Young Researcher Vision Camp

An international Career building Symposium

June 25th-27th, 2010

Castle Wildenstein
Leibertingen
Germany

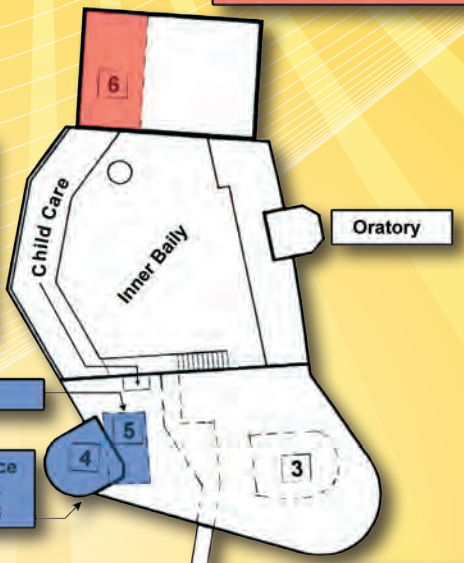
www.vision-camp.eu

Jugendherberge
88637 Burg Wildenstein

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

Lounges:

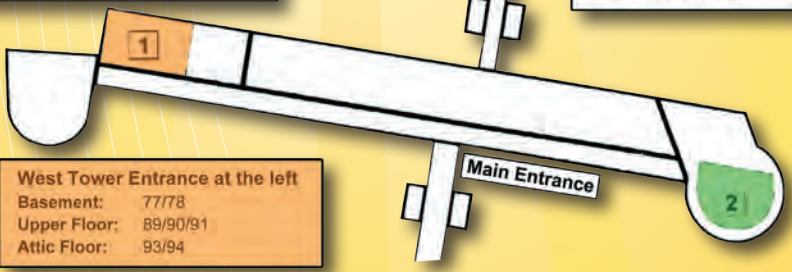
1 West Tower
2 East Tower
3 Alleyway Bastion: Casemate
4 Commander's Office: Former Chapel
5 Bastion: Common Hall
6 Manor House



Commander's Office
Upper Floor: 53/54
Attic Floor: 55/76

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

Bastion Attic Floor:
Main Lecture Hall



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

East Tower
1st Upper Floor: 63/64/65/66/67/68/69
2nd Upper Floor: 73/74
Attic Floor: 75/52

Contact:

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

www.leibertingen-wildenstein.jugendherberge-bw.de

Preamble

Dear Colleagues,
Dear Participants of the Young Researcher'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)

Christine Augustin
Manuela Baur
Michaela Bitzer
Sigrid Diether
Christian Grimm
Nikki Hafezi
Philipp Hunger
Rachel Metz
François Paquet-Durand
Thomas Schäberle
Lars Scheel

FRIDAY, JUNE 25TH, 2010

until 16:30	Arrival
18:00 - 19:00	**Keynote Lecture I "Stem Cell Research: Myth And Reality"** Dr. Dusko Ilic MD PhD - School of Biomedical and Health Sciences, Guy's Hospital, Great Maze Pond, London, UK
19:30 - open end	Open-air Barbecue (campfire outside the castle) courtesy supported by EVER www.ever.be

SATURDAY, JUNE 26TH, 2010

06:00 - 07:00	Early morning exercises
07:00	Breakfast
08:00 - 09:00	**SESSION I: "Function and Exploitation of Retinal microRNAs"** CHAIR: Arpad Palfi, Dublin, Ireland • "Data mining of miRNA expression from retinal gene expression microarray" Jun Yin, Bioinformatics Laboratory, Conway Institute, University College Dublin, Ireland • "MicroRNAs couple cell fate and developmental timing in retina" Sarah Decembrini, Developmental Biology Unit, Institute of Child Health, University College London, UK • "Regulation of microRNA expression in an in vitro model of retinal cell differentiation" Laura Harwood, Centre for Vision Science, Queen's University Belfast, UK • "Exploitation of microRNA-based gene repression in rhodopsin-linked Retinitis Pigmentosa" Claire Kilty, Smurfit Institute of Genetics, Trinity College Dublin, Ireland
09:00 - 10:00	**SESSION II: "Proteomics in Vision Research"** CHAIR: Stefanie Hauck, Munich, Germany • "Application of LC-MS/MS for microRNA Target Identification in Retinal Degeneration" Carol Loscher, Trinitiy College, Dublin, Ireland • "The retinal ciliopathy-associated protein homologs RPGRIP1 and RPGRIP1L are linked to (connecting) cilium dynamics through interaction with NEK4 serine/threonine kinase." Karlien Coene, Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands • "Differential protein expression in vitreous of autoimmune uveitis" Florian Hofmaier, Ludwig Maximilians University, Munich, Germany • "Identification of interaction networks relevant for retinal degeneration" Matteo Gorza, Helmholtz Zentrum München, Neuherberg, Germany
10:00 - 10:30	Coffee Break
10:30 - 11:30	**SESSION III "Clinical Trials in Vision Research"** CHAIR: Monika Fleckenstein, Bonn, Germany • "FAM-Study - a prospective longitudinal natural history study in AMD" Arno P. Göbel, University of Bonn, Germany • "Acceptance of offered cataract surgery in South India" David Kupitz, University of Bonn, Germany • "Vision mediated by a subretinal electronic implant" Katarina Stingl, Department of Ophthalmology Tuebingen, Germany • "The Offer of Cataract Surgery: Determinants of Acceptance following Outreach Eye Clinics in South India. Acceptance of offered cataract surgery in South India" Robert P. Finger, University of Bonn, Germany

11:30 - 12:30	**SESSION IV "Gene Therapy for Retinal Disease"** CHAIR: Naomi Chadderton, Dublin • "Evaluation of rAAV vectors for gene replacement therapy in a new canine model of Leber congenital amaurosis (LCA) : the RPGRIP1 deficient dachshund dog." Elsa Lheriteau, Nantes, France • "Protection of photoreceptor cells in a mouse model of RP10" Lawrence Tam, Smurfit Institute of Genetics, Trinity College Dublin, Ireland • "Restoration of cone vision in the CNGA3-/- mouse model of congenital complete lack of cone photoreceptor function" Stylianos Michalakis, University of Munich, Germany • "Delivery of anti-apoptotic agents to the degenerating retina by AAV-mediated modulation of the inner blood-retina barrier" Anh Thi Hong Nguyen, Smurfit Institute of Genetics, Trinity College Dublin, Ireland
12:30 - 14:00	Lunch
14:00 - 15:00	**SESSION V: "Nonmammalian Vision"** CHAIR: Edda Kastenhuber, Zuerich, Switzerland • "Expressional and functional analysis of mGluR6 in the zebrafish retina" Marion Haug, University of Zurich, Institute for Molecular Life Sciences, Zurich, Switzerland • "Touchdown: Visual Input Orchestrating Landings in Insects" Torill Kornfeldt, Lund University, Department of Cell and Organism Biology, Zoology, Lund, Sweden • "Extracellular recordings from polarization-sensitive interneurons in the central complex of the locust brain" Miklós Bech, Philipps University of Marburg, Department of Biology - Neurobiology/Ethology, Marburg, Germany • "Visual Behavior of Adult Zebrafish" Kaspar Müller, University of Zurich, Institute for Molecular Life Sciences, Zurich, Switzerland
15:10 - 15:40	**Roundtable discussion I** Career opportunities - Academia versus Industry Marius Ueffing - Department for Ophthalmology, Tuebingen Germany Arnd Carrette, Pharm-Allergan GmbH
15:50 - 16:20	**Roundtable discussion II** Research – Family - Career Anneke den Hollander - Radboud University Nijmegen Medical Centre, Department of Ophthalmology, The Netherlands
16:20 - 16:50	Coffee Break
16:50 - 17:20	**Roundtable discussion III** Career Development and Transferable Skills T. Wheeler-Schilling, S. Diether, M. Bitzer - Research Management Unit, Department for Ophthalmology, University Hospital Tuebingen
17:30 - 18:00	**Roundtable discussion IV** Postdoc time abroad – where to go? Per Ekstroem - Lund University, Lund, Sweden

SATURDAY, JUNE 26TH, 2010

18:15 - 19:00	**Keynote Lecture II "Vision of Innovation in Ophthalmologic Development"** Prof. Craig Smith, MD CSU Forum Chair Clinical Science Unit Head Ophthalmology Novartis Pharma AG
19:00 - 19:10	**Group Photo**
19:15 - open end	**Poster Session**
20:30 - open end	Music, dancing and buffet in the inner bailey

SUNDAY, JUNE 27TH, 2010

07:00 - 08:00	Early morning exercises
08:00	Breakfast
09:00 - 10:00	**SESSION VI "Cell Death Mechanisms in Inherited Retinal Degeneration"** CHAIR: François Paquet-Durand, Tuebingen, Germany • "The neuroprotective effect of retinal müller glial cell-derived neurotrophic factors in retinal neurons" Patricia del Rio Medina, Helmholtz Zentrum Munich, Neuherberg, Germany • "The effect of the administration of buthionine sulfoximine and glutathioneethyl ester in a model of retinitis pigmentosa." Violeta Sánchez Vallejo, University of Valencia, Spain • "The Role of PARP in Photoreceptor Cell Death" Ayse Sahaboglu, Institute of Ophthalmic Research, Tuebingen, Germany • "Epigenetic Markers in Retinal Degeneration" Pietro Farinelli, Lund University, Sweden
10:00 - 11:00	**SESSION VII: "Genes and mechanisms involved in myopia development"** CHAIR: Marita Feldkämper, Tuebingen, Germany • "The role of genetics in susceptibility to environmentally induced myopia" Paul Chen, Cardiff, UK • "Contrast adaptation and myopia" Arne Ohlendorf, Tuebingen, Germany • "Responses of Different Retinal Areas to Imposed Defocus in Chicks" Tudor Tepelus, Tuebingen, Germany • "Hormones in Myopia Development" Alexandra Marcha Penha, Tuebingen, Germany

11:00 - 11:30	Coffee Break
11:30 - 12:30	**SESSION VIII: "Axon glial interactions in the optic nerve"** CHAIR: Virginia Bay, Portsmouth, UK • "NMDA RECEPTORS and OPTIC NERVE INJURY" Tahani Huria, Cell Physiology and Pharmacology, University of Leicester, UK • "GSK3beta signalling and glial differentiation in the optic nerve" Andrea Rivera, Institute of Biomolecular and Biomedical Science, University of Portsmouth • "Impact of impaired oligodendrocyte differentiation on optic nerve function" Aiman Saab, Max Planck Institute of Experimental Medicine, Goettingen, Germany • "Assessing microstructural changes in the optic nerve in a spontaneously demyelinating mouse model using magnetic resonance imaging" Fredrik Gruenenfelder, Institute of Comparative Medicine, University of Glasgow, UK
12:30 - 13:30	**SESSION IX: "Genetics of Eye Diseases"** CHAIR: Kinga Bujakowska, London, UK • "Genotypes and phenotypes of the DB/FOAR syndrome" Tina Storm, Anatomisk Institut, Århus Universitet, Denmark • "IQCB1 mutations in patients with Leber congenital amaurosis" Alejandro Estrada, Nijmegen Centre for Molecular Life Sciences • "Novel Frameshift and Splice Site Mutations in the Complement Factor H Gene in Patients with Basal Laminar Drusen" John van de Ven, Nijmegen Centre for Molecular Life Sciences • "Alterations of the 5'untranslated leader region of SLC16A12 are associated with development of age-related cataract in a female patient." Jurian Zürcher, Institute of Medical Genetics University of Zuerich, Switzerland
13:30 - 14:30	FAREWELL Lunch

Data mining of miRNA expression from retinal gene expression microarray

Jun Yin (1), Ian B Jeffery (1), Stephen F Madden (1,2), Desmond G Higgins (1), Breandan Kennedy (3)

(1) School of Medicine and Medical Science, Conway Institute, University College Dublin, Belfield, Dublin 4, Ireland.
(2) National Institute for Cellular Biotechnology, Dublin City University, Glasnevin, Dublin 9, Ireland.
(3) School of Biomolecular and Biomedical Science, Conway Institute, University College Dublin, Belfield, Dublin 4, Ireland.

Purpose
MicroRNAs (miRNAs) are non-coding RNAs that regulate gene expression by binding to the messenger RNA (mRNA) of protein coding genes. They control gene expression by either inhibiting translation or inducing mRNA degradation. A number of computational techniques have been developed to identify the targets of miRNAs. In this study we used predicted miRNA-gene interactions to analyze mRNA gene expression microarray data. Gene expression data on zebrafish eye development was shown as an example to predict miRNA enrichment from gene expression microarray data sets.

Methods
miRNA target gene predictions were downloaded from the miRanda database. Here we combined several multivariate analyses, including correspondence analysis, between group analysis and co-inertia analysis to determine which miRNA regulating gene expression. The multivariate analysis methods were compared with other reported methods, such as Wilcoxon rank sum test, Ranked ratio and linear model Gene Set Enrichment Analysis. Microarray gene expression data on zebrafish eye development were analyzed. We aimed to identify miRNAs regulating zebrafish early eye development.

Results
In this study we showed the advantages of using combined multivariate analysis protocols over other methods to determine miRNA enrichment in zebrafish early eye development. A ranked list of miRNAs was generated, in which several miRNAs previously reported to be expressed in the eye were successfully identified. Real-time PCR validation on predicted miRNAs will be performed in future.

Conclusions
This study highlights miRNA data mining of gene expression microarray using multivariate analysis. It also provides an effective way to extract additional information from thousands of published gene expression microarray data.

Acknowledgement
We thank the Irish Research Council for Science Engineering and Technology (IRCSET) Graduate Education Programme (GREP), and Science Foundation Ireland (SFI) SFI 04/IN3/B559, and SFI 06/RFP/BIM052 for funding support.

MicroRNAs couple cell fate and developmental timing in retina

Sarah Decembrini a,1, Dario Bressan b, c, Robert Vignali a, Letizia Pitto b, Sara Mariotti a, Giuseppe Rainaldi b, Xiumei Wang d, Monica Evangelista b, Giuseppina Barsacchi a, and Federico Cremisi b, c.

a) Dipartimento di Biologia, Universita` di Pisa, Pisa 56100, Italy; b) Istituto di Fisiologia Clinica, Consiglio Nazionale delle Ricerche, Pisa 56100, Italy; c) Scuola Normale Superiore Pisa, Classe di Scienze, Pisa 56100, Italy; and d) Center for Brain and Cognitive Sciences, Institute of Biophysics, Beijing 100101, China 1) UCL Institute of Child Health and Great Ormond Street Hospital, 30 Guilford Street, London WC1N 1EH, UK.

Purpose
Cell identity is acquired in different brain structures following an established timing schedule, by coordinating the proliferation of multipotent progenitor cells and the generation of distinct types of mature nerve cells at precise times. However, the molecular mechanisms coupling the identity of a specific neuron and its birth date are poorly understood.

Methods
In the neural retina, only late progenitor cells that divide slowly can become bipolar neurons, by the activation of otx2 and vsx1 genes. In Xenopus, early retinal progenitors of st. 25–37 transcribe but only after st. 37–39, translate Xvsx1 and Xotx2 and the differentiations of bipolar cells occur. Thus, translational control in frogs plays a crucial role in establishing a specific cell type at a precise developmental time. Through expression and functional screenings, we selected 4 miRNAs, mir-129, mir-155, mir- 214, and mir-222 that are highly expressed in early fast dividing retinal progenitors which bind to the 3'UTR of Xotx2 and Xvsx1 mRNAs inhibiting their translation.

Results
The in vivo functional inactivation of these miRNAs releases the inhibition, supporting the generation of additional bipolar cells.

Conclusions
We propose a model in which miR-129, miR-155, miR-214, and miR-222 could be part of a mechanism coupling the determination of the bipolar cell identity with a low proliferation rate of retinal progenitors by inhibiting the translation of key homeobox genes Xotx2 and Xvsx1 early in development.

Acknowledgement
Our work was supported by grants from Scuola Normale Superiore, from Telethon, and from the Italian Ministry of University and Scientific Research (MIUR).

Regulation of microRNA expression in an in vitro model of retinal cell differentiation

Laura Harwood, J. Guduric-Fuchs, A. O'Connor, D.A. Simpson

Centre for Vision and Vascular Science, Queen's University Belfast, Belfast, United Kingdom.

MicroRNAs (miRNAs) are small non-coding RNAs which regulate mRNA expression at the post-transcriptional level. The potential functions of many miRNAs in retinal developmental are indicated by their unique stage-specific expression patterns. However, the specific roles of these miRNAs remain largely unknown. The purpose of this study was to optimise and characterise an in vitro model of retinal cell differentiation and to investigate changes in miRNA expression in this model.

Weri-Rb1 cells were treated with various combinations of laminin and all-trans retinoic acid (RA) on a poly-D-lysine/laminin substrate and assessed for morphological and molecular differentiation. Microarrays (Illumina HT-12) were performed to characterise induced changes in mRNA expression. Selected findings were validated by real-time qPCR. miRNA expression was analyzed using next generation sequencing (NGS) on an Illumina Genome Analyser. Reads were mapped to known miRNAs (mature, star and pre-miRNAs) using the miRAnalyser software.

The optimal protocol to induce both morphological and molecular differentiation of Weri-Rb1 involved consecutive treatment with laminin and 20µM RA. Cells formed ramifying processes, positive for the neuronal marker Map2. Upregulated genes were significantly enriched for genes associated with visual perception. Upregulated retina-associated genes included ARR3, Crx, GNAT2, RP1L1 and Nhlh1. Quantitative analysis of miRNA expression with NGS revealed high levels of known neuronal and retinal-expressed miRNAs, including miR-124. The expression of several miRNAs was altered during differentiation; most notably miR-9 and miR-181b were upregulated and miR-424 downregulated.

A combination of a laminin substrate and laminin and RA media supplementation over a 14 day period induces both morphological differentiation and expression of retina-associated genes in Weri-Rb1 cells. miRNAs potentially involved in retinal development were identified, to the best of our knowledge for the first time, using NGS. This model provides a platform for future studies of the interactions between miRNAs and mRNAs during retinal cell differentiation.

Funding: Department of Employment and Learning (DEL)

Exploitation of microRNA-based gene repression in rhodopsin-linked Retinitis Pigmentosa

Claire Kilty, O'Reilly M, Millington-Ward S, Chadderton N, Palfi A, Kenna PF, Humphries P, Farrar GJ

The Ocular Genetics Unit, The Smurfit Institute of Genetics, Trinity College Dublin

Purpose
Retinitis Pigmentosa (RP) categorises a collection of retinal diseases in which the photoreceptor cells degenerate. This often leads to complete blindness in the people affected. RP has many different inheritance patterns with autosomal dominant, autosomal recessive and X-linked forms of the disease occurring. One of the most common forms of RP is rhodopsin-linked autosomal dominant RP (adRP). Due to the heterogeneous nature of mutations in this gene, a mutation-independent suppression and replacement gene therapy strategy has been proposed. This approach involves utilising RNAi technology in order to suppress both the wild-type and disease rhodopsin alleles while simultaneously providing a codon-modified replacement rhodopsin gene refractory to the suppression agent. The purpose of the current study is the design and evaluation of artificial miRNA cassettes for use as suppression agents in vivo

Methods
Artificial miRNA-expressing cassettes were designed according to methods described by Invitrogen and Boudreau et al, 2009. These were cloned under the control of either the CMV or U6 promoters into pAAV-2/5 cassettes also containing an EGFP reporter gene and AAV-2/5 viruses were generated. Adult wild-type mice were subretinally injected with the artificial miRNA AAV suppression constructs. Ten days post-injection, transduced retinal cells were harvested and FACS sorted. RNA was analysed for rhodopsin expression using real-time RT-PCR analysis. Electeroretinography (ERG) was also performed (4-6 weeks post injection) in order to assess retinal function.

