to map out the individual defocus errors signals on the retina during natural vision, and will make it possible to study how they relate to myopia development and progression.

Statement on proprietary interests
No conflict of interest

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García Delgado, Ana Belén

Animal Models for the Study of Age-Related Macular Degeneration

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Purpose
Age-related macular degeneration is a neurodegenerative disease causing blurred or no vision in the center of the visual field. A key pathological feature of AMD is age-dependent, progressive degeneration of the outer retina including the retinal pigment epithelium, Bruch's membrane and the underlying choroid. Although major genetic variations have been identified as affecting the risk of developing AMD, the biological functions of the genes implicated remain largely elusive, and moreover, the complex pathogenesis of AMD involves genetic, environmental, and demographic factors. Different approaches are being used to model the disease, in this context, several animal models have been described which may provide an useful tool for the study of AMD.

Methods
In this study we have used Nfr2 deficient mice in different postnatal ages (1, 1'5, 2 and 4 months) and characterized morphologically and functionally the retinal damage. We have evaluated spatial vision (visual acuity and contrast sensitivity) with Optomotor test using different spatial frequencies. By electroretinography, we have evaluated scotopic vision and we have analyzed the structure of the retina using funduscopy.

Results
In this study, we have found deposits and drusen at earlier ages than those described by other authors. Although by electroretinography the dysfunction of the photorreceptors is not remarkable in young animals, the visual acuity is clearly affected in Nfr2 deficient mice compared to controls.

Conclusions
Even though our work is still ongoing, we consider Nfr2 deficient mice as a very useful animal model both for the study of AMD pathophysiology and for the preclinical testing of new therapies.

Acknowledgement of funding, if applicable
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Can L and M cone densities in the retina predict the susceptibility to myopia?

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Purpose
A big unknown in myopia research is why, even with the same visual experience, some subjects develop a lot of myopia and others none. At least 40 gene polymorphisms were mapped out by GWAS in the past that can enhance the probability of becoming myopic but the possibility of a full prediction is still far away. Based on a hypothesis proposed by Jay Neitz, Seattle, we have tested whether the abundance and ratio of L and M cones may be a predictor of myopia progression. We have studied this question in the model of the chicken. L and M cones were mapped out in the center and periphery and correlated to the amount of myopia that was induced by frosted diffusers in front of the eye.

Methods
Deprivation myopia was induced in 9 chickens by attaching occluders in one of their eyes for a period of 7 days. The other eye served as a control. The growth of the different ocular components was tracked by A-scan ultrasonography at the beginning and at the end of the treatment. Infrared photorefraction was used to measure the development of myopia. After the treatment, long and mid-wavelength sensitive cones were identified based on their oil droplets in retinal flatmounts. Since the counts of many thousands of cones were elaborate, software was written in Visual C++ for automated cone detection. Cones were mapped out both in the periphery and in the center of the retina.

Results
As might be expected, the density of cones declined when the eyes became myopic. The correlation with myopia developed was significant ($p<0.05$) only in the central visual field, and only for the L-cones. For M-cones, there was just a trend ($p=0.06$). The total number of cones did not decrease when eyes got longer. While L- and M-cone densities did not consistently decline in the center of the visual field when the eyes became longer and myopic, there are signs of a re-organization of densities across the visual field. Given the significant differences in densities in different animals, it was striking that the densities in left and right eyes were not correlated.

Conclusions
There was no correlation between cone densities in both eyes, even though it is known from previous studies that deprivation myopia is highly correlated in both eyes when both eyes wore diffusers. Given that the genetic information should be the same in both eyes, it seems as if the cones densities of cones are not tightly controlled by genes and therefore will probably not predict myopia development.

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HAMIEH, ABDALLAH

Characterization of pathological mechanisms during aging of the Prpf31-mutant mouse model of retinitis pigmentosa.

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Purpose
Mutations in the ubiquitously-expressed Pre-mRNA Processing Factors 3, 8, and 31 (PRPF3, 8 and 31) constitute the second most prominent cause of non-syndromic autosomic dominant retinitis pigmentosa (adRP) in humans. We previously determined that young Prpf-mutant animals display abnormal RPE functions and develop a phenotype with age. Despite the difference in pathological timing between patients and corresponding mouse models, human ARPE-19 cells down-regulated for PRPF31 and Prpf31-mutant primary RPE cells display a similar defect in retinal phagocytosis, suggesting this mouse model can be used as a paradigm to identify related pathological processes. Thus, we set out to characterize RPE-related stress pathways occuring during the aging of Prpf31-mutant mice.

Methods
Studies were conducted on 3 to 24-month-old animals in order to dissect the full series of molecular events. Gene and protein expression levels for the mitochondrial respiratory chain and detoxification pathways were assessed by qPCR and immunoblots. Accumulation of lipids was evaluated on histological sections by Oil-Red-O and Bodipy stainings.

Results
ND4 (complex I) expression is decreased from 6 months of age in the RPE/Choroid fraction, while CoxIV (complex IV) expression increases. ATP synthase (complex V) expression diminishes in young animals before augmenting after 12 months of age. Expression of the detoxifying enzyme SOD1 (CuZn-SOD, cytosol) increases with age in mutant mice in contrast to wildtype controls. SOD2 (Mn-SOD, mitochondrial matrix) shows less variation of its expression than SOD1. Lipids coloration were validated on control beta5 knockout integrin mouse sections that accumulate lipofuscin and lipids with age. Stainings on Prpf31-mutant sections confirm our hypothesis that Prpf31- mutant RPE cells accumulate more lipid droplets than wildtype littermates during aging.

Conclusions
Our results suggest that mitochondrial defects could contribute to the RPE phenotype. In addition, oxidative processes appear to take place in Prpf31-mutant RPE cells. Taken together, we strongly believe our data will help us decipher the etiology of tissue-specific adRP cases linked to ubiquitously-expressed splicing factors and could contribute to define a new potential common therapeutic approach for all PRPF genes.

Statement on proprietary interests
The authors declare they have no competing interest.

Acknowledgement of funding, if applicable
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Hölzel, Maj-Britt

A possible role for ON-bipolar cells in congenital nystagmus

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In the mammalian retina bipolar cells are the connections between the light-sensitive photoreceptors and ganglion cells, which relay the retinal information to higher visual centers in the brain. The cones, the horizontal cells and the bipolar cells contact each other via the ribbon synapse (Wässle, 2004). A defect in this synapse might leads to oscillations in the direction selective ganglion cells and thereby to altered signaling to the AOS and the vestibule-cerebellum. This causes symptoms that seems to be equal to which found in patients with Nightblindness-Associated Transient Tonic Downgaze (NATTD; Simonsz et al., 2009) and show mutations in genes found for congenital stationary nightblindness (CSNB, Bijveld et al., 2013).

To investigate the role of the On-Bipolar cells in NATTD three mice strains with different mutations in proteins of the ON- bipolar cell synapse will be used: nob (NYX; Gregg et al., 2003), nob2 (CACNA1F, Chang et al., 2006), nob3 (GMR6, Maddox et al., 2008). Furthermore, the ganglion cell response of the Cav1.4 IT mice, which present a model for the congenital stationary night blindness type 2 (Knoflach et al., 2013), will be determined. To identify the ON-dsGCs for the single cell recordings, the mice will be crossbreed with SPIG1 GFP mice (Yonehara et al., 2008; Yonehara et al., 2009).

This mouse models show, like the human NATTD patients, an altered ERG phenotype reflected in a reduced a-wave and an absent b-wave (Chang et al., 2006; Maddox et al., 2008; Mansergh et al., 2005). The measurement of the optokinetic reflex showed eye movement oscillations at a frequency of 5 Hz, which is also in line with the human patients (Winkelman et al., unpublished). MEA results show oscillations at about 5 Hz in nob mice (Gregg et al., 2007). It is therefore very likely that the oscillations of the ON-dsGCs lead to altered AOS signaling and therefore to the nystagmus mediated by the oculomotor neurons. The poster will overview the PhD-project “A possible role of ON- bipolar cells in congenital nystagmus”.

Kersten, Eveline

Molecular biomarkers in Age-related Macular Degeneration

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Purpose
Age-related macular degeneration (AMD) is a common progressive retinal disorder. Multiple environmental and genetic risk factors have been identified, however the exact etiology is not yet completely understood. Biomarker studies can contribute to the unraveling of disease mechanisms and identification of drug targets. Here, we aim to provide a literature overview of systemic or ocular fluid compounds measured in AMD patients and discuss their relevance as potential biomarker.

Methods
PubMed searches were performed using the following keywords: age-related macular degeneration, serum, plasma, blood, urine, tear, aqueous, and vitreous. Abstracts were screened for relevance and full texts of the selected articles were studied. Animal, ex vivo and in vitro studies were excluded, as well as articles that were not written in English.

Results
More than 100 different compounds involved in different pathways have been described in relation to AMD. Many studies reported lower antioxidant levels and higher levels of oxidation products (e.g. malondialdehyde) indicating oxidative stress in AMD. Also, products of complement activation and complement activation levels were consistently associated with AMD. Additionally, lipid metabolism is clearly involved in AMD as evidenced by genetic and molecular studies, however literature on systemic lipid measurements in AMD is conflicting.

Conclusions
The most promising biomarker candidates belong to the oxidative stress pathway, the complement system, and lipid metabolism. Until now, none of these biomarkers are implemented clinically. The majority of studies so far have targeted single candidate-biomarkers. Hypothesis-free techniques can offer new insights and discover novel biomarkers, but only limited studies have been performed.

In the future, established clinical examinations and diagnostic tests for AMD may be extended with molecular biomarkers, an area still in development.

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Rewiring of bipolar cells in congenital stationary night blindness type 2 mouse models

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Purpose
Cav1.4 L-type voltage gated calcium channels are predominantly expressed at photoreceptor terminals at outer plexiform layer (OPL) and most likely also in bipolar cells in the inner plexiform layer (IPL). They are localized at specialized ribbon synapses where they serve an important role for synaptic transmission as well as synapse formation and maturation. Mutations in the CACNA1F gene which is encoding Cav1.4 channels are linked to congenital stationary night blindness type 2 (CSNB2). CSNB2 is X-linked non progressive retinal disorder with symptoms of abnormal visual acuity, myopia, nystagmus, strabismus and impaired night vision. Cav1.4 gain-of-function mutation I745T serves as a good model for the human CSNB2 phenotype. Here we investigated the morphological aberrations in the retina of mutant mouse models (Cav1.4-KO, loss of function; Cav1.4-IT, gain of function).

