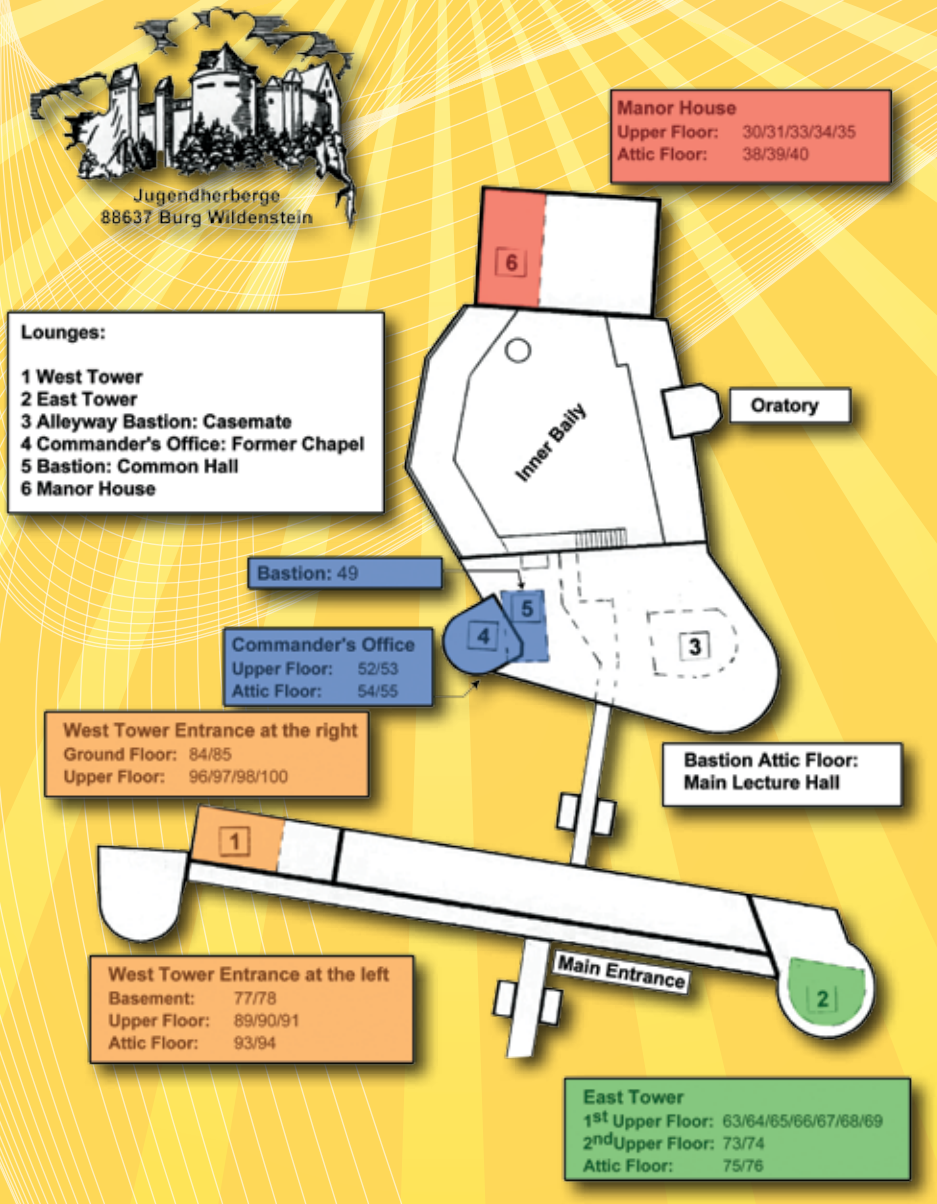
Young Researcher Vision Camp

An international Career building Symposium

Castle Wildenstein
Leibertingen
Germany

www.vision-camp.eu



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Preamble

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

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

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

ENJOY THE YOUNG RESEARCHER VISION CAMP

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

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

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

Thomas Wheeler-Schilling

on behalf of the organising committee (in alphabetical order)

Sigrid Diether
Philip Hunger
Norbert Kinkl
Francois Paquet-Durand
Lars Scheel
Timm Schubert

FRIDAY, JUNE 21TH, 2013

until 16:30	Arrival
18:00 - 19:00	**KEYNOTE LECTURE I** **"Photoreceptor Diversity Across Mammals: Adaptations to Lifestyle and Habitat?"** Leo Peichl Max-Planck-Institut for Brain Research, Frankfurt, Germany
19:30 - open end	Open-air Barbecue (campfire outside the castle)

SATURDAY, JUNE 22TH, 2013

06:00 - 07:00	Early morning exercises
07:00	Breakfast
08:00 - 09:00	**SESSION I: 'OPTICAL AND ADAPTATIONAL LIMITS OF VISION'** CHAIR: Juan Tabernero, Universidad de Murcia, Laboratorio de Óptica, Centro de investigación en Óptica y Nanofísica (CiOyN), Murcia, Spain • **"Adaptation to Simultaneuos Vision"** Aiswaryah Radhakrishnan (Madrid, Spain) • **"The Dark Side of the Eye"** Dimitrios Christaras (Murcia, Spain) • **"Adaptation in Peripheral Vision"** Abinaya Priya Venkataraman (Stockholm, Sweden) • **"Chromatic Effects on the Optics of the Eye"** Yun Chen (Tuebingen, Germany) OpAL is an Initial Training Network funded by the European Commission under the Seventh Framework Programme (PITN-GA-2010-264605)
09:00 - 10:00	**SESSION II: 'NEURON-GLIA INTERACTIONS IN VISION'** CHAIR: Antje Grosche, Paul Flechsig Institut for Brain Research University of Leipzig, Germany • **"Gliotransmitter release from retinal Müller glial cells"** Lysann Wagner (Leipzig, Germany) • **"Norrin mediates neuroprotective effects on retinal ganglion cells via the induction of leukemia inhibitory factor"** Stefanie Leopold, (Regensburg, Germany) • **"GDNF induces secretion of Cyr61 from retinal Müller cells – a novel neuroprotective factor in retinal degeneration"** Joanna Kucharska (Munich, Germany) • **"Stimulation and Analysis of Müller Glia and their Progeny"** Patrick Schäfer (Dresden, Germany) eduGLIA is an Initial Training Network funded by the European Commission under the Seventh Framework Programme ((PITN-GA-2009-237956)
10:00 - 10:30	Coffee Break

10:30 - 11:30	**SESSION III 'IDENTIFICATION AND CHARACTERIZATION OF GENES INVOLVED IN RETINAL DYSTROPHY'** CHAIR: Rob Collin, UMC St Radboud, Human Genetics, Nijmegen, The Netherlands • **"Genetic causes of retinal dystrophy in Indonesia"** Galuh D. Astuti, (Nijmegen, The Netherlands) • **"Identification of novel homozygous deletions in consanguineous pedigrees as a shortcut to candidate gene discovery in retinal dystrophies"** Kristof van Schil (Ghent, Belgium) • **"The farnesylated small GTPase RAB28 is mutated in autosomal recessive cone-rod dystrophy"** Susanne Roosing (Nijmegen, The Netherlands) • **"Crumbs homologues 1 and 2 restrict retinal progenitor cell proliferation and retina overgrowth"** Henrique Alves (Amsterdam, The Netherlands)
11:30 - 12:30	**SESSION IV 'INHERITED RETINAL DEGENERATIONS – CELL DEATH MECHANISMS AND PERSPECTIVES FOR THERAPIES'** CHAIR: Francois Paquet-Durand, Department for Ophthalmology,Tuebingen, Germany • **"Calcium dynamics in dying cone photoreceptors"** Manoj Kulkarni (Tübingen, Germany) • **"Retinal biomechanics in health and disease"** Linnéa Taylor (Lund, Sweden) • **"HDAC inhibition restores visual function in blind dying on edge (dye) mutants"** Lisa Shine (Dublin, Ireland) • **"Ethanol exposure induces cell death by oxidative stress in human RPE in vitro model"** Luis Bonet-Ponce (Valencia, Spain) DRUGSFORD is a Collaborative Project funded by the European Commission under the Seventh Framework Programme (HEALTH-F2-2012-304963)
12:30 - 14:00	Lunch
14:00 - 15:00	**SESSION V: 'TRANSLATIONAL RESEARCH IN OPHTHALMOLOGY'** CHAIR: Dominik Fischer, Nuffield Laboratory of Ophthalmology, University of Oxford, UK • **"Using extremes to identify rare pathogenic variants in age-related-macular degeneration"** Constantin Paun (Nijmegen, Netherlands) • **"Translating Induced Pluripotent Stem Cells from Bench to Bedside: Application to Retinal Diseases "** Alona O. Cramer (Oxford, United Kingdom) • **"Chimeric human opsins as potential optogenetic tools for vision"** Doron Hickey (Oxford, UK) • **"Fundus-controlled two-color adaptometry with the Microperimeter MP1"** Wadim Bowl (Gießen, Germany)

15:10 - 15:40	**EDUCATIONAL SESSION I** **Academic research versus industrial innovation: friends or foes?** Siegfried Wahl, Carl Zeiss AG, Germany
15:50 - 16:20	**EDUCATIONAL SESSION II** **The Balancing Act between Academia and Industry** Jan Kremers, University Erlangen-Nuernberg, Germany
16:20 - 16:50	Coffee Break
16:50 - 17:20	**EDUCATIONAL SESSION III** **Starting a Business** Jens Grosche, Effigos AG, Leipzig, Germany
17:30 - 18:00	**EDUCATIONAL SESSION IV** **Insights to Research Activities driven by the Industry** Markus Tiemann, Rodenstock GmbH, Munich, Germany
18:15 - 19:00	**KEYNOTE LECTURE II** **'Functional Assessment & Breadth of Application of Photoreceptor Transplantation Therapy'** Amanda Barber University of East Anglia, Norwich, UK
19:00 - 19:10	**Group Photo**
19:15 - open end	**Poster Session**
20:30 - open end	Buffet in the inner bailey

SUNDAY, JUNE 23TH, 2013

07:00 - 08:00	Early morning exercises
08:00	Breakfast
09:00 - 10:00	**SESSION VI 'RETINAL CIRCUITS/DATA PROCESSING'** CHAIR: Timm Schubert, Centre for Integrative Neuroscience, Tuebingen, Germany • "Probing visual receptive fields at single synapse resolution" Katrin Franke (Centre for Integrative Neuroscience, Tuebingen) • "Connexin interactions in the inner retina of the mouse" Arndt Meyer (University of Oldenburg, Germany) • "Retinal ganglion cell recordings in human retina" Katja Reinhard (Centre for Integrative Neuroscience, Tuebingen) • "Pericentrin in der Retina" Nathalie Falk (University of Erlangen, Germany)
10:00-11:00	**SESSION VII: 'PROTEIN TRANSPORT AND DYNAMICS IN PHOTORECEPTORS'** CHAIR: Karsten Boldt, Department for Ophthalmology, Tuebingen, Germany • "Determination of ciliary sub-modules by SDS-destabilization and correlation profiling" Yves Texier (Eberhard Karls University Tübingen, Germany) • "Ciliary transport in photoreceptor cells: the role of Usher syndrome related protein networks" Nasrin Sorusch (Johannes Gutenberg University of Mainz, Germany) • "Dissection of functional modules associated with retinal ciliopathies by affinity proteomics" Minh Nguyen (Radboud University Nijmegen Medical Center, Nijmegen, The Netherlands) • "NINL and DZANK1 cooperate in assembling the cytoplasmic dynein 1 motor complex, a process essential for photoreceptor outer segment formation in zebrafish" Margo Dona (Radboud University Nijmegen Medical Center, Nijmegen, The Netherlands) DYNAMO is a research network funded by the German Federal Ministry of Education and Research
11:00 - 11:15	Announcement of the Vision Camp Poster Price Awardees
11:15 - 12:00	**SESSION VIII 'INVERTEBRATE VISION'** CHAIR: Emily Baird, Lund Vision Group, Lund University, Sweden • "The effect of light intensity on flight control in bumblebees" Therese Reber (Baird-Dacke Group, Lund Vision Group, Department of Biology, Lund, Sweden) • "Seeing in the dark: higher-order visual processing in moths" Anna Stöckl (Warrant Group, Lund Vision Group, Department of Biology, Lund, Sweden) • "Vision in box jellyfish" Ronald Petie (Lund Vision Group, Department of Biology, Lund, Sweden)
12:00 - 12:45	**SESSION IX 'REGENERATING VISION'** CHAIR: Dominic Eberle, Center for Regenerative Therapies Dresden, Germany • "Cellular Reprogramming - An approach to drive cells towards retinal cell fate" Anna Seifert (CRTD, Dresden, Germany) • "Modes of photoreceptor migration in transplant and development" Katherine Warre-Cornish (UCL, London, UK) • "Cone-like photoreceptor transplantation into the mouse retina" Tiago Ferreira (CRTD, Dresden, Germany)
13:00 - 14:30	FAREWELL Lunch (optional)

Neural adaptation to Simultaneous Bifocal Vision

Aiswaryah Radhakrishnan, Lucie Sawides, Carlos Dorronsoro, Susana Marcos
Instituto de Optica, Consejo Superio de Investigaciones Cientificas, Madrid, Espana

Purpose
Simultaneous bifocal and multifocal corrections produce overlapping sharp and out of focus retinal images. These corrections are conventionally implemented as contact lenses or intraocular lenses based on the assumption that eventually neural adaptation takes place that suppress the degrading effect of the defocused image. We studied neural adaptation in the perception of simulated pure simultaneous bifocal images.

Methods
Simultaneous images were simulated for a 5mm pupil by adding a focused image with a blurred image (generated by convolving PSF corresponding to a given amount of defocus/near addition). These images were presented through a custom-developed Adaptive Optics system to ensure the same retinal images across subjects. Best Perceived Focus (BPF) was obtained by a 2 Alternative Forced Choice procedure after adaptation to bifocal images (different amounts of defocus, and sharp/blur image proportions). Perception was evaluated using an image scoring method for pure defocus and simultaneous 50%Sharp+50% Blur images. 4 young, normal subjects with spherical ametropia

Results
Simultaneous vision images produced shifts in BPF proportional to the magnitude and proportion of defocus after brief period of adaptation. There was highly significant correlation (r=0.99, p)

Conclusions
Subjects are able to adapt to the degradation imposed by bifocal images, as demonstrated by a shift of the best perceived focus,and an improvement in the perceived quality of simultaneous vision images following a brief period of adaptation. For the tested simultaneous vision design (pure simultaneous vision bifocality) an addition of more than 1D to 1.5D provides acceptable quality of vision.

Acknowledgement
This work has been supported by Spanish Government grant FIS2011-264605 and European Research Council Grant RC AdG and the European Community's 7th Framework Programme through the Marie Curie Initial Training Network OpAL (PITN-GA-2010-264605)

The dark side of the eye

Dimitrios Christaras
University of Murcia, Spain

Purpose
The study of the absolute threshold of vision and vision in dim light.

One of the most impressive features of the human vision is its high dynamic range, which may reach 8 orders of magnitude after long time adaptation, or 5 orders of magnitude in real time. A very interesting topic is the lowest extreme of this dynamic range of vision, or as is called the absolute threshold of human vision, which is the minimum amount of light one can see. In this talk, an experimental setup for the detection of the absolute threshold is described and some results are shown.

In particular, we have tried to build a more sophisticated version of the setup built by Pirenne and his group almost70 years ago, which would give us full control of the light pulse's intensity, wavelength and duration. Additionally, a Badal system and a cylindrical lens was incorporate to the system in order to compensate for the low order aberrations, namely the defocus and the astigmatism, in the periphery. The low order aberrations in the periphery were measured using a state of the art apparatus developed by our Laboratory. The effect of aberrations in the absolute threshold was also studied.

In the last part of the talk, the idea of using the eye as a quantum sensor, i.e. as a detector to study quantum correlations, is briefly explained. Based on the previous work of Nicolas Gisin and his team, the subject of the eye as a detector of quantum entanglement is tackled in a rather intuitive way, attempting to give a qualitative introduction to quantum optics.

Effects of Blur Adaptation in Peripheral Vision

Abinaya Priya Venkataraman, Linda Lundström, Peter Unsbo
Department of Applied Physics, Biomedical and X-Ray Physics, Royal Institute of Technology, Stockholm, Sweden

Purpose
The effect of blur adaptation on letter and grating acuity for foveal vision and its differences between the refractive groups have been reported already. However, what happens in the peripheral vision following blur adaptation is still unknown. This is a point of concern, as defocus in the periphery is known to influence the growth of the eye and thereby the development of myopia. In the current study, we investigated the effects of foveal blur adaptation on low contrast grating resolution in the periphery.

Methods
The foveal blur adaptation was given with +2.00D blur for the right eye, while the subjects were watching a movie for 30 minutes. 10% contrast grating resolution acuity was measured for clear and blurred (+2.00 D) condition in fovea, 10 degree in the nasal and temporal visual field in the testing eye and in the fovea of the fellow eye. The measurements were done pre and post adaptation and the difference were noted as blur adaptational effects.