Results
Significant rhodopsin suppression has been achieved in vivo in the laboratory using shRNA- suppression agents. Preliminary analysis of two different miRNA cassettes (based on miR-155 and miR-30a respectively) revealed that suppression of rhodopsin RNA by artificial miRNA cassettes was significantly less than by the shRNAs evaluated. This correlated to results at a functional level using ERGs. RNA analyses of retinas injected with the artificial miRNA constructs revealed that this is most likely due to lower levels of miRNA than shRNA expression in vivo.

Conclusions
Further evaluation of different artificial miRNA constructs, targeting different regions of the rhodopsin mRNA sequence is needed in order to accurately assess the use of these artificial miRNAs as safe and effective suppressors in vivo

Acknowledgement
We would like to thank the Irish Council for Research in Science, Engineering and Technology (IRCSET) 'Embark Initiative', Science Foundation Ireland (SFI), Fighting Blindness Ireland.

Application of LC-MS/MS for microRNA Target Identification in Retinal Degeneration

Carol Loscher1, Arpad Palfi (1), Stefanie M. Hauck (2), Karsten Hokamp (1), Marius Ueffing (2), G. Jane Farrar (1).

(1) Ocular Genetic Unit, Genetics Department, Trinity College Dublin, Dublin 2, Ireland.
(2) Department of Protein Sciences, Helmholtz Zentrum München - German Research Center for Environmental Health (GmbH), Ingolstädter Landstr. 1, D-85764 Neuherberg, Germany.

Purpose
Retinitis pigmentosa (RP), a group of inherited retinal degeneration, is characterised by a progressive loss of photoreceptor cells and consequent visual handicap. We have previously reported a common pan-retinal signature of altered microRNA (miR) expression in the P347S-Rhodopsin transgenic mouse model of RP; expression of miR-96, -182 and -183 decreased, while expression of miR-1, -133 and -142 was markedly up-regulated. As miRs are powerful modifiers of gene expression, it is of interest to identify retinal genes regulated by these miRs in RP. A dual approach was taken to identify retinal miR targets; changes of retinal protein expression in the P347S-RP were examined, in parallel with in silico predictions of miR targets.

Methods
Total protein extractions and membrane-enriched fractions were taken from both wild type and P347S-RP mouse retinas. These protein samples were digested with trypsin and analysed by liquid chromatography-tandem mass spectrometry (LC-MS/MS). All detected peptides were aligned and quantified, and the peptides and the corresponding protein IDs were identified by database search. In addition a combination of miR target prediction algorithms, Miranda, Targetscan and Diana, were used to predict retinal targets for miR-1, -133, -142, -96, -182, and -183. These in silico predictions were cross-referenced with proteins IDs altered in the mouse RP retina, to identify potential targets.

Results
A total of 1043 and 1233 proteins were identified from total protein and membrane-enriched fractions respectively. Of these proteins, 162 proteins were upregulated ($p<0.05$, >2fold) and 188 proteins were down-regulated ($p<0.05$, >2fold) in the RP retina. Of these, 36 proteins were predicted to be targets of the 6 miRs altered in expression in RP, in addition to exhibiting an inverse correlation to miR expression changes. Specifically, 22 proteins predicted to be targets of miR-182,-183 and -96 were increased in expression ($p<0.05$), while 14 proteins predicted to be targets of miR-1,-133, and -142 were decreased in expression ($p<0.05$). This inverse relationship between alterations in miR and protein expression in RP may reveal proteins that are negatively regulated by these miRs.

Conclusions
Comparison of proteins altered in expression in the P347S RP retina to in silico predicted miR targets, provides a list of candidate miR targets. However, further studies are needed to validate these miR targets.

Acknowledgement
Fighting Blindness Ireland and Health Research Board, Ireland.

The retinal ciliopathy-associated protein homologs RPGRIP1 and RPGRIP1L are linked to (connecting) cilium dynamics through interaction with NEK4 serine/threonine kinase.

Karlien Coene (1,2), D.A. Mans (1,2), S.J.F. Letteboer (1), K. Boldt (3), C.J. Gloeckner (3), M. Ueffing (3), F.P.M. Cremers (1,2), R.Roepman (1,2).

1. Human Genetics, Radboud University Nijmegen, Nijmegen, The Netherlands 2. Nijmegen Centre for Molecular Life Sciences, Nijmegen, The Netherlands 3. Center of Ophthalmology, Tübingen University, Tübingen, Germany

Purpose
Recent studies have established ciliary dysfunction as the underlying cause of a broad range of multi-organ phenotypes, often including retinal pathology. Although more and more 'ciliopathy' genes are identified, little is known about their actual function in the (connecting) cilium. Our aim is to gain more insight in the function of two retinal ciliopathy-associated protein homologs, RPGR interacting protein 1 (RPGRIP1), and RPGRIP1-like protein (RPGRIP1L). Mutations in RPGRIP1 lead to Leber congenital amaurosis, while mutations in RPGRIP1L were identified in patients with Joubert, Meckel, and COACH syndrome. The RPGRIP1L A229T variant is also a common modifier for retinal degeneration.

Methods
RPGRIP1 and RPGRIP1L were used as bait proteins in Strep-Flag tandem affinity purification (SF-TAP) experiments in human embryonic kidney (HEK293T) cells. The associated protein complexes were identified by mass spectrometry (LC-MS), and further validated by affinity biochemistry and immunohistochemistry. Genes were silenced in ciliated retinal pigment epithelium cells by transfection of small interfering RNA (siRNA) oligos.

Results
The SF-TAP procedure allowed the identification of functionally active protein complexes in cultured cells in vivo. We identified NEK4 serine/threonine kinase as a component of both the RPGRIP1- as well as the RPGRIP1L-associated protein complex. The interaction between this kinase and RPGRIP1/1L was confirmed in GST-pulldown and co-immunoprecipitation assays. In ciliated retinal pigment epithelium cells, NEK4 localized to basal bodies. In adult mouse retinas, the kinase was predominantly detected at the ciliary rootlet. Downregulation of the gene encoding NEK4 led to a significant decrease in cilium assembly.

Conclusions
We have identified NEK4 serine/threonine kinase as interacting partner of RPGRIP1 and RPGRIP1L. This kinase localizes to centrosomes and plays a role in cilium dynamics, possibly through regulation of cell cycle progression. Its direct association with the connecting cilium protein complex defines NEK4 as a candidate gene for (retinal) ciliopathies.

Acknowledgement
The Netherlands Organization for Scientific Research (NWO Toptalent-021.001.014)

Differential protein expression in vitreous of autoimmune uveitis.

Florian Hofmaier(1), Stefanie M. Hauck(2), Barbara Amann(1), Marius Ueffing(2,3),Cornelia A. Deeg(1)

1) Institute of Animal Physiology, Department of Veterinary Sciences, Ludwig-Maximilians University , Munich, Germany 2) Department of Protein Science, Helmholtz Center Munich, Neuherberg, Germany 3) Center of Ophthalmology, University of Tübingen, Tübingen, Germany

Purpose
Equine recurrent uveitis (ERU), the only spontaneous animal model for human autoimmune uveitis, is a T-cell mediated disease targeting the inner eye.
The aim of this study was the identification and quantification of differentially expressed proteins in the vitreous of horses suffering from ERU in comparison to unaffected control animals, enabling the identification of ERU-related functional protein-networks and affiliated molecular signalling pathways through protein network analysis.

Methods
Differential expression of vitreous proteins was detected by LC-MS/MS-based label free quantification. Western Blot was used to further validate and quantify expression of proteins of interest. Gelatin-Zymography was performed to examine Matrix-Metalloproteinase activity in vitreous samples. ERU-related functional protein networks were identified by protein network analysis with the Search Tool for Retrieval of Interacting Genes/Proteins (STRING, http://string.embl.de/). Co-expression of functional-related Proteins was examined by immunohistochemistry in the target tissue of ERU, the retina.

Results
A total of 119 vitreous proteins were identified of which 17 were found to be upregulated during ERU and 62 showed a decreased expression in samples derived from diseased animals. Protein network analysis of the differential regulated vitreous proteins led to the identification of four functional protein clusters. A cluster of proteins functionally associated with Matrix Metalloproteinase-2 stood out. These MMP-2 associated proteins have not yet been described in the context of ERU. For proteins related to this cluster, differential expression and co-expression was confirmed in the target tissue of ERU, the retina.

Conclusions
LC-MS/MS followed by protein-network analysis has proven to be a suitable tool for analyzing differentially expressed proteins and their associated pathways in the vitreous of horses suffering from ERU. Proteins functionally related to MMP-2 are differentially expressed in the uveitic state and might be involved in the destruction of ocular tissue.

Acknowledgement
SFB 571 / A5 Deeg

Identification of interaction networks relevant for retinal degeneration

Matteo Gorza1, Stefanie M. Hauck1, Elöd Körtvely 1, Marius Ueffing 1,2

1) Department of Protein Science, Helmholtz Center Munich, Neuherberg, Germany
2) Center of Ophthalmology, University of Tübingen, Tübingen, Germany

Purpose
Age-related Macular Degeneration (AMD) is a medical condition that results in the loss of sight in the center of the visual field (the macula), a region responsible for sharp vision. The accelerated deterioration of the central retina has been associated with single polymorphisms (SNPs) in several genomic regions harbouring complement factor H (CFH) and factor 3 (C3) genes, and in chromosomal region 10q26 (harboring Htra1, ARMS2, PLEKHA1). However, identification of highly disease-associated SNPs does not explain the pathomechanisms of disease development. A growing number of evidences indicates that the above mentioned mutations cause imbalance in a network of interacting proteins, which finally leads to the manifestation of the symptoms. Because of its peak association, we aimed at identifying interacting partners of ARMS2, in order to gain a better understanding on its role in healthy and diseased eye.

Methods
Due to the lack of ARMS2-specific antibodies applicable for immunoprecipitation, GST-ARMS2 protein (normal and "at risk" variants) was purified from bacteria, and used in pull-down experiments. Although ARMS2 is found only in primates, porcine retinas were used in this pilot study, because of the scarce availability of human material. Co-purified proteins were identified by LC-MSMS and evaluated by Scaffold (Proteome Software).

Results
A number of photoreceptor-specific proteins were found as binding partners for ARMS2. Many of them belong to the extracellular matrix or are involved in secretion. Comparing the interactomes of GST-ARMS2 wt and GST-ARMS2* (risk variant) indicated that the two forms differ in their protein binding characteristics. A protein implicated in autosomal dominant retinitis pigmentosa was found to interact exclusively with the risk-variant of ARMS2.

Conclusions
In this study we propose a proteomics strategy for analyzing protein-protein interactions from tissue as a prerequisite to assemble functional networks, even if antibodies for immunoprecipitation are not available. This approach was employed to gain an understanding on the physiological function of ARMS2 and to detect differences in the binding properties of ARMS2 variants corresponding to the risk vs. non-risk haplotypes.

Acknowledgement
This work was supported by EU grant RETNET (MRTN-CT-2003-504003 to M.U.)

FAM-Study - a prospective longitudinal natural history study in AMD

Arno Philipp Göbel Göbel AP[1], Fleckenstein M[1], Adrion C[2], Schmitz-Valckenberg S[1], Bindewald-Wittich A[1], Scholl HP[1], Mansmann U[2], Holz FG[1], for the FAM Study Group

[1]Department of Ophthalmology, University of Bonn [2]Department of Medical Informatics, Biometry and Epidemiology, University of Munich

Purpose
Age-related macular degeneration (AMD) is the leading cause of severe visual loss in the industrialized world beyond 50 years of age. Geographic atrophy (GA) represents the atrophic late-stage manifestation of "dry" AMD. To date there is no proven treatment for patients with GA. Several lines of evidence indicate that the accumulation of autofluorescent lipofuscin (LF) granules in retinal pigment epithelium (RPE) cells plays a key role in the pathogenesis of the disease. The purpose of the study is to determine GA progression rates over time and to identify predictive biomarkers.

Methods
The FAM-Study (Fundus Autofluorescence (FAF) in Age-related Macular Degeneration) as a prospective, multicenter, longitudinal natural history study uses FAF imaging with confocal scanning laser ophthalmoscopy (cSLO) to assess the topographic distribution of LF in GA patients over time. This allows for quantification of atrophic areas using semi-automated image analysis software and for identification of distinct phenotypic patterns around atrophy. GA progression rates were calculated with a two-steps-linear-mixed-effects model.

Results
In a recent analysis of 234 eyes the mean progression rate was 1,55 mm^2/year (95% CI [1,37-1,73]). However, progression rates between patients showed high variation while there is a high degree of intraindividual symmetry. Progression rates in eyes with the banded and the diffuse FAF pattern were significantly higher compared to eyes without FAF abnormalities and focal FAF patterns. Also bilateral manifestation of GA and multifocality of GA patches seem to represent high risk characteristics for fast GA progression.

Conclusions
The investigations performed in the FAM-Study contribute not only to better understanding of underlying pathophysiological mechanisms but also to the design of current and future interventional clinical trials in patients with GA. cSLO-based FAF imaging and consecutive semi-automated GA quantification is by now the standard method in monitoring therapeutic effects with GA progression as anatomical endpoint.

Acceptance of offered cataract surgery in South India

David Kupitz, Robert P. Finger 1,2, Frank G. Holz1, Seetha Chandrasekhar3, Bharath Balasubramaniam3, RV Ramani3

1 Department of Ophthalmology, University of Bonn, Germany
2 Centre for International Health, Curtin University of Technology, Perth, Australia
3 Sankara Eye Care Institutions, Coimbatore, India

Purpose
A large proportion of cataract surgical services is offered through outreach eye clinics in India. Against this background, the influence of regular outreach by the same provider in the same region on the uptake or non-uptake of cataract surgery services offered was investigated.

Methods
The study was conducted at the Sankara Eye Centre in Coimbatore in Southern India. A structured questionnaire was administered to groups of patients who had attended outreach eye clinics and either accepted or not accepted the offer of cataract surgery. Data regarding attendance and acceptance of service offered during outreach were gathered from the service provider's records.

Results
A total number of 398 participants were interviewed. Mean age of the overall sample was 60 yrs (SD 10) with equal gender proportions (51.8% males). Mean visual acuity of the better eye was 20/80 (LogMAR 0.63, SD 0.36). Persons who took up the offer of cataract surgery tended to come out of smaller households, where family support is not but financial support, transport and distance are a problem ($p<0.001$). Persons who did not take up the offered surgery at outreach clinics tended to live in larger households, where they experienced less family support ($p<0.001$). They mentioned fear of the operation and worry about necessary postoperative care more often as a concern, furthermore reported a worse overall health and more comorbidities ($p\leq0.004$). Regular outreach in the same vicinity lead to a higher rate of acceptance (95% versus 82%; $p<0.001$). Persons who took up the offer had participated in more outreach clinics ($p=0.003$) and had received more invitations for surgery ($p<0.001$) than those who did not take up the offer. Men showed a marked preference for their own gender when asked which gender should receive surgery first ($p<0.001$).

Conclusions
Findings indicate the importance of building trust by provision of regular outreach eye clinics in the same area. Some people, especially those who come out of large households, need more help to overcome a lack of family support. However, most patients may need to go through a certain number of outreach eye clinics before accepting surgery. Women could once more be shown to be disadvantaged when it comes to negotiate family support to take up offered cataract surgery.

Vision mediated by a subretinal electronic implant

Katarína Stingl1,. Bartz-Schmidt K.U. 1, Benav H.1, Besch D.1, Bruckmann A.1, Gekeler F.1, Greppmaier U.2, Harscher A.2, Kibbel S.2, Kusnyerik A.3 , Peters T.4, Sachs H.5 , Stett A.6, Wrobel W.2 , Wilhelm B.4, Wilke R.1, Zrenner E.1

1 University of Tuebingen, Centre for Ophthalmology, Tuebingen, Gemany 2 Retina Implant AG, Reutlingen, Germany 3 Semmelweis University, Department of Ophthalmology, Budapest, Hungary 4 STC Autonomous Nervous System and Safety Studies, Ofterdingen, Germany 5 Klinikum Friedrichstadt, Augenklinik, Dresden, Germany 6 NMI Natural and Medical Sciences Institute, Reutlingen, Germany

Purpose
Hereditary retinal degenerations (e.g. Retinitis pigmentosa) lead to progressive loss of retinal photoreceptors. They lead to blindness mostly in patients' middle age. Up to date, no effective treatment is available. We developed an active subretinal electronic device being implanted in 11 volunteers suffering from a hereditary retinal disease in an end-stage.

Methods
The active part of the subretinal implant consists of a chip carrying a microphotodiode array (MPDA) with 1500 light sensitive pixels. On the tip of the chip a 4 x 4 matrix of electrodes was placed for direct retinal stimulation and assessment of the tissue-electrode interface. The implant was connected to power supply and the control unit by a subdermal cable. The study period ranged from 3 weeks to 3 months.

Results
The direct stimulation of the retina was performed by single electrodes or patterns formed by the 4x4 matrix leading to percepts described as well defined dots. Stimulation by simple patterns could be reliable recognized and described in tested subjects.
Five of the eleven patients could use the subretinal MPDA to perceive and localize light sources or large bright objects. In two subjects spatial resolution up to 0.32 cycles/deg and 0.22 cycles/deg could be achieved by a stripe pattern recognition. In the subject, whose subretinal implant has been placed directly under the macula a visual acuity of 20/1000 could be measured by a standard test with Landolt C rings. He was able to recognize and read letters and words as well as identify objects of daily living under optimal contrast conditions.

Conclusions
For the first time we could show that subretinal implants can restore useful visual functions in blind patients with hereditary retinal diseases.

The Offer of Cataract Surgery: Determinants of Acceptance following Outreach Eye Clinics in South India. Acceptance of offered cataract surgery in South India

Robert P. Finger1,2, David G. Kupitz1, Frank G. Holz1, Seetha Chandrasekhar3, Bharath Balasubramaniam3, RV Ramani3

1 Department of Ophthalmology, University of Bonn, Germany
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Conclusions
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Evaluation of rAAV vectors for gene replacement therapy in a new canine model of Leber congenital amaurosis (LCA) : the RPGRIP1 deficient dachshund dog.