Methods
Animals: Eyes from 5 week and 12 to 15 week old Cav1.4 I745T (IT) and knock-out (KO) mice and controls (WT) were fixed with 4% paraformaldehyde for 10 and 20 minutes, cryoprotected in a graded sucrose series and embedded in OCT medium. 16µm horizontal retinal sections were cut on a cryostat. Primary antibodies were incubated at 4°C overnight, secondary antibodies were incubated for 1h at room temperature. Data Anaysis: Leica SP5 Confocal Microscopy, ImageJ.

Results
Immunostaining with PSD-95 showed that mislocated photoreceptor terminals were observed in the outer nuclear layer in Cav1.4-IT mice (5weeks) in contrast to WT where they were located in the outer plexiform layer and Cav1.4-KO retinas which lacked PSD-95 staining (WT, n = 3; IT, n = 4; KO, n = 3). Some rod bipolar cell dendrites of IT mice approached to displaced photoreceptor terminals in the outer nuclear layer. Some invaginating contacts were also observed in the outer nuclear layer of IT mice in 11 to 14 weeks (WT, n = 3; IT, n = 5). Mislocated photoreceptor terminals of IT retinas contained mostly immature synaptic ribbons. Immunoreactivity against to secretagogin to define Type 2-6 and possibly Type 8 cone bipolar cells illustrated that some type of cone bipolar cells in IT retinas also showed dendrites that were sprouting into the outer nuclear layer in 13-14 weeks (WT, n = 4 ; IT, n = 4).

Conclusions
The absence of invaginating contacts in the outer nuclear layer of IT mice suggested that these do not reflect mislocated existing contacts due to rod spherule migration. The synaptic organization seemed more severely affected in KO than in IT mice confirming that different mutations in the CACNA1F gene can cause different types of morphological aberrations. Sprouting cone BCs seen in IT mice indicated that bipolar cells in the cone pathway are also affected. Taken together differences similar to those seen in the two mouse models may also explain subtle variations in the clinical manifestation of CSNB2.

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KÜHN, SANDRA

Retinal and optic nerve damage was triggered via intravitreal injection of glial protein S100B

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Purpose
The glial protein S100B belongs to a calcium binding protein family. It is up-regulated in several neurological diseases, like multiple sclerosis or glaucoma. In previous studies, S100B immunization led to retinal ganglion cell (RGC) loss in an autoimmune glaucoma model. The intravitreal injection of S100B might also trigger a degeneration, but this mechanism based on the often seen glia response during neuronal damage processes. The direct impact of S100B on the retina and optic nerve was evaluated.

Methods
S100B or PBS (n=6/group) were intravitreally injected in one eye per rat. At day 14, neuronal protein level (β-III-tubulin), apoptosis level (cleaved caspase 3) and microglia amount (Iba1) was measured in retinae with Western blot analysis. RGCs (Brn-3a), amacrine cells (calretinin) and bipolar cells (PKCα) were histologically labelled. Furthermore, neurofilaments (SMI-32) of optic nerves were evaluated. Additionally, microglia cells (Iba1) and activated ones (ED1) were counted. The results were statistically analyzed (ANOVA, Dunnetts post-hoc test).

Results
In comparison to PBS group, S100B application reduced the neuronal protein level significantly ($p<0.001$) and activated apoptotic mechanisms ($p=0.014$) at day 14. Specifically, the number of RGCs was reduced in the S100B group ($p=0.007$), while the number of amacrine ($p=0.8$) and bipolar cell amount ($p<0.9$) remained comparable to the PBS group. Also, the optic nerve neurofilament structure was damaged ($p<0.001$). The protein level of microglia ($p<0.001$) and the number of Iba1+ ($p<0.001$) and ED1++Iba1+ microglia cells ($p<0.001$) was increased in S100B retinae.

Conclusion
At day 14, first signs of degeneration were noted for this novel retina degeneration model based on the intraocular injection of S100B. Apoptotic mechanisms and a microglia response were still in process, while RGCs and their axons were already damaged. S100B can trigger a direct effect on microglia via receptor binding. Therefore, the degeneration in this model might be induced by microglia activation.

LAPAJNE, LUKA

Effect of visual training with VEP biofeedback on visual outcome in amblyopia after the critical period

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Purpose
The purpose of the study was to assess the effect of visual training with Retimax vision trainer (CSO, Florence, Italy) on visual function in persons with amblyopia after the critical period of development.

Methods
Ten participants with monocular amblyopia of various aetiologies (4 strabismic, 5 anisometropic and 1 deprivation) between 8 and 16 years old (11.2±2.9) underwent 10-week, 20-session training. During each session the participants were instructed to be focused on the fixation point on the screen (the red square in the middle of the screen, surrounded by 60' VEP checkerboard with the frequency of 7.5 Hz) as much as possible. The visual response in terms of VEP was recorded through the scalp electrodes. In real time, the size of the fixation point and the frequency of the background sound were changing according to VEP parameters, thus providing the participants real time biofeedback of visual performances. ETDRS and FACT contrast charts were used to assess vision. Two and 12-month follow up were also performed.

Results
The average improvement of visual acuity on ETDRS chart between the baseline (logMAR 0.46±0.31) and the end of the treatment (logMAR 0.30±0.30) was 8 letters (range from 0 to 30, Paired sample t-test $p<0.05$). The contrast sensitivity function assessment demonstrated an increase of sensitivity across all spatial frequencies, although statistically significant only for 1.5, 3 and 12 cycle per degree (all $p<0.05$) and not for 6 and 18 cycle per degree ($p=0.052$ and $p=0.196$, respectively). The VEP data showed a rise of mean amplitude (2.27 and 2.39 μV, $p<0.05$) and a decline of mean fixation (7.25 and 7.13, $p<0.05$) between the first and the last two weeks of training. At the follow up at 2 and 12 months the visual acuity and contrast sensitivity remained roughly on the same level (within 3 letters on ETDRS) as they were at the end of the therapy in all tested individuals.

Conclusions
Our preliminary results do support the findings of other research groups on the matter of remained plasticity in the visual cortex after the critical period of development. The so called perceptual learning techniques may indeed improve vision and what is more, the effects seem to be long lasting. However, a larger sample and a proper control group is utmost desired in order to provide a reliable evidence of therapy effectiveness.

Statement on proprietary interests
None.

Lee, Meng-Jung

Electrically imaging retinal neurons using high-density multi-electrode arrays

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Purpose
Multielectrode arrays (MEAs) have been widely used in the study of neuronal physiologies. The advantages of high sampling rate, multiple recording sites and transparency make it an ideal tool to study not only single neuronal electrophysiology but also the networks of neural tissues. Here with the CMOS-based high-density microelectrode array (CMOS MEAs), we were able to further increase the spatial resolution of the MEAs to a level that enable us to electrically imaging retinal neurons, which will lead us to a better understanding of the networking signal transduction in the retina.

Methods
In this study, we adapted isolated healthy C57BL/6 retinae in vertical slices onto poly-l-lysine coated CMOS MEAs with 1 cm2 sensor area and record with 20 k sampling rate. We provided light stimulus with LED setup and record the light response. Recordings from these retinae were further analyze and visualized in the customized python-based software SOMA and Matlab.

Results
In the vertical slices from healthy mice retinae, we were able to electrically image the vertical signal transduction of light response from photoreceptor side to retinal ganglion side. In the same preparation, we also observed the horizontal signal propagation within the vertical slices. With longer light stimulation and pharmacological treatment, we further revealed the ON and OFF pathways separately.

Conclusions
Our results prove that with high-density CMOS MEAs, it is possible to electrically image retinal neurons in healthy retina to reveal the functional circuits. This technology will provide a fast and convenient way for neurological study in the future.

Acknowledgement of funding, if applicable
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Leyk, Janina

HDAC6 – a novel target for retinal degeneration?

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Progressive loss of photoreceptor cells is characteristic for retinal degenerative disorders, such as retinitis pigmentosa (RP). Recent studies suggest that histone deacetylases (HDACs) are involved in neurodegeneration and that inhibition of their enzymatic activity plays a protective role. Using the HDAC class I/II pan-inhibitor trichostatin A it was shown that HDAC inhibition could prevent photo-receptor cell death in mouse models for RP and cone dystrophies. HDAC6 is unique among the 18 members of the HDAC family since it mainly deacetylates non-histone proteins like α-tubulin, HSP90, cortactin and the redox regulatory protein peroxiredoxin 1 (Prx1). HDAC6 plays a role in cel-lular stress responses, redox regulation, and its inhibition has been implicated to be protective in certain neurodegenerative diseases. Our study aims to elucidate the role of HDAC6 and the influ-ence of its inhibition on 661W cells, a mouse derived cell line with characteristics of cone photore-ceptors, subjected to oxidative stress. To determine whether HDAC6 inhibition has a therapeutic effect on visual function, the dye zebrafish model of inherited sight loss was utilized. The enzymatic activity of HDAC6 was inhibited using the specific inhibitor tubastatin A (TST).

RT-PCR and immunoblot analysis revealed the presence of HDAC6 in 661W cells and in retinal ly-sates of C57/BL6 wildtype mice. In 661W cells HDAC6 inhibition by TST led to the acetylation of α-tubulin, the major component of microtubules. After oxidative stress, exerted by hydrogen perox-ide, TST promoted cell survival and the upregulation of heat shock proteins HSP70 and HSP25 by activation of heat shock transcription factor 1. Furthermore, H_2O_2 treatment led to strong overoxi-dation and thereby inhibition of Prx1. Preincubation with TST prevented the inactivation of Prx1 and its preserved activity may exert protective effects in photoreceptor cells. The dye zebrafish model is characterized by defects in visual behavior and retinal morphology. Using assays to evalu-ate optokinetic and visualmotor responses we demonstrated that TST treatment restored cone mediated visual behaviors. In agreement, TST improved retinal morphology and the qualitative appearance of photoreceptors.

In conclusion, our data suggest that among the family of HDAC enzymes, HDAC6 has a significant and specific role in neuroprotection.

VEGF overexpression in the rat mimics the ultrastructural changes of CNV with wet AMD

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Purpose
Vascular endothelial growth factor (VEGF) promotes choroidal neovascularization (CNV), an important component of subsequent vision loss in agerelated macular degeneration (AMD). Our aims were to develop a new efficient and reliable CNV model induced by overexpression of VEGF, to test its effects on extracellular matrix formation and to use the model to facilitate the study of anti-angiogenic and antiproliferative therapies for ocular diseases.

Methods
We developed a CNV model by subretinal injection of a highcapacity adenovirus vector encoding for human VEGF-A165 (HC Ad.VEGF) into 8 rats (Long Evans). Two rats which were injected with HC Ad.EGFP and five untreated rats were used as controls. Scanning laser ophthalmoscopy (SLO), indocyanine green angiography (ICG) and optical coherence tomography (OCT) were performed in all rats 2 and 4 weeks after the vector injection. Fluorescein angiography (FA) was performed in all rats 4 weeks after the vector injection. The eyes were enucleated 4 weeks after the injection and fixed for paraffin and EPON embedding. Sections were observed by light and electron microscopy (EM). HE staining and immunohistochemistry (IHC) were performed. Loss of retinal pigment epithelium (RPE) and choriocapillaris (CC) was quantified as well.