Results
After adaptation to blur, all four subjects showed improvements in foveal low contrast grating resolution under blurred condition and a decrease under clear viewing condition in the testing eye. There were no corresponding changes in 10 degree nasal and temporal visual field in the testing eye nor in the fovea of the fellow eye.

Conclusions
Foveal blur adaptational effects did not spread to the periphery or to the other eye. The decrease in the foveal vision under clear condition following blur adaptation could be a result of changes in the sensitivity for certain spatial frequencies. The effect of adaptation for peripheral blur will be investigated in future.

Acknowledgement
This project has received funding from the Marie Curie Research Training Network "OpAL". OpAL (PITN-GA-2010-264605) is an Initial Training Network (ITN) funded by the European Commission under the Seventh Framework Programme (FP7)

Crystalline lens thickness determines transverse chromatic aberration

Yun Chen and Frank Schaeffel
Section of Neurobiology of the Eye, Ophthalmic Research Institute, Tuebingen, Germany

Purpose
To describe transverse chromatic aberration (TCA) in the human eye, its variability, and the optical parameters that might determine its magnitude.

Methods
Two psychophysical procedures were used to quantify TCA. First, a red and a blue square, presented on a black screen, was matched in size by the subjects with their right eyes, using customized software. Second, a filled red and blue square, flickering on top of each other at 2 Hz had to be adjusted in brightness and size to minimize flicker. Biometric ocular parameters were measured with a commercially available low coherence interferometer, the Lenstar LS 900 (Haag Streit, Switzerland). Lens tilt and decentration was measured with a custom-built Purkinjemeter. Corneal power, thickness, anterior chamber depth, lens thickness, vitreous chamber depth and axial length were correlated to the psychophysically measured TCA. Twenty-one subjects with no ocular disorders, other than refractive errors, with ages ranging from 22 to 58 years were measured.

Results
In line with published data, TCA varied widely among subjects since the perceived differences between the red and the blue squares ranged from 0.0% to 3.6%. The two different psychophysical procedures to measure TCA provided highly correlated results, suggesting that no major confounders existed. Multivariate regressions to optical parameters in the subjects' eyes showed that only lens thickness was significantly correlated to TCA (p

Conclusions
The high inter-individual variability in TCA is largely determined by lens thickness. Since age is a major factor determining lens thickness, TCA increases inevitably with age.

Acknowledgement
Supported by OpAL, an Initial Training Network funded by the European Commission under the Seventh Framework Programme (PITN-GA-2010-264605)

Gliotransmitter release from retinal Müller glial cells

Lysann Wagner (1), Philip G. Haydon (2), A. Reichenbach (1), A Grosche (1)

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(2) Department of Neuroscience, University of Pennsylvania, Philadelphia, PA 19104, USA

Müller cells, the principal glial cells of the retina, are crucial for the homeostatic and metabolic support of retinal neurons. Furthermore, Müller cells are supposed to modulate neuronal function by glutamatergic and purinergic signalling. A prerequisite for such kind of glia-neuron interaction is a tightly regulated, fast release of transmitters from both neurons and glia cells.

Previously, we examined how transmitter release is involved in the volume regulatory signalling cascade of retinal Müller glial cells. Growth factor- stimulated release of glutamate, activation of metabotropic glutamate receptors and subsequent release of adenosine-5'-triphosphate (ATP) are key steps of this pathway.

Although the action of these transmitters on Müller cells is well described, little is known about release mechanisms. These experiments provide first evidence that Müller cells are capable to release glutamate via regulated exocytosis, while ATP is set free via alternative pathways. The most probable one appears to be the opening of connexin hemi-channels. To further characterize the glutamate release from Müller cells we used a fluorometric enzyme assay based on the Amplex® Red Glutamic Acid Kit to visualize glutamate release from acutely isolated murine Müller cells. In combination with a transgenic mouse line that expresses a dominat-negative SNARE (dnSNARE) protein to block the vesicular release of transmitters we demonstrate glutamate release via exocytosis and at least one alternative calcium-dependent pathway.

Abstract
Saturday, June 22th, 2013

Norrin mediates neuroprotective effects on retinal ganglion cells via the induction of leukemia inhibitory factor

Stephanie Leopold, Roswitha Seitz, Ernst R. Tamm, Andreas Ohlmann

Institute of Human Anatomy and Embryology, University of Regensburg, Regensburg, Germany.

Purpose
To investigate if and how leukemia inhibitory factor (LIF) is involved in the neuroprotective effects of Norrin on retinal ganglion cell (RGC) survival following excitotoxic damage. Norrin is a secreted protein that activates the classical Wnt/β-catenin pathway via specific binding to Frizzled-4. In albino mice, Norrin protects RGC from excitotoxic damage and increases the retinal expression LIF and endothelin-2 (EDN2), as well as that of neuroprotective factors such as fibroblast growth factor-2 (FGF2) and ciliary neurotrophic factor (CNTF).

Methods
Recombinant human Norrin was isolated and purified from conditioned cell culture medium of HEK 293-EBNA cells. To induce excitotoxic RGC death, 3 µl NMDA [10 mM] were injected into the vitreous body of both hetero- and homozygous LIF-deficient mice in a C57/Black6 genetic background. The fellow eye received 3 µl of combined NMDA [10 mM] and Norrin [5 ng/µl]. To determine the degree of RGC damage, TUNEL labeling was performed on meridional sections 24 h after injection, and the number of labeled nuclei was quantified. The expression of mRNA for Lif, Edn2, and Fgf2 was investigated by quantitative real-time RT-PCR of treated retinae.

Results
After injection of wild-type mice with combined NMDA/Norrin approximately 50% less TUNEL positive cells were observed in the RGC layer when compared to that of NMDA-treated littermates. The protective effect of Norrin was completely lost when homozygous LIF-deficient mice were treated with combined NMDA/Norrin. In addition, in LIF-deficient mice, NMDA induced a substantial increase in excitotoxic damage as 50% more apoptotic cells in the RGC layer were observed when compared to NMDA-injected wild-type littermates. By real-time RT-PCR for the expression of Lif and Edn2 mRNA in retinae of NMDA/Norrin-treated eyes, a significant increase was observed in wild-type mice when compared to eyes that received NMDA only. The Norrin-mediated effect was substantially reduced in heterozygous LIF-deficient mice. In contrast, only moderate changes were observed in the expression of Fgf2.

Conclusions
Norrin mediates its neuroprotective properties on retinal ganglion cells via an increased expression of Lif. In pigmented mice, the induction of LIF might involve an increased expression of Edn2 while Fgf2 plays no or only a minor role.

Acknowledgement
Supported by DFG Research Unit (Forschergruppe) FOR 1075

Cyr61 activates retinal cells and prolongs photoreceptor survival in retinitis pigmentosa mouse model

Joanna Kucharska (1,2), Patricia del Río (1), Blanca Arango-Gonzalez (2), Matteo Gorza (3), Annette Feuchtinger (4), Stefanie M. Hauck (1) and Marius Ueffing (1,2)

(1) Research Unit of Protein Science, Helmholtz Zentrum München, Germany, (2) Experimental Ophthalmology, Institute for Ophthalmic Research, University Tübingen, Germany, (3) Institute Human Genetics, Helmholtz Zentrum München, Germany, (4) Research Unit Analytical Pathology, Helmholtz Zentrum München, Germany

Purpose
Retinitis pigmentosa (RP) is hallmarked by progressive loss of vision caused by mutations affecting photoreceptors (PR) or retinal pigment epithelium (RPE). Subretinal injections with glial cell line-derived neurotrophic factor (GDNF) stimulate retinal Müller glial cells (RMG) which release factors rescuing morphology and function of rod cells in RP animal models. The aim of this study was to analyse changes in RMG protein secretion after GDNF stimulation and validation of novel factors with potential neuroprotective activity.

Methods
Protein expression changes in supernatant and lysate from GDNF stimulated mouse RMG cells were analysed using Proteome profile arrays. Following, Pde6brd1 (rd1) mice retinal explants were cultured with Cyr61, a factor chosen for further analysis, and PR survival rate was calculated based on TUNEL staining. Cyr61-induced signaling in the retina or isolated retinal cell types (primary photoreceptor cultures, MIO-M1 and ARPE19 cell lines) was monitored by Western blot technique.

Results
GDNF treatment of primary mouse RMG induced significant increase of Cyr61 an extracellular matrix protein with broadly documented prosurvival activity. Cyr61 significantly decreased the number of TUNEL positive cells in short and long term retinal explants from rd1 mice. Additionally, after 12 days in culture, retinal explants treated with Cyr61 showed a significantly higher amount of preserved PR in outer nuclear layer. In retinal explants tissue treated with Cyr61 we could observe time-dependent increase in phosphorylation of Erk1/2 and Stat3 signaling proteins. Phosphorylation of Erk1/2 as well as Akt was also induced by Cyr61 in human RMG and RPE cell lines (MIO-M1 and ARPE19, respectively) which indicated responsiveness of these cells. However, we couldn't observe changes in activation in any of the studied signaling pathways in isolated primary porcine photoreceptors.

Conclusions
Cyr61 prolonged PR survival ex vivo in retinal explants of rd1 mouse model of retinitis pigmentosa, most probably indirectly, through RMG and RPE cell stimulation. This qualifies it as a candidate for future neuroprotective therapeutic approaches in neurodegenerative retinal disorders.

Acknowledgement
This work was supported by the European Community's 7th Framework Program through Marie Curie Initial Training Network Edu-GLIA (PITN-GA-2009-237956) and the German Federal Ministry of Education and Research (HOPE - FKZ 01GM0852)

Abstract
Saturday, June 22th, 2013

Stimulation of Müller Glia Proliferation and Progeny Generation in the Mouse Retina

Patrick Schäfer, Konstantinos Anastissiadis, Mike O. Karl

(1) German Center for NeurodegenerativeDiseases (DZNE), Dresden, Germany, (2) Center for Regenerative Therapies Dresden (CRTD)

Purpose
In the human and mouse retina Müller glia (MG) are well known to undergo gliosis in all major types of retinal diseases – which sometimes may even lead to scar formation due to proliferative gliosis. Some studies suggest that in the mouse retina MG derived neuronal regeneration can be stimulated, but only to a very limited extent. Here, we started to find out, if conditional immortalization might stimulate MG derived proliferative gliosis and /or neuronal regeneration.

Methods
Juvenile retina were explanted at day postnatal 10 (P10), plated on membrane inserts in 6-well plates and cultured ex vivo with the mitogen EGF. Expression of the SV40 large T-antigen (cSV40LT) was induced for either 6 or 10 days by application of dexamethasone and doxycycline. EdU was applied for pulse chase 4h before fixation.

Results
Others and we reported that in the juvenile mouse retina, after retinogenesis is finished, some Müller glia shift from a quiescent differentiated state into a proliferative state upon damage of retinal explants ex vivo. We now observed that this process is differentially inducible by mitogens like EGF (epidermal growth factor). EdU (S-phase marker) pulse chase experiments revealed a tremendous increase in MG proliferation within the first two days, a peak of EdU positive cells at day 4 (14 +-2 SEM, N=4) and a massive decrease until day 6 (4 +-0.4 SEM, N=4) per 400µm of a central retina section.

Next, we used transgenic mice with tightly and temporally controlled expression of the proto-oncogene SV40 large T-antigen (cSV40LT). It is well reported that cSV40LT binds several proteins including the tumor suppressor p53 and retinoblastoma and bypasses cell cycle checkpoints. Induction of the cSV40LT for 6 days ex vivo led to an overall increase in proliferation compared to control. The number of EdU+ cells was 8.5 fold increased (cSV40LT: 23 +-6 SEM, N=4; control: 3 +-0.16 SEM, N=4; p

Conclusions
Our results so far suggest that induction of cSV40LT not only overcomes the proliferative restriction of Müller glia but also maintains its progeny in the cell cycle over extended period of time. Surprisingly, major parts of the generated cell progeny formed gliotic cell clusters, which were all located within the boundaries of the retina. Further, we present data of successful Müller glia isolation at high purity by FACSorting. In our current and future work we study the Müller glia and its derived progeny to find out the underlying mechanisms that enable neuronal regeneration and prevent gliotic scar formation.

Acknowledgement
PhD fellowship funded by the Herbert-Funke-Foundation, DFG CRTD, DZNE & TUD grants to mokalab.

Genetic causes of retinal dystrophy in Indonesia

Galuh D.N. Astuti (1,2,3), Anna M. Siemiatkowska (1,2), Kentar Arimadyo (3,4), Anneke I. den Hollander (1,5), Sultana M.H. Faradz (3), Frans P.M. Cremers (1,2), Rob W.J. Collin (1,2)

(1) Department of Human Genetics, Radboud University Medical Centre, Nijmegen, The Netherlands; (2) Nijmegen Centre for Molecular Life Sciences, Radboud University Medical Centre, Nijmegen, The Netherlands; (3) Division of Human Genetics, Center for Biomedical Research, Faculty of Medicine, Diponegoro University, Semarang, Indonesia; (4) Department of Ophthalmology, Faculty of Medicine, Diponegoro University/Dr. Kariadi Hospital, Semarang, Indonesia; (5) Department of Ophthalmology, Radboud University Medical Centre, Nijmegen, The Netherlands

Purpose
Retinitis pigmentosa (RP) consists of a clinically and genetically diverse group of inherited retinal dystrophies (IRDs). Despite the huge increase in knowledge about genes involved in RP and emerging therapeutic approaches, information regarding its underlying genetic defect in the Indonesian population is very limited. Our aim in this study is to describe the genetic defects responsible for non syndromic RP in Indonesia.

Methods
Familial and single RP cases were enrolled in this study. Blood samples of 42 families segregating RP (68 affected and 63 unaffected individuals) were obtained. Homozygosity mapping was conducted using Illumina 6k, Affymetrix 5.0 or 700k single nucleotide polymorphism (SNP) array analysis. Sanger sequencing was performed for genes known to be associated with autosomal recessive RP that resided in conspicuous homozygous regions and for X-linked RP genes in case X-linked inheritance was suspected. Frequency analysis was performed using DNAs from 149 ethnically matched healthy controls.

Results
Pathogenic variants were identified in three consanguineous and six non consanguineous families. Sequence analysis revealed eight novel homozygous mutations segregating in the respective families, in the following genes: ATP-binding cassette, sub-family A, member 4 (ABCA4), crumbs homolog 1 (CRB1), eyes shut homolog (Drosophila) (EYS), c-mer proto-oncogene tyrosine kinase (MERTK), nuclear receptor subfamily 2, group E, member 3 (NR2E3), phosphodiesterase 6A, cGMP-specific, rod, alpha (PDE6A) and retinitis pigmentosa 1 (RP1). Interestingly, the mutation in RP1 was found in a homozygous state in the last exon of this gene, while previously reported RP1 mutations are mostly inherited in an autosomal dominant manner.

Conclusions
In this study we were able to identify underlying genetic defects of RP in Indonesia by employing homozygosity mapping, both in consanguineous and non consanguineous families. Results of this study has provided information for genetic counseling, enables further patient management and, in selected cases, may enable the inclusion of patients in future therapy trials.

Acknowledgement
The research of G.D.N.A. is supported by the Directorate General for Higher Education (DIKTI) of the Ministry for National Education of Indonesia

Identification of Novel Homozygous Deletions in Consanguineous Pedigrees as a Shortcut to Candidate Gene Discovery in Retinal Dystrophies

Van Schil, Kristof (1); Meire, Françoise (2); De Ravel, Thomy (3); Leroy, Bart P. (4); Verdin, Hannah (1); Coppieters, Frauke (1); De Baere, Elfride (1)

(1) Center for Medical Genetics, Ghent University Hospital, Ghent, Belgium. (2) Department of Ophthalmology, Huderf, Brussels, Belgium. (3) Center for Human Genetics, Leuven University Hospitals, Leuven, Belgium. (4) Center for Medical Genetics, Department of Ophthalmology, Ghent University Hospital, Ghent, Belgium.

Purpose
To identify the underlying genetic cause in 25 pre-screened consanguineous families diagnosed with autosomal recessive retinitis pigmentosa (ARRP) or Leber congenital amaurosis (LCA) using identity- by-descent (IBD) mapping. To demonstrate the power of mapping of homozygous deletions as a shortcut to candidate gene identification in retinal dystrophies (RDs).

Methods
IBD mapping was performed by genome-wide SNP chip analysis (HumanCytoSNP-12, Illumina). For IBD data analysis we integrated PLINK with arrayCGHbase, a platform for data analysis of microarray based comparative genome hybridization. Deletions were confirmed and fine-mapped by conventional PCR. Segregation analysis was performed by qPCR (LightCycler, Roche; qBase Plus, Biogazelle).

Results
Homozygous deletions were identified in 3 out of 25 families. The first deletion (133 kb) was found in an ARRP patient and removes the first non-coding exon of the known gene EYS. The second deletion (112 kb) was found in a patient with early-onset RD. It disrupts the last 3 exons of RERG and the lncRNA RERG-AS1. The third one (416 kb) is a partial deletion of GRID2, found in a patient with LCA. It leads to an in-frame deletion (p.Gly30_Glu81del). All of them were located in the one but largest IBD region and their presence could be confirmed. The deletions involving the EYS and GRID2 gene were absent in our local database containing more than 3000 subjects. The partial GRID2 deletion was found twice, in heterozygous state however.