Elsa Lhéritea

Laboratoire de Thérapie Génique, INSERM UMR-649, Institut de Recherche Thérapeutique 1, Université de Nantes ,

Purpose
RPGRIP1 is a structure photoreceptor protein. Loss of RPGRIP1 in human retina causes retinitis pigmentosa or Leber congenital amaurosis, a severe form of photoreceptor degeneration. The current study was an investigation of whether gene replacement therapy could rescue degenerating photoreceptors in a canine model of LCA due to a defect in RPGRIP1.

Methods
The first objective of this study was to evaluate the RPGRIP1-deficient miniature long-haired dachshund (MLHD) dog as a potential candidate for gene therapy. For this purpose, six RPGRIP1-deficient dogs from our colony have been observed for two years using bilateral full-field electroretinograms (ERG), fundus photographs, optical coherence tomographs (OCT), behavioral tests and histological examinations.
The second objective of this study was to develop an rAAV vector with specific tropism for photoreceptor after subretinal injection. rAAV2/5 vector carrying the eGFP reporter gene under the control of the human rhodopsine kinase promoter (Rk) was evaluated in rats and dogs.
The third objective was to evaluate 3 photoreceptor-specific rAAV vectors carrying either the human RPGRIP1 cDNA or the canine RPGRIP1 cDNA for gene replacement therapy in RPGRIP1-deficient dogs, in order to prevent or delay the loss of rod function and to rescue cone function. Four dogs were injected subretinally with an rAAV2/5-Rk-humanRPGRIP1, one dog was injected subretinally with an rAAV2/5-Rk-canineRPGRIP1 and one dog was injected subretinally with an rAAV2/8-CMV-canineRPGRIP1.

Results
We have characterized the kinetics of the retinal degeneration and the disease phenotype in the RPGRIP1-deficient MLHD dog. The fact that there are close similarities between the clinical disease characteristics resulting from RPGRIP1 gene mutations in humans and in the dog make this RPGRIP1-deficient dog a valuable model for the evaluation of gene therapy. These results suggest that a treatment strategy should consist in initiating gene therapy as early as possible after birth.
Then, we showed that subretinal injection of rAAV2/5-Rk-eGFP led to specific expression in photoreceptor cells (rods and cones) in rats and dogs.
Unfortunately, gene replacement therapy using the vector carrying the human RPGRIP1 cDNA (rAAV2/5-Rk-humanRPGRIP1) did not allow to prevent rod degeneration or to rescue cone function.
Vectors carrying the canine RPGRIP1 cDNA (rAAV2/5-Rk-canine RPGRIP1 and rAAV2/8-CMV-canine) have been injected recently in 2 affected dogs.

Conclusions
The RPGRIP1-deficient MLHD dog is a valuable model for the evaluation of gene therapy. We have developed an rAAV vector with specific tropism for photoreceptors after subretinal injection in dogs.
The rAAV vector carrying the human RPGRIP1 cDNA did not rescue photoreceptors function.
rAAV vectors carrying the canine RPGRIP1 cDNA are currently under evaluation.

Protection of photoreceptor cells in a mouse model of RP10

Lawrence Tam, Anna-Sophia Kiang, Matthew Campbell, James Keaney, G. Jane Farrar, Marian M. Humphries, Paul F. Kenna, and Pete Humphries

The Ocular Genetics Unit, Department of Genetics, Trinity College Dublin, Dublin 2, Ireland

Purpose
Mutations within the inosine 5'-monophosphate dehydrogenase type 1 (IMPDH1) gene cause the RP10 form of autosomal dominant retinitis pigmentosa (adRP), an early onset retinopathy resulting in extensive visual handicap owing to progressive death of photoreceptor cells. Previous studies on the biochemical properties of mutant IMPDH1 proteins bearing the missense mutation, Arg224Pro, together with computational modeling simulations, have indicated that mutant IMPDH1 has a tendency to aggregate, and thus photoreceptor cell death may be caused as a result of this phenomenon. We investigate whether induction of a heat shock response by inhibition of heat shock protein 90 (Hsp90), could reduce the pathogenicity associated with insoluble mutant IMPDH1 protein aggregates in the RP10 mouse model.

Methods
Wild type mice were first sub-retinally inoculated with AAV expressing mutant human IMPDH1 bearing the Arg224Pro mutation. Subsequently, repeated doses of 17-AAG (Hsp90 inhibitor) were administered intravitreally into the same animals.

Results
We show here, in a murine model of autosomal dominant RP (RP10) involving expression of an Arg224Pro mutation within the IMPDH1 gene, that treatment with the low molecular weight drug, 17-AAG, activates a heat shock response in the murine retina, and protects photoreceptors against degeneration induced by aggregating mutant IMPDH1 protein.

Conclusions
These data suggest that a single low molecular weight drug has the potential to suppress a wide range of mutant proteins causing RP.

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Restoration of cone vision in the CNGA3-/- mouse model of congenital complete lack of cone photoreceptor function

Stylianos Michalakis1, Regine Mühlfriedel2, Naoyuki Tanimoto2, Vidhyasankar Krishnamoorthy3, Susanne Koch1, M. Dominik Fischer2, Elvir Becirovic1, Lin Bai1, Gesine Huber2, Susanne C. Beck2, Edda Fahl2, Hildegard Büning4, François Paquet-Durand5, Xiangang Zong1, Tim Gollisch3, Martin Biel1, Mathias W. Seeliger2

1Center for Integrated Protein Science Munich (CIPSM), Department of Pharmacy – Center for Drug Research, Ludwig-Maximilians-Universität München, Munich, Germany. 2Division of Ocular Neurodegeneration and 5Division of Experimental Ophthalmology, Institute for Ophthalmic Research, Centre for Ophthalmology, Eberhard Karls-Universität, Tübingen, Germany. 3Visual Coding Group, Max Planck Institute of Neurobiology, Martinsried, Germany. 4Department I of Internal Medicine and Center for Molecular Medicine Cologne, University of Cologne, Cologne, Germany.

Congenital absence of cone photoreceptor function is associated with strongly impaired daylight vision and loss of color discrimination in human achromatopsia. Here, we introduce rAAV-mediated gene replacement therapy as a potential treatment for this disease in the CNGA3-/- mouse model. We show that such therapy can restore cone-specific visual processing in the CNS even if cone photoreceptors had been non-functional from birth. The restoration of cone vision was assessed at different stages along the visual pathway. Treated CNGA3-/- mice became able to generate cone photoreceptor responses and to transfer these signals to bipolar cells. In support, we found morphologically that treated cones expressed regular CNG channel complexes and opsins in outer segments, which previously they did not. Moreover, expression of CNGA3 normalized cGMP levels in cones and reduced the inflammatory response of Müller glia cells that is typical of retinal degenerations. Furthermore, ganglion cells from treated, but not from untreated CNGA3-/- mice displayed cone-driven light-evoked spiking activity, indicating that signals generated in the outer retina are transmitted to the brain. Finally, we demonstrate that this newly acquired sensory information was translated into cone-mediated vision-guided behavior.

Delivery of anti-apoptotic agents to the degenerating retina by AAV-mediated modulation of the inner blood-retina barrier

Anh Thi Hong Nguyen M. Campbell, P. Kenna, M.M. Humphries, A.-S. Kiang, P. Humphries

The Ocular Genetics Unit, Smurfit Institute of Genetics, Trinity College Dublin, Dublin 2, Ireland.

Purpose
The inner blood-retina barrier (iBRB) is the main element preventing systemic delivery of low molecular weight therapeutic drugs for treatment of retinal diseases. We have shown that systemic injection in mice of siRNA targeting transcripts encoding claudin-5 (CLDN5), a tight junction protein of vascular endothelial cells, results in a transient modulation of the iBRB to molecules of up to 800 Daltons for a period between 24 and 72 hours post-injection. The process has no negative impact on neuronal transcription or visual function and does not induce neuronal or retinal oedema. Sub-retinal inoculation of AAV2/9 expressing a doxycycline-inducible shRNA targeting claudin-5 results in a similar barrier modulation for the period of time during which the inducing agent is administered, providing a means of systemic drug delivery specifically to the retina, avoiding systemic access to the brain. In this regard, we have undertaken a series of experiments delivering anti-apoptotic agents to the degenerating retina by AAV-mediated modulation of the inner blood-retina barrier using light induced retinal degeneration in Balb/c mice. As calpain activity has been highly implicated in light induced photoreceptor cell apoptosis, we selected the calpain inhibitor ALLM as the potential protective agent of this form of cell death. Calpain has been shown to cleave proteins involved in apoptosis such as pro-caspase-9, pro-caspase-3, PARP, Bax, p53 and p35. In addition, calpain also cleaves 240 kDa alpha fodrin, an abundant neuroskeletal protein, whose cleavage is an important early event in apoptosis, into 145 and 150 kDa fragments.

Methods
3 month old Balb/c mice were sub-retinally injected with CLDN5 AAV-2/9 in their right eye and NT AAV-2/9 in their left eye. Mice were supplemented with the inducing agent Doxycycline (2mg/ml) in their drinking water for up to 2 weeks. All mice received an intra-peritoneal injection of ALLM (20mg/kg); and were dark adapted for 24 hours before being exposed to white light. Immediately prior to light exposure, their pupils were dilated with 1 % cyclopentalate. Mice were then placed in cages with reflective sides and exposed to white light of 7900 lux for 2 hours. The mice were left in the dark and sacrificed by CO_2 asphyxiation 12, 24 and 48 hours that followed 2 hours of light ablation. The eyes were then sectioned and stain with TUNEL for photoreceptor cell death. Also, alpha fodrin cleavage level was measured using western blot method.

Results
Using the inducible system, we show that systemic administration of ALLM, which does not normally cross the inner blood-retina barrier, substantially protects murine retinas from light induced photoreceptor degeneration.

Conclusions
The technique could, therefore, be used as a minimally-invasive means of delivery of a wide range of drugs targeting molecular pathologies associated with common retinal degenerations.

Acknowledgement
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Expressional and functional analysis of mGluR6 in the zebrafish retina

Marion Haug, Ying-Yu Huang, Matthias Gesemann, Stephan C. F. Neuhauss
Institute of Molecular Life Sciences, University of Zurich, Winterthurerstrasse 190, CH-8057 Zurich, Switzerland

Purpose
Metabotropic glutamate receptors (mGluRs) have been identified at all synapses of the vertebrate retina, where they likely regulate neurotransmitter release. The only example of an mGluR functioning in direct synaptic transmission is mGluR6, which is expressed on ON-bipolar cell dendrites and is found to mediate the ON-response in the mammalian retina. However, data from lower vertebrates propose a division of labor of the ON-bipolar cell response. mGluR6 is suggested to be only responsible for the scotopic ON-response, whereas most likely a glutamate transporter fulfills a similar function in photopic vision. We use the cone dominant retina of zebrafish to assess this hypothesis and to define the functional role of mGluR6 in teleost vision.

Methods
Expression of mGluR6 in larval zebrafish retina was assessed by RNA in situ hybridization and immunohistochemistry. To functionally analyze mGluR6 paralogs, ERG and OKR measurements are currently performed on morpholino antisense injected larvae.

Results
Phylogenetic analysis of mGluR6 reveals the occurrence of two zebrafish mGluR6 paralogs, mGluR6a and -6b. The RNA of both paralogs is expressed in retinal ganglion cells of 5 day old fish, whereas the proteins are additionally detected in the IPL, INL and OPL, suggesting a higher sensitivity of immunohistochemistry. Preliminary data show that diminishing the mGluR6b protein level results in a decreased ERG b-wave but has, if any, only a slight effect on the optokinetic response. Depletion of mGluR6a is in progress.

Conclusions
In contrast to mammals, where mGluR6 is expressed in ON-bipolar cells, we locate both genes predominantly in retinal ganglion cells by in situ hybridization. This localization is confirmed by immunohistochemical analysis, however, we additionally detect both mGluR6 proteins in other layers of the inner retina and in the OPL. Behavioral analysis of mGluR6b deficient larvae provides evidence that this protein plays a role in the cone ON-bipolar cell pathway. Future work will include the depletion of mGluR6a to unravel its function in the teleost retina.

Touchdown: Visual Input Orchestrating Landings in Insects

Torill Kornfeldt

Despite it's appeared simplicity, a honeybee landing on a flower performs an impressive feat. Apart from the task of navigating to the flower and avoiding collisions with flying and stationary objects in its path, the bee must correctly judge the distance to the flower, and reduce her speed accordingly, to land safely and efficiently.

The flying insect has access to a repertoire of different visual cues to help it with the difficult task of distance estimation. To regulate flight speed, many insect rely on optic flow, the motion of the image across the retina. The use of motion parallax, where closer objects move faster over the retina than more distant ones, can help insects determine relative distances. Bees have been shown to use this technique in recognizing and choosing artificial flowers of a preferred height. The effect of looming is used by several insects to help them estimate how fast they are approaching an object.

Those tools have all of them been proven useful and necessary among insects, but are they enough to give insects the fine-tuned measurements of distances needed to land on a swaying flower?

Many vertebrates use stereovision to provide depth information, but stereovision in insects has been questioned as it is dependent on large interoccular separation and small interommatidial angles. At present, the only insect that has been demonstrated to use stereovision is the praying mantis (Rossel 1983), who use it to estimate the distance to prey before striking.

To investigate whether insects with a smaller interoccular distance could utilise stereovision for close range tasks, such as landings, the behaviour of honeybees landing on a platform was studied. Bees were trained to land on a platform, subsequently caught, and one of their eyes was painted with semi-opaque nail varnish. The landings of binocular and monocular honeybees were compared.

Binocular individuals consistently extended their legs in preparation for landing at approximately 15 mm from the platform. In contrast, monocular individuals showed a much more erratic landing behaviour, with no consistent distance for leg extension and several colliding with the platform. Monocular bees also moved laterally to a larger extent, possibly to compensate for the loss of stereovision with the use of motion parallax and did not decrease their speed during the approach to the platform to the same extent as binocular bees. Control bees, painted around the base of the antenna, exhibited behaviour comparable to the unpainted binocular bees. These results indicate that honeybees, despite their small interoccular separation, apply and utilise stereovision to assess depth and distance during landing.

References

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Extracellular recordings from polarization-sensitive interneurons in the central complex of the locust brain

Miklós Bech, Uwe Homberg

Philipps-University of Marburg, Department of Biology, Animal Physiology, Karl-von-Frisch-Str. 8, 35032 Marburg

Purpose
The desert locust Schistocerca gregaria is able to perceive polarized light with a specialized dorsal rim area of the eye and potentially uses the polarization pattern of the blue sky as a compass cue for spatial navigation. In the brain, polarization information is passed through the optic lobe and the anterior optic tubercle to the central complex which most likely serves as an internal sky compass.
Because the electric field vectors (E-vectors) of the blue sky are arranged in concentric circles around the sun, the sky polarization pattern changes during the day like the position of the sun. Therefore, for long range navigation the processing of polarized light information has to be time-compensated. To gain insight into mechanisms underlying time-compensation in the processing of sky polarization at neuronal level it is necessary to establish a method which allows the performance of long term recordings from polarization-sensitive (POL-) neurons in the locust brain.

Methods
Extracellular recordings with electrodes made of copper wires were performed in the lower division of the locust central body (CBL). The CBL is densely innervated by polarization-sensitive neurons and was, therefore, chosen in order to establish this method.
The electrode was inserted into the brain while the locust was fixed vertically in the experimental setup. The animal was stimulated with polarized blue light (465 nm), obtained by an LED. Blue light passed a motor-driven rotating polarizer, which was set on a perimeter apparatus that allowed stimulation with a rotating E-vector from different elevations of the visual field. The angular extent of the stimulus at the locust eye was ~13,6°. The receptive field of the animal was tested every hour by stimulation through a clockwise and counterclockwise rotating polarizer (rotating speed of 30°/s).

Results
We obtained for the first time long term recordings lasting for several hours from POL-neurons. Single units were identified through template matching combined with a cluster analysis. The units showed polarization-opponency and had wide receptive fields over a range up to 180° along the right-left meridian. Within the visual field, E-vector tuning was not constant but changed considerably along the right-left meridian. In addition, changes in response amplitude and E-vector tuning depending on the time of day were observed.

Conclusions
First evidence is presented that E-vector tuning of individual units depends on the time of day and increases in the evening. Corresponding changes were observed in response amplitude which decreased in the evening. Differences in E-vector tuning depending on the stimulus position along the right-left meridian might significantly contribute to differentiate between the solar and antisolar hemisphere in the sky when relying on sky polarization for azimuth coding.

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Visual Behavior of Adult Zebrafish

Kaspar Müller, Stephan C.F. Neuhauss
University of Zurich, Institute of Molecular Life Sciences, Winterthurerstr. 190, 8057 Zurich, Switzerland

The zebrafish (Danio rerio) model system is increasingly used for behavioral studies. While for larval fish a set of well developed and automated assays suitable for high-throughput screening is available, automated behavioral tests for adult fish are largely missing. In the past, the need for such tests has been minimal because of the sparseness of available adult mutant strains. New methods allow now knocking out specific genes of interest, and transgenic technology in this species is constantly improving. Thereby, generation of adult mutant strains is greatly simplified, and, consequently, the demand for behavioral tests for adult fish is on the rise.

Our lab currently tries to adapt behavioral tests for zebrafish larvae to adult fish and to automate assays to make them amenable to high- or at least medium-throughput screens. The larval tests we adapted so far include the optokinetic response assay (OKR) and the visual motor response assay (VMR), both allowing for a fast and quantifiable evaluation of visual function in adult fish. The optomotor response assay (OMR), previously used to e.g. determine the wavelength dependence of motion vision in adult fish, was also automated by using video tracking, allowing for a preciser and less time consuming quantification of the response. To complement these assays, we sought for an additional method independent of motion vision (as OKR and OMR are), but allowing a more versatile evaluation of visual system function than merely the reaction to increments or decrements of illumination (as it is the case for the VMR). Visually guided choice disrimination training satisfies these conditions, although the method is extremely time consuming when done manually. Using video tracking, stimulus presentation on a LCD screen and food delivery by peristaltic pumps, we also automated this method. Potential distractions and disturbances caused by human intervention are thereby minimized, and the apparatus could easily be extended to enable training of multiple fish in parallel.

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The neuroprotective effect of retinal müller glial cell-derived neurotrophic factors in retinal neurons

Patricia del Rio1, S. M. Hauck1, M. Irmler2, P. de la Villa4, M. Götz3, J. Beckers5, U. Bartsch6, E. Vecino7, M. Ueffing1,8

1Protein Science, 2Experimental Genetics, 3Stem Cell Research, Helmholtz Zentrum München, Munich, Germany; 4Dept. of Physiology, Univ. of Alcalá, Alcalá de Henares (Madrid), Spain; 5Ctr. of Life and Food Sciences, Technise Univ. München, Weihenstephan, Germany; 6Dept. of Ophthalmology, Univ. Med. Ctr. Hamburg-Eppendorf, Hamburg, Germany; 7Dept. of Biol. and Histology, Univ. of the Basque Country, Bilbao, Spain; 8Ctr. of Ophthalmology, Univ. Med. Ctr., Tübingen, Germany

Purpose
Neuroprotection is a promising strategy to develop therapies against neurodegenerative diseases. Therefore it is crucial to investigate the neuroprotective mechanisms through glia-neuron interactions in the nervous system. Glial-Cell-Line-Derived Neurotrophic Factor (GDNF) is a neuroprotective factor which enhances the survival of neurons. In the retina, the protection of photoreceptors (PR) is transmitted indirectly via Retinal Mueller Glial cells (RMG) (Hauck et al., 2006). We aimed at identifying GDNF-induced factors from RMG and at validating their neuroprotective potential in mouse models of PR degeneration.