Results
The hyperfluorescent areas, shown with FA and ICG in 81.25% of the VEGF treated eyes, were possibly caused by alterations of the CC and subretinal neovessels. OCT showed a marked subretinal edema-like change in most eyes. In the EM, we found newly formed blood vessels with perivascular cells and fenestrations between Bruch's membrane (BM) and RPE or between RPE cells, multi-layered RPE, loss of photoreceptors, loss of RPE and CC compared to controls ($p<0.05$), thickened BM and collagen accumulation in CNV areas, resembling human CNV. We can't confirm the existence of leakage in the model, because of the lack of fibrin around newly formed blood vessels. IHC verified human VEGF expression, occurrence of pericytes (alpha SMA) and multi-layered RPE (RPE 65) in CNV areas.

Conclusions
Based on the results of examinations, especially EM, this rat model developed by overexpression of VEGF resembles human CNV with wet AMD.

Acknowledgement
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Ljungholm, Mikael

Simulating vision in an invertebrate eye

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Purpose
Optical simulations can be a useful compliment to behavioral test and provide information on the visual performance of an eye that can be hard to achieve by other means. By creating optical filters, we can also provide ecologists with a powerful tool that can simulate what the eyes see of the world. In this case, we have applied the process to the E-Rowelli (Onychophoran). The E-Rowelli has an asymmetrical eye that at first looks to be heavily under focused. We want to see if we can provide additional information to the behavioral tests and gain information on why the eye is shaped as it is.

Methods
The computer model of the eye was created by reconstructing images of microscopy slices to form a 3D model. The optical properties of the different structures were measured and added to the model. Light was then simulated using a ray-tracing approach. The absorption signal was used to create an estimation of the spatial response function for the eye. This information was also used to create a filter that was applied to images of the E-Rowelli natural habitat to create an image of what the world looks like though this simulated eye.

Results
The simulation estimates the spatial resolution of the eye to be around 0.02-0.08 cycles/deg with the higher resolution in the forward direction of the head.

Conclusions
The estimated resolution is slightly better than indicated by behavioural test of 0.02-0.03 cycles/deg. This was not unexpected since the behavioural tests require the animal to respond to a stimulus. And it is entirely possible that the animal could see the stimulus without providing a statistical significant result. The asymmetry of the eye could possibly be to provide the Rowelli with higher resolution in a critical direction (similar to a fovea), while still keeping the eye compact, reducing both energy and computational cost for the rest of the eye.

LUŽNIK, ZALA

Dendritic cell subtypes in preserved and cultured cadaveric human corneolimbal tissue on amniotic membrane

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Purpose
Limbal allograft rejection is the leading cause of graft failure. Resident dendritic cell (DC) maturation plays a critical role in the initiation of host allosensitization. There are two lineages: a myeloid (mDC) and a lymphoid (pDC), with different biological properties. The aim was to analyse the distribution of DC subtypes in human cadaveric corneal tissue and in limbal explant cultures on amniotic membrane (AM).

Methods
The expression of CD11c (for mDC) and CD303/CD123 (for pDC) was evaluated by flow cytometry on limbal explant cultures cultivated on either the epithelial or stromal side of the AM and compared with directly isolated cells from cadaveric whole corneoscleral tissue, divided into specific areas for comparison. Additionally, the expression of co-stimulatory molecules CD80, CD86, and activation markers HLA-DR, CD83 was investigated, as well as the expression of corneal epithelium marker CK12 and ABCB5, a new epithelial stem cell marker.

Results
Expression of pDC markers, mDC and stem cell ABCB5 marker was significantly higher ($p < 0.05$) in non-cultured cadaveric corneolimbal tissue compared to limbal explant cultures cultivated on AM. Cells positive for pDC and mDC markers were found in all examined areas with a prevalence of pDC ($p < 0.05$), but with no statistically significant difference between activated pDC and mDC. In contrast, in limbal explant cultures the percentage of pDC and mDC was similar, with no statistically significant difference between both sides of AM.

Conclusions
The DC content was significantly lower after ex vivo limbal explant cultivation. DC subtypes and ABCB5 positive cells were found in all tested conditions.

Statement on proprietary interests
None

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Primary Cilia in the Visual System

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Abstract

Cilia are hair-like projections found on almost all cells in the human body. Originally believed to function merely in motility, the function of solitary non-motile (primary) cilia was long overlooked. Recent research has demonstrated that primary cilia function as signaling hubs that sense environmental cues and are pivotal for organ development and function, tissue hoemoestasis, and maintenance of human health. Cilia share a common anatomy and their diverse functional features are achieved by evolutionarily conserved functional modules, organized into sub-compartments. Defects in these functional modules are responsible for a rapidly growing list of human diseases collectively termed ciliopathies. Ocular pathogenesis is common in virtually all classes of syndromic ciliopathies, and disruptions in cilia genes have been found to be causative in a growing number of non-syndromic retinal dystrophies. The reason for this is that the photoreceptor cells in the retina contain a highly modified primary cilium, the connecting cilium and the outer segment. Therefore any disturbances in ciliary function quickly manifest in loss of photoreceptors. However there are many other ciliated cell types in tissues of the eye (e.g. lens, RPE and Müller glia cells), which have only recently come to light. This talk will highlight their biological function and possible contribution to disease progression. Progress in basic research on the cilia function in the eye is paving the way for therapeutic options for retinal ciliopathies.

Characterization of Visual Function after Excitotoxic Damage of Retinal Cells

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Purpose
In the previous years, Kainic acid (KA) and N-methyl-D-aspartic acid (NMDA) have been used to induce excitotoxic damage in neurons of the inner retina in animal models. Although structural characterization of retinal damage after the effect of KA/NMDA has been shown, no effective functional tests have been developed to characterize the degree of visual impairment. In the present work we demonstrate the utility of pattern Electroretinogram (pERG) and the optomotor test to fully characterize the degree of visual deficit in animal models.

Methods
Retinal cell damage was induced by injection of 1 µl of PBS containing different doses of KA/NMDA into the right eye. Same volume of PBS was injected in the left eye. Binocular recordings were performed by the pERG technique prior to, immediately after and one week after the induction of excitotoxic retinal damage. Different spatial frequencies were tested. In addition, we performed optomotor test in the same animals. Different spatial frequencies and light contrasts were tested in clockwise and counterclockwise directions.

Results
The pERG recordings from eyes injected with KA/NMDA showed a significant decrease in amplitudes of the P50 and N95 trace components one week after retinal damage. The pERG showed that at increasing concentrations of KA/NMDA, the amplitude of the P50 and N95 components were diminished, being almost null for highest excitotoxic concentrations. Results from the optomotor test corroborated all data obtained by pERG. At high concentrations of excitotoxic agents, the visual acuity of the animals showed a significant decrease when the optomotor stripes moved in counterclockwise direction, indicating the damage of the right eye.

Conclusions
We demonstrate that the pERG and optomotor test are effective for evaluate the visual impairment induced in retinal cells. This work opens the possibility to test the effect of therapeutic agents on retinal damage in living animals.

Moniruzzaman, MD

Dissection of the ciliary function of Lebercilin and its dysfunction in Lebers Congenital Amaurosis

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Purpose
Leber's congenital amaurosis (LCA) is an early form of inherited blindness, which is the most common cause of blindness in children. Patients suffer from a severe loss of sight within the first year after birth. Mutation in LCA5 gene (responsible for encoding lebercilin protein) causes LCA. To date, four mutations causing LCA and affecting either the protein sequence or its expression were described. These mutations either lead to a loss of expression or to the expression of a truncated form of Lebercilin. However, the exact function of lebercilin and the mechanism by which the mutation lead to LCA are unknown. In this study, we want to describe the molecular function of lebercilin using cellular models in order to understand the LCA5 dysfunction in disease.

Methods
We used CRISPR/Cas9 genetic engineering tools for knocking out LCA5 as well as adding the FLAG tag in two different cell lines. Human embryonic kidney (HEK293T) cells are used for generating LCA5 knock-out (KO) and endogenously FLAG tagged lebercilin to analysis LCA5 dependent protein complexes by mass spectrometry. Wildtype and LCA5 KO retinal pigment epithelium (RPE1) cells are used for phenotypic analysis by immunostaining and microscopy.

Results
Based on Flag-Ip and mass spectrometric analysis we found several lebercilin interactors (e.g. ALMS1, IFT proteins, motor proteins). Immunohistochemistry staining showed normal ciliogenesis in both control and LCA5 KO cell lines. Interestingly, the LCA5 deficient cell line exhibited slower cilia disassembly compared with the control cell line.

Conclusions
We knew it before that lebercilin is not essential for cilia assembly. Now we can suggest that it is involved in transport processes.

NAESSENS, SARAH

Antisense oligonucleotide-based splice correction of two neighboring deep-intronic ABCA4 mutations causing Stargardt disease

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Purpose
Stargardt disease (STGD1) is one of the most frequent autosomal recessive retinal dystrophies. STGD1 can be caused by over 600 mutations in ABCA4, which explain 70% of the cases. We and others hypothesize that the remaining 30% can be largely explained by non-coding mutations of the ABCA4 locus. Recently, we identified two neighboring, deep-intronic mutations in intron 30 of ABCA4 that are predicted to create a new cryptic splice donor site.

Methods
We analyzed potential splice defects resulting from the deep-intronic mutations by employing minigene assays, by transfecting constructs containing ABCA4 exon 30 to 31, with and without the mutations, in HEK293T cells, followed by RNA-isolation and RT-PCR analysis. Subsequently, we designed 2'-O-methyl phosphorothioate antisense oligonucleotides (AONs) targeting the cryptic donor site in intron 30. AONs were tested by co-transfection of AONs together with the minigines. Finally, patient-derived fibroblasts were tested for the presence of the pseudo-exon and subsequently treated with the AONs, including a dose-response analysis.

Results
Both deep-intronic mutations indeed activate a cryptic donor site, resulting in the use of a nearby strong acceptor site and the inclusion of a 68-bp pseudo-exon in the ABCA4 mRNA. AONs directed against SC35 motifs in this pseudo-exon prevent insertion of this exon by blocking the binding of splicing factors. A nearly full restoration of normal splicing was observed in the minigene assay. The inclusion of the 68-bp pseudo-exon was confirmed on patients' fibroblasts and the designed AONs led to a nearly full restoration of normal splicing, in a dose- dependent manner.