Non-coding deletions of EYS have not yet been described. As to the RERG and GRID2 genes, both are regulated by the transcription factor CRX (Corbo et al., 2010). Although the function of RERG remains unclear, it was found in several retinal transcriptome datasets (Gamsiz et al., 2012 and Booij et al., 2009). The RERG deletion was found in a homozygous state in two unaffected siblings however, whose clinical status needs to be revisited. GRID2 encodes a neurotransmitter receptor that plays an important role in the brain, a homozygous mutation in mouse was found to be lethal (Zuo et al., 1997). The in-frame GRID2 deletion found here might represent a hypomorphic allele leading to a milder phenotype. Further in-depth analysis of siblings, characterization and functional validation of these deletions is underway.

Conclusions
This study revealed involvement of a homozygous 5'UTR deletion of EYS in ARRP, and uncovered potential novel candidate genes for RD. We demonstrated that homozygous deletion detection in consanguineous families might be a powerful approach for gene discovery of RDs.

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Mutations in RAB28, encoding a farnesylated small GTPase, are associated with autosomal recessive cone-rod dystrophy

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Purpose
The majority of genetic causes for autosomal recessive (ar) cone-rod dystrophy (CRD) are currently unknown. Therefore, we employed a combined approach of homozygosity mapping and exome sequencing to identify new genes for arCRD.

Methods
In a German arCRD family with three affected siblings, homozygosity mapping was performed using SNP microarrays. DNA samples of two affected individuals underwent exome sequencing. Sanger sequencing of the RAB28 gene was performed in 617 additional unrelated individuals with CRD or cone dystrophy, and in families with large homozygous regions. Identified mutations were screened in ethnically matched controls.

Results
Exome sequencing revealed a homozygous nonsense mutation in RAB28 (c.565C > T; p.Q189*) in all three affected individuals of the German arCRD family. In addition, a homozygous nonsense mutation (c.409C > T; p.R137*) was identified in two affected members of a consanguineous arCRD family of Moroccan Jewish ancestry. Both mutations were not identified in 176 and 118 ethnically matched controls, respectively. The five affected individuals of both families presented with hyperpigmentation in the macula, progressive loss of the visual acuity, atrophy of the retinal pigment epithelium, and severely reduced cone and rod responses on the electroretinogram.

Conclusions
RAB28 encodes a member of the Rab subfamily of the RAS-related small GTPases. Alternative RNA splicing yields three predicted protein isoforms with alternative C-termini, which are all truncated by the nonsense mutations identified in the arCRD families in this report. Opposed to other Rab GTPases which are generally geranylgeranylated, RAB28 is predicted to be farnesylated. Staining of rat retina showed localization of RAB28 to the basal body and the ciliary rootlet of the photoreceptors. Analogous to the function of other RAB family members, RAB28 might be involved in ciliary transport in photoreceptor cells. This study reveals a crucial role for RAB28 in photoreceptor function, and suggests that mutations in other Rab proteins may also be associated with retinal dystrophies.

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Crumbs homologues 1 and 2 restrict retinal progenitor cell proliferation and retina overgrowth

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Purpose
Loss of CRB1 protein function results in blindness such as Leber congenital amaurosis and progressive retinitis pigmentosa. Leber congenital amaurosis retinas lacking CRB1 are 1.5 times thicker than normal retina and lack the distinct lamination of normal adult retina. The abnormal retinal architecture suggested an immature lamination pattern of the human retina. Mice lacking CRB1 develop a relatively mild phenotype compared to the phenotype observed in patients. However, there is no clear genotype–phenotype correlation for CRB1 mutations in humans and mice, which suggests that other components of the CRB complex may influence the severity of retinal disease. We hypothesized that CRB2 might be an important player.

Methods
We generated and analyzed conditional knockout mice for Crb2 as well as Crb1Crb2 double mutant mice. Loss of retinal activity was measured by electroretinography and correlated with decreased levels of CRB proteins in the retina.

Results
Mice lacking CRB2 in the retina showed an increase in retinal neural progenitor cells at postnatal day 3. Surprisingly, there was an increase in number of late-born differentiated retinal cells (rod photoreceptors, Müller glia cells), concomitant with increased programmed cell death, suggesting retina overgrowth. Mice lacking CRB1 as well as CRB2 showed a thicker retina, as observed in human patients. These retinas lacked a separate photoreceptor layer, and, whereas all cell types were produced (including photoreceptors), a substantial number of each cell type was localized in the wrong layer. We detected an increase in retinal neural progenitor cells from embryonic day 15.5 till postnatal day 5, concomitant with increased programmed cell death. There was an increase in number of late-born differentiated retinal cells (rod photoreceptors, Müller glia cells, bipolar cells, late-born amacrine cells) suggesting overgrowth of the retina.

Conclusions
The data suggest that CRB1 and CRB2 control the retina size by preventing the overproliferation of progenitor cells during retinal development through regulation of P120-catenin-Kaiso signalling pathways.

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Calcium dynamics in degenerating cone photoreceptors

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Photoreceptor degeneration is a pathological state in which an inherited mutation leads to loss of photoreceptors and subsequently to blindness. Even though models of photoreceptor degeneration have been studied extensively and some players involved have been mapped, an exact pathway along which photoreceptor cell death proceeds has not been established to date. Previous studies demonstrated an accumulation of cGMP in outer segments of photoreceptors suggesting an elevation of intracellular Ca^{2+} via activation of CNG channels. Accordingly, a rise in Ca^{2+}dependent, calpain-type proteolytic activity has been detected, which indirectly indicates a contribution of Ca^{2+} to photoreceptor cell death. Similarly, rd1/CNG channel double mutant animals illustrated a temporal delay and overall decrease of photoreceptor deterioration also suggesting an involvement of Ca^{2+}. To test this, we are using retinal degeneration lines, rd1 and cpfl1, crossbred with TN-XL mice in which cones are labelled with a fluorescent ratio-metric Ca^{2+} biosensor (Wei et. al., 2012). The cpfl1 and rd1 mice carry analogous mutations in the phosphodiesterase 6 gene, in cone and rod photoreceptors, respectively. Consequently, the cpfl1 model is characterized by cone degeneration whereas rd1 retina shows primary rod degeneration followed by secondary cone degeneration.

Immunohistochemical results of cpfl1/TN-XL animals displayed co-localisation of cone photoreceptors with TUNEL (a cell death marker) as well as cGMP, concomitant with cone degeneration. rd1/TN-XL animals also exhibited co-localisation of TUNEL with cone photoreceptors after post natal day (PN) 20 indicating secondary cone degeneration. In contrast, at PN20 co-localisation of cGMP with cone photoreceptors was not detected in rd1/TN-XL animals. Analysis of wt/TN-XL animals showed a cone density comparable to wt-animals until PN24. In parallel, we established an approach to measure Ca^{2+} levels in cone pedicles, using two-photon microscopy, in ex-vivo retinal slices of TN-XL crossbred animals. Preliminary results of Ca^{2+} measurement in rd1/TN-XL crossbreds exhibited a steady decline in Ca^{2+} resting level with age. On the other hand, cpfl1/TN-XL animals revealed higher Ca^{2+} resting level than rd1/TN-XL animals of comparable age.

Altogether, our results demonstrate that TN-XL animals crossbred into degeneration lines can be effectively utilized to study Ca^{2+} dynamics in dying cones. Further studies will be focused on the measurement of Ca^{2+} at different time points to test whether alterations in Ca^{2+} dynamics take place before or after degeneration onset.

Retinal Biomechanics in health and disease

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The central nervous system (CNS), which includes the retina, resides in a highly mechanical milieu. The importance of biomechanical homeostasis for normal CNS function has not been extensively explored. Diseases in which normal mechanical forces are disrupted, such as retinal detachment or myopic degeneration of the eye, are highly debilitating and the mechanisms underlying disease progression are not fully understood.

Using an organotypic culture model of adult porcine retina to explore the effects of lateral tension on retinal tissue, we have obtained results confirming that biomechanical tension is a vital factor in the maintenance of retinal tissue integrity, and suggest that mechanical cues are important components of pathological responses within the CNS.

HDAC inhibition restores visual function in blind dying on edge (dye) mutants.

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We have identified and characterised a novel, blind zebrafish mutant with a mutation in the gene atp6v0e1, which encodes subunit V0e of the vacuolar ATPase (v-ATPase) proton pump. These mutants display a reduced or absent OKR and a reduced ERG b-wave. A striking phenotype is the predominant apoptosis which is largely restricted to the ciliary marginal zone and the brain. The retinal pigment epithelium (RPE) is also hypopigmented and photoreceptors are shorter and disheveled in appearance.

Our data shows that atp6v0e1 is expressed in the dorsal and ventral retinal pigment epithelium of 3 and 5 dpf zebrafish larval eyes and we also demonstrate that the V0e subunit is required for the proton pumping function of the v-ATPase complex. Notably, we can restore vision and improve retinal histology in dye mutants by treating with the HDAC inhibitors trichostatin A or scriptaid.

Ethanol exposure induces cell death by oxidative stress in a human RPE in vitro model

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Ethanol exerts deleterious effects involving free radical production and lipid peroxidation, potentially leading to an imbalance between oxidants and antioxidants, resulting in an increased oxidative stress.

Our group demonstrated that chronic ethanol consumption induces toxicity by increasing oxidative stress in rat retina associated with an impairment of ERG and Bcl-2 overexpression. These alterations proposed the existence of a potential “alcoholic retinopathy”. Hence, we tested the effect of alcohol exposure to a specific retinal cell type, a human Retinal Pigment Epithelium cell line (ARPE-19).

Cells were plated at confluence and then exposed to 3 different ethanol concentrations (250 mM, 500 mM and 750 mM). Using MTT and Alamar blue assays, we found out that cell viability was affected by the 3 different concentrations. We checked these results either by flow citometry and fluorescent microscopy. Furthermore, different cell death and oxidative stress markers were overexpressed.

Thus, our results indicate that alcohol is metabolized by ARPE-19 cells, leading to an increase of oxidative stress markers and an impairment of cell viability.

Using extremes to identify rare pathogenic variants in age-related-macular degeneration

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Purpose
Age-related macular degeneration (AMD) has a strong genetic component, and it has been estimated that 50% of the heritability of AMD is explained by common variants in the complement system. Despite the progress that has been made in identifying common variants in AMD, few studies have assessed the role of rare variants in the pathogenesis of AMD. The purpose of this study was to identify rare variants in AMD patients with high levels of complement activation.

Methods
Complement component C3 and the activation fragment C3d were measured in serum samples of 197 AMD patients and 150 unaffected age-matched controls. The C3d/C3 ratio was calculated as an indicator of C3 activation. DNA samples of patients with high C3d/C3 ratios (>3), were screened for mutations in the CFH gene by Sanger sequencing. DNA samples of 8 patients with the highest C3d/C3 ratios (> 4) were analyzed by exome sequencing.

Results
AMD patients displayed an increased median C3d/C3 ratio compared to controls (p3 were observed in 37 AMD patients, but only in 4 control individuals. Sequence analysis of the CFH gene revealed a heterozygous frameshift mutation (c.1769_1779del; p.Gly590fsX) in a patient with a C3d/C3 ratio of 4.4. Exome sequencing in 8 patients with C3d/C3 ratios >4 revealed rare variants in 5 patients in key components of the complement pathway.

Conclusions
High levels of complement activation in AMD patients can be caused by rare variants in components of the complement pathway, which have previously not been associated with AMD. High complement activation may therefore direct a genetic search toward complement gene abnormalities. This genotype-serotype correlation approach is able to identify new risk factors in AMD.

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Abstract
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Translating Induced Pluripotent Stem Cells from Bench to Bedside: Application to Retinal Diseases

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Induced pluripotent stem cells (iPSc) are a scientific and medical frontier. Application of reprogrammed somatic cells for clinical trials is in its dawn period; advances in research with animal and human iPSc are paving the way for retinal therapies with the ongoing development of safe animal cell transplantation studies and characterization of patient-specific and disease-specific human iPSc. The retina is an optimal model for investigation of neural regeneration; amongst other advantages, it is the most accessible part of the central nervous system (CNS) for surgery and outcome monitoring. A recent clinical trial showing a degree of visual restoration via a subretinal electronic prosthesis implies that even a severely degenerate human retina may have the capacity for repair after cell replacement through potential plasticity of the visual system. Successful differentiation of neural retina from mouse and human stem cells in-vitro increase the feasibility of generating an expandable and clinically suitable source of cells for clinical trials. Challenges in utilizing iPSc derived tissue for visual repair in humans will be discussed, and a murine model employing transplantation of genetically modified photoreceptor precursors will be presented as a potential step towards application of cell replacement therapies for Retinitis Pigmentosa (RP).

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Chimeric human opsins as potential optogenetic tools for vision

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Purpose
In the preceding decade optogenetics has become a powerful biological tool, enabling researchers to probe the function of specific cells by using light to induce controlled changes in cell physiology. The application of this technology to the degenerate retina holds significant promise. Our research utilises novel human opsins as potential optogenetic tools for restoring sight.

Methods
Opsins are light-sensitive G-protein coupled receptor proteins that modify cellular function by activating G-protein second messenger systems. Rod and cone opsins activate Gαt, while melanopsin (OPN4), activates Gαq/11. The Gαq/11 pathway is ubiquitous in neurons, while the Gαt is exclusive to photoreceptors. If bipolar cells were modified to become light sensitive, these cells could act as substitute photoreceptors in patients who have lost their photoreceptors. Chimeric opsins were created as potential candidates for gene therapy targeting bipolar cells in degenerate retinae.

Results
Eight visual opsin/melanopsin chimeras were cloned: four based on rhodopsin (RHO) and four based on long wavelength sensitive (LWS) opsin. Immunohistochemistry was performed on transfected HEK293T cells. Calcium kinetics were imaged using Rhod-2 fluorescent dye. Wild type and chimeric opsins were purified and underwent UV-visible spectroscopy.

Immunohistochemistry showed chimeric opsins to be correctly transported to the plasma membrane. Four of the eight chimeras induced increases in intracellular calcium in response to light, suggesting activation of the Gαq/11 pathway. Spectroscopy of wild type LWS opsin produced a λmax of 558 nm. However, spectroscopy of chimeric opsins has hitherto been unsuccessful.

Conclusions
Chimeric opsins based on RHO or LWS with OPN4 are transported to the plasma membrane, where some of them function to produce light-induced increases in intracellular calcium. Such responses suggest that changing the intracellular surface of a Gαt signalling human opsin to that from a Gαq/11 signalling human opsin, can change the signalling pathway. Such a novel opsin, could be utilised as gene therapy to patients who have lost photoreceptors.

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Fundus-controlled two-color adaptometry with the Microperimeter MP1

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Purpose
We developed a two-color fundus-controlled adaptometry with the commercially available microperimeter MP1 (Nidek, Padova, Italy).It provides a quick and easy examination of rod and cone function during dark-adaptation at defined retinal loci.

Methods
Our modification includes external filters that affect background, stimulus intensity and spectral composition of the stimulus in the MP1. After a standardized bleach, a pattern covering 3 blue spots at 12° nasal and 3 red spots at the centre is presented in a dark room. The test sequence is repeated every 2 minutes until 45 minutes of dark-adaptation. Under mesopic conditions, light increment sensitivity (LIS) for blue stimuli is determined by the rod pathway. LIS for red stimuli is mediated by the cone pathway.

Results
LIS for the red stimuli showed a slight increase during the first 5 min and remained thereafter unchanged at about 7 dB. LIS for the blue stimuli increased considerably during the first 10 min and then reached a plateau at 19 dB. These results are in accordance with published dark adaptation curves.

Conclusions
Two-color fundus-controlled adaptometry with a commercial MP1 without internal changes to the device provides a quick and easy examination of rod and cone function during dark adaptation at defined retinal loci. Under the mesopic lighting conditions chosen, LIS for blue stimuli is mediated by the rod pathway, and for red stimuli by the cone pathway. This innovative method will be helpful to measure rod vs. cone function at known loci of the posterior pole in early stages of retinal degenerations.

Probing visual receptive fields at single synapse resolution

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Purpose
The retina is a powerful image processor, that sequentially decomposes spatio-temporal photoreceptor activation patterns into increasingly specific parallel channels. As a result, only a small fraction of all available visual information is sent to the brain by retinal ganglion cells. Key to the restriction of retinal neuron response features to highly specific visual patterns are synaptic interactions in the retina's two synaptic layers, the outer and inner plexiform layer (OPL and IPL). Here, individual synaptic connections are subject to powerful modulation by lateral inhibitory interneurons, the horizontal cells in the OPL and the amacrine cells in the IPL. As a result individual synapses may exhibit specific spatio-temporal tunings that differ both pre- and post-synaptically even for neighboring synapses belonging to the same cell, as previously demonstrated in select types of amacrine cells (e.g. Euler et al., 2002; Grimes et al., 2010).