Methods
Three strategies for identification neuroprotective factors were followed: I) FACS-isolated RMG from hGFAPeGFP transgenic mice were treated with GDNF for 24 hours their transcriptome changes studied by microarray analysis. II) Similar experiments were performed with explanted total retinas from 20 mice. III) As a third strategy, isolated RMG were taken into short term culture and after GDNF treatment, the secretion of molecules was monitored directly from the cell culture medium by an antibody-based array approach. From these approaches, several molecules were selected to test their functional neuroprotective effect in primary PR cultures. The positively tested factors for PR survival in vitro were then applied in rd1 mouse organotypic cultures as well as injected intravitreally into rd1 and rd10 mouse models of retinal degeneration to analyze the effect of these factors for the maintenance of PR function in vivo.

Results
GDNF treatment induces distinct changes of RMG transcriptome and proteome. Combining those transcripts with the secreted proteins directly detected on culture medium with the candidate array approach, we accumulated 35 proteins increasedly secreted from RMG after GDNF stimulation. Osteopontin (OPN), a well known secreted glycoprotein, was one of the candidates expressed in RMG and upregulated after GDNF induction, both on the transcript level as well as on the protein level in lysates and supernatants of primary mouse RMGs. OPN and as well as additional candidate factors were confirmed to promote PR survival in vitro trough the activation of PI3-K pro-survival pathway. In organotypic retinal cultures from rd1 mice, OPN significantly increased survival of PR.

Conclusions
Among the GDNF-induced molecules secreted from RMG, we have discovered novel candidate factors for neuroprotection. The survival effect of these factors in animal models for inherited retinal degeneration, encourages further studies on their therapeutic properties towards future clinical application.

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The effect of the administration of buthionine sulfoximine and glutathioneethyl ester in a model of retinitis pigmentosa.

Violeta Sánchez Vallejo, M Miranda,R Alvarez-Nölting, E Arnal2; Fj Romero1

1 Universidad CEU Cardenal Herrera. dept fisiología, farmacología y toxilcología.
2 Fundación oftalmológica del mediterraneo

Purpose
We have administered Buthionine sulfoximine (BSO) an inhibitor of gamma-glutamylcysteine synthetase to wild type and rd1 mice, to study its influence over retina development and retinal pathology. We have also administered glutathione ethyl ester to wild and rd1 mice. Since by itself GSH is not effectively transported into cells, several alternatives are available to increase GSH within cells and GSH esters are hydrolyzed to GSH in cells.

Methods
Two groups of wild type and rd1 mice received an intraperitoneal (ip) injection of BSO (1,5 g/kg body weight) once daily for eight consecutive days starting from postpartum day 3. In addition, another two group of wild type and rd1 pups received ip injections of BSO and a combination of antioxidants orally or glutathione ethyl ester. Malondialdehyde (MDA) and glutathione (GSH) concentratioms as well as glutahione peroxidase (GPx) and glutathione reductase (GSSG-R) activities were measured and TUNEL, as well, as GSH inmunostaining were performanced.

Results
BSO treatment decreased GSH in wild type and rd1 retinas as well as the ratio GSH/GSSG, the administration of antioxidants to BSO treated animals produced an small but non significant increase in both parameters. When BSO was administered daily antioxidants failed to increase retinal glutathione concentration or to decrease the number in TUNEL positive cells. In all groups BSO induced cataracts.

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The Role of PARP in Photoreceptor Cell Death

Ayse Sahaboglu1, Theo van Veen 1,2, Eberhart Zrenner 1, Francois Paquet-Durand1

1Division of Experimental Ophthalmology, Centre for Ophthalmology, Tuebingen, GERMANY.
2 Clinical Sciences Lund, Lund university, Lund, SWEDEN.

Purpose
Retinitis pigmentosa (RP) is a neurodegenerative disease that affect photoreceptors and cause blindness in humans. The retinal degeneration (rd) 1 mouse is a well studied model for RP. In this model, the beta subunit of the rod photoreceptor cGMP phosphodisterase 6 (PDE6) gene is mutated and non-functional. Dysfunctional PDE6 causes accumulation of cGMP. It has been shown that cGMP accumulation associated with excessive poly (ADP-ribose) polymerase (PARP-1) activation and oxidative stress in the rd1 mouse. The aim of this study was to further investigate the role of PARP activity in photoreceptor cell death. To investigate role of PARP for photoreceptor cell death and survival, we studied retinal morphology and function in PARP knockout (KO) mice.

Methods
PARP knockout (KO), rd1 and corresponding wild-type (wt) animals at post-natal day (P) 11 and P30 were used. PARP KO and wt mouse retinal explants were cultured with/without Zaprinast, a selective inhibitor of cyclic GMP-specific phosphodiesterase, to simulate in vitro a situation comparable to the rd1 mouse model at P11. Immunofluorescent detection of cGMP staining, TUNEL staining, poly-ADP-ribose-polymer (PAR) immunohistochemistry were performed for analysis of Zaprinast effects, dying cells and PAR accumulation, respectively. Hematoxylin-Eosin staining was performed for analysis of retinal structure at P30.

Results
Immunofluorescence showed that in wt and PARP KO retinal cultures zaprinast treatment induced an accumulation of cGMP in the outer nuclear layer (ONL). Detection of degenerating cells using the TUNEL assay showed a zaprinast induced increase in the number of positive cells in the ONL of both PARP KO and wt retinae. However, the number of dying cells in PARP KO ONL was smaller than in corresponding wt. Similar results were obtained when PAR immunohistochemical assay was used for detection of PAR accumulation. Hematoxylin-Eosin staining showed a similar structure and retinal thickness for PARP KO and wt at P30.

Conclusions
PARP-1 may have important functions in retinal photoreceptor cell death in the rd1 mouse and could be a novel target for drug developments. An understanding of causative roles of PARP-1 in retinal degenerations could shed new light on the existence or absence of alternative pathways for degradation of poly-ADP-ribose-polymers.

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Epigenetic Markers in Retinal Degeneration

Pietro Farinelli, Blanca Arango-Gonzalez, Jasvir Kaur, Francois Paquet-Durand, Per Ekström

University of Lund, Department of Ophthalmology, Clinical Sciences; University of Tübingen, Centre for Ophthalmology, Institute for Ophthalmic Research, Division of Experimental Ophthalmology

Purpose
Gene repression by DNA methylation is a well documented epigenetic phenomenon, that seems to be involved in a number of cellular responses. However, so far there is only little information whether DNA methylation is also a component of the mechanisms that lie behind retinal degenerations, such as Retinitis Pigmentosa. Here we wanted to see whether there were any indications of altered DNA methylation in the degenerating photoreceptors that characterize this disease and therefore investigated several animal models for Retinitis Pigmentosa.

Methods
Retinas were collected from either rd1, rd2 or wild type background C3H mice, as well as from either P23H-1 and S334ter-3 rhodopsin mutant rats or wild type background CD rats at relevant age (postnatal day 11, 21, 16, 12 respectively for rd1, rd2, P23H-1, S334ter-3). The specimens were fixed, cryo-sectioned and immunolabeled for 5-methyl cytosine (sheep polyclonal, 1:200, Novus Biological NB 100-744), either alone or in combination with TUNEL staining for dying cells (Roche Diagnostic) and for Acetylated-Lysine (rabbit polyclonal, 1:200, Cell Signaling 9441) or against DNMT-1 (rabbit polyclonal, 1:1000, Abcam – ab16632).

Western blot analysis were performed as follows: retina from PN11 rd1 mice were removed in dissecting buffer supplemented with protease inhibitors. Tissues were homogenized and proteins were extracted in sample buffer (2% SDS). Samples were separated by SDS-PAGE loading 10 ug of protein per well. Membrane were incubated with antibody against DNMT-1 (1:5000)

Results
Methylated DNA was only very rarely found in the photoreceptor layers of wild type retina either from mouse or rat. By contrast, the outer nuclear layers in all of the four models showed distinct staining in a subset of photoreceptors, that corresponded to the expected number of degenerating cells of the respective age. Furthermore, colabelling in all of the four different mutant retina showed that the staining for methylated DNA overlapped with TUNEL positive cells and gaps of lysines acetylation to a major extent. At the same time, DNMT-1 analysis was perfomed in rd1 and ++ mice (PN11) in immunofluorescence and western blot. Interestingly, DNMT-1 staining looks slightly increased in the rd1 inner nuclear layer as compared to the control, but the same result was not confirmed in western blot where in ++ was significantly increased.

Conclusions
DNA hypermethylation was detected in degenerating photoreceptors regardless of the different kinds of mutations in the various models, and with characteristics that strongly suggested a relation with the cell death mechanism. Which enzyme is responsible of this massive DNA methylation is yet to be found out. These findings are therefore compatible with increased DNA methylation as an important and maybe also a general step in the cell death processes that occur in photoreceptors burdened with inherited degeneration.

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The role of genetics in susceptibility to environmentally induced myopia

Paul Yen-Po Chen, Jeremy A. Guggenheim

School of Optometry and Vision Sciences,
Cardiff University, Wales, UK.

Purpose
To test whether the inter-animal variability in susceptibility to form-deprivation myopia (FDM) is genetically determined.

Methods
Monocular form deprivation (FD) was induced in 232 outbred White Leghorn chicks aged 4 days old. After FD for 4 days, the susceptibility to FDM of each chick was quantified by the relative changes in ocular component dimensions measured by high-frequency A-scan ultrasonography and refractive error by retinoscopy. Chicks in the extreme high and low distribution of susceptibility to FDM were kept and paired for breeding. This selection process was carried out over 3 generations to test for a divergence in susceptibility to FDM between the high-susceptibility and low-susceptibility selected lines. In addition, for chicks in the final generation, FD was also carried out for 10 days, to investigate the time-course of myopia development in the two lines.

Results
After two rounds of selective breeding, chicks whose parents had been selected for high susceptibility to FDM developed approximately twice as much myopia as those whose parents had been selected for low susceptibility to FDM (High line, –15.27 ± 3.47 D; Low line, –6.88 ± 3.35 D; Mean ± SD; P

Conclusions
Susceptibility to FDM in White Leghorn chicks has a strong genetic component (additive polygenic effects explained more than 50% of the inter-animal variability in response to FD). Moreover, the difference in susceptibility to FDM between the two selected lines is not simply due to a delayed response in low-susceptibility chicks. This study provides pivotal evidence for the idea of genetic predisposition to environmentally induced myopia.

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Contrast adaptation and myopia

Arne Ohlendorf, Frank Schaeffel
Institute for Ophthalmic Research Tuebingen, Section of Neurobiology of the Eye

Purpose
To describe the time course of contrast adaptation induced by positive and negative spherical defocus and its possible relationship to myopia development.

Methods
Contrast adaptation was induced by presenting a movie to the subjects on a computer screen at one meter distance for 10 minutes, while the right eye was defocused by a trial lens (+4D; -4D; -2D). Supra-threshold contrast sensitivity was measured by a method of interocular adjustment with a Garbor patch of 1 deg angular subtense, filled with 3.22 cyc/deg sine wave gratings presented on a computer screen at one meter distance on gray background.

Results
The supra-threshold contrast sensitivity in the right eye was raised by about 30% after imposed myopic defocus and remained elevated for at least 2 minutes. Changes in supra-threshold contrast sensitivity were observed after myopic not after hyperopic defocus.

Conclusions
Transient contrast adaptation was found only following 3 D of myopic defocus. The unexpected difference between the signs of imposed defocus is potentially important, since it might reflect differences in retinal processing of positive or negative defocus and could lead to a yet unknown mechanism that can distinguish the sign of imposed defocus.

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Responses of Different Retinal Areas to Imposed Defocus in Chicks

Tudor C. Tepelus 1, Frank Schaeffel 1

1. Institute for Ophthalmic Research, Section of Neurobiology of the Eye, University of Tuebingen, Calwerstrasse. 7/1, 72076, Germany

Purpose
Recent experiments in monkeys suggest that defocus imposed in the periphery of the visual field can affect the development of foveal/central refractive errors. For designing spectacle lenses making use of this observation, it is important to know whether certain retinal areas are more responsive or whether changes in eye growth are just proportional to the defocused area. We have studied this question in chickens.

Methods
In total, 54 male white leghorn chickens were used. From day 7 after hatching, they were treated for 5 days either with full field negative (-7D) or positive (+7D) lenses, or with hemi-field lenses of the same powers, or with 2 different types of radial refractive gradient (RRG) lenses which were provided by the industrial partner, Rodenstock, Munich. A macro file was written for „ImageJ" (a publicly available image processing platform) to trace the outlines of excised eyes. Shapes of fellow control eyes and lens-treated eyes were compared in both the horizontal and vertical meridians. Refractions were determined over the horizontal visual field, at the beginning and at the end of experiments, using automated infrared photoretinoscopy.

Results
(1) Eye length, as determined by the tracing technique, was highly correlated to A-scan ultrasound axial length data.

(2) Full field -7D lenses increased eye size in axial direction by 0.21mm (from 9.98±0.28 to 10.20±0.35mm), with an increase in eye volume by 3.7%. Full field +7D lenses slowed down the eyes' growth rate, resulting in a decrease in axial direction by 0.08 mm (from 9.84±0.22 to 9.77±0.17mm) with a decrease in eye volume by 2.5%.

(3) First type of RRG lenses had no apparent effect on central refractions but induced small hyperopic shifts in the periphery which were significant only in the temporal retina (+1.83±1.87D, $p<0.0001$). Second type of RRG lenses induced peripheral hyperopia also changing the central refraction (TR +1.50±1.17D $p<0.0001$, CR +0.77±1.15D $p<0.05$, NR +1.47±1.35D $p<0.0001$)

(4) Morphology of the anterior segment of the eye remained unchanged during the lens treatments, except for the case of hemi-field lenses.

Conclusions
Different types of RRG lenses (different refractive profiles) are very differently effective in changing the central refraction.
After 5 days of treatment with RRG lenses imposing a myopic defocus in the periphery there was little change in external eye shape – although hyperopia could be induced. Thus we consider the refraction changes are largely choroidal.

***** Extra previous work (already published) will be presented as well.

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Hormones and Myopia Development

Alexandra Marchã Penha, Marita Feldkämper, Frank Schaeffel

Myopia is a refractive condition of the eyes in which an image is focused in front of the retina instead of on the retina. Among the refractive errors, myopia is a leading cause of visual impairment throughout the world and its prevalence is increasing. It is known that both, genetic and environmental factors play a role in myopia development. Most probably, a number of factors causing myopia development interact with each other, i.e. a change in one of them can trigger deviations of the others.

(1) The day-/night-rhythm of the body is controlled by incident light which stimulates the pineal gland to secrete melatonin when it is dark. Consequently, melatonin release is reduced in light, while cortisol and dopamine levels increase. Disturbance in the melatonin-dopamine system have a negative impact on eye growth regulation. This was demonstrated by melatonin injections in chicks that generated changes in the growth of several ocular tissues, in both eyes with normal visual experience and eyes that were covered with diffusers which are known to produce „deprivation myopia". Extensive near work goes along with extensive in-door work, often with lower levels of illumination. Long-term low light causes low levels of dopamine and cortisol, as well as reduced vitamin D levels. Dopamine (released from the retina) was shown to have a protective effect against myopia development in animal models, since dopamine antagonist injections increased ocular growth, while dopamine agonists reduced the development of deprivation myopia.

(2) Experimental myopia in animal models was shown to increase oxidative stress, interference with the normal function of the immune system, changes in serum and eye ion turnover, and in nitric oxide (NO) levels. Recent, substantial changes in nutrition, i.e. considerably increased intake of food rich in refined carbohydrates and sugar, increase blood glucose levels and, consequently, increase insulin and decrease glucagon levels. Retinal glucagon was shown to represent a stop signal for axial eye growth in chicks, whereas insulin, applied intravitreally, has been shown to have a powerful myopigenic effect in the chick, in particular when the retinal image was defocused in addition. Moreover, insulin levels can influence the central nervous system and the transport of dopamine. Furthermore, hyperglycemia, when the blood sugar rises, causes swelling of the crystalline lens, and refractive (not axial) myopia.

(3) Some deviations from the normal status of sexual hormones, as testosterone, 17-beta-estradiol, were also found in myopes. Extended estrogen treatment was shown to increase the activity of lysysl oxidase, an enzyme essential for the cross-linking of collagen, the main component of the sclera.
In conclusion, it is clear that there are most likely multiple environmental factors that are involved in the development of myopia (in addition to genetic predisposition), and they partially interact with each other.

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NMDA RECEPTORS and OPTIC NERVE INJURY

Tahani Huria, R Fern

Department of cell physiology and pharmacology ,University of Leicester, University road, Leicester, LE1 9HN, UK.

There are conflicting opinions regarding the role of glutamate and its receptors in CNS white matter that has suffered irreversible injury when subjected to anoxia / ischemia. Some reports shown that glutamate can be toxic to white matter oligodendrocytes, myelin and axons via NMDA-receptor-mediated excitotoxicity, leading to the accumulation of Ca2+ inside the cell and activation of Ca2+-dependent pathways which end in cell death 1 2-4. However, others find no evidence for a role for NMDA receptors 5 6. One explanation may be a species difference, since the latter groups of studies were performed on mice while axonal injury has only been examined in rat.

We have examined the effect of acute exposure of adult rat optic nerves to oxygen-glucose deprivation (OGD: a model of ischemia). Axon viability were monitored electrophysiologically by measuring the area under the compound action potential (CAP) during 15 min of control followed by 60 min OGD and then a 60 min recovery period. Extracellular CAPs were evoked and recorded with suction electrodes 7.We found that CAPs were stable during control and fell gradually during 60 min OGD to recover to 30 ± 5% of control during the recovery (n=15). When OGD was supplemented by the addition of NMDA + Glycine to over-stimulate NMDA receptors, CAPs recovered to 10 ± 5 %, after wash-out, confirming that NMDA-type glutamate receptors play a role in white matter injury in rat. We applied the same procedure to mouse optic nerves that surprisingly showed their OGD-injury is not aggravated by over-stimulation of NMDA receptors (n=8).

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GSK3beta signalling and glial differentiation in the optic nerve

Andrea Rivera, Arthur M. Butt

Institute of Biomedical and Biomolecular Science (IBBS), School of Pharmacy and Biomedical Science University of Portsmouth, UK

Purpose
GSK3beta is the target of numerous receptor-mediated pathways that regulate cell differentiation, however its effects on oligodendrocytes and astrocytes have not been understood. We examined the effects of GSK3beta inhibition on glial populations in the postnatal forebrain and the optic nerve: a typical CNS white matter tract.