Conclusions
The designed AONs induced skipping of the aberrant pseudo-exon and resulted in restoration of normal ABCA4 pre-mRNA splicing. Overall, we demonstrated the efficacy of AON-based splice correction of two different, neighboring deep-intronic mutations, using the same AON.

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Nemitz, Lena

Horizontal cells are essential for photoreceptor ribbon synapse formation in the mouse retina

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Purpose
Horizontal cells represent a class of interneurons in the mammalian retina that form triad synapses with photoreceptors and ON bipolar cells in the outer plexiform layer (OPL). During retinal development, two horizontal cell processes invaginate into the photoreceptor terminal and occupy the positions lateral to the presynaptic ribbon. Subsequently, one or two central ON bipolar cell dendrites are added and complete the triad configuration. To study the role of horizontal cells in the formation of the photoreceptor ribbon synapse, we used a mouse line which expresses the primate diphtheria toxin receptor (DTR) under the control of the connexin57 (Cx57) promotor. This mouse line enabled us to specifically ablate horizontal cells during early postnatal development via the injection of diphtheria toxin (DT).

Methods
Cx57+/+ and Cx57+/DTR mice were intraperitoneally injected with DT at postnatal day 4 (P4) and P5. To investigate the development of the triad synapses, retinas from control and horizontal cell-ablated mice were analyzed by immunohistochemistry and electron microscopy at different postnatal stages (P8, P15, P21 and P56).

Results
Our immunohistochemical analysis revealed that photoreceptor terminals and bipolar cell processes were evident in the OPL of DT-treated Cx57+/DTR and Cx57+/+ mice at P8. Labeling for CtBP2, a component of the synaptic ribbon, showed a punctate pattern within this layer in both genotypes. However, at P15, when the formation of triad synapses is largely completed in wild-type mice, horizontal cell-ablated mice showed abnormal ribbon structures, a reduced number of photoreceptor terminals in the OPL and ectopic photoreceptors terminals and bipolar cell dendrites in the outer nuclear layer. Rod spherules only partially retained their association with rod bipolar cells. Immunoreactivity for the mGluR6 macromolecular complex was strongly reduced at all tested developmental stages, suggesting that the formation of functional synapses between photoreceptor terminals and ON bipolar cells is disrupted. In contrast, we found GluR5-positive band-like structures at the base of cone pedicles which indicates that flat contacts between cones and OFF bipolar cells are formed and maintained in the absence of horizontal cells.

Conclusions
Taken together, our results suggest that horizontal cells are required for the proper formation of triad synapses in the OPL, possibly acting as guide rails for ON bipolar cell dendrites to invaginate into the photoreceptor terminal.

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Nowakowska, Dominika

Changes in the concentration of tryptophan, L- kynurenine and activity of IDO in the retina and serum in DBA/2J mouse model of optic neuropathy

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Purpose
Optic neuropathy is caused by the degeneration of retinal ganglion cells' axons in the optic nerve. The aim of this study was to evaluate changes in the concentration of tryptophan, L-kynurenine and activity of indolamine 2,3-diooksygenaze (IDO) in retinas and serum from DBA/2J mouse model of damage retinal ganglion cells.

Methods
Five DBA/2J and five C57/BL6(control group) mouse were held under specific pathogen-free conditions at room temperature and a 24-h light/dark cycle. The water and nourishment were supplied ad libitum. In DBA / 2J rodents is genetically determined ocular hypertension. A number of disorders of the anterior segment of the eye (dispersion dye, iris atrophy, adhesions front and rear, ciliary body atrophy) leads to an increase in intraocular pressure and secondarily to changes in the retinal ganglion cells and optic nerve. The loss of ganglion cells in these mice is reduced after using antiglaucoma medication to lower intraocular pressure . Age-matched control retinas were collected from mice of the C57/BL6 strain, which do not display any ocular abnormalities. Retinas and serum were collected from mice at the ages of 6 and 10 months. Animals were anaesthetized with an intraperitoneal injection of chloral hydrate and carprofen. After toracotomy and right ventriuculum punction the vessel blood was collected. The eyes were enucleated immediately. Following hemisection of the eye along the ora serrata, the cornea, lens and vitreous body were removed. Eyecups were shock frozen in liquid nitrogen and then lyophilised. The lyophilisate was suspended in 80 µ l of water and the proteins were precipitated with 20 µl of 10% (w/v) sulfosalicylic acid. After centrifugation, the supernatant was applied on an amino acid analysator based on separation by ionxchange chromatography followed by postcolumn derivatization using ninhydrin for detection. In retinas and serums obtained from animals levels of TRP and L-KYN by high performance liquid chromatography (HPLC) with fluorescence and electrochemical detectors were determined. Statistical analysis was performed using unpaired t-test and U-Mann Withney test (STATISTICA 10 PL, Stasoft). $p< 0.05$ was considered as statistically significant.

Results
In 6 months old DBA/2J mice retinas, the concentration of tryptophan was lower than in control group (C57 mice) (19,13±3.06 vs 37,23±3.01pmol/mg). The concentration of L-kynurenine in 6 and 10 months old mice DBA/2J retinas was lower than in control group and hasn't changed over the time(1,54±0.37 and1,3±0.26 vs 2,33±0.63 and 3,08±0.58). IDO activity hasn't changed over the time, in contrast to C57 mice retinas where statistically significant increase was found(0,06 and 0,2). The same L-KYN changes were found in serum analysis(105,49±10.24 and 100,34±7.46 vs 138,09±23.37 and139,23±7.85). Comparing to control group, the tryptophan level was lower in the serum of 10 months old mice (872,84±215.27vs 598,87±71.59). Both, in control group and DBA/2J mice, IDO activity hasn't changed.

Conclusions
The tribe mouse DBA/2J can be useful model of low tryptophan concentration laboratory animal.

Statement on proprietary interests
The authors declare that there is no conflict of interest regarding the publication of this paper.

Papadogiannis, Petros

Effect of chromatic aberration on detecting the sign of defocus

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Purpose
It has earlier been reported that myopes are generally more affected by myopic (positive) defocus than by hypermetropic (negative) defocus, fovealy as well as in the periphery. Therefore, signed defocus detection appear to have an asymmetric profile in nearsighted individuals whereas nonmyopes are more symmetric. This study aims to determine the role of the chromatic aberration in that asymmetry, by investigating how the eye detects the sign of defocus in the periphery with and without chromatic aberrations present.

Methods
The resolution threshold in low contrast was measured off-axis (20 degrees nasal visual field) for induced defocus between -5<D<2 and -5.5<D<3 for the right eye of one myopic and one emmetropic subject respectively. Stationary gratings were used (leaning to the left or right in a 2 alternative-forced-choice paradigm) in white and monochromatic(green) light. Peripheral monochromatic lower order aberrations were corrected by trial lenses and the chromatic aberration was eliminated by using a narrow band pass filter (550nm; bandwidth 25nm giving an average luminance over 20 cd/m2). No cycloplegia was used and there was no correction for higher order aberrations in the periphery. A lab-based Hartmann-Shack sensor was measuring the defocus values continuously during the vision evaluation, thus any changes in accommodation were taken into consideration during the data analysis. The data was analyzed with custom Matlab scripts which were compensated for the spectacle magnification of the trial lenses and all measurements were repeated 3 times.

Results
Peripheral resolution varied with defocus similarly for white and green light. In both conditions there was an asymmetry to the sign of defocus for the myopic as well as the emmetropic subject. For the myope the average slopes for white and green light were 0.07 and 0.09 logMAR/D for negative defocus and 0.12 and 0.11 logMAR/D for positive defocus respectively, and for the emmetrope the slopes for white and green light were 0.11 and 0.12 logMAR/D for negative defocus and 0.20 and 0.17 logMAR/D for positive defocus, respectively. For the nearsighted subject the slopes were steeper for the positive defocus values, which is in agreement with a previous study (Rosén et al. Invest. Ophthalmol. Vis. Sci., 52:318-323, 2011) suggesting that myopes are less sensitive to hypermetropic (negative) defocus in the periphery. Note that, although less common, some of the emmetropic subjects in that previous study showed an asymmetric profile similar to the emmetrope of this study.

Conclusions
For both white and monochromatic light, the myopic and the emmetropic subject had the same degree of asymmetry, proving that the asymmetry is not caused by the chromatic aberrations of the eye. Thus, this asymmetric profile in myopes and emmetropes deserves further investigation.

Pool, Fran

Mathematical Modelling of Biological Systems

Frances Pool

UCL Institute of Ophthalmology

Purpose
A brief review the advantages and disadvantages of mathematical modelling of biological systems with examples of specific eye models.

Methods
Examples will be drawn on models developed in Matlab, and visualised in Cell Designer.

Conclusions
Predictive models of complex biological systems provide important tools for the understanding of processes and on how therapeutic intervention may affect them.

Statement on proprietary interests
none.

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Eye Risk

Power, Michael

Simultaneous imaging of calcium and calcium-dependent enzymatic activity in degenerating cone photoreceptors

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Purpose
Calcium levels are strictly regulated within neurons and, thus, calcium dysregulation has been proposed to be linked to cell death in the nervous systems. Recent research has highlighted how various mechanisms of calcium dysregulation may be connected to both primary and secondary cone photoreceptor cell death in the mammalian retina (e.g. Arango-Gonzalez et al., PLoS One, 9:e112142, 2014).

Methods
Here, we examine the activation patterns of the calcium-dependent cysteine protease calpain in comparison with cell death patterns seen in mouse models of photoreceptor degeneration. To link calpain activation to calcium dynamics within the cones, we use the transgenic HR2.1:TN-XL mouse line that expresses a calcium biosensor in cones to study calcium levels and, where applicable, light-evoked responses in an acute retinal slice preparation (Kulkarni et al., J Vis Exp. 6:e52588, 2015).

Results
We determine the spatial activation of calpain at several time points in the secondary cone degeneration model rd1 (and later rd10), and the primary cone degeneration model cpfl1. Our preliminary data suggest that calpain activity is markedly increased in the rd1 and rd10 models (P30: rd1, 1.01 ± 0.113 calpain-positive cells in ONL/1000 µm2, n=45 observations from 3 mice $p < 0.0001$ compared to wt; rd10, 0.869 ± 0.068, n=45 observations from 3 mice; wild-type, 0.086 ± 0.023 n=45 observations from 3 mice $p = 0.0001$ compared to wt). We use two-photon microscopy to measure calcium dynamics in retinas of HR2.1:TN-XL mice as our wild-type control, as well as in the retinas of rd1 and cpfl1 which have been stably crossbred with HR2.1:TN-XL mice. By adapting an approach used commonly on thin, unfixed retinal sections, we have developed a protocol for the combination of detecting enzymatic activity of calpain and live-cell calcium imaging.