Methods
Based on earlier work (Briggman & Euler, 2011), we have developed a technique to sparsely label individual retinal neurons with synthetic calcium indicators in the ex-vivo intact retina, preserving full connectivity. Using two-photon imaging we now probe the visual response properties at the single synapse level both within bipolar cell presynaptic terminals and retinal ganglion cell (RGC) dendrites. This allows to gain detailed insight into how visual signals are processed and integrated within RGCs to ultimately yield the specific response profiles observed at the level of the RGC spike output that is relayed to the brain. We study how the spatio-temporal receptive fields of single synapses differ along the length of RGC dendrites, in particular with respect to the impact of dendritic spikes and backpropagating somatic spikes on dendritic integration in different types of RGCs.

Results
Using spatial dense-noise stimuli, our preliminary data confirmed that receptive fields of single synapses can be reliably estimated using our imaging approach both at the level of bipolar cell terminals and RGC dendrites. Consistent with the morphology of the RGC dendritic arbor, synapses and dendritic segments closer to the soma systematically exhibit larger receptive fields. Moreover, different dendritic sectors of the same RGC selectively extract visual information presented in different retinal positions. Conversely, within the spatial resolution of our experimental approach, individual presynaptic terminals belonging to the same bipolar cell exhibit highly overlapping spatial receptive fields, but often differ in their temporal tuning properties.

Conclusions
In conclusion, spatio-temporal receptive fields can be estimated at the single synapse level in the intact retinal network using two-photon calcium imaging. This technique will allow the study of synaptic integration in the inner retina in great detail, yielding to a refined understanding of how complex trigger features observed at the level of the RGC spike train sent to the brain are established through synaptic interactions in the retina's IPL.

Connexin interactions in the inner retina of the mouse

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Results
Electrical coupling via gap junctions is an abundant phenomenon in the mammalian retina and occurs in photoreceptor and horizontal cells, cone bipolar (CB), amacrine (AC) and ganglion cells (GC). Gap junction channels are assembled from different connexin (Cx) subunits, whereby the Cx composition of the channel confers specific properties to the electrical synapse such as permeability, its dynamic modulation, or targeted assembly in cell type-specific circuits. In the inner retina, gap junctions have been shown to couple similar (AC-AC, GC-GC) and dissimilar cell types (AC-CB, AC-GC) within highly specified circuits. In many cases coupling crucially depends on the expression of either Cx36 or Cx45 (Bloomfield and Völgyi, 2009, and therein; Pan et al., 2010). Recently, we demonstrated the expression of a novel connexin, Cx30.2, in a number of ganglion and amacrine cells (Pérez de Sevilla et al., 2010).

In the present study we tried to shed light on the role of Cx30.2 in amacrine cells. Tracer coupling experiments using a Cx30.2-deficient mouse line (Kreuzberg et al., 2005) demonstrated that coupling is not necessarily disrupted in circuits formed by Cx30.2-expressing cells. This suggests that further Cxs are involved in the formation of electrical synapses in those cells. Furthermore, electroretinogram recordings did not reveal any major differences between wildtype and Cx30.2-knockout retinae. There are two possible explanations for that: 1) other Cx may compensate for the missing Cx30.2, and/or 2) Cx30.2 does not form channels on its own in these cells but instead is integrated into channels made of Cx36 or Cx45. To determine, whether the formation of channels comprising Cx30.2 and either of the other neuronal Cxs is possible, the formation of large intercellular gap junction clusters was investigated in transiently transfected HeLa cells. Our results indicate that interactions with both, Cx36 and Cx45, are possible, but that Cx30.2 cluster formation was especially enhanced when Cx36 was cotransfected.

Those results are consistent with previous findings showing that the deletion of Cx30.2 was largely compensated by Cx36 in other brain areas (Kreuzberg et al., 2008). Consequently, it seems likely that the specific integration of Cx30.2 into subsets of Cx36-containing gap junctions enables cells to discriminate between different synaptic sites in order to specifically modulate the electrical synapses corresponding to distinct circuits. Current experiments therefore focus on the modulation of electrical synapses involving Cx30.2 using various connexin knockout mouse models.

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In-vitro model to study human retina function on cell and system level

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Purpose
Most treatment options for retinal diseases are developed in animal models and then directly tested in clinical trials. We believe that there is a step missing in between where novel treatment approaches are probed in-vitro on human tissue. Further, the Eye Clinic of Tübingen made it its goal to decrease the number of animals sacrificed for research on retinotoxicity and ischemia. We thus propose an in-vitro model to study human retina function on cell and system level in order to (1) characterize the functional retinal ganglion cell (RGC) types in the healthy human retina, and (2) to evaluate the efficacy and side-effects of treatment approaches by comparison of the functionality of treated and healthy retinas.

Methods
(1) Human retinal tissue was donated by patients who had to undergo enucleation. We recorded the RGC responses with a 60-electrode multi-electrode array (MEA). Various light stimuli were projected onto the photoreceptors of retinal patches, such as flashes of different contrast, white-noise-flicker, bars moving in eight directions, drifting sine wave gratings, and natural movies. We then characterized the response properties of each recorded RGC such as their polarity, latency, and tuning to spatial and temporal frequencies.

(2) Novel treatment options can be tested on post-mortem tissue which is not light-sensitive anymore. The health condition of post-mortem donations obtained from the local cornea bank was evaluated with the same experimental paradigm as in 1.

Further, culturing of human retina – a prerequisite for the application and testing of treatment approaches – has been established.

Results
(1) In 14 months we obtained 12 donated retinas from enucleations. In 7 out of 10 recorded retinas, we could measure light responses. The recorded cells showed various properties such as different polarity (ON, OFF, ON-OFF), and distinct spatial and temporal tuning.

(2) Out of 4 post-mortem donations, 3 could be used for experiments. 2 of them showed abundant RGC activity after 14 and 25h of ischemia, which indicates that RGCs were still alive. RGCs could further be kept alive for up to 3 days (the longest period tested so far) in organotypic culture. All experiments have also been performed with pig retina obtained from the Department of Experimental Medicine, which we used to further improve the in-vitro model.

Conclusions
(1) Different functional types of ganglion cells can be recorded in donated human retinas from patients. In the future, acquired expertise, improved collaboration with the eye clinic, and the recent implementation of a high-density MEA system (11011 electrodes) will allow further quick and comprehensive characterization of human RGC types.

(2) RGCs in post-mortem human retina donations can survive ischemia times of up to 25h, and these retinas are thus a promising tool for testing of novel treatments. We are currently establishing the evaluation of optogenetic approaches for the treatment of blindness in such post-mortem tissue.

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Abstract
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Pericentrin, linked to a growing list of human disorders, identified at the basal-body complex in mammalian photoreceptor cells

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Purpose
TPericentrin (Pcnt), a conserved protein of the pericentriolar material (PCM), serves as a multifunctional scaffold for numerous proteins and plays an important role in microtubule organization. To date, three Pcnt splice variants from orthologous genes in mice and humans are known. We generated a specific Pcnt antiserum detecting all known Pcnt splice variants, and examined the cellular and subcellular distribution of Pcnt in ciliated tissues of the mouse, such as the olfactory epithelium and the retina. For the first time, we identified Pcnt and its known centrosomal interaction partners at the basal body complex of mouse photoreceptors. Photoreceptors are morphologically and functionally subdivided into the light sensitive outer segment and the metabolic active inner segment, which contains the typical energy producing and protein synthesizing components of an eukaryotic cell. The two compartments are linked via a modified, non-motile primary cilium, the connecting cilium. Here, Pcnt colocalizes with the whole protein machinery responsible for transport processes between the two photoreceptor compartments. Surprisingly, photoreceptors express a small Pcnt splice transcript, which is not present in receptor neurons of the olfactory epithelium.

Recent studies indicate that Pcnt mutations are associated with a range of diseases including primordial dwarfism (like Majewski/microcephalic osteodysplastic primordial dwarfism type II - MOPD II) and ciliopathies. Diseases associated with mutations in the PCNT gene display heterogeneous clinical manifestations, making it difficult to pinpoint the functional role of Pcnt.

In order to get more insights on the function of Pcnt in general we tried to identify new interaction partners of Pcnt using Strep/FLAG tandem affinity purification. Therefore we fused a TAP tag, consisting of a tandem Strep-tag II and a FLAG tag to the N- and C-terminus of Pcnt. Tandem affinity purification combines two affinity purification steps and allows isolation of high-purity protein complexes under native conditions (Gloeckner et al., 2009). Apart from that we constructed cDNA libraries from the mouse retina and olfactory epithelium and performed a yeast two-hybrid screen utilizing specific parts of the Pcnt protein as baits.

Our findings suggest so far several novel Pcnt interaction partners, emphasizing the importance of Pcnt in microtubule organization, cell division, cell cycle progression, assembly of cilia and probably in various other fundamental cellular processes. Identification of new Pcnt interaction partners may help us to understand Pcnt function in general and provide new insights into human disorders related to defects in ciliary function.

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Determination of ciliary sub-modules by SDS-destabilization and correlation profiling

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Purpose
A major drawback of protein complex analysis is the fact that a mixture of different complexes is purified and it is virtually impossible to detect sub-modules therein.

Methods
To get access to sub-modules, we developed a novel toolset that combines controlled destabilization of protein complexes by application of low concentrations of SDS with label-free quantitative mass spectrometry and bioinformatic analysis of the generated relative abundances.

Results
As proof of principle we selected the well described complex of the Leber Congenital Amaurosis-associated protein Lebercilin. It was described to associate with distinct sub-modules of the intraflagellar transport machinery, essential for the transport of cargo along ciliary structures. Loss of this interaction and thereby disruption of the intraflagellar transport leads to photoreceptor degeneration and blindness.

By applying the SDS-destabilization method, we could perfectly confirm the known complex composition by clearly separating IFT-A from IFT-B complexes. Furthermore, we were able to detect novel sub-modules and new members of the complexes, which we further validated by alternative methods.

Conclusions
We demonstrate that this tool is absolutely suitable for the detection of sub-modules within protein complex mixtures. In contrast to previously existing methods that rely on blue-native gel electrophoresis, this method is easier to perform and clearly less time consuming and labor intensive.

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Ciliary transport in photoreceptor cells: the role of Usher syndrome related protein networks

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Purpose
Human Usher syndrome (USH) is the most common hereditary form of combined deaf-blindness. USH type I (USH1), the most severe form, is characterized by profound congenital deafness, constant vestibular dysfunction and pre-pubertal onset of retinitis pigmentosa (RP). Previous studies have indicated that USH proteins participate in the ciliary transport of photoreceptor cells. To gain further insights into this mechanism we focused on USH1G protein SANS interactions and the molecular composition of the transport machineries of photoreceptor cells.

Methods
We adopted yeast-2-hybrid (Y2H) screens of retinal cDNA libraries and validated putative interactions by complementary assays, namely GST-pull downs, co-IPs and co-transfection assays. We tested SANS binding to microtubules in microtubule destabilization and spin-down assays. Finally we analysed the spatial distribution of the identified proteins and complexes in photoreceptor cells by immunofluorescence, immunoelectron microscopy and proximity ligation assays (PLA).

Results
Spin-down assays demonstrated the direct binding of SANS to microtubules. In photoreceptor cells, the localization of SANS is altered after microtubule depolymerisation. Complementary interaction assays demonstrated the interaction of SANS with components of the dynein-dynactin motor-complex. Light and electron microscopy revealed the co-localization of SANS with dynactin and canonical cytoplasmic dynein components along the microtubule transport tracks in inner segments and at the periciliary region of photoreceptor cells. Furthermore, PLAs demonstrated that in these cell compartments, SANS and the investigated motor components are assembled into protein complexes.

Conclusions
The present data strengthen our hypothesis that USH protein networks participate in cargo transport to its ciliary destination. There is liable evidence that SANS is part of the cytoplasmic dynein motor complex, a molecular machine, translocating outer segment cargoes along microtubules through the inner segment to the periciliary region of photoreceptor cells. Defects in this molecular transport machinery may lead to the photoreceptor dysfunctions introducing the degenerative processes underlying USH.

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Dissection of functional modules associated with retinal ciliopathies by affinity proteomics

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Purpose
Leber Congenital Amaurosis (LCA) is an inherited retinal degenerative disease characterized by severe loss of vision at birth. It occurs in 2 to 3 per 100.000 newborns and is one of the most common causes of blindness in children. LCA is usually inherited in an autosomal recessive manner and is genetically heterogeneous. Twenty genes have been identified to be associated with LCA to date (according to RetNet, last update 4th Feb 2013), and approximately 70% of the LCA cases can be explained by a mutation in any of these genes. Most LCA candidate genes encode proteins with a wide range of retinal functions, including photoreceptor development, phototransduction, retinoid metabolism, ciliary transport, guanine synthesis, and outer segment phagocytosis by the retinal pigment epithelium. One of the genes which function has remained a mystery is Spata7, encoding a spermatogenesis associated 7 protein

Methods
In order to unveil the functional module in which Spata7 participates, which may be dysfunctional upon genetic mutation, we have taken an affinity proteomics approach. We have used tandem affinity purification in HEK293T cells followed by mass spectrometry to identify the Spata7 interactome, and yeast two-hybrid screening to identify binary protein-protein interactions. Results were validated by biochemistry and immunohistochemistry.

Results
Protein-protein interaction study using tandem affinity purification approach suggested that Spata7 might be involved in the intracellular trafficking of protein cargos toward cilium via its links to dynein motor complex components. Our first localization data showed that fluorescent-tagged Spata7 associated with cytoskeleton microtubule structure and was enriched at the basal body of cilium, a microtubule-based organelle protruding from cell's surface, in hTERT-RPE cells. To further validate these results, a knock-down experiment will be performed and transport of specific interactors of Spata7 will be studied.

Conclusions
Spata7 is a ciliary protein, most likely involved in transport towards the ciliary base. Dysfunction of such transport may explain the early-onset detrimental effects in the retina of the gene mutations associated with LCA

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Abstract
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NINL and DZANK1 cooperate in assembling the cytoplasmic dynein 1 motor complex, a process essential for photoreceptor outer segment formation in zebrafish

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Purpose
In order to gain insight into the pathogenic mechanisms underlying three retina degenerative disorders: Leber Congenital Amaurosis, Bardet-Biedl syndrome and Usher syndrome. The role of NINL, a molecular connector of these hereditary disorders, is scrutinized using a combination of (affinity) proteomics and morpholino-induced knockdown studies in zebrafish,

Results
A bovine retina cDNA library screen identified DZANK1 as an important interaction partner of NINL. Subsequent morpholino-induced knockdown of either ninl, dzank1,or both ninl and dzank1 in zebrafish embryos resulted in defective photoreceptor outer segment formation, accumulation of trans Golgi-derived vesicles, and rhodopsin mislocalization. In addition, retrograde melanosome transport was impaired in these morphant zebrafish. Affinity proteomics revealed that NINL and DZANK1 associate with complementary subunits of the cytoplasmic dynein 1 complex. These subunits together are essential for the assembly and proper functioning of this motor unit.

Conclusions
Our results support the hypothesis that the NINL-DZANK1 protein module is essential for the proper assembly of the cytoplasmic dynein 1 motor complex in photoreceptor cells of the zebrafish retina. Absence of either NINL or DZANK1 will result in a defective transport of molecules necessary for photoreceptor outer segment formation, maintenance and/or functioning towards the base of the connecting cilium.

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The effect of light intensity on flight control in bumblebees

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Purpose
The purpose of this study is to investigate the visual limitations of flight in animals. In particular, how dim can light levels become before flight performance collapses? To answer this, we use insects as model animals, since it is known that they rely primarily on vision to control their flight. However, most studies on insect flight control have been carried out in bright light, so the effects of dim light are still unknown. By examining flight behaviour in different light intensities, we can better understand the tolerances and limits of vision and visual flight control. This would not only enhance our knowledge about insect vision and flight performance under natural conditions, but may give us some insight into visual limitations of flight performance in other animals as well.

Methods
To study the effect of light intensity on flight control, we filmed flights of bumblebees, Bombus terrestris, while flying in an experimental tunnel under different light intensities. The experiment was performed at 6 different light intensities, ranging from 600 lux (the light level of sunrise or sunset on a clear day) down to 3.4 lux (the dark limit of civil twilight under a clear sky). The flight paths were then digitised and analysed using an automated tracking program.

Results
Even in very dim light, bumblebees are still able to stay centred around the midline of the tunnel, which is normal behaviour in bright light. Moreover, when examining the overall straightness of their flight trajectories, measured as path length, we see no effect until the light intensity is very low. Together, these results indicate that a decrease in light intensity does not have a very strong effect on the precision of the bumblebees' flight control system. However, as light levels fall, we observe a significant decrease in flight speed.

Conclusions
Our observations indicate that bumblebees still see enough visual information in dim light to fly safely, but do so at a reduced flight speed. This suggests that bumblebees compensate behaviourally, by flying slower, for the lower temporal and spatial resolution that most likely occur in their visual system as light levels fall. This behavioural adaptation to falling light levels allows bees to continue to fly safely right up until the point when they do not have enough visual information to fly at all. It is likely that other flying animals that use vision to control their flight have similar adaptations.

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Higher-order visual processing in hawkmoths

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Purpose
Even though humans see poorly at night, the majority of the world's animals – both on land and under water – are active in dim light and many of them have excellent vision. Despite possessing tiny eyes and brains, nocturnal insects can distinguish colour, avoid obstacles during flight and find their way home using learned visual landmarks. But how is this remarkable visual performance possible? How have the properties of the visual circuitry been optimised to maximize visual performance in dim light conditions?