Methods
All procedures were in accordance with the Animals Scientific Procedures Act (1986). Optic nerves (ON) aged postnatal day (P) 15-20 were dissected with the retina intact and maintained in organotypic culture for 3-7 days in vitro (DIV), either in normal medium or medium containing a GSK3β inhibitor (20uM ARA-014418, 50mM Lithium Chloride or Wnt3a 2uM).

Results
We compared Wnt and GSK3beta effects in our ex vivo culture. GSK3beta inhibition increased astrocytes, oligodendrocytes and oligodendrocyte precursors (OPs) whereas Wnt increased OPs but inhibited their differentiation into oligodendrocytes. GSK3beta inhibition and Wnt differentially affected GFAP+ cells, dramatically increasing their cell number and morphology.

Conclusions
Our findings show that GSK3beta and Wnt differentially regulate oligodendrocyte and astrocyte differentiation ex vivo. GSK3beta is a key negative regulator of glia differentiation and we are currently examining the mechanisms of these novel GSK3beta pathways.

Impact of impaired oligodendrocyte differentiation on optic nerve function

Aiman S. Saab1,2, Bianka Götze3, Lena Schmid1, Wiebke Möbius1, Wenhui Huang2, Nicolas, Snaidero1, Siegrid Löwel3, Frank Kirchhoff1,2 , Klaus A. Nave1, Moritz Rossner1

1) Department of Neurogenetics, Max Planck Institute of Experimental Medicine, Göttingen, Germany; 2) Molecular Physiology, University of Saarland, Homburg/Saar, Germany; 3) Institute of General Zoology and Animal Physiology, Friedrich-Schiller-University Jena, Jena, Germany

Purpose
Oligodendrocyte maturation and myelin formation are crucial for rapid impulse propagation, the prerequisite for proper information processing. The molecular mechanisms underlying the differentiation steps from glial precursors to mature, myelinating cells are currently intensely studied.

It has been shown that the basic helix-loop-helix (bHLH) transcription factor Olig1 is essential for oligodendrocyte maturation and myelination. Olig1 regulates the transcription of myelinspecific genes such as Mbp, Plp1 and Mag. Mice deficient of Olig1 show a profound failure in myelin formation, hence exhibit severe neurological deficits and die in the third postnatal week. Herein, we describe a distinct Olig1 mouse mutant that also failed to establish proper myelination, but interestingly, revealed no signs of neurological symptoms, was viable and grew into adulthood showing no obvious behavioural abnormalities. However, the deficient myelinogenesis in these knockouts was alleviated compared to the previously described Olig1-null mice. The optic nerves of the adult mouse mutants comprised more than 70% of unmyelinated axons and were therefore well suited to study the impact of impaired or restrained oligodendrocyte myelination on optic nerve function in vivo. As expected, the lack of accurate axonal ensheathment led to a profound decrease in nerve excitability and conduction velocity. To evaluate the impact of diminished optic nerve function at the systems level, we investigated the visual system in vivo by assessing visual acuity, contrast sensitivity and cortical map activities in adult Olig1 deficient mice. Interestingly, both, visual acuity and contrast sensitivity were aggravated while visual cortical responses were unaltered. We conclude that the development and maintenance of visual cortical maps are not hampered by slower conduction velocities, however, normal visual capabilities seem to be more dependent on rapid impulse propagation.

Assessing microstructural changes in the optic nerve in a spontaneously demyelinating mouse model using magnetic resonance imaging

Fredrik Gruenenfelder1, W. Holmes2, M.R. Lopez-Gonzalez3, J. A. Barrie1, T. J. Anderson1, J. Penderis1, J. M. Edgar1

1. Applied Neurobiology Group, Faculty of Veterinary Medicine, UK; 2. Division of Clinical Neuroscience, Faculty of Medicine, University of Glasgow, UK; 3. Department of Clinical Physics, SINAPSE Collaboration, Faculty of Medicine, University of Glasgow, UK

Purpose

Conventional magnetic resonance imaging (MRI) is highly sensitive in detecting pathology in brain tissue. However, the specificity for determining the lesion type is low1. Diffusion weighted imaging (DWI), an advanced MRI technique, detects changes in diffusion of water molecules caused by tissue damage. Extension of DWI with multiple increasing b-values (a factor sensitising the tissue to diffusion) in normal nerve fibre tracts has shown that diffusion of water in nerve fibres can be divided into fast (D1) and slow (D2) components2. We hypothesised that this would provide a means of distinguishing lesion types within nerve fibre tracts. To test this, we examined the optic nerve of the Plp1 overexpressing mouse (line #72; a model of Pelizaeus-Merzbacher disease3), which is characterised by progressive central nervous system demyelination. Two spatially and pathologically distinct regions are present along the optic nerve at post-natal day 120 (P120); a completely demyelinated, non-inflamed rostral region and a demyelinated, but highly inflamed caudal region4. This model is therefore useful for assessing if DWI with multiple b-values can distinguish acute from chronic demyelinating fibre tract lesions.

Methods

After fixation in 4% paraformaldehyde, isolation and incubation in a contrast agent (PBS/gadobenate dimeglumine; 2.5mM final concentration; 6 days) 12 pairs of P120 homozygous #72 and 6 pairs of P120 wild type adult optic nerves were scanned in a purpose-built receiver/transmitter coil using DWI at two regions (rostral and caudal). One parallel and two perpendicular diffusion directions (relative to the direction of orientation of optic nerve fibres) were measured at sixteen increasing b-values (from 94.6 to 6304.6 s/mm2). Subsequently, nerves were frozen and cryosections for immunohistochemistry were obtained from the scanned regions. The nature and densities of optic nerve cells were quantified. The DWI data was analysed using ImageJ MatLab was used for curve fitting, and to calculate parallel and perpendicular diffusivity for both D1 and D2.

Results

Histological analysis showed a significant increase in cell density ($p < 0.001$) within the caudal region of the demyelinated optic nerve of the #72 mouse compared with the rostral region of the nerve and compared with the wild type mouse nerve. This was mainly due to a significant increase ($p < 0.001$) in the inflammatory cell (microglia/macrophage) population. A marked astrocytosis, evidenced by a significant increase ($p < 0.001$) in GFAP immunoreactivity was present in the homozygous #72 optic nerve compared to the wild type optic nerve. There was no significant difference between the two regions of the homozygous mouse optic nerve.

DWI using a single b value (b1 = 1153.3 s/mm2) showed that the fractional anisotropy (FA, a measure of diffusion directionality) was reduced by 10% and 25% respectively, in the rostral and caudal portions of the #72 optic nerve compared to corresponding regions of the wild type optic nerve. Using all sixteen b-values and curve fitting, fast and slow diffusion compartments became distinguishable. Differences between #72 and control tissue were mainly evident within the slow diffusion compartment. The slow diffusivity in both parallel and perpendicular directions was significantly higher ($p < 0.01$) in the #72 optic nerve compared to the wild type optic nerve. There was also a difference in the slow parallel diffusivity between the two regions of the #72 optic nerve, with diffusion in the rostral region being significantly greater ($p < 0.001$) than in the caudal region.

Conclusions

The histological findings confirmed the value of this mouse as a model to investigate the ability of DWI to distinguish silent demyelinated and active, inflammatory demyelinating lesions. In terms of DWI analysis, the small reduction in the FA value between the myelinated optic nerve and the demyelinated #72 optic nerve suggests that axons themselves are the main contributor to the anisotropic diffusion of water in CNS white matter, in agreement with other studies2.

By applying multiple increasing b-values, DWI was more sensitive at detecting subtle diffusion changes caused by the altered microstructure (particularly changes in cell density) with the potential to distinguish between distinct lesion types. The development and application of similar techniques in the clinical setting would be predicted to facilitate the characterisation of lesion phenotypes, thereby enhancing diagnosis and establishment of prognosis.

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Genotypes and phenotypes of the DB/FOAR syndrome

Tina Storm (1), Lisbeth Tranebjaerg (2) Carina Frykholm (3) Tryggve Nevéus (4) Jens Michael Hertz (5) Erik I. Christensen and Rikke Nielsen (1)

1) Department of Anatomy, Aarhus University, Denmark. (2) Department of Audiology, Bispebjerg Hospital and Wilhelm Johannsen Centre of Functional Genomics Institute of Cellular and Molecular Medicine, ICMM, University of Copenhagen, The Panum Institute, Denamrk. (3) Department of Audiology/Clinical Genetics, University Hospital, Uppsala, Sweden. (4) Uppsala University Children's Hospital, Uppsala, Sweden (5) Department of Human Genetics, Aarhus University, Denmark.

Purpose
Donnai-Barrow (DB) and facio-oculo-acustico-renal (FOAR) syndromes (OMIM 222448 and OMIM 227290) were recently confirmed to be allelic entities. The syndrome is a rare, autosomal recessive syndrome characterized by facial and ocular abnormalities, sensorial hearing loss, proteinuria and developmental delay. Studies of multiplex families, carried out by Kantarci and coworkes, led to the mapping of the disorder to the LRP2 gene on chromosome 2q23.3-31.1. The LRP2 gene encodes megalin, a multiligand scavenger-receptor involved in endocytosis of numerous ligands in absorptive epithelia as well as signalling. This highly correlates with diverse and seemingly unrelated malformations and functional deficits observed in affected individuals. The purpose of this study is to identify novel disease causing mutations of the LRP2 gene and elucidate the underlying molecular background of the renal and ophthalmological phenotypes observed in these patients.

Methods
Direct sequencing, immunoblotting and immunocytochemical analyses.

Results
In this study we investigated the underlying genetic cause for the syndrome in two unrelated families. A novel splice site mutation of the LRP2 gene was identified in one of the families and analyses of the urinary excretion pattern exhibited increased excretion of established megalin ligands supporting megalin proximal tubular dysfunction.

Conclusions
Identification of a novel splice site mutation in the LRP2 gene established the genetic background for the DB/FOAR syndrome observed in one of the families included in this study. Increased urinary excretion of megalin ligands furthermore supports proximal tubular megalin dysfunction. Additionally, megalin expression in the ciliary epithelium of the eye indicates that megalin dysfunction could play a role in the observed high myopia in these patients. We therefore, plan to investigate the role of megalin in the high myopia observed in DB/FOAR syndrome through localization studies using immunocytochemical analyses of eye tissue obtained from a megalin knock out mouse model.

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IQCB1 mutations in patients with Leber congenital amaurosis

Alejandro Estrada

Leber congenital amaurosis (LCA) represents the most severe retinal dystrophy and becomes evident in the first year of life. The disease is genetically heterogeneous with 15 genes identified so far, accounting for 70% of LCA patients. In our search for new LCA genes, we performed homozygosity mapping using high-density SNP microarrays in a large group of LCA patients of worldwide origin. In three LCA patients we identified homozygous regions containing the IQCB1 gene. Mutations in IQCB1 cause Senior Loken syndrome (SLSN), an autosomal recessive entity with nephronophthisis and a severe retinal dystrophy. As mutations in the CEP290 gene cause LCA and SLSN, and since the CEP290 protein interacts with IQCB1, we reasoned that IQCB1 would be an excellent candidate gene for LCA. Mutation analysis in 226 LCA patients identified frameshift and nonsense mutations in 12 patients of 10 families. Upon re-inspection of the patient's disease status, five were found to have developed SLSN, while seven maintained the diagnosis of LCA as the kidney function remained normal. One patient presented one heterozygous frameshift mutation in IQCB1 and one heterozygous mutation in CEP290, suggesting digenic inheritance. Our results show that the onset of renal failure in patients with IQCB1 mutations is highly variable, and that mutations are also found in LCA patients without nephronophthisis, rendering IQCB1 a new gene for LCA. However, these patients are at high risk to develop renal failure, which in early stages is often not recognized and can cause sudden death from fluid and electrolyte imbalance. We therefore recommend that all LCA patients be screened for IQCB1 mutations, in order to follow them more closely for kidney disease.

Novel Frameshift and Splice Site Mutations in the Complement Factor H Gene in Patients with Basal Laminar Drusen

John van de Ven1, A.I. den Hollander1, C.J.F. Boon1, D. Smailhodzic1, B.J. Klevering1, C.C.W. Klaver2, S. Fauser3 and C.B. Hoyng1

1Department of Ophthalmology, Radboud University Nijmegen Medical Center, Nijmegen, the Netherlands
2Department of Ophthalmology and Department of Epidemiology and Biostatistics, Erasmus Medical Center, Rotterdam, the Netherlands
3Department of Ophthalmology, University of Cologne Medical Center, Cologne, Germany

Purpose
The present study was conducted to identify novel complement factor H (CFH) mutations in patients with the basal laminar drusen (BLD) phenotype.

Methods
All subjects were derived from a large German-Dutch database for age-related macular degeneration (AMD). In brief, ophthalmic examinations, nonstereoscopic 30° color fundus photography and fluorescein angiography were performed. Venous blood samples were drawn for genomic DNA extraction from peripheral blood leukocytes. The CFH gene was analyzed by direct sequencing.

Results
In three unrelated families we identified novel heterozygous mutations in the CFH gene: p.Ile184fsX, p.Lys204fsX and c.1697-17_-8del. One patient received a renal transplant because of an end stage membranoproliferative nephritis type II (MPGN II). A second patient has an end-stage kidney disease of yet unknown cause.

Conclusions
Our findings confirm that the BLD phenotype belongs to a spectrum of diseases associated with either monogenic or multifactorial inheritance of variants and mutations of the CFH gene. These insights provide us with tools for risk estimation in the near future by genetic screening.

Alterations of the 5'untranslated leader region of SLC16A12 are associated with development of age-related cataract in a female patient.

Jurian Zürcher
Institute of Medical Genetics University of Zuerich, Switzerland

Recently we reported a nonsense mutation in SLC16A12 to lead to juvenile cataract. We hypothesized that this monocarboxylate transporter may also play a role in the complex form of age-related cataracts. Now, we identified a female patient with age-related cataract who is heterozygous for SNP rs3740030 and for a novel site (c.-17A>G), both within the 5'UTR of /SLC16A12/. The patient carries both alterations, the minor G-allele as well as the c.-17G mutation, /in cis/. Testing the effect of the alterations in a luciferase reporter system we found significant upregulation of enzyme activity caused by the patient's haplotype. Interestingly, the minor G-allele of the SNP alone caused also significant upregulation of enzyme activity compared to the major T-allele, but both to a lower extent compared to the patient's haplotype. Analysis of SLC16A12 transcripts in surrogate tissue demonstrated striking SNP allele-specific heterogeneity with respect to amount and sequence identity of the 5'UTR, while transcripts containing the coding region were not affected. Furthermore, an allele-specific predisposition to age-related cataract was found in a Swiss population (odds ratio of 2.058; 1.2025-3.5221). These data are in accordance with the allele-specific effects on gene expression and together may help to explain a mechanisms for development of age-related cataract.

CHANGES IN THE ERG OF STZ INDUCED DIABETIC RATS AND ITS UTILITY TO DETECT OTHER CEREBRAL ALTERATIONS

Raquel ÁLVAREZ-NÖLTING1, M. MIRANDA1, J.M. GENOVES1, E. ARNAL2, F. BOSCH-MORELL1,2, F.J. ROMERO1,2.

1 DEPARTMENT OF PHYSIOLOGY, UNIVERSIDAD CEU CARDENAL HERRERA, MONCADA, VALENCIA, SPAIN; 2FUNDACIÓN OFTALMOLÓGICA DEL MEDITERRÁNEO, VALENCIA, SPAIN.

Purpose
Diabetes induces changes in retinal function; indeed, functional changes occur in the retina prior to clinical symptoms of the disease. The electroretinogram (ERG) has been used for decades to uncover the mechanisms of retinal physiology and their alterations in disease. The purpose of this work was to study changes in the ERG in a model of diabetes along time and to observe if these changes can be used to detect other cerebral alterations.

Methods
Animals were treated in accordance to the ARVO statement for the use of animals in ophthalmic and vision research. Male, Wistar rats of approximately 60 days of age, corresponding to approx. 250 g body weight, at the beginning of the experiment, were used in the study. Diabetes was induced in animals by a single intraperitoneal injection of STZ (65 mg/ kg) in 0.1 M citrate buffer, pH 4.5. Fasting blood glucose levels were measured 72 h after STZ injection. Animals having blood glucose levels >200 mg/dL were considered diabetic. Animals were sacrificed after 2,5, 4 and 12 weeks of diabetes. Immediately before sacrifice ERG, visual evoked potentials (VEP) and morris water maze were performed. ERG and VEP were carried out in scotopic conditions and registered in McLab software. Latency time and a- and b-wave amplitude were measured, as well as latency and amplitude of VEP. Statistical significances were assessed by means of the Student's t-test, and correlation was studied using the Pearson method.

Results
No changes are observed in a-wave amplitude (Figure 1), and though we could observed an increase in a-wave latency after 2,4 and 4 weeks of diabetes, this change was reversed after 12 weeks of diabetes (Figure 2). Statistical reduced b-wave amplitude was observed after 4 and 12 weeks of diabetes, and increased b-wave latency time were detected as early as 2,5 weeks after diabetes induction, though again this change was also reversed after 12 week (Figures 3 and 4).
Increased in latency time in VEP were also detected after 12 weeks of diabetes (Figure 5 and 6).
Changes in Morris Water maze test were only detected after 12 weeks of diabetes (Figure 7), interestingly there was no difference in the time needed to reach a visible platform (Figure 8). There was a weak negative correlation between accumulated latency in the Morris Water Maze and latency time of the b-wave in the ERG (Figure 9), though we need a greater number of animals to confirm this correlation and to investigate the role of glycemia in this correlation.

Conclusions
Changes in retinal function in diabetes occur prior to changes in hippocampus. These results suggest that further studies are needed to know if retinal functional tests can be used to monitorize other cerebral alterations in diabetic patients.

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KERATOCONUS AFFECTS TO CORNEAL ANTIOXIDANT CAPACITY AND NITRIC OXIDE CONCENTRATION

Emma Arnal(1), S Johnsen-Soriano(1), L Gómez(1), D Martínez(1), C Peris(1), FJ Romero(12).

1. Fundación Oftalmológica del Mediterráneo. Valencia. Spain.
2. Universidad CEU-Cardenal Herrera. Departamento de Fisiología. Moncada (Valencia). Spain.

Purpose
The aim of this study was to compare antioxidant capacity in corneas with keratoconus versus normal corneas and to study the presence of oxidative stress and an elevated amount of total nitrites in keratoconus corneas.