Conclusions
In using this approach, we are able to simultaneously image two different parameters – here calcium and calpain activity – likely involved in cone cell death, in real-time. Future experimental manipulations (e.g. calpain inhibitors, Ca2+ channel blockers) will then allow us to establish if and how these factors are linked to each other and cell death. Data generated in this project may be used to rationally design new therapeutic approaches for the treatment of cone degeneration.

Statement on proprietary interests
Retinal Degeneration linked to calcium levels and calcium activated proteins

Acknowledgement of funding, if applicable
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Reinehr, Sabrina

Increase of intraocular pressure and loss of retinal ganglion cells in a new transgenic mouse model

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Purpose
Primary open-angle glaucoma is one of the most common causes for blindness worldwide. Although an elevated intraocular pressure (IOP) is the main risk factor, the exact pathology remained indistinguishable. In the presented study, we analyzed a transgenic glaucoma mouse model (βb1-CTGF) to elucidate new possible mechanisms of the disease focusing on the early onset of the axonal loss driven by the increased IOP.

Methods
IOP was measured in βb1-CTGF and wildtype (Co) mice at 5, 10, and 15 weeks. At 15 weeks, retinal flatmounts and cross-sections were prepared. Immunhistology was performed with markers against retinal ganglion cells (RGCs; Brn-3a), apoptosis (cleaved caspase 3), microglia (Iba1), activated microglia (ED1), and the complement system (C3 and membrane attack complex = MAC). Cells were counted via ImageJ. Groups were compared using Student's t-test.

Results
At 5 and 10 weeks, the IOP in the βb1-CTGF and Co group was comparable ($p>0.05$). After 15 weeks, a significant elevated IOP was measured in βb1-CTGF mice ($p<0.001$). On flatmounts, a significant reduction of Brn-3a+ RGCs was observed in the βb1-CTGF group ($p=0.02$). These results were confirmed on retinal cross-sections, where a significant loss of RGCs was also noted in the βb1-CTGF mice ($p<0.001$). Additionally, significant more cleaved caspase 3+ RGCs were revealed in the βb1-CTGF group ($p=0.002$). Neither the total number of microglia ($p>0.05$) nor the number of activated microglia ($p>0.05$) was altered in 15 weeks old mice. Additionally, no changes were observed in regard to the complement components C3 and MAC at the investigated points in time.

Conclusions
A previous study could show a loss of axons in optic nerves of the βb1-CTGF mouse model. Here, we observed a loss of RGCs after IOP elevation. At this point, no alterations could be noted regarding the microglia and the complement system. We assume that the CTGF mouse could serve as a good model for better understanding the pathomechanisms in primary open-angle glaucoma.

Statement on proprietary interests
The authors declare no conflict of interests

RODRÍGUEZ BOCANEGRA, EDUARDO

A Swine Model of Selective Geographic Atrophy Mimicking Atrophic AMD

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Barcelona Macula Foundation

Purpose
To establish the dose of subretinal sodium iodate ($NaIO_3$) in order to create a toxininduced large animal model of selective circumscribed atrophy of outer retinal layers, the retinal pigment epithelium (RPE), and photoreceptors, by spectral-domain optical coherence tomography (SD-OCT) and immunocytochemistry.

Methods
Fifteen male and female healthy Yorkshire pigs received unilateral subretinal escalating doses of $NaIO_3$ under general anesthesia. In all the animals, volumes of 0.1 to 0.2 mL $NaIO_3$ were injected into the subretinal space of the area centralis through a 23/38-gauge subretinal cannula. Control SD-OCTs were performed 1 and 2 months after the surgery, at which time pigs were euthanized and eyes enucleated. Globes were routinely processed for histologic and immunohistochemical evaluation.

Results
Spectral-domain OCT and immunohistochemistry revealed circumscribed and welldemarcated funduscopic lesions, limited to the outer retinal layers in pigs treated with 0.01 mg/mL subretinal sodium iodate.

Conclusions
The swine model of a controlled area of circumscribed retinal damage, with welldelimited borders, and selectively of the outer layers of the retina presented herein shows several clinical and histologic features of geographic atrophy in AMD. Therefore, it may represent a valuable tool in the investigation of new emerging regenerative therapies that aim to restore visual function, such as stem cell transplantation or optogenetics.

Statement on proprietary interests
None

Ruiz Lopez, Ana

Pro-survival redox signalling in progesterone-mediated retinal neuroprotection

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Purpose
Retinitis pigmentosa (RP) is a group of hereditary retinal diseases, characterised by photoreceptor cell loss. Despite a substantial understanding of the mechanisms leading to cell death, an effective therapeutic strategy is sought. Our laboratory has previously demonstrated the neuroprotective properties of Norgestrel, a progesterone analogue, in the degenerating retina, mediated in part by the neurotrophic factor basic fibroblast growth factor (bFGF). In other retinal studies, we have also presented a pro-survival role for reactive oxygen species (ROS), downstream of bFGF. The aim of the current study was to study the role of ROS production in survival responses following Norgestrel treatment.

Methods
The 661W photoreceptor cell line and the rd10 mouse model of RP were used in the experiments performed. Serum deprivation was used to induce stress for the time indicated. Flow cytometry was used to measure ROS levels, while bFGF levels were measured by rtPCR and western blotting. Cell viability and proliferation were analyzed using MTS assay in cells and TUNEL staining in retinal explants.

Results
Norgestrel enhances an early pro-survival burst of ROS up to 1h in the stressed cells. No changes in ROS are seen in the absence of bFGF action through either blocking its receptor (PGRMC1) or using siRNA, suggesting this burst is mediated by the up-regulation of bFGF at 30 min following serum starvation (SS). Norgestrel-driven protection was attenuated in the presence of antioxidants. This may in part explain its protective effects on the 661W cells. Using the rd10 mouse model of RP, we confirm that Norgestrel induces a similar early pro-survival increase in retinal ROS.

Conclusions
This study therefore presents an essential role for ROS signalling in Norgestrel-mediated neuroprotection in vitro and demonstrates that Norgestrel employs a similar pro-survival mechanism in the degenerating retina.

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

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Saari, Paulus

Co-expression of multiple opsins underlies broad spectral sensitivity of cockroach photoreceptors

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Abstract

Abstract We have recently created the retinal transcriptome of Periplaneta americana and successfully used gene-specific in vivo RNA interference (RNAi) to suppress expression of several proteins involved in phototransduction. Here, by using intracellular and patch-clamp recording methods we provide functional analysis of transcriptional silencing of green opsins achieved by injecting long (596 bp) double-stranded RNA into head hemolymph. Recordings were performed during 10–22 days post-injection. We measured basic electrical properties, spectral and absolute sensitivities to light, elementary current and voltage responses (bumps), macroscopic light responses and voltage-activated K+ currents. In 8 out of 9 treated animals, absolute sensitivity of green-sensitive photoreceptors decreased by 2 to 5 orders of magnitude in comparison to controls. No effect was observed on current quantum bumps or anything else except spectral sensitivity, indicating that disruption of opsin synthesis did not affect phototransduction and membrane properties. Almost all photoreceptors in control showed broad spectral sensitivity with maximal responses to green and substantial responses to UV light. However, opsin RNAi cockroaches demonstrated exclusive sensitivity to UV light albeit not exceeding that in control. These results imply that some of cockroach photoreceptors express at least two opsins, green and UV. Considering that the transfer of UV-specific information over a mixed channel is not possible, and that cockroaches are mainly active at dusk and dawn, when the fraction of UV in ambient light is maximized, this co-expression has likely evolved to fully utilize the available spectral range and through this optimize motion detection and recognition of conspecifics.

Sadeghi, Mahdi

Retina Ganglion Cell Visual Characterization toolbox

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Purpose
To improve visual coding strategies for the next generation of the Tübingen subretinal implant, retinal ganglion cell (RGC) coding diversity shall be quantified by the development of a visual characterization toolbox, building on previous work from Baden et al. [2016, Nature].

Methods
Visual stimuli building on the Euler Lab's stimulus set: 'chirp', moving bars, spatiotemporal noise, and on-off flash are presented via a LightCrafter patterned light projector to induce RGC spike trains. RGC responses are recorded with a 60-channel planar microelectrode array (MEA). The stored raw data are processed using commercial spike sorting software and custom cell validation methods.

Results
A large database of RGC responses to the visual stimuli are sorted into cell types by adapting the analysis of Baden et al. to our dataset. Sorting is achieved by using principal components analysis (PCA) for feature extraction and using machine learning to fit a Mixture of Gaussians model on these features to identify clusters. Our toolbox takes as input trigger times, spike times and response annotations and outputs the RGC type(s) that best correspond to each data sample. These classifications are compared to each other and published classifications to identify types that are easily and reliably identifiable. Finally, our toolbox calculates the probability of assigning each RGC sampled to each of the specific RGC types – clustered according to their functional diversity.

Conclusions
This toolbox is an enabling method for identifying RGC type-specific activation through the presentation of specially designed electrical stimulation patterns.

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SANZ DIEZ, PABLO

Adaptation to contrast and its influence on accommodation

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Purpose
Research in various animal models of myopia has shown that axial eye growth is continuously fine-tuned to achieve the best match of the average position of the focal plane and the retinal plane. A possible retinal process that could provide a measure of image defocus is the level of contrast adaptation. The research aimed to analyze the influence of adaptation to contrast onto the slope of the accommodative response curve.

Methods
This was a prospective, randomized and monocentric study. Natural grayscale filtered images were used to evaluate the role of contrast adaptation onto the slope of the accommodative response at a distance of 2.0D. Blur and contrast of the natural images were manipulated digitally in the Fourier domain by filtering with a Sinc function and modifying the slope of the amplitude spectrum, respectively. Furthermore, a Sinc filter mask was applied in order to smooth the sharp edges of the images to avoid any kind of visual cue. Finally, filtered images were adjusted to balance the same RMS contrast and mean luminance as the original. The experiment was performed using a 4-step process to reach the purpose of the study: (1) demonstration that Sinc-blur filtered image would not drive accommodation when lenses were positioned in front of the eye, (2) verification that accommodation responds to the non-blur filtered image when lenses were placed in front of the eye (-4 to +1 in steps of 1 D), (3) increasing logarithmically the details of the Sinc-blur filtered version until accommodation was evoked and (4) examination of the behavior of the accommodative response function, depending on whether the subject previously had undergone contrast adaptation by looking at high or low contrast versions of the image. The accommodative response was continuously monitored using an eccentric infrared photorefractor.