Methods
In order to approach these questions, we are studying the visual motion processing circuitry in hovering hawkmoths – a group of fast-flying insects renowned for their impressive visual abilities, in which closely related species are active under completely different light conditions, ranging from bright sunlight to starlight.

Results
One way of increasing visual performance in dim light is to spatially pool signals from neighbouring visual channels – a process called spatial summation. Alternatively, signals can be integrated over longer periods of time, a process called temporal summation.

In the first optic neuropil of insects (the lamina), the major relay neurons possess an anatomy that makes them well suited to the summation of visual signals: their lateral processes are long and extend to neighbouring processing units. Using the Golgi staining method, we compared the lengths of these lateral processes between the day-active hawkmoth Macroglossum stellaratum and the night-active Deilephila elpenor. As predicted for a possible function in spatial summation, the lateral processes of D. elpenor are longer than those of M. stellaratum and could potentially pool information from cartridges further away.

Wide-field motion-sensitive (HS-like) neurons in the third optic neuropil of hawkmoths, the lobula complex, are tuned to the optic flow experienced during flight and are an essential part of the flight control system. We recorded from these neurons to quantify their spatial and temporal response characteristics in the nocturnal hawkmoth species D. elpenor in different light intensities.

Going from dusk light intensities down to starlight levels, the spatial and temporal optima of their lobula plate neurons both decreased, well below the limits set by the optics and photoreceptor dynamics.

Conclusions
We therefore conclude that the visual system of D.elpenor sums visual signals both in space and time as light levels decrease and we identified the first optic neuropil as a possible location of spatial summation.

Vision in box jellyfish

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Purpose
Demonstrate the visual processing capacity of an animal with a relatively limited nervous system.

Methods
Behavioural studies, optical modelling and electrophysiology.

Results
- The box jellyfish Tripedalia cystophora can display a number of remarkably complex behaviours.
- High spatial frequencies are filtered out by the underfocused optics.
- Vision is slow.
- Box jellyfish are colorblind

Conclusions
The behavioural complexity seen in the box jellyfish Tripedalia cystophora can only be supported by the nervous system if the information input is limited by applying matched filters.

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Cellular Reprogramming – An approach to drive cells towards retinal cell fate

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Purpose
Our goal is to reprogram mouse embryonic fibroblasts (MEF) into retinal progenitor cells. For that purpose we want to use eye field transcription factors (EFTF). They are known to be necessary and in some cases sufficient to induce ectopic eye development. We see the ex vivo generation of retinal progenitors as a promising step towards providing retinal cells to develop new strategies to study retinal disease pathomechanisms and treat retinal diseases.

Cell reprogramming using a defined set of transcription factors has been shown to be able to induce pluripotent stem cells from fibroblasts. Further studies investigated the possibility to reprogram fibroblasts into any desired cell type by finding the right factor combination and culture condition. This has been shown to be possible for some cell types as for example for neural stem cells or cardiomyocytes, but so far it was not achieved for retinal progenitor cells.

Methods
As gene transfer system we use a replication-incompetent vector system based on the human immunodeficiency virus type 1 (HIV-1), which employs the modulated envelope protein of the vesicular stomatitis virus (VSV-G) for pseudotyping. Single EFTF were cloned into the transfer vector, which also encodes for an EGFP reporter gene. Generation of the viral particles and infection was done as previously reported by the Lindemann group. Mouse embryonic fibroblasts (MEF) were isolated from E13.5 mouse embryos, propagated in culture and virus infected. Three days past infection the medium was changed to media for embryonic retina culture conditions. MEF were cultured for 20 days and analyzed with fluorescent microscopy and reverse transcriptase-PCR.

Results
We designed a screening strategy to investigate cell reprogramming of MEF into retinal progeny by defined combinations of transcription factors. To facilitate the screening for reprogrammed retinal progenitors we generated MEF from transgenic reporter mice (Chx10-Cre::Rosa26-AI9 mice). Chx10 is a transcription factor expressed at the onset of neurogenesis in retinal progenitors. Induction of AI9, a red fluorescent protein, will be the first read out to find successful reprogrammed cells. Further, we cloned the desired reprogramming factors into the p6Nst50 transfer vector and generated infectious retroviral particles. The infection efficiency of the particles transferring the transcription factors is similar to the control virus, which yields up to 85%. The eye field transcription factors in the infected cells are expressed, which we confirmed on mRNA and protein level.

To test, whether our exogenous genes are functional, we overexpressed Otx2 in mouse embryonic retinal progenitor cells in culture. Otx2 is known to be able to drive cells towards rod fate. And indeed, Otx2 overexpression led to an upregulation of recoverin positive cells, proving its functionality.

Conclusions
Since we now have established our reprogramming method, as a next step, we want to screen combinations of factors to identify effective ones to generate retinal progeny. To further characterize the resulting cells we defined several levels of analysis including a RT-PCR screen, gene array analysis and cell differentiation analysis to investigate the potential induction of progenitor cells.

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Photoreceptor transplantation: modes of integration into recipient adult retinas

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Following transplantation, post-mitotic photoreceptor precursors are able to migrate into recipient adult retinas, assume mature photoreceptor morphologies and contribute to vision. Whilst the optimal stage of development for the donor cells has been identified, the properties conferring their ability to integrate remain unknown. Here, we aim to elucidate the mode of donor cell migration into recipient adult retinas and compare this with analogous migration processes during development.

Neonatal Nrl.GFP mouse retinas were dissociated enzymatically. Nrl.GFP+ve rod precursors were FACS-sorted and injected subretinally into adult wild-type mice. Recipients were sacrificed between 48 hrs and 3 weeks post-transplantation. Eyes were cryosectioned, immuno-stained and confocally imaged. Migration of Nrl.GFP+ve rod precursors during normal development was assessed using time-lapse 2-photon microscopy on explanted retinas. The interaction between rods and Müller glia was assessed in transplant recipients by immunostaining for glial markers and also in vitro in tissue culture, where co-cultured cells were imaged in real-time.

Transplanted rod photoreceptors integrate into the recipient retina throughout the first week post transplant. Confocal imaging and analysis of transplanted cell morphologies revealed that integrated cells possess an inner segment-like structure from the earliest stages post transplant, with synaptogenesis taking place subsequently. 2-photon imaging of explanted neonatal Nrl.GFP retinas revealed that, during development, rod photoreceptors follow a bidirectional migratory path and maintain an apical connection to the developing outer limiting membrane (OLM) whilst migrating. The spatial interaction between transplanted rod photoreceptors and recipient Müller glia was confirmed by nearest neighbour analysis. Time-lapse imaging in tissue culture revealed rod photoreceptors elongating and migrating along the surface of Müller glial cells.

Analysis of transplanted cell morphologies over a range of time-points post transplant has revealed a sequence of events in which outer segment formation precedes synaptogenesis. An apical connection to the OLM was seen in migrating rod photoreceptors both in transplant and in development. Furthermore we have demonstrated that Müller glial cells act as scaffolds along which rods migrate, both by nearest neighbour analysis in retinas post transplant and by time-lapse microscopy in rod-Müller cell co-culture.

Cone-like photoreceptor transplantation into the mouse retina

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Purpose
Vision impairment affects around 314 million people worldwide. In diurnal organisms, day vision depends on cone photoreceptors (PRs) and several eye diseases including age-related macular degeneration, lead to cone PR degeneration. Several therapeutic approaches, such as gene and cell-therapy, are currently being developed mainly focusing on rod dystrophies, leaving cone-dystrophy therapies not well studied. Thus, we evaluated the feasibility of cone-like PR transplantation into wild type and diseased mouse retinas and the possibility of functional recovery.

Methods
Cone PRs account for only 3% of the cells in the mouse retina. Hence, a more comprehensive source of cone PRs was developed. We crossed Nrl-/- mice, that contain no rods but only cone-like photoreceptors, with an actin GFP reporter line (aGFP). The resulting line tg(Nrl-/-,aGFP) was used as a source for cone-like PRs. Cone-like cells were sorted using Magnetic Associated Cell Sorting (MACS) using CD73 as a cell surface marker. Enriched CD73+ cells were transplanted into the subretinal space of adult wildtype (WT) retinas. Integration efficiency was analyzed 2 weeks after transplantation. Cone-like PRs were then transplanted into age matched cone dystrophy model (Cpfl1 mutant mice) and WT retinas. Integration rate and functional recovery (ERG measurements) were analyzed 4 weeks after transplantation.

Results
The generated reporter line showed rosette-like structures typical of a rodless retina and expressed cone-specific markers. tg(Nrl-/-,aGFP) mice showed comparable ERG measurements to Nrl-/- mice. Cone-like PRs expressed CD73, which was used as a cell surface marker. MACS-CD73 sorted cone-like cells were able to integrate into WT hosts, having a peak of integration at postnatal day 4 (P4). Integrated cone-like cells are able to acquire mature photoreceptor morphology. P4 MACS-CD73+ sorted cells were then transplanted into cpfl1 hosts, showing similar integration rates as in WT retinas and increased a-and b-wave amplitudes under mesopic and photopic conditions.

Conclusions
Cone-like cells can integrate in different types of host retinas having a peak of integration at PN4. Cone-like cells express cone specific markers, acquire mature photoreceptor morphology and partially rescue daylight vision. Hence, cone-like cell transplantation might represent a promising strategy for the restoration of vision in cone-dystrophies.

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Photoreceptor Diversity Across Mammals: Adaptations to Lifestyle and Habitat?

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All mammalian retinae contain rod photoreceptors for lowlight (night) vision and cone photoreceptors for daylight and colour vision. Most mammals have 'dichromatic' colour vision, based on two spectral cone types containing a middle-to-longwave-sensitive (commonly green-sensitive) L opsin and a shortwave-sensitive (commonly blue-sensitive) S opsin, respectively. However, superimposed on this basic similarity there are remarkable quantitative and qualitative differences between species. Cone-to-rod ratios vary strongly and are loosely correlated with the activity pattern of the species, from ~1:100 in very nocturnal mammals to ~1-3:10 in most diurnal mammals and ~10:1 in a few diurnal species (e.g., ground squirrels and tree shrew). Against expectation, subterranean mammals have comparatively low rod densities and high cone proportions (~1:10). The proportions of L and S cones and their topographies also vary considerably. They range from a conventional ~10% proportion of S cones across the retina to a dominant or even exclusive expression of S cone opsin in the ventral retina of some species. In some mammals, e.g. some rodents, bats and insectivores, the S cones are UV- rather than blue-sensitive. Obviously most mammals, including nocturnal and subterranean ones, have the photoreceptor prerequisites for colour vision, but their colour vision characteristics differ considerably. Whales and seals have no S cones at all, they are L cone monochromats and presumably colour-blind. It is widely assumed that the above variations represent adaptations to specific visual needs associated with particular habitats and lifestyles. However, in many cases we have not yet identified the adaptive value of a given photoreceptor arrangement (review: Peichl, Anat. Rec. Part A 287A: 1001-1012, 2005).

In the rods of nocturnal mammals we found a unique 'inverted' nuclear architecture, whereas the rods of diurnal mammals have the conventional nuclear architecture present in nearly all other eukariotic cells (Solovei et al., Cell 137: 356-368, 2009). In the 'inverted' rod nuclei, the inactive heterochromatin localizes in the nuclear centre, whereas the active euchromatin lines the nuclear border. This appears cell-biologically disadvantageous, but advantageous for vision. The inverted nuclei with the optically denser heterochromatin in their centre act as collecting lenses, and columns of such nuclei channel light effectively through the outer nuclear layer (ONL). The retinae of nocturnal mammals have a higher rod density for improved night vision and hence a thicker ONL, which on the other hand would lead to increased light scatter so that fewer of the precious photons reach the outer segments. Light-guiding by the inverted rod nuclei seems a suitable evolutionary solution to this dilemma. The retinae of diurnal mammals have a thinner ONL and operate at higher light levels, so their rods can have the conventional nuclear architecture. We recently showed that the conventional architecture with heterochromatin attached to the nuclear envelope requires either a lamin-B-receptor (LBR)-dependent or a lamin-A/C-dependent tether, or both; the inverted rod nuclei of nocturnal mammals lack both LBR and lamin-A/C, hence their heterochromatin localizes to the nuclear interior (Solovei et al., Cell 152: 584–598, 2013).

Functional Assessment and Breadth of Application of Photoreceptor Transplantation Therapy

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Purpose

Cell transplantation is a potential strategy for treating blindness caused by the loss of photoreceptors. Although transplanted rod-precursor cells are able to migrate into the adult retina and differentiate to form mature photoreceptor cells phenotypes, a fundamental question remains as to whether transplantation of photoreceptor cells can actually improve vision. In the first part of this study we assess whether transplanted cells are functional and able to contribute to meaning vision. Secondly, photoreceptor replacement by transplantation has been proposed as a broad treatment strategy applicable to all degenerations. Considering the heterogenous nature of many retinal degenerative disorders, it is yet unknown whether photoreceptor replacement is equally effective in different types and stages of degeneration. In the second apart of this study, we assess photoreceptor transplantation in several clinically relevant models of retinal degenerative diseases.

Methods

The Gnat1-/- recipient, which lacks rod function and is a model of congenital stationary night blindness, was used to assess rod-mediated vision of transplanted rod photoreceptor precursor cells. We assess synaptic connectivity using immunohistochemistry and electron microscopy. Single cells recordings from the newly integrated photoreceptor cells were employed to demonstrate light-responsiveness. Intrinsic imaging of the visual cortex was performed to assess whether visual signals generated in scotopic lighting conditions were projected to higher visual areas. Optokinetic head tracking responses and visually guided behaviour was tested to demonstrate whether the visual signal generated by transplanted photoreceptor cells were able to contribute to higher visual functions.

In order to assess the breath of photoreceptor transplantation therapy, we assess rod-photoreceptor transplantation, both in terms of efficiency and morphology, across six murine models of inherited photoreceptor degeneration at early, mid and late degenerative stages. We also look to assess and manipulate factors of changing recipient microenvironment to enhance integration efficiency.

Results

We demonstrate that transplanted rod-photoreceptor cells can respond to light and form functional connections with second order neurones. Haemodynamic responses were restored in the visual cortex of recipient mice and rescue of optokinetic head-tracking responses and visually guided behaviour in watermaze testing was demonstrated.

Secondly, we demonstrate that transplantation is feasible in several models of retinal degeneration but disease type has a major impact on outcome, as assessed both by the morphology and number of integrated rod-photoreceptors. Robust integration is possible even in late-stage disease in some disease types. Glial scarring and outer limiting membrane integrity, features that change with degeneration, significantly affect transplanted photoreceptor integration and a combined breakdown of these barriers in the rho-/- recipient leads to an eightfold increase in integration and restoration of optokinetic head-tracking responses.

Conclusions

Here we provide evidence of functional rod-mediated vision after photoreceptor transplantation in adult Gnat1−/− mice. We also demonstrate that it is possible to achieve robust integration across a broad range of inherited retinopathies. Moreover, transplantation outcome can be improved by administering appropriate, tailored manipulations of the recipient environment. Together, these results demonstrate the feasibility of photoreceptor transplantation as a therapeutic strategy for restoring vision after retinal degeneration.

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Starting a Business

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The proper communication of scientific results is more and increasingly becoming important in the research area. Life science content in particular is often very complex, so that didactically and visually appealing animations and illustrations can give an effective support for the knowledge transfer. However, for scientists the technical means and time reserves required for producing useful visualizations are available only to a limited extent. A way out of the conflict is to assign this job to specialized teams bundling scientific, graphical and IT expertise. The presentation of the company Effigos AG intends to provide an insight how this concept can be made to work successfully.

The Balancing Act between Academia and Industry

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The major part of Research and Development is performed by private companies. Nevertheless this research seems to be less visible in the public. However, research in industry should lead to products that in some way may have impact on human wellbeing and therefore are subject to strict requirements. On the other hand academics are considered to be very unreliable by industrial researchers. Having experience and insights in academic and industrial research, this presentation will give a personal overview of the differences and similarities of research in the two.

1 Securing the work

2 The output and its evaluation

3 Daily work

4 Career and family

Differences	Similarities
2 The output of research is evaluated in different currencies: money in industry and publications in academia	3 If performed well, there is huge amount of pressure on the individual researchers.
	1 Acquisition of support from investors and contact with clients in industry and grant applications in academia have similar goals and structures.
2 Research in industry should lead to a product; In academia to gain of knowledge	3 Huge amount of idealism is necessary. Personal vanities play a role.
3 More restricted intellectual freedom in industry.	3 Decisions and the evaluation of achievements are based on subjective and quasi-objective criteria.
2 Intellectual property and patents are more important than scientific publications in industry; in academia it is reversed. This is a matter of major concern for possible collaborations.	3 Deadlines are strict and important in industry. If basic research is well performed then deadlines (for application or publication submission) are very important as well (but often there is less external control and is largely based on self-discipline). As a result, time pressure is high in both fields.
4: Careers in industry are often discontinuous. Continuation of careers (doctorate; post-doc; tenure track etc.) in academia although there is a possibility of losing all perspectives.	4 Flexibility in career and family planning is required.
	2 research in industry should lead to products that in some way may have impact on human wellbeing and therefore are subject to strict requirements. Research in academia is subjected to peer review.