Methods
First, the antioxidant capacity was measured with a commercial kit (Antioxidant Assay Kit, Cayman) that is used to measure the total antioxidant capacity of the different samples. The assay relies on the ability of the antioxidants in the sample to inhibit the oxidation of ABTS to ABTS+ by metmyoglobin. The amount of ABTS+ can be monitored by reading the absorbance at 405 nm. To measure the total nitrites, a commercial kit was used (Parameter, Total Nitrite, R&D Systems). The principle of this assay is the determination of nitric oxide concentrations based on the enzymatic conversion of nitrate to nitrite. The amount of total nitrites can be monitored by reading the absorbance at 540 nm wich is proportional to the nitric oxide concentration.

Results
The antioxidant capacity in keratoconus corneas was decreased significantly (0.2 ± 0.07 mM/mg prot) when compared to normal corneas (0.8 ± 0.47 mM/mg prot, $p < 0.05$ vs control). Moreover, the total nitrites were significantly elevated in the corneas with keratoconus (0.87 ± 0.27 umol/mg prot) when compared with the control (0.42 ± 0.15 umol/mg prot, $p < 0.05$ vs control).
CONCLUSIONS: The increased levels of total nitrites and the decreased antioxidant capacity in the keratoconus corneas indicate that oxidative stress is involved in the development of this pathology and may provide new insights for e prevention and treatment of this disease in the future.

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Electrophysiological measurements in DBA/2J mice as an animal model for glaucoma: The long term influence of memantine

Jenny Atorf1, J. Kremers1

1) Department of Ophthalmology, University Hospital Erlangen, Erlangen, Germany

Purpose
Memantine is a partial blocker of N-methyl-D-aspartate (NMDA)-type glutamate receptors that blocks the receptor in its open configuration. This means that the blockade is mainly effective at high glutamate concentrations that have been suggested to cause neurodegenerative disorders including glaucoma and retinal disease. At physiological glutamate concentrations, however, the blockade of the receptor is ineffective. Hence, memantine is able to prevent excitotoxic cell death due to excessive activation of the glutamate receptors but preserves normal neuronal function. Previous studies have shown that memantine might be protective against experimental glaucoma (Hare et al., 2004a; Hare et al., 2004b) and glaucomatous retinal neurodegeneration related with glutamate excitotoxicity (Schuettauf et al., 2002; Lagrèze et al., 1998; Hare & Wheeler, 2009). A functional study of this potentially protective effect was, however, lacking. It is the purpose of this study to provide electrophysiological data on the long-term functional effects of memantine on retinal degenerations in the DBA/2J mouse.

Methods
15 DBA/2J mice received intraperitoneal injections of 5 mg/kg memantine twice a day, five days per week (weekends excluded) over a period of 7 months (at ages between 3 and 10 months). Five untreated DBA/2J mice and five C57Bl/6 mice served as controls. Measurements of intraocular pressure (IOP) and electroretinographical (ERG) recordings were performed at age 3, 6 and 10 months. To study rod function ERG responses to scotopic flash stimuli were recorded. To assess cone function the photopic flash and photopic flicker ERG was performed.

Results
The IOPs were initially lower in the DBA/2J mice in comparison with the C57Bl/6 mice. But the IOPs increased more strongly in the DBA/2J mice. There were no differences between treated and non-treated DBA/2J mice. In agreement with previous data, the amplitudes of the scotopic a- and b-wave were similar at the age of 3 months. In the DBA/2J mice the amplitudes decreased more strongly with age than in the C57Bl/6 mice. There were no differences between the treated and untreated DBA/2J animals as regards the a-wave amplitude. However, the decrease of the b-wave amplitude was less strong in treated animals. The data for the photopic flicker ERG were similar to previous measurements (Harazny et al., 2009): all response amplitudes and phases of the 1st and 2nd harmonic component were altered in DBA/2J mice in comparison with the C57Bl/6 mice, except the 12 Hz 1st harmonic amplitudes at age 3 months which were similar for both strains. There were no differences between treated and non-treated DBA/2J animals.

Conclusions
The IOPs were very similar to those reported previously in C57Bl/6 and DBA/2J mice. Memantine did not have influence on the measured IOP. Abstract Young Researcher Vision Camp 2010 The scotopic flash ERGs are similar to previous results. The response amplitudes continuously decrease with age in both C57Bl/6 and DBA/2J mice, whereas this decrease is larger in DBA/2J mice. Memantine seems to have a small protective effect on the b-wave. This indicates that memantine may have a functionally protective effect at a post-receptoral level.
The data of the photopic flicker ERG are in agreement with earlier conclusions that the photopic flicker ERG reveals a different degenerative mechanism in DBA/2J animals than the scotopic flash ERG. This functional deficit occurs already at a young age (three months) at which the scotopic flash ERG is still normal in DBA/2J mice. This means that the photopic flicker ERGs were already altered when memantine treatment was started. It is therefore not surprising that memantine does not have any influence on this type of functional deficit of the retina.

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Discovery of allelic expression differences in retinal (disease) genes

Sukirthini Balendran, Bernd Wissinger, Simone Schimpf-Linzenbold
Molecular Genetics Laboratory, Institute of Ophthalmic Research, Tuebingen, Germany

Purpose
Reduced penetrance and variability in disease expression with respect to onset, course, and severity is a well-documented feature in retinal dystrophies and hamper solid and reliable genotype/phenotype correlations as well as individual disease prognosis. Although the basis of this variability is largely unknown, it is commonly accepted that secondary genetic factors (modifier-genes) are key factors for the determination of the development, severity, and course of a disease. A good example is the Prpf31 gene. Mutations in Prpf31 cause retinitis pigmentosa (RP) with reduced penetrance. Two independent studies showed that the transcript level of the non-mutant allele is highly correlated with the risk of expression of the disease and that higher expression of the non-mutant transcript protect asymptomatic carriers from RP. Based on such known examples and high heritability of gene expression regulation, we hypothesize that cis-acting variants governing gene expression levels play a crucial role in phenotypic variation and disease penetrance in hereditary retinal disorders. The principle aim is the identification of such cis-acting gene variants and the determination of their impact on disease expression.

Methods
Five different mouse inbred strains (C57BL/6, BALB/c, CAST/Ei, CBA/Ca & LP) were crossbred in all possible combinations because we want to achieve a heterozygous but genetic identical F1 generation with a high genetic variability.
15 different retinal disease genes were screened for heterozygous cSNPs applying PCR and sequencing. We applied Pyrosequencing assays on RT-PCR amplified retinal cDNAs generated from retinal RNA to determine allelic expression differences based on the identified cSNPs. Results were calibrated for equimolar ratios by used genomic DNA as a control.

Results
Using the Pyrosequencing technology we detected an allelic imbalance (AI) in four (Pde6c, Ush2a, Cerkl and Tlr4) out of 15 retinal disease genes. The AI in all four genes was identified only on cDNA level. Screening of the Pde6c gene revealed a 116-bp insertion on cDNA level that results in a premature termination codon leading, due to the nonsense mediated mRNA decay, to a downregulation of the mutant transcript. For the remaining genes the cause of the AI has to be verified by determining the promoter regions and identify putative cis-acting variants applying reporter gene assays.

Conclusions
Until now, only in few cases cis-variants which cause an allelic imbalance could be identified. Several mechanisms like promoter variants, cis-regulatory elements, alternative splicing, variants in the UTR, copy number variations or nonsense-mediated mRNA decay can cause such an allelic imbalance. Our results demonstrate that allele-specific differences in gene expression are common in retinal expressed genes.

Statement on proprietary interests
Genregulation, RNA

Gene expression changes in aged rat eyes following oral zinc supplementation

Neda Barzegar-Befroei1, Jane Flinn (2), Grtechen Knaack (2), Christer Hogstrand (3)and Imre Lengyel (1)

1. Ocular Biology and Therapeutics, UCL institute of opthalmology, London UK
2. Psychology Department, George Mason University, USA
3. Nutritional Sciences Division, School of Biomedical and Health Sciences, King's College London, UK

Purpose
Zinc is a multifaceted trace element which plays a role in many metabolic pathways, either as a signalling molecule or as a component of proteins. It is involved in DNA replication, transcription, protein synthesis and signalling pathways, influencing cell division and differentiation. Zinc is found in unusually high concentrations in ocular tissues with the highest amount concentrated within the retinal pigment epithelium and choroid. As decreased zinc levels are associated with age related macular degeneration (AMD) supplementation with zinc is proposed to slow the progression of the disease with no clear understanding of how this might be beneficial at the cellular level. In the present study we supplemented the drinking water of aged rats with zinc and monitored changes in gene expression in the eye.

Methods
Three groups of 12 months old female rats were given supplemented drinking water for 4 months as follows: 1) normal lab water; 2) +10 mg L-1 zinc sulphate; and +10 mg/L zinc sulphate + 0.2 mg/L copper sulphate. Following sacrifice, one eye of each animal was used to isolate RNA for microarray analysis. Gene expression (n=4 per group) was determined using GeneChip Rat Gene 1.0 ST (Affymetrix, CA) microarrays. Significant changes in gene expressions ($p<0.05$) were selected using Qlucore Omics Explorer and overrepresented pathways among regulated genes were selected using Ingenuity Pathway Analysis (IPA; Ingenuity Systems). For gene expression changes the control group was compared to the pooled supplementation groups. Differential gene expressions were validated by real-time quantitative polymerase chain reaction for 17 genes. The second eyes were fixed for assessing morphological changes.

Results
From the zinc affected genes 738 were represented in the Ingenuity database. Networks involved in oxidative stress, inflammatory response, DNA replication, recombination, and repair had high scores. Amongst the top molecular and cellular functions that were affected by zinc supplementation were: cell death, carbohydrate metabolism, small molecule biochemistry, cellular compromise and cell morphology. A number of interesting and significant molecules are being currently further investigated. There are no gross morphological changes observed following long term zinc supplementation but electron microscopy might highlight ultra structural changes.

Conclusions
Oral zinc supplementation is a choice of treatment to slow the progression of AMD. Here we show the first time that oral zinc supplementation has the potential to modulate molecular level changes in the eye and provide evidence that zinc supplementation can modify essential processes within ocular tissues to combat the major cause of blindness in the elderly. It is especially important to emphasize that our supplementation study was carried out on aged animals and for an extended period of time, mimicking closely the use of zinc supplementation in AMD.

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Henry Smith Charity, Bill Brown Charitable Trust, Mercer Fund from Fight for Sight, Moorfields Eye Hospital Special Trustees

Mueller glial cells in autoimmune uveitis Altered expression of potassium channel Kir 4.1 and Aquaporin 4 protein

Christina Eberhardt1, Barbara Amann1, Stefanie M. Hauck2, Cornelia A. Deeg1

1 Department of Veterinary Sciences, LMU Munich, Munich, Germany
2 Department of Protein Science, Helmholtz Center Munich, Neuherberg, Germany

Purpose
Equine recurrent uveitis (ERU) is a spontaneous disease with high prevalence in horses, leading to blindness. It is the only spontaneous animal model for human autoimmune uveitis. Ongoing inflammation in course of disease leads to changes in morphology of Mueller glial cells. To further investigate the role of Mueller cells in ERU pathogenesis we studied expression patterns of potassium channel Kir 4.1 and Aquaporin 4 protein.

Methods
Immunohistochemistry was performed on paraffin embedded eye sections to investigate expression patterns of potassium channel Kir 4.1 and water channel Aquaporin 4 (AQP4) in comparison to the specific Mueller cell intermediate filament Vimentin in horses with ERU and negative controls. We used monoclonal goat antibody specific for Kir 4.1 (Santa Cruz) and monoclonal mouse antibody specific for AQP4 (Santa Cruz) for candidate detection in tissue. Mouse-anti-Vimentin antibody (Sigma Aldrich) served as Mueller cell specific marker. Expression of candidates as well as further Mueller cell markers such as Glutamine synthetase (GS) and Glial fibrillary acidic protein (GFAP) were quantified using Western Blot analysis. Rabbit-anti-GFAP antibody (Dako Cytomation) and mouse-anti-GS antibody (BD Biosciences) were used.

Results
Mueller cells of ERU cases showed an activated phenotype with upregulation of GFAP and/or Vimentin, depending on the stage of inflammation while GS was downregulated. Immunohistochemistry revealed a decreased expression of Kir 4.1 protein in diseased horses compared to controls. Western Blot analysis confirmed a significant downregulation of potassium channel Kir 4.1 to 40% in diseased cases. Aquaporin 4 was expressed throughout Mueller cell bodies in retinas of healthy controls. This expression pattern changes in uveitic state to cell nuclei of inner and outer nuclear layer. Overall expression of AQP4 increases in ERU.

Conclusions
Our data underscore the importance of Mueller glial cells in the pathogenesis of ERU. The changed expression of Mueller cell membrane channels in the uveitic state might lead to imbalance of cell homeostasis and contribute to the ongoing cell damage, resulting in edema and loss of function.

Acknowledgement
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Ultrastructural analysis of photoreceptor outer segments using rhoGFP transgenic mice

Dominic Eberle, Thomas Kurth, Marius Ader#
Center for Regenerative Therapies, TU Dresden

Purpose
Retinitis pigmentosa (RP) as one of the most common forms of inherited retinal degeneration is, beside age-related macular degeneration, diabetic retinopathy, or cone-rod dystrophy, characterised by a significant progressive loss of photoreceptor cells. Currently, there is no effective treatment available. Many current studies are focussing on a cell transplantational approach to replace degenerated photoreceptor cells using stem or progenitor cells. Up to now the successful transplantation and integration of cells into the outer nuclear layer (ONL) has been shown as well as some first hints that some of these cells manage to connect to bipolar cells and form inner and outer segments. Nevertheless, the key feature of every photoreceptor, to have a functional outer segment with properly aligned disc membrane staples has not been studied well so far. Here we show an ultra-structural analysis of outer segment integrity which might be useful for further transplantational studies.

Methods
For our studies we used rhodopsin-GFP fusion construct mice (rhoGFP).
Transplanted cells were gained from postnatal day 4 rhoGFP retinas and transplanted into the sub-retinal space of wild-type mice using a Hamilton syringe. Further analysis was done by immunohistochemical staining of ultra-thin cryosections with anti-GFP antibody. For electron microscopy (EM) analysis a protein A gold staining was applied at labelled cryosections.

Results
We were able to detect the reporter protein on ultra-thin cryosections in very good quality. The anti-GFP antibody we used showed a very specific staining and nearly no background. After labelling with protein A gold particles and observation using an transmission electron microscope (TEM) we observed a very specific and well visible staining of outer segments with no background. Membrane disc staples were clearly visible in high contrast. Additionally we were able to rediscover transplanted rhoGFP cells. Their outer segments are specifically stained whereas the surrounding wild-type cells show no signal at all. The transplanted cells often start to develop membrane disc staples but we were not able to find a transplanted cell with properly developed and aligned discs. Surprisingly, a lot of cells which didn't manage to integrate and staying in the subretinal space seem to be successful in forming outer segments with intact and well organised disc membrane staples.

Conclusions
We could show that our method is very suitable for detailed examination of outer segments of transplanted photoreceptor cells which might be very useful for further transplantational studies.

Statement on proprietary interests
The authors hereby state that there are no commercial of proprietary interest of any kind of any drug, device, or equipment mentioned in this study. Neither author has any financial interest of this study. This poster was prepared solely by the authors listed.

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Examining the molecular basis of light adaptation at the photoreceptor ribbon synapse

Michaela Fuchs, Johann Helmut Brandstätter
Department of Biology, Animal Physiology, University of Erlangen-Nuremberg, Erlangen, Germany

Photoreceptor ribbon synapses transmit signals over a wide dynamic range and they continuously adjust their synaptic output to changes in light intensity. Such a level of performance requires a specialized presynapse and complex adaptive mechanisms. It is well known that the synaptic ribbon is a highly dynamic organelle, which changes its size and shape depending on illumination and thus activity. However, the molecular basis of these adaptational changes is unknown. The focus of this project is to identify the molecules at the photoreceptor ribbon synapse, which are involved in the light-dependent, adaptational processes.
With immunocytochemistry, high resolution light and electron microscopy, western blotting and with laser microdissection in combination with quantitative realtime-PCR, we study the localization and expression of ribbon and active zone associated proteins during the regular 24h cycle and under various light and dark regimes in the retina of C57BL/6 mice.
From what is known in the literature, we were surprised to find no obvious differences between the various experimental conditions with respect to photoreceptor ribbon structure and the localization of ribbon and active zone associated proteins, e.g. Piccolo, Bassoon, RIM1, RIM2, Syntaxin 3, etc. with the exception of the main ribbon constituent RIBEYE. In retinae acutely exposed to light and stained for RIBEYE, the outer plexiform layer (OPL) appeared disorganized and the photoreceptor ribbon structure less compact, compared with the OPL in dark-adapted retinae. First results from quantitative realtime-PCR experiments suggest a decreased RIBEYE expression in the light and an increased expression in the dark.
As most of the data concerning illumination dependent changes in ribbon structure originate from experiments using albinotic BALB/c mice, our data suggest a much less pronounced capacity for adaptational changes in pigmented C57BL/6 mice.

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Bruch's membrane changes in the APP/PS1 transgenic mice model of Alzheimer's disease

Ashraf Gango1, Sabrina Cahyadi1, Zhi-Hong Chi2, Zhan-You Wang2 Tunde Peto3 and Imre Lengyel1

1 UCL Institute of Ophthalmology, London, UK; 2 Key Laboratory of Medical Cell Biology of Ministry of Education, China Medical University, Shenyang, P.R. China; 3 Moorfields Eye Hospital, London, UK.

Purpose
Age-related macular degeneration (AMD) and Alzheimer's disease (AD) share many similarities. Almost identical genetic and environmental risk factors and similar molecular processes are involved in the resulting neurodegeneration. The purpose of this study was to examine whether deposit formation in the brain is associated with increased sub-RPE deposit formation in the Bruch's membrane in a transgenic animal model for AD and to assess whether excess zinc deposition is associated with Bruch's membrane changes.

Methods
The well known APP/PS1 [B6C3-Tg (APPswe, PSEN1dE9) 85Dbo/J] transgenic mice model of AD were used to study ultrastructural changes in the Bruch's membrane. Animals were sacrificed at 4 and 12 months of age and changes in Bruch's membrane thickness was compared to those of 4 and 12 months old wild type mice. Following terminal anaesthesia animals were injected with 10 mg/kg sodium selenide then the animals were perfused with 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4) for 20 min. Eyes were removed and postfixed in the same buffer before processing for autometallography (Danscher, 1982) and electron microscopy. Half of the ultrathin sections were stained with 1% uranyl acetate and lead citrate and the other half was left unstained. Images were acquired using JEOL JEM-1010 TEM. Care was taken to measure Bruch's membrane thickness at segments of pictures where the choriocapillary lumen was both open and lined by a single layer of endothelium. Intercapillary, far periphery and optic nerve regions were avoided. Ten measurements were made in segments of equal length form 10 pictures from each mouse. The number and size of zinc granules were measured using ImageJ 1.43 by cropping Bruch's membrane areas and using the semi-automated counting function.