Results
A total of 6 subjects with ages ranging from 23 to 29 years (mean age of 25.33 ± 1.97 years) participated in this experiment. Part (1) and (2): the non-response or the response of the accommodative system eye was confirmed when different lenses were positioned in front of the eye, using a Sinc-blur or non-blur filtered image, respectively. Part (3): different levels of blur were required to stimulate the accommodative system in the different subjects. Part (4): analyzing each subject individually, an increase of the accommodative response was observed in half of the subjects involved ($p<0.05$). The other half showed a similar trend in the behavior of the accommodative response but with no statistical significance ($p>0.05$). The increase in accommodative response seems to be affected according to the level of contrast shown previously.

Conclusions
A possible increase in accommodation could represent an attempt by the accommodative system to restore image contrast. A large sample size is necessary to reach more solid conclusions.

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Sapeta, Sabina

In vivo ultrahigh-resolution imaging of aqueous outflow structures in healthy subjects and glaucoma patients after canal expander implantation.

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Purpose
To investigate the capability of ultrahigh-resolution OCT (UHR-OCT) for visualizing the microstructure of the Schlemm's canal (SC) and providing a better insight into the aqueous outflow system (AOS).

Methods
SC cross-sectional area was measured using the UHR-SDOCT, providing a theoretical axial resolution of 1.2 µm in tissue, in 10 healthy volunteers during 2 conditions: before and after topical instillation of 2% Pilocarpine. To validate the precise anatomical location of SC, 2 POAG patients were included, who underwent canaloplasty procedure combined with implantation of Stegmann Canal Expander (SCE) into SC.

Results
10 healthy volunteers (5 female, 5 male) with an average age of 32.1 ± 11.0 years (range 21 – 53 years) were included in the study. Following instillation of one drop pilocarpine, mean IOP in the study eye decreased from 13.3 ± 2.9 mmHg to 11.5 ± 3.1 mmHg (p = 0.03). The mean SCLA of all subjects at baseline was 3620 ± 589 µm2 nasally and 4075 ± 335 µm2 temporally. After administration of pilocarpine, SCLA increased to 7772 ± 994 µm2 (p = 0.002 versus baseline) on the nasal and to 7760 ± 857 µm2 on the temporal side (p = 0.002 versus baseline), respectively. No correlation was found between change in IOP and change in SCLA after instillation of pilocarpine (nasal: r = -0.092, p = 0.86; temporal: r = 0.084, p = 0.88).

Conclusions
UHR-OCT allowed detailed visualization of limbal and AOS morphology and precise measurement of SCLA in healthy individuals and POAG patients.In comparison with conventional OCT systems, UHR-OCT provides non-invasive in-vivo high-resolution optical biopsy of AOS microstructure, close to the visibility in histologic methods and could be consider as a valuable tool in glaucoma management, including clinical decision making and follow-up. It is a promising technique for the use in clinical practice in assessment of the SC and its regional alterations caused by pathologic conditions and may enable better selection of patients with predicting and optimizing the outcomes of pharmacological, laser or surgical treatment targeting outflow structures.

Statement on proprietary interests
No conflict of interest.

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Schmitt, Sabrina I.

Characterization of endothelin receptor type b mediated signalling in Müller cells and photoreceptors

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Purpose
The endothelin receptor type B (Ednrb) is expressed in Müller cells and neurons and is activated after binding of its ligands, including the neuroprotective molecule Endothelin 2 (Edn2). In this study, we characterized the expression of Ednrb and Edn2 in the mentioned cell types and their potential neuroprotective effects on the retina in vivo and in vitro.

Methods
We enriched Müller cells and neurons of wildtype mice by magnetic activated cell sorting and characterized mRNA expression levels of Ednrb and Edn2. To learn about the physiological function of the endothelin signalling pathway, we generated mutant mice with a conditional deletion of Ednrb in neurons and Müller cells (α-Cre;EDNRBfl/fl). We analysed the retinal morphology, performed morphometric analyses and investigated the molecular expression levels of Ednrb, Edn2, fibroblast growth factor 2 (Fgf2) and leukaemia inhibitory factor (Lif). To examine the function of Ednrb/Edn2 signalling selectively in photoreceptors, we designed three guide RNAs to generate a Crispr/Cas mediated deletion of Ednrb in the photoreceptor cell line 661W. The mRNA expression levels of Ednrb, Edn2 and Lif were analyzed.

Results
The mRNA expression level of Ednrb was higher in neurons than in Müller cells. The deletion of Ednrb in neurons and Müller cells (α-Cre;EDNRBfl/fl) was confirmed by western blotting and realtime RT-PCR. There were no differences of the outer and inner nuclear layer morphology between α-Cre;EDNRBfl/fl and control mice. The mRNA expression levels of Edn2 and Fgf2 were increased in the α-Cre;EDNRBfl/fl mice compared to control littermates. The deletion of Ednrb in vitro via Crispr/Cas, selectively in photoreceptors, resulted in an up to 87% decreased mRNA level of Ednrb. Furthermore, real-time RT PCRs showed a decreased mRNA level of Edn2 and Lif.

Conclusions
The deletion of Ednrb in neurons, Müller cells and photoreceptors influences the expression levels of Edn2, Fgf2 and Lif in a cell type specific manner. Our findings highlight the importance of Ednrb mediated signaling in the regulation of the expression of neuroprotective factors like Edn2, Fgf2 and Lif.

Statement on proprietary interests
This might point towards a new therapeutic avenue to attenuate the loss of neurons in diseases like hereditary retina degenerations or age-related macular degenerations.

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Schneider, Sandra

Complete ablation of the primary cilium in the Retinal Pigment Epithelium using condi-tional transgenic mice

Sandra Schneider, Jun. Prof. Dr. Helen May-Simera

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Purpose
Cilia, microtubule-based organelles, are present on almost every eukaryotic cell type and play important roles in many physiological and developmental processes. A defect in the function or assembly of cilia leads to a wide range of syndromic and non-syndromic dis-orders, amongst which retinal degeneration is the most common phenotype. One reason for this is that the connecting cilium and outer segment of retinal photoreceptors is com-prised of a highly specialized primary cilium. However, the eye also has other ciliated cell types. The retinal pigment epithelium (RPE), a monolayer of pigmented epithelial cells, intercalates between the photoreceptors and the choriocapillaris. It is essential for the development and function of the retina. Virtually nothing is known about the role of pri-mary cilia in the RPE, nor how cilia defects in the RPE may affect vision. We set out to investigate whether the primary cilium is required for RPE development, and how this may influence visual function.

Methods
First we characterized a novel RPE specific Cre recombinase. We used a tamoxifen induc-ible Cre driver under transcriptional control of tyrosinase (Tyr::CreERT2), an RPE specific gene expressed early in the development of the RPE. Tyr::CreERT2 mice were crossed with Rosa-dsRed reporter mice, containing a gene for the red fluorescent protein tdTomato. After treatment with tamoxifen eyes were dissected and monitored for red florescence of the cells, either at E18.5 or in adult mice. Next we generated RPE conditional mutants in which the cilium is only ablated in the developing RPE. For this we crossed the Tyr::CreERT2 mice with IFT20floxed mice to generate conditional IFT20 knock-outs (IFT20 cKO). IFT20 is critically required for ciliogenesis. At E16.5 eyes from IFT20 cKO and lit-termate controls were dissected and stained for immunofluorescence using antibodies against IFT20 and Arl13b (cilia marker).

Results
After crossbreeding the Tyr::CreERT2 mice with Rosa-dsRed mice, recombinant expression of tdTomato was observed in embryonic and adult RPE tissue. Recombination was only observed specifically in the RPE, suggesting that the tyrosinase Cre driver is RPE specific. In embryonic tissues expression was high and uniform (70-90%), whereas adult mice ex-hibited only sparse and patchy expression (1-5%). Recombination of the IFT20floxed locus was not as efficient, although were able to see reduced expression of IFT20 and ablation of primary cilia. In our IFT20 cKO mice approx 70% of the RPE cells were still IFT20-positive and continued to display a primary cilium.

Conclusions
We were able to characterize a novel tamoxifen-inducible RPE specific Cre driver (Tyr::CreERT2) that is adapted for excision of genes specifically in the RPE. Since IFT20 knock out was not as high as we had expected, based on the read out of our reporter gene, we conclude that recombination of the IFT20floxed allele is not as efficient as recom-bination of the Rosa-dsRed allele. Therefore we will test additional Cre drivers to ablate the cilium in the RPE. However, further investigations into a possible RPE phenotype in our current IFT20 cKO mice are ongoing. In addition to characterization of the RPE phe-notype, we will test the visual function of these mice.

Statement on proprietary interests
Not applicable

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Sen, Merve

Effects of Sustained Delivery of VCP Inhibitors in Animal Models of Retinitis Pigmentosa

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Numerous human diseases, including inherited retinal degenerations, arise from defects in protein homeostasis. Retinitis Pigmentosa (RP) is a degenerative disease in humans primarily characterized by progressive loss of photoreceptor neurons leading to blindness. Photoreceptor cells within the retina are especially vulnerable to defects in protein homeostasis, as huge amounts of the visual pigment are newly synthesized every day. Several mutations leading to RP affect proper folding of proteins, which in turn causes intracellular stress. Cells are equipped with different physiological mechanisms to detect misfolding and deal with stress caused by misfolded proteins. This process, which includes a biochemical quality control, intracellular sorting and removal of defective proteins, is called proteostasis. Proteostasis is critical for cell survival, as an imbalance in proteostasis for prolonged periods of time results in cell death.

Experiments performed in HEK293 cells expressing mutant Rho^{P23H} and Rho^{wt} indicate that the mutant protein retain the WT protein in the ER illustrating induced ubiquitination and insoluble aggregates and was found in a complex with p97/VCP (Griciuc et al. 2010). Moreover, recent evidence suggests that genetic depletion or chemical inhibition of p97/VCP suppresses photoreceptor cell death in Drosophila expressing Rho^{P37H} (the fly equivalent of Rho^{P23H}) (Griciuc et al, 2010b; Griciuc et al, 2011; Griciuc et al, 2012).

These results suggest that sustained pharmacological manipulation of protein homeostasis (e.g. small molecule inhibitors of p97/VCP) may reduce intracellular stress, and decrease photoreceptor cell degeneration in RP.

Sothilingam, Vithiyanjali

New insights into the mechanisms of retinal degeneration due to Phosphodiesterase 6 (PDE6) deficiency

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Purpose
PDE6, a tetrameric complex composed of one alpha (Pde6a) and one beta catalytic subunit (Pde6b) combined with two gamma inhibitory subunits (Pde6g), is a central protein of the phototransduction cascade in rod photoreceptor cells of the retina. Loss-of-function mutations in the Pde6 gene lead to a premature retinal degeneration clinically manifesting as retinitis pigmentosa (RP), an ultimately blinding eye disease. In genetically homologous models of the human disease, a key feature is the massive accumulation of cyclic guanosine monophosphate (cGMP) together with a substantial deviation of the rod Ca^{2+} metabolism.