Although it is not always easy to combine work for industry and academia this should be a plea for a closer collaboration of research in both environments: the combination of the academic innovation and the industrial application is highly rewarding in every sense of the word. It requires, however, an attitude of mutual trust and respect.

Insights to Research Activities driven by the industry

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The talk gives an overview on how the research activities in industries are planned and shows the way of an innovative idea to a final product. There are different topics in industry, which needs to be covered by the R&D department. Next to the development of rather simple adjustments in a production process or a product, there can be bigger improvements by research based on new technologies or at the highest degree there are "revolutions" driven by complete new insights which lead to complete new processes or products (sometimes leading also to new market opportunities for a company). In general the research in industry is driven by the need of new products or cost savings in the production process. Some example of these types of research activities and also the background frame of company based research will be presented.

Academic research versus industrial innovation: friends or foes?

Siegfried Wahl
Carl Zeiss AG, Germany

It is not possible to speak from THE academic research or THE industrial innovation. There is not the one and only academic approach or the one and only industrial process. Both have many facets and in fact they are not completely different. The basic drivers of each process are different, but both deal with guard rails where in between at least some freedom exists. In the talk are examples given in regard to these guard rails, e.g. in academic research you will find financing models mainly directed to public funding, or in regard to personal profiles, e.g. in the industry normally personal profiles or competencies in an innovation project are from many different departments. Interdisciplinary approaches are essential for an smart product introduction.

But also in academic research you have since years interdisciplinary departments dealing with different aspects of an research area with great inventions as outcome. Bringing these inventions into the real life that is innovation or with the words of J.A. Schumpeter: "Innovation is the implementation of a technical or organizational novelty, not just its invention.". Further examples are given regarding targets, career opportunities, intellectual property and drivers. One of the major drivers for innovative products or even disruptive and radical innovative products are global megatrends, but also the cooperation with basic research provides value add for companies in the industry and therefore academic research and industrial innovation are complementing each other.

HIF1 dependent upregulation of Stanniocalcin-2 in chronic and acute hypoxia in the retina

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Purpose

A hallmark of retinal blinding diseases is degeneration of photoreceptors. Often, oxidative stress influences cell survival and the disease process. Hypoxia may differentially affect retinal integrity-chronic hypoxia leads to cell death but acute hypoxia is neuroprotective. Hypoxia stabilizes hypoxia inducible transcription factors (HIFs) expressing several target genes involved in diverse physiological processes. In normoxia HIF-alpha subunits are rapidly degraded by a Von Hippel Lindau (VHL) protein complex dependent pathway. To elucidate molecular mechanisms involved in hypoxia - mediated degeneration and / or protection, we aimed at the identification of the gene network controlled by HIF1 in rod photoreceptors in vivo.

Methods

Mice with a Cre-mediated deletion of Vhl specifically in rod photoreceptors were generated (VhlΔrod). These showed a hypoxia-like response with the stabilization of HIF1α and HIF2α in normoxia. The retinal transcriptome of these mice was compared with the transcriptome of mice that additionally lack HIF1A in their rods (Vhl;Hif1aΔrod). Expression of individual genes and proteins was analyzed an by qPCR and western bloting, respectively. Retinal function was tested by ERG and morphology analyzed by light microscopy and immunofluorescence (IF).

Results

Retinal morphology of VhlΔrod and Vhl;Hif1aΔrod mice did not grossly differ from controls at 11 weeks of age, but at 1 year of age VhlΔrod mice lost about 75% of photoreceptors, indicating that long term stabilization of HIF1A results in an age-dependent degeneration. IF stainings for markers of Müller glia cells, rods, cones and microglia at 11 weeks of age showed slight variations in the knockdown mice. Gene chip analysis indicated that HIF1 directly or indirectly controls expression of a large number of genes in rod photoreceptors. One of those, stanniocalcin-2 (Stc2), showed a 14 fold upregulation on stabilization of HIF1A. Retinal Stc2 expression was also elevated in acute hypoxia, with a fast return to basal levels upon reoxygenation. Surprisingly, IF results suggests a strong expression of STC2 in the nerve fibres.

Conclusions

STC2 is a secreted glycoprotein shown to be regulated by HIF1A in other systems. Stc2 has been shown to protect HeLa cells and neuroblastoma cells against UPR induced cell death. It's role in the retina remains to be explored. One of the intriguing aspects of STC2 is that our data suggests localization of the protein in the retinal ganglion cell axons even though the gene might be strongly expressed in the photoreceptors and probably other cell types. This raises the question why photoreceptors produce a protein required by ganglion cells. Our future investigations will thus focus on the function of STC2 in the GCL and regulation of Stc2 by hypoxia.

Wavelength Dependence of the Ocular Straylight

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Impaired neural retina development in the DNA double-strand break repair pol mu mutant mouse

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Purpose
During neural development, the distinctive cytoarchitecture and connectivity of the nervous system are achieved by an orchestrated balance of proliferation, differentiation and cell death. Several genetically-modified mouse model systems defective in DNA double-strand break repair present a dramatic phenotype during neural development, suggesting a possible function of DNA repair in the process. Hereby, we try to demonstrate in vivo the requirement of Pol mu, a DNA polymerase involved in a DNA double-strand break repair pathway, for proper neural development.

Methods
We have analyzed retinal development and visual function in the Polμ deficient mouse, a murine model defective in DNA repair, as well as in its wild type counterpart. Whole mount and dissociated retinas were processed for immunohistochemistry and western blot to determine the effect of the mutation on neuronal differentiation, cell-adhesion molecule distribution, axonogenesis and cell death. Optic nerve architecture was visualized by electron microscopy.

Results
The pol μ deficient mouse presented increased apoptotic cell death that selectively affected young retinal ganglion cells. Moreover, the intraretinal pattern of axonal growth, as well as optic nerve fasciculation and decussation at the optic quiasma were altered. These abnormalities correlated with alterations in axonal-guidance proteins and an increased ectopic neural localization.

Conclusions
Our results show that deficiency of DNA polymerase mu affects both survival and distribution of embryonic retinal neurons. Further work is required to integrate DNA double-strand break generation and repair during retinal neurogenesis and proper retinal function.

Acknowledgement
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Ectopic activation of Wnt/β-catenin signaling in lens fiber cells results in cataract formation and abberant fiber cell differentiation

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Proper regulation of Wnt/β-catenin signaling during eye and lens development is critical for its formation. Abberant Wnt/β-catenin signaling activation in the lens placode stage supress the subsequent lens development.

In order to study the output of ectopic Wnt/β-catenin signaling activation during later stages of lens development we used transgenic mouse line called CLEF in which transactivation domain of β-catenin is fused to DNA-binding protein LEF and the expression of transgene was controled by αA-crystallin promoter. Constitutive Wnt/β-catenin signaling activation via expression of transgenic protein CLEF in fiber cells by E12.0 leads to cataract development and micropthalmia in adulthood. Lenses of transgenic CLEF mice were as well characterized by lower amount of γ-crystallins in adulthood, caused by decreased γ-crystallin mRNA transcription in adult lenses. Examination of eye phenotype during embryonic development of CLEF mice revealed abnormal and delayed fiber cells differentiation.

Our results demonstrate that exact regulation of activation of Wnt/β-catenin signaling in later stage of lens development and differentiation has crucial impact on its proper differentiation that results in lens transparency.

Establishment of a retinal ischemia organ culture model

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Purpose
Ischemia plays an important role in several ophthalmologic diseases. To investigate neuroprotective agents and therapies against these diseases we developed an easy-to use chamber for 6-well plates with inserts for organotypic cultures. We decided to use organotypic cultures, because in-vivo models or primary cultures are very time-consuming, expensive and several therapies or agents cannot be tested in these models.

Methods
We incubated retinas at 37°C for different durations (45, 60, 75, 90 and 120 minutes) under ischemic conditions. Briefly, the chamber was streamed with N2 for 5 minutes, then the chamber was immediately sealed and the retinas were incubated for the rest of the designated time. After the incubation the 6-well plate was adjusted to normal air conditions and incubated for 24, 48 or 72h in an incubator under standard conditions. To analyze the amount of RGCs immunohistology was performed with a Brna3a-antibody. Apoptotic cells were visualized via TUNEL-staining and overall cell amount via DAPI-staining. Furthermore, Western-Blot analyses with GFAP- and Thy-1-antibodies were performed. Moreover, OCT measurements of the organ cultures were performed for up to one week. Additionally, comparisons with retinas treated with 0.5mM and 1mM glutamate were performed.

Results
A time- and ischemia duration-dependant decrease in the amount of RGCs after 24, 48 or 72 h was observed. Moreover, the amount of TUNEL-positive RGCs was also ischemia duration- and time-dependant. The damage to the RGCs through 75 minutes of ischemia was comparable to the amount of damage by 1mM glutamate incubation for 24h (20.27 vs. 19.69) and 48h (13.41 vs. 14.41). In contrast, in glutamate treated retinas, only few apoptotic RGCs were found. The thickness of the retina significantly decreased ischemia duration- and time-dependant as observed with OCT-measurement.

Conclusions
We successfully established a cheap, reliable, reproducible, ease-to-use organotypic culture model for retinal ischemia. Any therapy can now be tested under ischemic organotypic conditions. We selected 75 minutes of ischemia for further studies, because approximately 50% of the RGC died compared to the control after 48h. Moreover, the RGC-loss after 75 minutes of ischemia is comparable to the loss with 1mM glutamate. Results of a neuroprotective treatment with our chamber are shown on another poster from our group (Dorfi et al.).

Probability of acquired myopia progress in dependence of morphometric parameters and clinical data

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Purpose
On an etiology myopia can be congenital and acquired (school myopia),and on character of development – stable and progressing. According to experimental works (Ai L, Li J, Guan H, Wildsoet CF, 2009, Nickla DL, Wildsoet CF, Troilo D., 2002) it is known, that the thickness of a retina varies at eyeball growth, and also at defocus change. The probability of myopia growth in each patient remains difficult to predict. The purpose was to reveal differences in patients with acquired myopia and divide them in dependence of studied indices.

Methods
Children of 7-15 years old (53) with myopia were observed, a criterion of inclusion in research were children with acquired myopia. Parameters which were studied: visual acuity, refraction, axis length of an eye, accommodation reserves, intraocular pressure, retina layer thickness and peripapillary fiber layer thickness, optic nerve disk indices by optical coherent tomography under standard protocols by SOCT Copernicus, Optopol Technology Sp.z o.o., Poland.

Results
Results Optimum points of division of acquired myopia on stable and progressing forms were established by ROC analysis. The discriminate analysis with high reliability has shown, that the progressing form of myopia will be observed in children with average thickness in a zone 3mm more than 246,3mk, with a refraction more than 4,5 dptr and axes more than 25,3mm (p=0,0001). Fisher's discriminating functions were defined and the equations for definition of the stable and progressing forms of acquired myopia were offered. Effect of classification is 80,3%.

Conclusions
Results have allowed suggesting model of classification for the stable and progressing forms of myopia in dependence of morphometric indices, axis length and refraction in patients with acquired myopia confirmed in 80,3% of cases.

Retinal astrocytes in the mouse model of oxygen-induced retinopathy

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Purpose
Retinal neovascularization has been intensively investigated in the mouse model of oxygen-induced retinopathy (OIR). The role of astrocytes for vascular loss and reactive angiogenesis, however, is not fully understood. Due to their stellar morphology, only semi-quantitative analysis has yet been possible. This study presents data on the kinetics of retinal astrocytes in relation to changes in the vascular bed during the OIR model based on a quantitative approach.

Methods
In the OIR model, mice are exposed to 75% oxygen from post-natal day 7 (P7) to P12 (hyperoxic phase). After return to room air, the avascular area of the retina becomes hypoxic and responds with physiologic and pathologic revascularization from P12 to P21. In this study, reporter mice expressing histone-bound GFP under the control of the Pdgfra promoter were used to identify astrocyte nuclei in the murine retina. The astrocytic density across the retina was determined at different times during the OIR model using the automated ImageJ macro AuTOCellQuant.

Results
The inner surface of the murine retina is densely populated with astrocytes at P7. From P8 to P10 during the hyperoxic phase of the OIR model, astrocytic density strongly decreases in the avascular zone due to apoptotic cell death compared to controls not exposed to 75% oxygen. The low astrocytic cell density persists throughout the hypoxic phase. An increase in cellular density to a normal level of 800 cells/mm² can only be observed in revascularized parts of the avascular zone.

Conclusions
Reporter mice expressing nuclear GFP in retinal astrocytes can be used to examine the kinetics of astrocytes during physiological angiogenesis and the OIR model on a quantitative level. Our study found a strong decrease of astrocytic cell density in the avascular zone during the hyperoxic phase, as opposed to earlier studies describing loss of astrocytic Gfap expression only during the hypoxic phase. These results indicate that astrocyte damage might occur early in proliferative retinopathy, even before the onset of retinal hypoxia. In turn, in the OIR model, interventional treatment to protect retinal astrocytes has to take place before the hyperoxic phase.

Differential expression patterns of semaphorin 3F in the murine and human retina and its role in choroidal neovascularization

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Purpose
Semaphorines (Sema) play an important role in neuronal guidance and as inhibitory mediators for vascular development. We investigated retinal expression of Sema 3F during normal retinal development in mice and in the adult human retina. The influence of Sema 3F on choroidal neovascularization was investigated in vitro.

Methods
Eyes from wild type C57BL/6 mice were analyzed using immunohistochemistry, laser capture microdissection, qPCR and Western Blot for Sema3F at postnatal day P3, P17 and P60. Immunohistochemistry for Sema3 F was performed in adult human retinas. For functional in vitro analysis, choroidal explants (CE) from wild type C57BL/6 mice were placed in collagen matrix and stimulated with VEGF 165.

Results
qPCR expression analysis following laser capture microdissection revealed that Sema3F was mainly expressed in photoreceptors and retinal pigment epithelium (RPE). Western Blot analysis confirmed strong Sema3F expression in RPE. This expression pattern was confirmed by Immunohistochemistry where robust staining of Sema3F localized to the photoreceptor/RPE interface. These expression patterns were stable at all timepoints investigated in mice and were confirmed by immunohistochemistry in adult human retinas. Consistent with a role in outer retinal homeostasis, Sema 3F significantly reduced angiogenic sprouting in VEGF-stimulated CE ($p = 0.01$).

Conclusions
Our data suggests that deregulation in Sema3F levels may play a role in diseases affecting the outer retina, e.g. age-related macular degeneration. The reduced angiogenic sprouting in Sema3F- stimulated CE suggests that Sema3F may play a role in maintaining the physiologic avascularity of the outer retina.

Calcium waves and calcium-dependent activation of rhodopsin gene transcription in cultured retinal precursors cells of chicken embryo

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Purpose
A striking feature of photoreceptor differentiation in rodent and avian retinas is the 8-10 days lag between the exit from mitosis and the expression of opsin genes. In the embryonic chicken retina, photoreceptor precursors exit mitosis around embryonic day 6 (E6) and they immediately express visinin (a photoreceptor-specific calcium-binding protein), suggesting they are rapidly committed to a photoreceptor fate. Yet, opsin gene expression cannot be detected before E14 (LWS opsin) or even E16 (rhodopsin). It is not known whether this time lag reflects the implementation of a differentiation program acquired at the exit from mitosis, or if it reflects a standby state of the committed precursors, waiting for a signal to activate opsin gene transcription.

Methods
Chicken retinal precursor cells were placed in culture either at E8 or at E13. Transcriptional activation of the rhodopsin gene in the cultured cells was monitored by real-time RT-PCR and by rhodopsin promoter-driven luciferase activity from a transiently transfected plasmid. Oscillations in Ca^{2+} concentration in cultured retinal precursors were monitored by Fluo8 fluorescence on a spinning disc laser microscope. Cell cultures were treated with drugs that modify calcium signalling and pacemaker channel activity.

Results
In retinal precursors isolated at E8, rhodopsin gene transcription was not spontaneously activated during a 4-day culture, but was efficiently stimulated by membrane depolarization (KCl or veratridine). Retinal precursors isolated at E13 spontaneously activated rhodopsin gene transcription in 4-day cultures. This spontaneous expression of the rhodopsin gene was efficiently blocked by calmodulin-dependent kinase II inhibitors (KN62 and KN93) and by general and T type-selective Ca^{2+} channel blockers (Cd^{2+}, Ni^{2+}, bepridil, mibefradil, ML218). In contrast, Ca^{2+} channel blockers of other selectivities had no effect (cilnidipine, nifedipine, ω-conotoxin GVIA, ω-agatoxin IVA, SNX482). Inhibition by L-cis-diltiazem suggested a contribution of CNG channels. The primary event in Ca^{2+}-dependent activation of rhodopsin gene transcription could be the pacemaker activity of HCN channels because it was blocked by ZD7288. Correlatively, Ca^{2+} oscillations were observed in retinal precursors cultured at E13 and were inhibited by ZD7288.