Results
Our data confirms earlier reports showing a thickening of Bruch's membrane with normal ageing. At 4 month the average thickness of BM in wild type animals was 0.345 ± 0.047 µm that increased to 0.63 ± 0.12 µm by 12 months. However, in the transgenic groups Bruch's membrane was already thickened at 4 month (0.53 ± 0.04 µm). At 12 months there was a dramatic increase in BM thickenss (1.21 ± 0.07 µm) accompanied by the appearance of sub-RPE deposits. Preliminary data shows that there is substantially more zinc in BM in TG animals compared to WT. Injection of DEDTC (a zinc selective chelator) completely abolished autometallographic labelling of zinc.

Conclusions
The APP/PS1 transgenic mice have an elevated level of amyloid beta through over production and altered processing. Simultaneously thickening of Bruch's membrane and sub-RPE deposit formation is facilitated in the back of the eye. This thickening is associated with increased zinc deposition in the BM. These might be relevant to understand how sub-RPE deposits are formed but also important in strengthening further the association between AMD and AD. Importantly, this transgenic model for AD may represent a good model to study early deposit formation that lead to retinal degeneration and blindness in AMD.

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Development of M/L-cones in organotypic retinal cultures of Syrian golden hamster and Norwegian rat

Gergely Halász, Viktória Doma, Arnold Szabó, Ákos Lukáts

Department of Human Morphology and Developmental Biology, Semmelweis University, Budapest, Hungary

The retina of most mammalian species contains two types of cones, one being sensitive to shorter- (S-cones), and another one to the middle- or long wavelengths (M/L-cones). According to theory of transdifferentiation, these two populations do not develop independently. All cones first express the S-opsin only, and some of them continue to do so till adulthood. The rest switch on M/L-opsin production as well, coexpresses both pigments, and then S-opsin disappears from their outer segments. Despite intensive studies, little is known about the factors influencing this pigment switch. The putative candidates are numerous; their precise role however is mostly unknown.

Hereby, we report a new model, an in vitro organotypic retinal culture, to study the possible regulatory factors of M/L-cone differentiation in Norwegian rats and Syrian golden hamsters. In the latter species, all cones express the M/L-pigment only, an ideal situation for developmental investigations. The retina of both species exhibits full differentiation in vitro, under control conditions. Analyzing and comparing the retinal developments, using different culturing conditions, allow us to study the regulation in two different systems. The experiments reported here, focused on thyroid hormones - that play a decisive role in the mouse -, but other factors, such as serum and vitamin-E substitution were also considered.

Our results show, that if the retinas were explanted after the 4th postnatal day (P4), the composition of the culturing media had no effect on cone development. M/L-pigment expression was observed in all culturing conditions. This confirms the results of PCR experiments in which the mRNA was first detectable at P4.

In both species studied, serum supplementation alone (10% FCS), was sufficient to induce M/L-cone differentiation in cultures explanted prior to P4, indicating that the serum contained all the necessary factors in suitable concentrations.

In serum free conditions however, differences were detected between the species used. In the rat, thyroid hormone substitution was enough to induce transdifferentiation, no other factor studied, had any significant effect in cultures initiated between P1-P4.

In the same time window, in the Syrian hamster, thyroid hormone substitution alone, - even if added in excess – was unable to produce detectable M/L-pigment expression, unless the media contained sufficient quantities of vitamin-E as well. The effect of vitamin-E seems to be specific, as no significant macroscopic difference was observed between cultures supplemented or devoid of it.

These results prove that retinal culturing could be used as a reliable tool to study the possible effect of soluble factors in retinal differentiation. In both species studied, thyroid hormone seems to be necessary for M/L-cone development, but other factors like vitamin-E could also play additional roles.

From bench to scientific poster: Classical proteomics in autoimmune uveitis –an undergraduate training program

Stefanie Hauck3, Konstantin Lutz1, Martin Gerdes2, Sabine Geuder2, Sabrina Hangen2, Jessica Klemmer2, Carina Renner2, Sophia Walter2, Sophie P. Watz2, Johanna K. Zipplies1, Cornelia A. Deeg1

1 Institute of Animal Physiology, Department of Veterinary Sciences, Ludwig-Maximilians University, Munich, Germany
2 Participants of "Wahlpflichtfach Proteomik – klassische Methoden der Proteomanalyse", in alphabetical order
3 Department of Protein Science, Helmholtz Zentrum München – Center for Environmental Health, Neuherberg, Germany

Purpose
Equine recurrent uveitis (ERU), the only spontaneous animal model for human autoimmune uveitis, occurs in the horse at a prevalence of ~10 percent. The etiology of the disease, however, has yet remained elusive. The aim of this study was to investigate a relevant disease model with methods of classical proteome analyses in the context of undergraduate students training. In order to train for research, the results from the practical course should be translated into a scientific poster and presented at an international conference.

Methods
Samples from ERU as well as healthy horses were collected and processed for 2D-gel analyses. IPG strips (pH 3-11) were loaded with equal total protein amounts (75 µg) and proteins were isoelectrically focused until steady state. Before second dimension, strips were equilibrated under reducing conditions followed by alkylation with iodacetamid. Second dimension was performed on small self cast SDS-PAGE gels and gels were stained silver. Stained and dried gels were scanned and images loaded into graphic analysis software and qualitatively evaluated.

Results
During the practical course, different samples were investigated: sera from ERU and healthy horses, enriched peripheral leukocytes (PBL) from both conditions as well as vitreous from the ERU and healthy condition. A total of 12 samples underwent 2D gel analyses. As a result from the qualitative analyses of the silver stained protein patterns after separations, several conclusions were apparent:
Healthy vitreous is less complex as compared to ERU vitreous. Many changes in the 2D protein pattern could be observed. In contrast, PBL patterns are similar between the healthy and the ERU state, and no obvious changes are apparent from the qualitative analysis. In serum likewise no apparent changes between both conditions are detected, serum is presenting on the 2D gels with very little complexity, due to high abundance of view serum proteins. Vitreous of diseased horses contains a similar protein pattern to serum.

Conclusions
Classical 2D gel analyses can be easily used in students training and proves to be a stimulating and motivating topic when applied to real samples. Results-driven systematic analysis enables preparation of scientific posters and teaches effectively scientific work style.

Degenerating photoreceptors in the RCS rat can be rescued by an AAV2/4-RPE65-Mertk vector

Peter Heiduschka1, Sylvie Julien1, Focke Ziemssen2, Anna Günther1, Sigrid Schultheiß1 and Ulrich Schraermeyer1

Centre for Ophthalmology, 1Section for Experimental Vitreoretinal Surgery, 2University Eye Hospital, Schleichstr. 12, D-72076 Tübingen, Germany

Purpose
The RCS rat with its mutation in the Mertk gene and therefore the loss of phagocytic function of the retinal pigment epithelium (RPE) is an established model for retinitis pigmentosa. We checked whether photoreceptors can be rescued from degeneration by introduction of an intact copy of the rat Mertk gene by an adeno-associated viral vector.

Methods
We used an AAV2/4-RPE65-Mertk vector with a high specificity for RPE cells and a high effectiveness of Mertk expression. A solution of the vector (2 µl with 1.2 x 1011 vg/ml) was injected subretinally into the eyes of 13 20-day-old RCS rats. As a control, PBS alone was injected in three rats, and sham surgery or no treatment was performed in 14 rats. After one or two months, protective effects were evaluated by checking retinal function by electroretinography (ERG), inspection of the eyes by optical coherence tomography (OCT) as well as histological and ultrastructural evaluation of sections of the eyes.

Results
Enhanced ERG amplitudes compared to the control eyes could be recorded in 8 out of 13 eyes injected with the AAV Mertk vector one month after the injection of the vector, and in 7 out of 10 eyes two months after the injection. Histological inspection of the eyes revealed a well-preserved photoreceptor layer, though restricted to a part of the eye, in 8 out of 12 eyes one month after the injection of the vector, and in 6 out of 8 eyes two months after the injection, whereas normal degeneration was found in 11 out of 13 eyes injected with PBS. Enhanced survival of photoreceptors was found in particular in those eyes where increased ERG amplitudes had been measured before. An increased thickness of the retina was also found by OCT, simultaneously with a decreased autofluorescence in the rescued area. Moreover, phagosomes could be detected in the RPE by electron microscopy two months after an injection of the vector.

Conclusions
We found that degenerating photoreceptors in the RCS rat can be rescued and their function can be preserved by a subretinal injection of an AAV Mertk vector. Further studies will target the issues of long-lasting effects and a broader distribution of the injected vector in the eye. These results encourage the search for a gene therapy for retinitis pigmentosa.

Proinsulin: A putative novel therapy for retinal degenerations.

Alberto M Hernandez-Pinto, Gomez-Vicente, V. and de la Rosa, E. J.

3D Lab (Development, Differentiation and Degeneration). Centro de Investigaciones Biológicas – CSIC. SPAIN.

Purpose
Retinitis Pigmentosa (RP) is a general term for a disparate group of hereditary retinal dystrophies characterized by a progressive loss of vision and photoreceptor cell death. The aim of the present work is to characterize molecular and cellular changes leading to photoreceptor loss and to explore the therapeutic effect of proinsulin in attenuating visual decline.

Methods
Two RP mice models, rd1 and rd10, have been employed. The putative therapeutic effect of proinsulin in both models was evaluated by means of either constitutive transgenic expression of human proinsulin (hPi) or by treatment with AAV vectors coding for hPi. Rd1 and Rd1/tghPi mice were euthanized at postnatal days 12 and 14, whereas Rd10, Rd10-AAV(hPi) and Rd10-AAV(Ø) were euthanized between postnatal days 20 and 32. Eyes were rapidly enucleated and retinas processed to determine the molecular and cellular response to proinsulin.

Results
In this work, we have focused our research in characterizing the occurrence of oxidative and endoplasmic reticulum stress during RP-associated retinal degeneration. In parallel, we are characterizing proinsulin effect in these processes.

Conclusions
Neuroprotection induced by proinsulin may provide a mutation-independent therapy for hereditary retinal dystrophies.

Statement on proprietary interests
EJ de la Rosa is co-author of a presented patent and founder of a Spin-Off company.

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Soft contact lenses as a basis for the cultivation of the fetal fibroblasts.

Olga Ivanova, Drozhzhina G.I
The Filatov Institute of Eye Deseases and Tissue therapy Odessa, Ukraine

Purpose
The purpose of study was possibility use the different on composition soft contact lenses as a basis for the cultivation of the fetal fibroblasts. Used two types of soft contact lenses (Akvalan, Ukraine). The first group of lenses contained a hydrogel 87% and polyacrylamide - 13%, second group 87% is included hydrogel 87%, polyacrylamide 12% and 1% collagen (rat).

Conclusions
Forming of monolayer of the fetal fibroblasts is possible on the internal surface of soft contact lens at cultivation in the conditions in vitro. In the group of the cultural lenses, composition of which had 1% of collagen, monolayer of the cells covering practically the entire area of the lenses on the 5ht day of cultivation.

Statement on proprietary interests
aplying corneal stem cells

Blue light collagen cross linking to treat progressive myopia

Nicole Körber1,2, Qing Liu2, Christian Koch1, Katrin Günter1,2, Peter Wiedemann3, Andreas Reichenbach2, Hans Peter Iseli4 and Mike Francke1,2

1 TRM, University of Leipzig, 2Paul-Flechsig Institute of Brain Research, University of Leipzig, Germany, 3 Eye Clinic, University of Leipzig, 4 Institute of Refractive and Ophthalmic Surgery, Zuerich, Switzerland

Purpose
The aim of our study is to develop an effective and applicable method to treat progressive myopia. Myopia is the most common eye disorder of the world, with an incidence of 20-30 % in Western Europe and up to 90 % in Asian regions, respectively. About 50 % of people with myopic eyes suffer from progressive (degenerative) myopia characterized by excessive eye growth and severe pathological changes of the retina. The biomechanical weakness of the sclera enables an excessive axial elongation of the eye, driven by intraocular pressure. We use the method of Riboflavin/blue light collagen cross linking to increase scleral stiffness and thereby, to reduce or stop eye elongation.

Methods
The sclera/eye of adult and young postnatal rabbits was treated with Riboflavin/blue light. The exact parameters for the adequate laser intensity, irradiation time, Riboflavin concentration and soaking time were determined to avoid degenerative side effects. The animals were monitored ophthalmologically over a period of 3 weeks and subsequently, the eyes were isolated and examined by means of histology, immunohistochemistry and electron microscopy. Additionally, isolated scleras from adult rabbits were examined by biomechanical measurements.

Results
Biomechanical measurements of isolated scleras from adult rabbits revealed an increased stiffness/rigidity after collagen cross linking. The examination of treated eyes of adult rabbits indicate a critical treatment intensity of 400mW/cm². Higher irradiation intensities induced neurodegeneration and glial cell reactivity in affected retinal areas. Young animals treated with a suitable laser energy of about 10mW/cm² displayed a clear reduction of their eye growth as compared to the fellow control eyes. Immunohistochemical examinations showed no neurodegenerative side effects or signs of glial reactivity if using an intensity lower than 200mW/cm².

Conclusions
The results confirm the idea that collagen cross linking by Riboflavin/blue light irradiation is a suitable method to increase scleral stiffness and therefore, it might be an adequate treatment for progressive myopia.

Decreased Ezrin Expression in Peripheral Blood-derived Lymphocytes of Horses with Spontaneous Recurrent Uveitis

Roxane L. Kramer(1), Stefanie M. Hauck(2), Barbara Amann(1), Marius Ueffing(2, 3), Cornelia A. Deeg(1)

1) Department of Veterinary Sciences, LMU Munich, Munich, Germany
2) Department of Protein Science, Helmholtz Center Munich, Neuherberg, Germany
3) Centre of Ophthalmology, University of Tübingen, Tübingen, Germany

Purpose
Equine recurrent uveitis (ERU) is a spontaneous, periodic recurrent and T-cell mediated disease leading to blindness. It affects 10 to 15 percent of the equine population worldwide and is the only spontaneous model for human autoimmune uveitis. In ERU, autoaggressive lymphocytes manage to migrate through the blood-retinal barrier (BRB). Differences in abundance of lymphocyte proteins between healthy and diseased state potentially reflect reasons for crossing of the BRB by these cells. Thus the aim of this study was the identification of differentially expressed proteins in peripheral blood-derived lymphocytes of ERU affected horses compared to non-diseased animals.

Methods
In this study, peripheral blood-derived lymphocytes (PBL) of 37 healthy and 33 ERU diseased horses were examined.
After cell lysis, proteins were labelled with fluorescent CyDyes and 2D Difference Gel Electrophoresis (2D DIGE) was performed comparing healthy and diseased state. The resulting gels were then scanned at different wavelengths. Through spot detection and quantification using DeCyder 6.5 software we generated a spot map with identical spot boundaries for all gel images. Differentially expressed spots were then excised and subsequently identified either by MALDI/TOF-TOF or by LC-MS/MS. Expression differences of identified candidates were verified by Western Blots as well as immunocytochemistry of PBL cytospin preparations and immunohistochemistry of ERU specific ocular lymphoid follicles.

Results
We created a map of immune cell proteins and determined 20 differentially expressed proteins comparing PBL of healthy and diseased state. Eight of the possible candidates were identified by mass spectrometry and analyzed in their function and relevance. Two of the identified proteins showed higher abundance in ERU and 6 proteins were decreased in their expression. Among the latter was Ezrin. Ezrin is involved in cell adhesion, motility and morphogenesis and plays a role in signal transduction. In phosphorylated state, Ezrin is also associated with T-cell activation. Decreased Ezrin expression in lymphocytes of ERU diseased horses points to changes in immune response, cell motility and transmigration of PBL in ERU.

Conclusions
Differences in complex immune cell protein expression patterns can be efficiently studied by proteome analysis involving fluorescent protein labelling and 2D DIGE.
Diminished expression of Ezrin in PBL of ERU diseased horses merits further exploration and characterisation.

Acknowledgement
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Annexin 2 is a new component of the RPE phagocytic machinery

Ah-Lai Law (1, 2), Clare E Futter(1), Matthew J Hayes(1), Stephen E Moss(1)

(1) UCL Institute of Ophthalmology, Division of Cell Biology, 11-43 Bath Street. London. EC1V 9EL, UK
(2)Current address: Institut de la Vision, Team of E.F. Nandrot, Genetics Department, INSERM UMR_S968, Université Pierre et Marie Curie-Paris6, CNRS UMR_7210, 17 rue Moreau, 75012 Paris, France

Purpose
Annexin 2 is implicated in many cellular internalisation systems but its role in the retinal pigment epithelium (RPE) has not been studied. Annexin 2 is enriched in the apical microvilli of RPE cells that mediate photoreceptor outer segment (POS) phagocytosis. RPE phagocytosis is absolutely essential for the function of the photoreceptors and for vision. The purpose of this study was to ascertain if annexin 2 has a role in the RPE phagocytic machinery.

Methods
ARPE-19 cells were challenged with POS for confocal microscopy, biochemical and kinetic studies. To consider the circadian aspects of POS phagocytosis, eyes from annexin 2 knockout (Anxa2-/-) and wild-type mice were harvested before and after light onset. Eyes were processed for transmission electron microscopy (TEM), phagosomes were quantified along the RPE and were then mapped from Bruch's membrane. Separate eyes were processed for flat mounting and labeled with 1D4 rhodopsin antibody, which identifies early phagosomes, and ZO-1 to identify RPE cell junctions.

Results
In ARPE-19 cells annexin 2 localised to the phagocytic cup during POS internalisation and dissociated from it once internalisation was complete. Down-regulation of annexin 2 using siRNA decreased phagocytosis suggesting an important role of annexin 2 for phagocytosis completion. In vivo, Anxa2-/- animals lacked the characteristic burst of phagocytosis one hour after light onset exhibited in wild-type animals. Phagosomes were retarded in the apical processes of RPE cells, when they should have been already internalised. Furthermore, annexin 2 was phosphorylated on tyrosine at the time of POS phagocytosis. Concomitantly, the downstream signaling molecules c-Src and FAK were phosphorylated in wild-type retina, whereas phosphorylation of FAK and c-Src was delayed in Anxa2-/- retinas.

Conclusions
This study demonstrates that annexin 2 is required for efficient internalisation of POS both in vitro and in vivo by RPE cells. Annexin 2 appears to be necessary as an early step for the timely activation of FAK and c-Src that initiates the signaling pathway for POS internalisation.

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Genetic determinates of Keratoconus identified with SNP mapping

Judith Lechner, Prof Anne Hughes, Mr Colin Willoughby
Queen's University Belfast

Purpose
Keratoconus is a bilateral, non-inflammatory progressive corneal ectasia that is the leading cause of corneal transplantation in the Western World. It is an inherited disorder of the cornea or the transparent outer part of the eye. The pathogenesis of keratoconus is unknown and no specific treatment exists except to replace the corneal tissue by surgery (corneal transplantation) which is not without risk – graft rejection, infection, transmission of CJD and recurrence of original disease can occur. Keratoconus has a strong genetic basis and the application of DNA microchips (SNP arrays) can accelerate gene identification. The key objective is to identify genetic determinants associated with keratoconus. Identifying the genetic basis of this common corneal condition in young adults will allow the development of newer therapies.