As the underlying mechanisms are not yet satisfactorily resolved, our goal in this work was to investigate the disease mechanisms via a separation of the direct impact of high rod cGMP levels from potential downstream effects mediated by the cyclic nucleotide gated (CNG) channels in the outer segment membrane. In case elevated intracellular cGMP levels alone were sufficient to evoke the disease phenotype, the additional loss of CNGB1 would be without much of a consequence. In contrast, if a dysfunction of CNG channels was the key for the induction of the degeneration, the additional mutation would have a protective effect.

Methods
Pde6a- and Pde6b mutant models of cGMP-related RP were cross-bred with Cngb1-/- mice lacking any CNG channels in rod photoreceptors. Two specific mouse lines were examined: a) Cngb1-/- x Pde6brd1/rd1 (Y347ter nonsense mutation, severe phenotype) b) Cngb1-/- x Pde6anmf282/nmf282 (V685M missense mutation, severe phenotype). These double mutants were then evaluated in vivo using non-invasive diagnostic techniques (ERG functional analysis and SLO/OCT imaging) and in vitro by way of a histological work-up.

Results
Our data show that the additional removal of CNG channels from rod photoreceptors by cross-breeding with Cngb1-/- does indeed protect respective individuals from cGMP-related RP. Importantly, cone function in both lines of mice remains detectable for a long period of time. This result means that the main deleterious effect in Pde6 mutations in both the rd1 mouse (Paquet-Durand et al., 2011) and the V685M mutant requires functional CNG channels, suggesting that the loss of Ca^{2+} influx control may be causative for the development of the disease.

Conclusion
In both Pde6a (V685M) and Pde6b (rd1) mutants, the removal of CNG channels led to an excellent rescue of the retinal phenotype for a protracted period of time. These findings reinforce the hypothesis that the deleterious calcium (Ca^{2+})-influx, mediated by CNG channels, is the cause of rapid rod cell death in Pde6-deficiency, and highlight the importance of CNG channels in this process. Therefore, a specific modulation of rod CNG channels, but not cone channels, is a most promising symptomatic approach to treat otherwise incurable forms of cGMP-related RP, and may also widen the therapeutic window for gene therapeutical approaches.

Srivastava, Prerna

Altered bipolar cell function in the degenerated retina.

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Purpose
Rod photoreceptor degeneration leads to severe anatomical and functional remodeling of the synaptic connections between retinal neurons. In particular, the outer retinal network, composed of remnant cone photoreceptors (cones), bipolar cells and horizontal cells, shows dendritic retraction, formation of ectopic synapses and changes in neurotransmitter levels (Strettoi et al., 2002; Marc et al., 2007; Chua et al., 2009) while generating spontaneous oscillatory activity (Haq et al., 2014). While morphological alterations in the inner retina are minor, the absence of light-driven input does affect inner retinal function, with ganglion cells, AII amacrine cells and cone bipolar cells showing spontaneous oscillatory activity bursts (Stasheff, 2008; Goo et al., 2011). The involvement of bipolar cells in both networks suggests a central role for this cell class (reviewed in Euler and Schubert, 2015). The mechanisms underlying the spontaneous activity in the inner and outer retinal networks are still unclear. Here, we aim to understand (i) how spontaneous activity in both networks changes as degeneration progress, (ii) whether the outer retinal network drives/modulates the inner retinal network via glutamatergic transmission of bipolar cells.

Methods
We used the rd10 mouse model of retinitis pigmentosa (RP), where rod degeneration starts at postnatal day (P) 18 (Gargini et al., 2007) and major synaptic remodeling occurs between P30 and P60 (Kalloniatis et al., 2016). To record spontaneous Ca2+ signals from remnant cones, horizontal and bipolar cells in the outer and amacrine cells and ganglion cells in the inner retina, we electroporated whole-mounted retina with a fluorescent calcium indicator and performed two-photon calcium imaging. To record glutamate signals, we ubiquitously expressed the AAV-encoded glutamate biosensor iGluSnFR and imaged the synaptic input and output of bipolar cells in the outer and inner retina, respectively.

Results
We recorded spontaneous oscillations in both outer and inner retina at P30 and P60. In the outer retina, activity did not change between P30 and P60, while in the inner retina, activity at P60 contained slower oscillations than at P30, suggesting some independence between two networks at later degeneration stages. To analyze whether bipolar cells relay activity from the outer to the inner retina, we pharmacologically blocked ON-bipolar cells using the mGluR6 receptor agonist L-AP4. In addition to reducing spontaneous activity in the outer retina (Haq et al., 2014), L-AP4 also modulated oscillations in the inner retina, suggestive of the outer retinal network modulating inner retinal activity. To study this possible vertical connection between the networks in more detail, we recorded glutamate release from cone and bipolar cell axon terminals. Our preliminary data add evidence to the notion that the outer retinal activity can be relayed from remnant cones via bipolar cells to amacrine cells and ganglion cells.

Conclusions
In summary, our data show that in the same retinal tissue, two oscillatory networks with different properties co-exist in the degenerated retina. Rod bipolar cell dendrites in the outer retina undergo severe remodeling and receive spontaneous synaptic input from remnant cones, and inner retinal activity has been shown to be generated in the AII amacrine cell network and then transmitted to cone bipolar/ganglion cells (Trenholm et al., 2012; Choi et al., 2014). Therefore, it is tempting to speculate that the rod bipolar cell – AII amacrine cell synapse is crucial to connect outer and inner retinal activity.

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Swiatczak, Barbara

„In vivo markers of myopia development: changes in fundal reflectance.“

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Purpose
Preliminary findings have shown that fundal reflectance declines when humans are exposed to outside illuminances for a few minutes. The biochemical basis of these changes is unknown, but it suggests that different retinal processing modes may be visualized as changes in reflectance that could easily be measured in vivo. I measured fundal reflectance in the UVB range in chickens (Gallus gallus domesticus), hoping that changes in retinal proteins that are associated with experimentally induced myopia would be detectable. Chickens have ultraviolet vision and UV transmitting ocular media.

Methods
Changes in fundal reflectance in UV were mapped out with a UV sensitive camera with a single UV emitting LED (366 nm) fixed in the center of camera lens. Fundus reflectance was determined from pixel brightness in the pupil (measured in average pixel grey levels), and after correction for changes in the f/number. Images of pupil were collected from myopic eyes and controls and analyzed with respect to brightness distribution in the horizontal profile, absolute pupil brightness and pupil diameter. Myopia was induced in 2 weeks post-hatch male chickens (n=3) by placing frosted plastic occluders in front of one eye for eleven days. Fellow eyes had normal vision and served as control. Myopia development was monitored by A-scan ultrasonography under corneal anesthesia (axial length) and infrared photorefraction (refraction error).

Results
All three chicken developed high myopia (-9.7D ± 1.1D) in eyes treated with occluders. Myopic eyes reached axial lengths of 9.66mm (± 0.55mm), compared to control eyes which had axial lengths of 8.93mm (± 0.36mm). Fundus reflectance in near UV was significantly higher in myopic eyes than in control eyes ($p<0.001$). Pupil brightness of myopic eyes reached pixel values of 68.3 (± 0.42px), and controls reached 58.72px (± 1.18px). Since myopic eyes had higher f/numbers, darker pupils would have been expected. Since they were brighter, fundal reflectance must have increased even more.

Conclusions
Since less bright pupils were expected in myopic eyes based on their higher f/numbers, it is clear that fundal reflectance must increase considerably when myopia is induced. The underlying changes in the retina are not yet know but they could include (1) changes in reflectance of retinal proteins, (2) changes in photoreceptor outer segments and light absorbance, (3) changes in photoreceptor alignment, and other yet unknown factors. In the future, the abundance of some retinal proteins that absorb in the wavelength range will be studied and histological studies will be performed to compare photoreceptor outer segment lengths in myopic and normal eyes. Furthermore, I will determine the time course of the fundal reflectance changes and relate them to changes in eye growth.

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Clinical and genetic characteristics of patients with RPGR-associated retinal dystrophies: a long-term follow-up study

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Purpose
To describe the phenotype, long-term clinical course, and genotype of patients with RPGR-associated retinal dystrophies.

Methods
Retrospective, observational cohort study.

Results
Fifty-two patients had a retinitis pigmentosa (RP) phenotype (70%), and 5 (7%) and 17 (23%) patients had a cone dystrophy (COD) or cone-rod dystrophy (CORD), respectively. The median follow-up time was 11.6 years (range 0-57.1). The median age at symptom onset was 5.0 years (range 0-14 years) for RP patients and 23.0 years (range 0-60 years) for COD/CORD patients. Survival curves showed a 20% and 55% probability of being blind (BCVA <0.05) at the age of 40 in RP and COD/CORD patients, respectively. High myopia was associated with a faster BCVA decline in RP ($p<0.001$) and COD/CORD ($p=0.03$) patients. RP patients with mutations in ORF15 had a faster visual field decline ($p=0.01$) and thinner central retina ($p=0.03$) than patients with mutations in exon 1-14.

Conclusions
Mutations in the RPGR gene can cause a spectrum of retinal dystrophies from RP to COD. Based on the BCVA survival, the intervention window for gene therapy for RPGR-associated retinal dystrophies is relatively broad in RP patients. High myopia is a risk factor for faster BCVA decline in RP and COD/CORD. Mutations in the ORF15 region of the RPGR gene were associated with a more severe phenotype in RP.

Statement on proprietary interests
No proprietary interests.

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Urbasik, Alexander

Examining the synaptogenesis and synapse maintenance factor Bassoon as a putative factor for the differential sensitivity of photoreceptors to late-onset degeneration

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Purpose
Progressive photoreceptor degeneration is a hallmark of a group of retinal degenerations referred to as retinitis pigmentosa (RP). Cone photoreceptors display particular sensitivity to degeneration as they rely on the integrity of the rod photoreceptor population for function and survival. The cell biological and molecular basis of the cone photoreceptor sensitivity remains unclear but may involve synaptic pathways and proteins. Indeed, differential sensitivity to degeneration is observed in mutant mice deficient for the presynaptic protein Bassoon (Bsn mt). These mice display a severe structural and functional photoreceptor synaptic phenotype (Dick et al., Neuron, 2003; tom Dieck et al., JCB, 2005; Regus-Leidig et al., EJCB, 2010). Surprisingly, although the synaptopathogenic effect of Bsn-deficiency affected both photoreceptor types equally, only the cone photoreceptors underwent late onset degeneration, while rod photoreceptors survived and reacted with structural and functional remodeling (Specht et al., EJN, 2007). The goal of the project is to shed light on the putative role of Bsn in conferring differential vulnerability of photoreceptors.