Conclusions
Rhodopsin gene expression in cultured retinal precursors is activated by Ca^{2+} signalling. The oscillations in Ca^{2+} fluorescence are reminiscent of the "retinal waves" previously described in immature rodent and avian retinas. Destabilization of the membrane potential by an HCN channel may be the origin of the Ca^{2+} signalling cascade that activates rhodopsin gene transcription. It is not known at this point whether the entire cascade is cell-autonomous or if it requires intercellular communication.

P23H-rhodopsin causes ER stress and cell death in a model mouse of retinal degeneration

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Purpose
Retinitis pigmentosa (RP) is a group of inherited neurodegenerative diseases that result in selective cell death of retinal photoreceptors. Most of the genes affected are genes specific for the function, structure or metabolism of rods. Indeed, usually the mutations first lead to degeneration of the rod photoreceptor cells followed by a mutation-independent secondary degeneration of cone photoreceptors. Mutations in the Rhodopsin gene account for about 10% of all cases of RP. In particular, one of the most common rhodopsin mutations in USA produces a proline-to-histidine change at codon 23 (P23H). This mutation causes misfolding and retention in the endoplasmic reticulum (ER), leading to ER stress and activation of unfolded protein response (UPR). The UPR signalling pathway re-establishes homeostasis but if the ER stress persists it eventually leads to apoptosis. In this study, we characterized activation of the three ER pathways during rod cell death in the P23H transgenic retina. Elucidating mechanisms of rod cell death is important because at the moment there are no treatments available for these diseases.

Methods
We studied the P23H transgenic mouse model by immunohistochemistry on retinal sections from mutant and wt mice. We evaluated the expression and the cleavage of caspase-3, caspase-7 and 12 through Real Time qPCR and Western blot. We also analyzed Ire1, Perk and Eif2α phosphorylation and nuclear translocation of Xbp1, Atf6 and Atf4 as well as calpain activity. We also performed in vivo treatments with calpastatin, a calpain inhibitor to correlate the activity of calpain with cell death, caspases inhibitor, zVAD, and with salubrinal, an inhibitor of eIF2α phosphatase, to evaluate the influence of ER stress on apoptosis.

Results
Using Real Time qPCR and Western blot, we showed a significant increase in transcription and cleavage of caspase-7, caspase-12 and caspase-3 in retinas of P23H mice compared to wild type. Increased calpain activity can also be observed in the P23H transgenic mice. There is, however, only a partial correlation between calpain positive cells and cell death detected by TUNEL. Otherwise we observed a strong correlation of cell death with the three ER stress pathways that can activate caspases. To define the correlation between ER stress and photoreceptor cell death we treated P23H transgenic mice by intraperitoneal injection with salubrinal to conteract ER stress and we found a protective effect for cell death.

Conclusions
Here we demonstrated the activation of ER stress in P23H mutant photoreceptors and its role in activation of the caspase cascade. Contrary to other model of Retinitis Pigmentosa, we observed a key role of caspases and not of calpains even if both proteases are activated. Therefore, this study highlights pathways that, in the future, could be targets for gene therapy or pharmacological treatments for these diseases.

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Hypothermia protects retinal ganglion cells against ischemia induced cell death

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Purpose
Hypothermia has been shown to be neuroprotective in the therapy of ischemic stroke. Furthermore the retina is easily accessible for inducing hypothermia in an acute onset of ischemia like during central retinal artery occlusion. By using a cooled irrigation solution during pars plana vitrectomy the retina could be easily cooled down for a certain time span. The best temperature for cooling the retina is so far unknown.

Methods
To investigate potential neuroprotective therapies like hypothermia we developed an easy-to use chamber for 6-well plates with inserts for organotypic cultures (see Poster of Blak M). To determine the optimal neuroprotective temperature we incubated retinas at 20, 30 and 37°C for 75 minutes under ischemic conditions. For hypothermia testing the chambers were cooled to the aspired temperature for 4h, then transferred to an incubator at 37°C in an environment containing 5% CO2 for 44h. For comparison other organotypic cultures were treated with 1mM glutamate instead of ischemia but underwent the same hypothermia protocol. To analyze the amount of RGCs and apoptotic RGCs, the retinas were frozen and processed for cutting. RGCs immunohistology was performed with a Brn3a-antibody. Apoptotic cells were visualized via TUNEL-staining and overall cell amount via DAPI -staining. Cells were counted manually.

Results
Quantification of the cells after 48h of cultivation showed that hypothermia with 20°C or 30°C increased the number of RGCs with ischemic treatment from 13.4% to 23.6% and 22.3% RGCs and with glutamate treatment from 14.4% to 26.6% and 28.6% RGCs. In case of ischemia hypothermia decreased the number of apoptotic RGCs from 15.4% to 3.7% and 1.4%. In the whole mounts treated with glutamate more apoptotic cells were detected in cultures after hypothermia (1.9% and 2.6% vs. 0.0% apoptotic RGCs).

Conclusions
In conclusion, we successfully tested a first neuroprotective treatment in a new established organotypic culture model for retinal ischemia. Hypothermia rescued RGCs from cell death in both models. The reduction of the temperature to 20°C or 30°C did not cause any significant differences. Therefore, a reduction from 37°C to 30°C is sufficient to induce a neuroprotective effect. Through the detection of apoptotic RGCs in glutamate treated retinas after hypothermia it might be concluded, that hypothermia slows down the death of RGCs offering possibilities for further neuroprotective treatments. Therefore, by a cooling the retina during pars plana vitrectomy the tolerance time against ischemia or glutamate stress could be increased.

Retinal Degeneration in the Royal College of Surgeons Rat – A Study on Electrophysiological Properties

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Purpose
Retinitis pigmentosa is a neurodegenerative disease that is designated by the degeneration of photoreceptors, finally leading to blindness. Analysis of animal models with a genetically caused retinal degeneration, e.g., the retinal dystrophic Royal College of Surgeons (RCS) rat, showed that photoreceptor degeneration induces a remodeling process of remaining neurons. As an effect of this process, the spontaneous spiking activity of retinal ganglion cells (GC) changes. In this study we analyzed in vitro the spontaneous firing activity of GCs in the RCS rat in a time-dependent manner and compared the results with recordings of coeval wild-type control animals

Methods
Retinae of RCS rats between postnatal months 1 to 5 were analyzed. As control the activity of isolated wild-type Wistar rat retinae was recorded. For detection of GC activity, the isolated whole mount retinae were placed on 8x8 three dimensional multi electrode arrays (3D MEAs). After transfer, the retinae were permanently perfused with carbogen (95% O2, 5% CO2) bubbled Ames Medium for at least 30 minutes. For spike detection, raw data of 3 to 5 minutes continuous recording were evaluated with a 300 Hz high pass filter. To distinguish different spike waveforms recorded from one electrode, spikes were sorted computer-based as well as manually with spike-sorting software before statistical analysis was done.

Results
The spiking activity of the RCS ganglion cells changed during different time-points of retinal degeneration. Within the first month, the firing rate was similar to the activity of the respective wild-type ganglion cells. In GCs of 3 months and older RCS rats the spontaneous mean spiking frequency was significantly lower than in the first month and in the respective control GCs. Our results revealed a decrease in the spiking rate over age in the RCS rat, which was in contrast to the control.

Conclusions
The decrease in spiking activity during retinal degeneration in RCS rats showed that the photoreceptor loss leads to changes of spontaneous spiking activity in retinal GCs. Furthermore, the changes in RCS GCs activity depended on the stage of retinal degeneration. Thus, the decreased spontaneous spiking frequency is potentially caused by changes of the synaptic plasticity in the inner retina. In further experiments we will analyze the effects of retinal remodeling after various voltage and current stimuli plus effects of synaptic blockers on electrophysiological properties.

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Effect of Jimpy Mutation on Retinal Structure and Function

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Purpose
The Jimpy mutant mouse has a point mutation in the proteolipid protein (PLP) gene, rendering a non-functional PLP protein resulting oligodendrocyte death. This results in myelin destruction and failure to produce adequately myelinated neurons in the central nervous system (CNS) [1,2].The physiological process of myelin formation has been a subject of intense investigation to understand the pathological process of demyelination diseases [3]. However, the effect of demyelination on neuronal functioning and development has been overlooked. Hence, it is not known how the absence of normal myelination during development influences the functioning of neurons. For this reason we characterized the Jimpy mouse retina in order to find out if lack of myelination during development has an effect on normal functioning and morphology of the retina.

Methods
We have used P20 to P22 day old male Jimpy mutant and wild type mice. We performed morphological (Electron microscopy) and histological measurements, RT-PCR, MEA recordings, optokinetic reflex measurements.

Results
In the optic nerve, plp mRNA was detected (RT-PCR) in wild-type and Jimpy mouse, but PLP antibody staining was negative in the Jimpy mouse. However, neither axon count nor axon morphology were significantly different (EM micrographs). In the retina, there was no evidence for PLP expression on the protein or mRNA level. Nevertheless, cell body sizes in the inner nuclear layer were on average bigger, and the number of rod bipolar cells, horizontal cells and astrocytes was increased in Jimpy mice. The overall thickness of the retinal layers and the number of cells in outer nuclear layer and inner nuclear layer were unchanged. Physiological recordings using microelectrode arrays didn't show any significant change of ganglion cell response properties. Optokinetic reflex measurements indicate that the visual system of Jimpy mice is functional.

Conclusions
In conclusion, the Jimpy mutation has moderate influence on retinal morphology and function, although it severely affects phenotype and CNS myelination of the mouse.

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Using artificial neural networks to model long-term adaptation to changes in wavefront aberrations

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Purpose
The project aims to build upon existing experience in the team in developing customized models of visual acuity and it dependence on retinal image quality using artificial neural networks. Each artificial neural network is trained based on a set of single valued metrics of retinal image quality and the corresponding responses of an individual to a visual acuity task. In this manner the network is trained to respond to blur as the individual examined during training. Such artificial neural networks model the particular individual's immediate response to blur. A number of networks will be trained for subjects undergoing an acute change in their optical system (e.g. LASIK patients). The subject's response post-operatively (for example 1 year after the procedure) will be compared to the same subjects' response as modeled by their neural network "record" created based on their responses in the past.

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DYNAMO: Molecular dynamics of membrane-associated protein complexes

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Dynamics of multifunctional protein complexes originate from signal-dependent adaptations that are materialized as structural alterations, post-translational modifcations, proteolytic cleavages, protein translocation and inter-exchange in binding partners. Dynamic changes in a given protein complex are connected to protein regulatory networks and result in systemic and integrated quantitative signal outputs. Towards systemic analysis of macromolecular membrane-associated protein complexes, the DYNAMO consortium will further develop methodology and mathematical models that allow quantitatively defining composition, molecular dynamics and functional properties of protein complexes and relating these qualities to higher order biological activities within cells.

The aim of this consortium is a systems biology approach to analyze dynamics of membrane-associated protein complexes. Those protein complexes usually execute their function by transporting either a signal or a molecule. Two different model systems covering these different dynamic aspects will be studied:

1. The light sensitive G-protein coupled receptor rhodopsin – transducing light to protein networks
2. The transport of proteins across membranes: Protein translocation through mitochondrial protein import

Fly safe – Plan ahead. The visual angle over which bumblebees measure optic flow for centring.

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Purpose
Most flying insects are highly aerobatic and elegantly navigate through open as well as highly complex environments. Information extracted from the pattern of image motion generated on the retina during flight (known as optic flow), is crucial for many flight control behaviours. When flying through confined spaces, such as along narrow corridors, honeybees and bumblebees avoid collisions by keeping an equal distance to the nearby surfaces, a behaviour known as the 'centring' response. This is achieved by balancing the rate of optic flow experienced in each eye. However, it remains unclear how this centring strategy is affected when the distance to nearby surfaces changes and how early these insects are able to detect and respond to such changes.

The distance at which a change in the proximity of the environment can first be detected depends upon the minimum viewing angle (the angular deviation from the frontal direction of the longitudinal axis of the insect) at which optic flow is measured. The greater this angle, the later changes in the environment will be detected and the greater the risk of collision.

Methods
To estimate the minimum viewing angle at which bumblebees measure optic flow for centring, we recorded the flight trajectories of bumblebees flying along a corridor where the apparent proximity of the walls changes half way along. This was achieved by abruptly reducing (axial stripes) or increasing (chequerboard) the optic flow cues on the walls. We analysed the lateral position of the bees in the tunnel to determine when they reacted to this change.

Results
Our results suggest that the bumblebees' minimal viewing angle over which they measure optic flow for centring is quite low, allowing the bumblebees to change their flight path well ahead before encountering changes in the apparent proximity of the environment.

Conclusions
The biological significance of being able to detect changes in the proximity of the environment early is clear; it allows the bumblebees to change their flight path in response to approaching obstacles, enabling them to safely avoid collisions, even in cluttered environments.

Disease modeling of retinal distrophies using iPS cells

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Purpose
Retinal distrophies are genetically higlhy heterogeneous diaseases with largely unknown molecular mechanism of pathogenicity. The existing animal models do not reproduce the diasese faithfuly and in addition, the genetic backgroung is known to inlfuence largely the disease clinical outcome. The ability to develop disease models in vitro which are disease specific and patient specific will enable to get insight of the disease mechanism and also screen potential therapeutic agents.

Methods
Skin punch biopsies for LCA and RP patients with known mutations have been obtained from Dr. C. Ayuso (FJD; Madrid) and reprogrammed in induced pluripotent cells from by nonintegrative approach using Sendai virus. The iPS cells have been characterizad for their pluripotency by immunocytochemistry, and ability to differentiate into all three germ layers. The iPS cells have been subjected to differentiation protocol to obtain RPE and fotoreceptor progenitors.

Results
IPS cells were successfully generated for LCA (Cys89 Arg) patient and RP (Ser 331fs) patients and characterized for pluripotency and differentiation capacity. These cells are submitted to differentiation protocol using DKK-1 and Lefty A molecules and spontaneous differentiation to obtain RPE and fotoreceptors.

Conclusions
We have successfully generated iPS cells from LCA and RP patients and an unaffected patient. After subjecting these cells to different characterization protocols the obtained progenitors will be extensively phenotyped for both RPE and photoreceptor marker expression and functional characterization. The differences with the normal cells will allow the identification of molecular pathways affected by the disease.

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Dissection of functional modules associated with retinal ciliopathies by affinity proteomics

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Purpose
Leber Congenital Amaurosis (LCA) is an inherited retinal degenerative disease characterized by severe loss of vision at birth. It occurs in 2 to 3 per 100.000 newborns and is one of the most common causes of blindness in children. LCA is usually inherited in an autosomal recessive manner and is genetically heterogeneous. Twenty genes have been identified to be associated with LCA to date (according to RetNet, last update 4th Feb 2013), and approximately 70% of the LCA cases can be explained by a mutation in any of these genes. Most LCA candidate genes encode proteins with a wide range of retinal functions, including photoreceptor development, phototransduction, retinoid metabolism, ciliary transport, guanine synthesis, and outer segment phagocytosis by the retinal pigment epithelium. One of the genes which function has remained a mystery is Spata7, encoding a spermatogenesis associated 7 protein

Methods
In order to unveil the functional module in which Spata7 participates, which may be dysfunctional upon genetic mutation, we have taken an affinity proteomics approach. We have used tandem affinity purification in HEK293T cells followed by mass spectrometry to identify the Spata7 interactome, and yeast two-hybrid screening to identify binary protein-protein interactions. Results were validated by biochemistry and immunohistochemistry

Results
Protein-protein interaction study using tandem affinity purification approach suggested that Spata7 might be involved in the intracellular trafficking of protein cargos toward cilium via its links to dynein motor complex components. Our first localization data showed that fluorescent-tagged Spata7 associated with cytoskeleton microtubule structure and was enriched at the basal body of cilium, a microtubule-based organelle protruding from cell's surface, in hTERT-RPE cells. To further validate these results, a knock-down experiment will be performed and transport of specific interactors of Spata7 will be studied

Conclusions
Spata7 is a ciliary protein, most likely involved in transport towards the ciliary base. Dysfunction of such transport may explain the early-onset detrimental effects in the retina of the gene mutations associated with LCA

Acknowledgement
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The role of Properdin in the development and progression of age-related macular degeneration

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Purpose
Age-related macular degeneration (AMD) is the leading cause for irreversible vision loss in the aging population in industrialized countries. The growing population of elderly emphasizes the need for effective treatment options and the development of preventive programs. Currently, monoclonal anti-VEGF antibody therapy slows down neovascular age-related macular degeneration, a late complication in about 10-15% of AMD patients. Importantly, there is no effective therapy option for atrophic AMD, the most common phenotype with slow bilateral progression of the disease. The various phenotypes of AMD result from contributions of genetic, environmental and aging factors. Complement factor H, a known risk-associated genetic factor in AMD, is a negative regulator of the alternative complement pathway while Properdin is the sole positive regulator.

The aim of the present study is to establish Properdin detection systems from human and murine samples and to investigate the functional role of Properdin in AMD.

Methods
BALB/c mice were immunized either with purified human Properdin or Properdin-specific sequences in virus-like particles to generate highly specific and affine monoclonal antibodies (mAb) against Properdin. Different immunological methods ELISA, Western Blot analysis and intracellular FACS staining were applied to characterize specific hybridoma clones. Commercially available and in house monoclonal antibodies were used for sandwich immunoassays to detect Properdin from buffer and serum samples.