Methods
Generation of conditional knockout mice of Bsn in rod and cone photoreceptors, structural (immunocytochemistry, light and electron microscopy), functional (ERG, patch-clamp) analyses, and investigation of cell death pathways. In an explorative part, comparative expression profiling from rod and cone photoreceptors in the context of Bsn-deficiency will be performed to identify candidates for vulnerability factors.

Results
The Bsn mt mouse model used in the referred publications lacks a functional Bsn, but still expresses a rest protein of 180 kDa. We are now provided with a new Bsn mouse model with a complete loss of Bsn (Bsn ko). First immunocytochemical analyses show that the retinal phenotype of the Bsn ko mouse is similar to the Bsn mt mouse. Currently, ERG recordings and detailed ultrastructural examinations are performed to gain a more comprehensive insight into the structural and functional phenotype of the Bsn ko mouse.

Conclusions
Switching from the Bsn mt to Bsn ko mice is applicable for our future experiments examining the putative role of Bsn conferring differential vulnerability of photoreceptors.

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Wagner, Franziska

The status of DNA repair mechanisms in the healthy and degenerating retina

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Purpose
Genome editing using the cell's own proteins for non-homologous end joining (NHEJ) or homology directed repair (HDR), which are the two major pathways for DNA double strand break (DSB) repair, represents a therapeutic option for inherited retinal dystrophies. Using HDR, a correct exogenous template can replace the mutated DNA sequence through the use of highly specific nucleases, which induce a specific DSB at a target site. NHEJ functions without a template through direct religation of the cleaved ends. Retinal explant cultures can be used to optimize gene therapeutic approaches prior to testing in animals in vivo. Therefore, the purpose of this study was to detect DSB repair proteins in control retinae and retinal explant cultures of wildtype mice as well as a degenerative mouse line to establish it as a model for gene editing.

Methods
Neuroretina explants were prepared from 3 month old wild type C57Bl6 mice and a mouse model for X-chromosomal retinitis pigmentosa (B6J.SV129-Rpgrtm1stie). Total RNA was isolated. Expression levels of DNA repair proteins for NHEJ (Ku80, 53BP1, DNA-PKcs) and for HDR (RAD50, CtIP, BRCA1) were evaluated after 0 to 8 days in culture by qPCR. To investigate the DNA repair protein expression in individual retinal layers, samples were collected via laser capture microdissection. We used 8 µm thin unfixed retinal slices which were stained with haematoxylin. Subsequently, RNA isolation was conducted in the same way as for whole retina samples.

Results
The tested DNA repair genes for NHEJ and HDR showed only small changes in their expression levels during the cultivation of the whole retina. KU80, a protein for NHEJ, as well as RAD50 and CtIP (HDR) showed an upregulation during the culture. The differences between the DNA repair gene expression patterns of wildtype and RPGR mice were not significant, as expected. In contrast to this, there were prominent differences in the DNA repair protein expression between individual retinal layers, i.e. outer nuclear layer (ONL) versus inner nuclear and ganglion cell layer (INL+GCL). We showed a higher expression of KU80 in the ONL compared to the INL+GCL. This is consistent with our findings in retinal sections treated with antibodies against DNA repair proteins.

Conclusions
Our results on culturing retinal explants and their DNA repair protein expression profiles, yet without any manipulations, were the first step to establish retinal explant culture for gene editing as an intermediate step between cell culture and animal experiments. These are the first gene expression studies of DNA repair proteins in the mammalian retina; therefore, representing a valuable source of information for subsequent gene therapeutic manipulations. Further experiments will include the modification of retinal explant culture conditions by external drug applications.

Statement on proprietary interests
None

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WEIXIN, WANG

Comparative effects of Transforming growth factor-β1 and Heparin-binding epidermal growth factor on the production of anti-oxidants by human Müller glial cells

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Purpose
Müller glia account for the majority of glial cells in the retina. They play a major role in providing metabolic support to the neural retina and as such, they are known to produce anti-oxidant molecules to protect and promote the survival of retinal neurons. During retinal degeneration, cytokines such as transforming growth factor-β1 (TGF-β1) and heparin-binding epidermal growth factor (HB-EGF) are upregulated, which may have either pro-inflammatory or neuroprotective function. Oxidative mechanisms also accompany degeneration and Müller glia release neurotrophic and anti-oxidant molecules to protect against oxidative stress damage during retinal degeneration. The antioxidants heme oxygenase-1 (HO1) cleaves heme ring to biliverdin, whilst NAD(P)H:quinone acceptor oxidoreductase 1 (NQO1) is a known anti-oxidant molecule that protect neurons against quinone-induced damage by competing with potentially toxic one-electron pathways. This is very important in process where neurons have been damaged and the retina needs to be repaired. The aim of this study was therefore to investigate whether TGF-β1 and HB-EGF may modify the production of anti-oxidant molecules by Müller cells in vitro.

Methods
The Müller glial cell line MIO-M1 cell generated in our lab was cultured in Dulbecco's Modified Eagle's medium +10% fetal calf serum and 1% Penicillin/Streptomycin in the presence of TGF-β1 (50ng/mL) or HB-EGF (50ng/ml) for 3 and 6 days. Cells and culture supernatants were collected for RT-PCR and ELISA assays respectively. Cells cultured in medium alone were used as controls. Cell pellets were collected and RNA was extracted using a commercially supplied kit (Qiagen, UK). RNA extracted from cell pellets was subjected to RT-PCR to examine the expression of mRNA coding for HO1 and NQO1. mRNA expression was normalised to β-actin expression. The expression of these molecules was then confirmed by ELISA analysis to assess the level of HO1 protein and the enzymatic activity of NQO1. Protein concentration and enzymatic activity of these antioxidants were determined by extrapolation to standard curves.

Results
MIO-M1 cells express mRNA and proteins coding for the anti-oxidant molecules HO1 and NQO1. TGF-β1 causes a significant upregulation in the production and release of HO1. In contrast, HB-EGF significantly reduced the release of this molecule by Müller cells in culture. A significant down-regulation of NQO1 enzyme activity was observed when cells were cultured in the presence of TGF-β1 and HB-EGF for 3 days. No changes were observed when Müller cells were cultured with these two cytokines for 6 days.

Conclusions
The results suggest that HO1 might act at an early phase after exposure to these cytokines to inhibit intracellular reactive oxidative species (ROS) that may be released upon retinal injury. Whilst both TGFβ1 and HB-EGF are produced during retinal gliosis, HB-EGF has also been associated with the regenerative response of Müller cells, suggesting that HB-EGF might be acting as a protective cytokine in response to retinal degeneration, and may prevent ROS-induced damage in neural retinal cells. The observation that HO1 release is upregulated by TGF-β1 whilst the NQO1 activity is decreased by this cytokine, suggests that Müller glia may possess compensatory mechanisms to regulate ROS production in response to inflammatory stimulus, and this merits further investigations.

Zanetti, Lucia

Light induced ganglion cell responses in Cav1.4 mutant mouse retinas

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Purpose
To examine the ganglion cell (GC) activity of Cav1.4 I745T mouse retinas under both dim light (scotopic) and bright light (photopic) conditions

Methods
10-13 weeks old mice carrying the mutation Cav1.4 I745T in the Cacna1f gene and wild type (WT) were used for Multielectro array recordings. Mice were dark adapted for 2h, eyes were removed and retinas were dissected under red dim light and kept in oxygenated aCSF at room temperature. Retinas were then placed ganglion cell side down onto the perforated MEA chamber containg 120 electrodes, and continuously super-fused with 30°C oxygenated aCSF. Visual stimuli were generated through a DLP projector and ganglion cell responses were recorded. Photopic and scotopic light conditions were achieved with the use of different ND filters. Ganglion cell spikes were extracted and sorted into single units using a custom matlab script.

Results
IT retinas show impaired LFP upon frequency-modulated sinusoid stimulus under scotopic and photopic light conditions. IT GCs show a higher spontaneous firing rate in the absence of visual stimulation and delayed response upon full-field flash in both dim and bright light conditions. Moreover, only 25% of IT GCs responding in dim light responds also to bright light stimulation.

Conclusions
These preliminary data indicate that, although scotopic and photopic pathways show similarly impaired responses, in the IT CSNB2 model photopic responsiveness is more severely affected; similar to what is seen in electroretinograms of CSNB2 patients.

Statement on proprietary interests
none

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Zimmermann, Maxime

Chromatic processing in the zebrafish inner retina

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Purpose
The zebrafish retina comprises four distinct cone photoreceptor types, each expressing one of four opsins that can be categorised as Red-, Green-, Blue- and UV-sensitive. In the outer retina, different types of bipolar cells inherit differential chromatic response properties through selective or non-selective contacts with different cone types and horizontal cells. These signals are then sent to the Inner Plexiform Layer (IPL) to drive inner retinal circuits. Here, we surveyed the functional diversity of the zebrafish bipolar cell synaptic terminals across the IPL for chromatic processing.

Methods
To survey the chromatic response properties of bipolar cell terminals in the IPL, we used transgenic lines expressing GCaMP6 or iGluSnFR under the RibeyeA promoter [Dreosti et al. 2009]. Fluorescence responses to flashes of light and white noise stimuli delivered by a custom-built tetrachromatic stimulator emitting narrow spectra optimised for each cone type were recorded under 2-photon.

Results
We indiscriminately recorded from all bipolar cell terminals across the IPL at different positions in the eye (n=13 fishes, n=59 scans, n=4127 terminals). This revealed that the IPL is highly organised into chromatic and achromatic layers across both traditional ON and OFF sublaminae. Chromatic responses were found in at least a third of bipolar cell terminals, and included both spectral preferences as well as full chromatic antagonism. Furthermore, depending on their relative position in the eye, bipolar cell terminals shift their chromatic and achromatic response properties to match natural scenes (red ground, green horizon, blue and UV sky, etc.). A chromatic map of the eye was then constructed highlighting amongst others, a clear preference to ultra-violet light in the ventral region (fish looking upwards). Further analysis of this map reveals that where chromatic diversity is the most important, the functional distribution of different polarity responses in the IPL is altered, with the ON sublamina taking over 80% of the IPL depth compared to the usual ~60% in all other regions.

Conclusions
The chromatic signals collected by the photoreceptors are refined at the bipolar cells level before being further distributed and processed in the inner retina. Here, we demonstrated that the IPL sublaminae are highly specialised into chromatic and achromatic layers with a defined topographic sensitivity and specificity. Moreover, we show that this organisation is far to be uniform across the entire IPL; this functional diversity of the bipolar cells terminals and their relative position the eye likely serve specific behavioural purposes.

Statement on proprietary interests
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