Results
Newly generated monoclonal anti-Properdin antibodies mAb149 and mAb1340 detect highly sensitive native Properdin in an indirect ELISA format. Validation of sandwich ELISA assays show a specific detection of human Properdin from serum with a limit of detection in the lower ng/mL range. Consequently, we compared Properdin concentrations in serum samples from AMD patients and a matched control group. Murine Properdin was not detected from serum samples with the methods used for human specimen.

Conclusions
These initial results encourage further investigation into the functional role of Properdin in the development and progression of age-related macular degeneration. Further attempts are necessary to generate anti-Properdin antibodies against human and murine Properdin, which then can be used in AMD animal models and humans in parallel.

A novel compact optical instrument for the clinical measurement of intraocular light scattering

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Purpose
To develop a compact instrument to measure light scattering in the human eye for angles in the range between 3 and 9 degrees of visual angle.

Methods
The instrument is based on a previous laboratory setup using extended light sources in a double- pass (DP) configuration (Ginis et al., J of Vision, 2012). The light source is an array of green (528±10 nm) light emitting diodes (LEDs) spatially homogenized by light shaping diffusers. The field is separated in 2 zones: a central area (corresponding to visual angles from 0 to 3 degrees (in radius) and an annular area (3 to 9 degrees). In both zones LEDs are square-wave temporally modulated at 483 Hz and 769 Hz for the central and peripheral areas respectively. Two annular diaphragms conjugated with the cornea and the lens allow the projection of the source on to the retina while leaving the central part of the pupil free of back scattered light and reflections. Light reflected from the fundus is sensed through a circular diaphragm conjugated with the center of the pupil with no overlapping of the illumination and measurement paths. A pupil camera controls the alignment. The light reflected from the central retinal area (15-arcmin) is selected through a circular diaphragm and a pinhole by a photodiode. The Fourier transform of the signal reveals the contribution of each annulus and therefore the average intensity of scattered light for the corresponding angles. The total measurement time is 200 msec.

Results
Functionality, sensitivity and repeatability of the method were demonstrated with an artificial eye and two different previously characterized diffusers. The equivalent logarithm of the straylight parameter measured for the diffusers were 0.67 (SD=0.005) and 0.84 (SD=0.003), values not statistically significantly different than the anticipated. Pilot measurements in human eyes were also obtained. A careful analysis of the artifacts associated to alignment and refractive errors was also performed.

Conclusions
A new compact instrument suitable for routine or clinical measurements of light scattering in the eye was developed. It builds on previous experience with multi-wavelength, high-sensitivity, imaging double-pass system for the measurement the wide-angle point-spread function of the eye.

Statement on proprietary interests
Patent; Ginis, Harilaos S.; Perez, Guillermo M.; Bueno, Juan M.; Artal, Pablo

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Mice with a D190N mutation in the gene encoding rhodopsin: a model for human autosomal dominant Retinitis Pigmentosa

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Purpose
Rhodopsin initiates signal transduction in rod photoreceptors upon the arrival of the photon. D190N, a missense mutation this protein, causes adRP in humans. Affected patients present hyperfluorescent retinal rings and progressive rod photoreceptor degeneration. Studies in humans cannot reveal the molecular processes causing the earliest stages of the condition, thus necessitating the creation of an appropriate animal model. A knock-in mouse model with the D190N mutation was engineered to study the pathogenesis of the disease. The animal was then validated as an appropriate model by performing fundus autofluorescence (FAF), electroretinogram (ERG), and histology studies. D190N cell death pathway was studied by crossing these animals with others which molecular pathway is well known: GCAP deficiency and rd12 (RPE65) mice.

Methods
Three adRP patients with a D190N mutation in gene encoding rhodopsin were analyzed by FAF, ERG, and SD-OCT. D190N knock-in animals containing the D190N rhodopsin were generated. D190N/+ animals were the objects of FAF and ERGs studies, which were performed in the very same way as in humans. Paraffin sections were prepared and hematoxilin/eosin staining was conducted to visualize the thickness of the retina and compare it to the SD-OCTs in humans. Rhodopsin localization was studied by immuno-histochemistry and western blot. D190N/GCAP and D190N/RPE65 animals were generated by crossing. FAF, ERGs, immunohistochemistry, and blotting analysis were also performed in these animals.

Results
Affected humans and mice revealed higher fundus autofluorescence. At post-natal day (P) 21, scotopic a- wave was diminished in D190N/+ animals at different stimulus intensities: -1.2 log cd/m2: -62.3 µV (wt) vs. -25.7 µV (mutant), p= 0.007; 0 log cd/m2: -135.4 µV (wt) vs. -75.6 µV (mutant), p= 0.013; -0.9 log cd/m2: -127.8 µV (wt) vs. -75.6 µV (mutant), p= 0.022. At this age, scotopic b-waves did not show significant changes. At P100, the differences were more pronounced, and the scotopic b-wave amplitudes in D190N mouse were lower than in control, especially at dimmer intensities: -3.2 log cd/m2: 136.5 µV vs. 50.9 µV, p= 0.015; -2.4 log cd/m2: 224.65 µV vs. 91.31 µV, p= 0.018; -1.6 log cd/m2: 270.9 µV vs. 141.9 µV, p= 0.042. Retinal sections of mutant animals presented with less nuclei of photoreceptors in the outer nuclear layer (ONL). Human patients also showed a thinning of the ONL. Immunohistochemistry showed correctly localized rhodopsin in the outer segments of d190n/+ animals at P21 and P210.

Conclusions
Electrophysiological and histological findings in the mouse were similar to those observed in human patients, and the hyper-fluorescence pattern was analogous to that seen in humans, confirming that the D190N mouse is an accurate model for the study of adRP. Experiments are being performed to figure out the mechanisms of cell death is this animal model, but observing the proper localization of rhodopsin, a trafficking problem might be excluded.

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Cellular Reprogramming – An approach to drive cells towards retinal cell fate

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Purpose
Our goal is to reprogram mouse embryonic fibroblasts (MEF) into retinal progenitor cells. For that purpose we want to use eye field transcription factors (EFTF). They are known to be necessary and in some cases sufficient to induce ectopic eye development. We see the ex vivo generation of retinal progenitors as a promising step towards providing retinal cells to develop new strategies to study retinal disease pathomechanisms and treat retinal diseases.

Cell reprogramming using a defined set of transcription factors has been shown to be able to induce pluripotent stem cells from fibroblasts. Further studies investigated the possibility to reprogram fibroblasts into any desired cell type by finding the right factor combination and culture condition. This has been shown to be possible for some cell types as for example for neural stem cells or cardiomyocytes, but so far it was not achieved for retinal progenitor cells.

Methods
As gene transfer system we use a replication-incompetent vector system based on the human immunodeficiency virus type 1 (HIV-1), which employs the modulated envelope protein of the vesicular stomatitis virus (VSV-G) for pseudotyping. Single EFTF were cloned into the transfer vector, which also encodes for an EGFP reporter gene. Generation of the viral particles and infection was done as previously reported by the Lindemann group. Mouse embryonic fibroblasts (MEF) were isolated from E13.5 mouse embryos, propagated in culture and virus infected. Three days past infection the medium was changed to media for embryonic retina culture conditions. MEF were cultured for 20 days and analyzed with fluorescent microscopy and reverse transcriptase-PCR.

Results
We designed a screening strategy to investigate cell reprogramming of MEF into retinal progeny by defined combinations of transcription factors. To facilitate the screening for reprogrammed retinal progenitors we generated MEF from transgenic reporter mice (Chx10-Cre::Rosa26-AI9 mice). Chx10 is a transcription factor expressed at the onset of neurogenesis in retinal progenitors. Induction of AI9, a red fluorescent protein, will be the first read out to find successful reprogrammed cells. Further, we cloned the desired reprogramming factors into the p6Nst50 transfer vector and generated infectious retroviral particles. The infection efficiency of the particles transferring the transcription factors is similar to the control virus, which yields up to 85%. The eye field transcription factors in the infected cells are expressed, which we confirmed on mRNA and protein level.

To test, whether our exogenous genes are functional, we overexpressed Otx2 in mouse embryonic retinal progenitor cells in culture. Otx2 is known to be able to drive cells towards rod fate. And indeed, Otx2 overexpression led to an upregulation of recoverin positive cells, proving its functionality.

Conclusions
Since we now have established our reprogramming method, as a next step, we want to screen combinations of factors to identify effective ones to generate retinal progeny. To further characterize the resulting cells we defined several levels of analysis including a RT-PCR screen, gene array analysis and cell differentiation analysis to investigate the potential induction of progenitor cells.

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Role of the voltage-gated calcium channel subunit α2δ-3 in retinal function

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Purpose
Little is known about the role of the auxiliary α2δ subunits of voltage-gated calcium channels (VGCC) in the retina. Only α2δ 4 has yet been conclusively shown to be involved in a specific retinal function at ribbon synapses of photoreceptors and bipolar cells. In a knockout mouse model of α2δ-3, we investigate the role of this subunit in retinal processing.

Methods
In the mouse model used, α2δ-3 was knocked out by insertion of LacZ. The cell types expressing α2δ-3 were determined by colocalizing LacZ expression with immunohistochemical markers of cell types. We assessed retinal morphology by whole-mount immunostainings of horizontal cells (HC) and quantitative analysis of the HC mosaic. We performed extracellular recordings from ganglion cells using microelectrode arrays (MEA), employing a set of visual stimuli to probe retinal processing.

Results
Strong expression of α2δ-3 could be localized to horizontal cells, but we found no change to the horizontal cell mosaic in α2δ-3 knockout mice. In our MEA recordings we found subtle differences in responses to contrast-modulated Gaussian white noise full-field flicker. While firing rates of ganglion cells at low-contrast flicker was unchanged, firing rates at high-contrast flicker were lower for ganglion cells in the α2δ-3 knockout.

Conclusions
The auxiliary VGCC subunit α2δ appears to be expressed specifically in certain subsets of retinal cells, as illustrated here for α2δ-3. The expression of α2δ-3 within horizontal cells was remarkably strong, yet there was no deleterious effect of the α2δ-3 knockout on the horizontal cell mosaic. However, the change in firing rates to the high-contrast but not the low-contrast white noise stimulus that we found in our MEA recordings suggests that α2δ-3 might affect contrast adaptation.

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Electrophysiological investigation of the regenerative state of RGCs using high-density microelectrode arrays

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Purpose
Optic nerve injury leads to degeneration of the retinal ganglion cells (RGCs) and subsequent to visual loss. Apart from the morphological changes after nerve injury, remaining RGCs also change their physiological properties (Menzler, 2011, PhD Thesis, LMU München).

To overcome the destructive processes of nerve injury, experimental approaches have been developed to transform RGCs into an active regenerative state allowing these neurons to survive nerve injury and to regenerate axons into the injured optic nerve. Findings from morphological and molecular studies suggest Pam3Cys as suitable regeneration inducer (Hauk et al., 2010, IOVS). Here we investigated the regenerative state of RGCs in an electrophysiological manner using microelectrode arrays. We adressed the question wether Pam3Cys is sufficient to restore the functionality of a neuron after nerve injury.

Methods
Extracellular recordings were performed with two different types of sensor arrays – the MEA-256 and the Neurochip. The Neurochip is a high-density multitransistor array consisting of 16384 sensor pixels in an area of 1 mm^2 allowing recordings on a sub-cellular level.

RGC activity was recorded from ex vivo retinae of SD rats. The retinae were extracted from rats which either received unilateral axotomy of the optic nerve or unilateral axotomy and additional Pam3Cys injection into the corresponding eye ball. To analyse the light response latency full field flashes of 1 Hz flicker frequency were presented during recording.

Results
Retinal ganglion cells in axotomized retinae showed a reduced maintained activity, decreased axonal conduction velocities and increased light response latencies as compared to control RGCs. Pam3Cys treatment of the retina resulted in elevated axonal conduction velocities not only compared to the RGCs in axotomized retinae but also to RGCs in control retinae. Apart from that axotomized RGCs, which were treated with Pam3Cys respond faster to light stimuli than without appropriate treatment. On the other hand, the maintained RGC activity is not affected by Pam3Cys injection.

Conclusions
The results of the present study show that Pam3Cys is capable to preserve certain physiological properties of the RGCs after nerve injury. This is the first functional evidence supporting the hypothesis of Hauk et al. (2010) that Pam3Cys acts as a possible regeneration inducer in the CNS.

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Human retina tissue culture – towards an optogenetic treatment of blindness

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Purpose
There are no existing treatments for blindness caused by retinal degeneration. Animal studies suggest that optogenetic approaches (i.e., expression of light-sensitive proteins in retinal neurons) hold a lot of promise to restore light sensitivity to the retina and therefore vision to the affected individual. Here, we established tissue culture conditions with the goal to evaluate optogenetic approaches in the human retina in-vitro. This is an important step towards the translation of this promising new treatment strategy for blindness.

Methods
We cultured retina from three different species: mouse, pig and human. Human retina was donated by patients that had to have an enucleation. We evaluated the degree to which culture conditions preserved the state of the retina by recording light-evoked and spontaneous ganglion cell activity on multi-electrode arrays (MEA).

Results
Mouse and pig retina that was brought into culture immediately after enucleation, stayed light responsive for at least 48h (mouse) and 72h (pig), and showed spontaneous ganglion cell activity for at least 120h (pig). Human retina showed spontaneous ganglion cell activity after at least 48h in culture.

Conclusions
MEA date from mouse, pig and human retina suggest that our culture condition maintain the retina in healthy physiological state for at least 48 hours. This time period should be sufficient for lentivirus-mediated plasmid expression after transfection, such that optogenetic approaches can be assessed in this in-vitro system.

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Characterization of Light Lesion Paradigms and Optical Coherence Tomography as Tools to study Adult Retina Regeneration in Zebrafish

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Purpose
Light-induced lesions are a powerful tool to study the amazing ability of photoreceptors to regenerate in the adult zebrafish retina. However, the specificity of the lesion towards photoreceptors or regional differences within the retina are still incompletely understood.

Methods
We therefore characterized the process of degeneration and regeneration in an established paradigm, using intense white light from a fluorescence lamp on swimming fish ([1], diffuse light lesion). We also designed a new light lesion paradigm where light is focused through a microscope onto the retina of an immobilized fish (focused light lesion). Focused light lesion has the advantage of creating a locally restricted area of damage, with the additional benefit of an untreated control eye in the same animal.

Results
In both paradigms, cell death is observed as an immediate early response, and proliferation is initiated around 2 days post lesion (dpl), peaking at 3 dpl. We furthermore find that cones are more sensitive than rods towards intense bright light. Thus, the cone-depleted area exceeds the size of the rod-depleted area. We also observed specific differences within light lesioned areas with respect to the process of photoreceptor degeneration: UV cone debris is removed later than any other type of photoreceptor in light lesions. Apart from macrophages and Müller glia, also the retinal pigment epithelium seems to be involved in clearing cellular debris. Unspecific damage to retinal neurons occurs at the center of a focused light lesion territory, but not in the diffuse light lesion areas. We simulated the fish eye optical properties using software simulation, and show that the optical properties may explain the light lesion patterns that we observe. Furthermore, as a new tool to study retinal degeneration and regeneration in individual fish in vivo, we use spectral domain optical coherence tomography (OCT).

Conclusions
Collectively, the light lesion and imaging assays described here represent powerful tools for studying degeneration and regeneration processes in the adult zebrafish retina.

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The Bardet Biedl syndrome mouse model Bbs12-/- exhibiting retinal degeneration phenotype: a promising model for eye gene therapy

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Purpose

Bardet–Biedl syndrome (BBS) is a genetically heterogeneous ciliopathy, with 17 different BBS genes identified to date. It is clinically characterized by retinitis pigmentosa, obesity, polydactyly and renal dysfunction. The classical clinical manifestations of the retinal degeneration are the early-onset attenuated electroretinograms responses, severe vision loss before adulthood and abnormal pigmentation in the fundus. In order to study the mechanism leading to this severe phenotype, we studied the cellular mechanism leading to the death of photoreceptor cells in our Bbs12-/- mouse model. We could put in evidence that BBS12 inactivation resulted in defective intraciliary transport of proteins such as Rhodopsin and Arrestin through the photoreceptors' connecting cilium. This resulted in a stressed endoplasmic reticulum ultimately activating a pro-apoptotic unfolded protein response characterized by the activation of the endoplasmic reticulum molecular chaperone Bip, the phosphorylation of eIF2α and the activation of Caspase12. Concurrently, the Bbs12-/- mice suffered from severe visual impairment evidenced by drastic decrease in electroretinogram recordings. Although we have been able to device and optimize a pharmacological treatment to slow down retinal degeneration, this therapeutic approach does not treat the cause of the disease.

Methods

In order to restore BBS12 expression in the photoreceptors of the Bbs12-/- mice, we will use an adeno-associated virus based approach to insert a working copy of Bbs12 in the photoreceptors. This approach should allow us validate the proof of essential concept before clinical tests.

Abstract
Poster Session

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