Methods
SNP microarrays, microsatellite markers, long range PCR, next generation sequencing

Acknowledgement
Fight for Sight

Choroidal blood flow and retinal ganglion cell function in early glaucoma

DARIO MARANGONI, COLOTTO A, SALGARELLO T, STIFANO G PARISI V, RIVA CE, CAMPOS EC, FALSINI B.

Institute of Ophthalmology, Catholic University (Rome)
Ophthalmology, GB Bietti Eye Foundation-IRCCS (Rome)
Department of Ophthalmology, University of Bologna (Bologna)

Purpose
To assess subfoveal choroidal blood flow in patients with early manifest glaucoma (EMG) and to compare blood flow with functional measures of retinal ganglion cell (RGC) integrity

Methods
Subfoveal choroidal blood flow was determined by confocal, real-time laser Doppler flowmetry in 25 patients with EMG (< -6 dB Humphrey mean deviation, age range: 42-64 years, visual acuity: 0.8- 1.0) and in 20 age-matched control subjects. All patients had a therapeutically (topical beta-blockers with or without a prostaglandin) controlled intraocular pressure (IOP < 20 mm Hg). Subfoveal choroidal blood volume (ChBVol), velocity (ChBVel) and flow (ChBF) were determined as the average of three 60 sec recordings with changes in the DC < 10% between the recordings (the DC measures the intensity of the light scattered by the tissue and red blood cells in the illuminated volume). In all patients and controls, pattern electroretinograms (PERGs) were also recorded according to a standardized protocol

Results
In EMG patients, average ChBVel and ChBF were reduced by 31 and 35%, respectively ($p < 0.01$) compared to control values. No significant difference in ChBVol was found between the two groups. PERG amplitudes were reduced by 40% ($p < 0.01$) in EMG patients compared to controls. No correlation was found between anyone of the choroidal flow parameters and PERG data or IOP values

Conclusions
The results suggest a significant alteration of subfoveal choroidal hemodynamics in EMG patients, involving both ChBVel and ChBF. These changes do not appear to be associated with the severity of functional retinal ganglion cell loss. Our findings may have implications for the pathophysiology of early glaucomatous damage and its treatment

Study photoreceptors without a retina: Cone-like 661W cells in a neuroprotection screening system

Stine Mencl, Dragana Trifunovic, Stefan Kustermann, Francois Paquet-Durand
Division of Experimental Ophthalmology, Centre for Ophthalmology, Tübingen, GERMANY

Purpose
Human vision is strongly depending on cone photoreceptors. But in many eye diseases like age-related-macula-degeneration (AMD), diabetic retinopathy, retinitis pigmentosa, achromatopsia, and cone-rod-dystrophies cone degeneration is taking place. For all of these diseases no or only insufficient therapies are available. An effective neuroprotective substance that prevent or delay vision loss disease independently would be a great improvement. To search for a neuroprotective compound require a reliable, high-throughput model system. By now only living animals or retinal explant cultures are available. As an alternative we want to establish a cell culture based screening system using the cone-like 661W cell line (Al-Ubaidi et al. 1992). The advantages of a cell culture model system are an increased experimental outcome by using multi well plates and no animals are needed.

Methods
In the cone-photoreceptor-function-loss-1 (cpfl1) mouse line the cone specific phosphodiesterase 6 (PDE6) is mutation dependent not functional. This function loss is leading to an increase of cGMP, the activation of the cGMP dependent protein kinase (PKG) and to cone degeneration. With Immunohistochemistry the cone-like nature of the cells was validated, using different cone markers like opsins, glycogen phosphorylase or the cone PDE6. Also a staining against PKG1 and 2 was done. As a cone degeneration paradigm the PDEs in 661W were inhibited by the specific PDE inhibitors or the PKG was activated with 8-pCPT-PET-cGMP. At different time points the cell viability was measured with alamarBlue, Live/Dead assay, or cGMP accumulation was analyzed with Immunohistochemistry.

Results
The expression for the cone opsins, glycogen phosphorylase and the cone specific PDE6 in 661W cells were positive. Additionally the 661W cells are expressing PKG1 and 2. After IBMX treatment for 6h and 24h were no differences in cell viability visible but after 24h the cGMP level was increased. PKG activation was leading to a decrease in cell viability and an increase in cell-death after longer treatment.

Conclusions
The 661W cell line is cone-like and expresses also photoreceptor proteins like PDE6 and PKG, which are important for the cone degeneration paradigm we performed. IBMX treatment increased the cGMP level but showed no significant difference in the cell viability assay. Longer treatment with different concentration might show reduced cell viability. Treatment with the PKG activator leads to reduced cell viability and increased cell-death, which proofs that cells behaving like the degenerating cpfl1 cone photoreceptors.

Acknowledgement
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BBS12 knock down induces photoreceptor cells apoptosis mediated by Endoplasmic Reticulum stress and PERK activation

Anaïs Mockel, Schlicht Dominique,, Dollfus Hélène, Marion Vincent

Laboratoire de physiopathologie des syndromes rares et héréditaires – EA 3949
11 rue Humann, 67089 Strasbourg Cedex, France

Purpose
Bardet-Biedl Syndrome (BBS) results in retinal degeneration, obesity, polydactyly, and nephropathy BBS is an emblematic member of a class of rare genetic disorders called ciliopathies. BBS proteins are involved in primary cilium biogenesis and function. The primary cilium is a microtubule-based organelle present at the surface of almost all cell types in the human body, acting like the cell's antenna. Primary cilium is a mecha-no-sensor in kidney epithelial cells, or a spatio-sensor during development. In the retina, the connecting cilium of the photoreceptor is a modified primary cilium. To understand how a ciliary protein dysfunction can lead to retinal degeneration, we performed a functional study of BBS proteins in photoreceptor cells by gene knock down in mice retinal explants.

Methods
We performed organotypic retina culture from 15 days old mice using co-culture of pigment epithelium and retina. BBS12 knockdown was induced by infection of lentiviral particles carrying shRNA sequences. We analyzed induced cell stress using quantivative PCR and the apoptotic cascade by TUNEL assay. We perfor-med immunostaining of phototransduction proteins after light treatments to investigate light-dependent bidirectional movements of these proteins to analyze photoreceptors functionality.

Results
We were able to deplete 40% of Bbs12 RNA in the whole retinal explant. This depletion lead to an up-regu-lation of both Caspase 12 and Chop10 mRNAs, indicating endoplasmic reticulum stress. BBS12 knock down induces an increase of apoptotic cell death demonstrated by TUNEL positive nucleus. After BBS12 depletion, phototransduction proteins are mis-localized and photoreceptor outer segments are shorter.

Conclusions
BBS12 knock down in photoreceptor cells triggers apoptotic pathways mediated by Endoplasmic Reticulum stress and PERK activation probably due to a photoreceptor dysfunction because of transduction proteins mislocalization.

The genetic basis of keratoconus (KTCN) can be determined with gene expression profiling.

Dorota Muszynska, Dr David Simpson, Mr Colin Willoughby
Queen's University Belfast

Purpose
Objective 1. To perform gene expression microarray on normal and keratoconic corneas and cultured corneal epithelium and stromal keratocytes.
Objective 2. To analyse the expression, in normal cornea and changes in keratoconic tissue, of genes in regions identified from whole genome case-control association studies (GWAS) in KTCN. This approach will identify novel genes associated with keratoconus pathogenesis.
Objective 3. To knock down and/or over-express candidate genes in corneal cell cultures; cell wounding and migration studies.

Methods
Three types of sample will be analysed: 1) Whole corneal tissue: approximately 15 samples banked in RNAlater® from Canada; 2) Dissected corneal epithelium and stroma from ~ 20 corneal samples will be collected from KTCN patient following corneal transplantation, Belfast; 3) Tissue culture: cornea epithelium and stromal keratocytes will be cultured separately from fresh corneas.
Normal tissue (control): donor corneo-scleral rims subject to identical manipulation as groups 1-3.
Microarrays: Gene expression will be analysed in the above samples using Illumina® BeadChip whole genome gene expression arrays. The raw data will be analysed using the ' Genomics Workbench 'software (CLCbio) 'in-house'. Expression of selected genes will be validated using qPCR. Furthermore, data will be correlated with DNA genotyping array data and mapped loci to identify novel candidate genes for KTCN. Finally, preliminary investigations of the function of genes determined above will be initiated by in vitro knock-down/over- expression studies, as a prelude to future therapeutic approaches.

Conclusions
The genetic basis of KTCN provides an opportunity to apply molecular genetic approaches to elucidate the mechanisms responsible for the pathogenesis of keratoconus. The key objective is to identify the genetic determinants of keratoconus to enable the development of new therapies which may help to prevent progression of the condition.

Role of endogenous Dicer dependent processes in the development of mouse retina and telencephalon

Tomasz Jan Nowakowski, Pratt T., Price D.J.

The University of Edinburgh, Centre for Integrative Physiology, Hugh Robson Building, George Square, Edinburgh EH8 9XD, United Kingdom

Purpose
Dicer is a type III endoribonuclease necessary for the maturation of micro RNAs. RNA interference is a biological process by which small RNA molecules can induce Post Transcriptional Gene Silencing (PTGS) by inhibiting translation of specific messenger RNAs or targeting them to degradation in the RNA Induced Silencing Complex RISC.

The aim of this study was to investigate the role of Dicer dependent processes in cell division, death and differentiation during early development of mouse retina and telencephalon.

Methods
Using the cre-LoxP recombination system, we mutated Dicer in mouse embryonic dorso-nasal retina and the telencephalon and assessed the expression of markers of the proliferative and differentiating pools using immunohistochemistry.

Results
We found that the progenitor population was strongly affected by the loss of Dicer with profound loss of nestin positive radial processes both in the mutant retina and in the telencephalon. Mutant retina and lens were hypomorphic and underwent extensive apoptosis, although overall tissue morphology was not as strongly affected as in the mutant telencephalon. Retinal Ganglion Cell projections were disorganised. Additionally, we found evidence that the level of expression of Pax6 is affected by the loss of Dicer dependent processes.

Conclusions
Taken together, our results suggest that Dicer dependent processes are required for proliferation and survival of early neural progenitors in the mouse embryonic retina and telencephalon, as well as differentiation of early born neurons

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Retinal Tissue Oxygenation Mapping Based on Multispectral Images

Eran Ohel [a],[b], David Rosenblatt [b] ,Stanley Rotman [a], Dan Blumberg [c]

a - Ben-Gurion University of the Negev, Beer Sheva, Israel.
b - NeD - Next Dimension, Ofakim, Israel.

Purpose
We will describe a new method, in order to produce Retinal Tissue Oxygenation Mapping (RTOM) based on multi-spectral images, while each image is based on a few spectral bands, between 550-600nm.The main problem while doing RTOM is the complex optical environment: the retinal is spherical and the illumination is very dynamic between areas.

Methods
We will describe a method with two main steps: a- modeling of the illumination on the retinal, b- mathematic analysis based on optical behavior of the blood, so we can map the oxygen saturation and the blood concentration.

Results
The results will be compared to the traditional Flourescein Angiography (FA) imaging, to show reliability.

Conclusions
The RTOM can give the doctor unknown information, so it will enable early detection of several retinal diseases.

A New Interpretation of Components in the ERG Signals to Sine Wave Luminance Stimuli at Different Temporal Frequencies and Contrasts

Gobinda Pangeni, J. Kremers

Department of Ophthalmology, University Hospital Erlangen, Erlangen, Germany.

Purpose
To describe constituent components in the full-field luminance flicker ERGs in normal human subjects using a novel analysis.

Methods
Full-field stimuli were produced using a ganzfeld bowl with arrays of Light Emitting Diodes (LEDs) as light sources. ERG responses were recorded using DTL electrodes from five subjects (mean age ± SD: 34.4 ± 8.8) using luminance sine wave stimuli (white light; mean luminance 250cd/m^2) at different frequencies (1-120 Hz) and contrasts (25-100%). Signal analysis was performed using self-written programmes and spreadsheets in MATLAB and Excel.

Results
In agreement with previous studies, Fourier analysis on the original responses revealed that the amplitude of the fundamental component displayed a dip at about 12 Hz, coinciding with a maximum in the second harmonic component, indicating frequency doubled responses. By including measurements at low contrasts, it was possible to identify two distinct components. We introduced a new analysis method that separated the two components. We found that the waveform of a spike-like ("peaky") component was independent of frequency, but could vary in amplitude and time of occurrence. This component was fitted to the original response. Subtraction of the fitted "peaky" component from the original response revealed a second component with a waveform that closely resembled the waveform of the stimulus. This "sine-wave" component depends linearly on contrast and is prominent at low temporal frequencies, absent above 16 Hz and resembles the ERG in non-human primates after pharmacological blocking of signal transmission from photoreceptors to bipolar cells. The "peaky" component has a more non-linear contrast relationship and is more prominent in the high frequency region peaking at about 40 Hz. At about 12 Hz the two components have similar amplitudes.

Conclusions
We propose that the "sine-wave" component is mainly driven by activity of the photoreceptors (but does probably not reflect the modulation of the photoreceptor excitation) and the "peaky" component mainly represents activity of the inner retina. The interaction between these components at about 12 Hz results in a frequency doubled response.

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Effect of zinc on fenestra formation in cultured endothelial cells

Bhavika Patel, Sabrina Cahyadi, Meihua Ju and Imre Lengyel
UCL Institute of Ophthalmology, 11-43 Bath Street, EC1V 9EL, UK

Purpose
The retinal pigment epithelium (RPE) contains the highest concentration of zinc/g of tissue in the body. We hypothesize that the release of zinc from RPE cells will affect neigbouring tissues including the choroidal microcapillaries. This, consequently, may influence the exchange of nutrients and waste material. To prove this hypothesis we monitored the effects of extracellular zinc on fenestrae formation in a cellular model.

Methods
For our experiments a murine endothelioma cell line, bEND5, was used. These cells have been shown to form fenestrae when treated with 1.5 uM Latrunculin A (LA), an actin depolymerising agent, for 3 h. Cells were seeded onto 1% gelatine coated covers slips and maintained in high-glucose DMEM including 10% FBS and antibiotics. Zinc treatment started following an overnight incubation to adhere cells to the cover slips for 20 hours. Results were compared to 3 hours treatment with LA. Rhodamine phalloidin was used to visualize F-actin rearrangement. Sieve-plate (fenestrae enriched areas) formation was monitored by the use of diaphragm protein PV1 specific immunostaining. Immunolabelling was visualized using Zeiss LSM700 confocal microscope. Transmission electron microscopy (TEM) was used to confirm the formation of fenestrae. For this cells were grown on formvar grids coated with 1% gelatine in PBS, postfixed and dehydrated for imaging using JEOL 1010 TEM. Biologically available zinc concentration in the culturing medium was determined by using a zinc selective fluorescence indicator (ZnAF2) and fluorometry.

Results
The cultur medium was able to fully buffer zinc to up to 75 uM external zinc. At 100 uM the available zinc concentration was 100 nM (3 order of magniture lower than that of added zinc!). Cells exposed to up to 150 uM extracellular zinc alone showed very minor F-actin rearrangements but clear signs of sieve-plate formation without LA. Cells exposed to concentrations of zinc higher than 175 uM showed signs zinc-induced toxicity perhaps related to the depletion of zinc buffering capacity of the culturing medium. TEM showed the presence of fenestrae in areas of sieve-plates.

Conclusions
Due to the high zinc binding capacity of components in culturing mediums it is essential to determine the concentration of bio-available zinc to assess its efficiency to modify any biological processes. Too high concentration of added zinc can be toxic, too little will be buffered by molecules like albumin. In support of our hypothesis, we found that nanomolar levels of bio-available zinc can induce the formation of fenestrae in vitro. Therefore, we propose that zinc might be involved in regulating fenestrae formation in vivo.

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FATP1 and Vision

Marie O. Pequignot, Karim Chekroud Laurent Guillou, Emilie Brun, Gilles Ducharme, Christian Hamel, Philippe Brabet
Inserm U583, Institute for Neurosciences of Montpellier

Purpose
The visual cycle, occuring in the neuroretina and RPE, is the set of enzymatic reactions leeding to the regeneration of the visual chromophore, 11cis retinal. Isomerization of the all-trans retinylester, carried out by RPE65 in the RPE, is the limitating step of the cycle.We previously demonstrated in vitro that FATP1 (fatty acid transport protein 1) interacts physically with RPE65, and that the overexpression of FATP1 in Sf9 and HEK cells inhibits 11-cis-retinal production. Here we evaluate the visual activity in vivo of the FATP1-/- mouse.

Methods
Neuroretina and RPE morphology and function have been studied by optic and electron microscopy, and electroretinography. The visual cycle activity has been evaluated by light/dark adapted retinoid dosage, electroretinography recordings and lipofuscin measurment. Q-PCR assays have been setup to quantify the FATP family transcripts and those of the major actors of the visual cycle, in RPE and retina of wt and ko mice.

Results
We analyze the structure and function of the retina of young adult FATP1-/- mice as well as the visual cycle activity and show slight differences compared to the wild-type mice. Thus the absence of FATP1 does not perturbate strongly the vision mecanisms. Even with a slight dysregulation, an accumulation effect could be observed, and FATP1-/- mice may present abnormalities in oldness. However, we found again only slight differences between old (20-24 months) KO and wt mice in retina morphology, lipofuscin content, ERG or chromophore regeneration. To resolve this apparent contradiction between in vitro and in vivo results, we compared the expression of the major actors of the visual cycle between the two genotypes. We show differences in transcript content for rhodopsin (2-fold decrease), but no differences in protein content, and no differences for RPE65 and the other visual cycle proteins. We observed a modification of the transcription profile FATP4, another member of the FATP family in KO retina.

Conclusions
The lack of FATP1 does not affect the visual cycle activity in a mouse model. This could be due partly to a compensation phenomenon of FATP1 activity by FATP4. The slightly lower visual function in the FATP1-deficient mice could be explained by the implication of FATP1 in the lipid transport and in the energetic metabolism.

Acknowledgement
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Hue changes of amplitude-modulated flicker used to dissect visual pathways into early and late stages

Daniela Petrova, G. B. Henning, A. Stockman
Institute of Ophthalmology, University College London, U.K.

Purpose
It is well-known that monochromatic lights change colour and/or brightness when flickered; lights near 560 nm, for example, appear brighter, whereas lights near 650 nm appear yellower. These effects are usually attributed to nonlinearities, possibly located in different visual pathways. We used the nonlinearities to dissect the temporal characteristics of the two pathways into early (pre-nonlinearity) and late (post-nonlinearity) stages.

Methods
We used amplitude-modulated flicker with a high carrier frequency (fc=7.5–50 Hz) and a low amplitude-modulation frequency (fam=0.5–5 Hz). The observer set the modulation threshold for detecting the colour or brightness change at fam either as a function of fc (to determine the early temporal characteristics) or as a function of fam (to determine the late characteristics).

Results
The early characteristics were bandpass for both colour and brightness changes, peaking at about 10-15 Hz and falling-off in sensitivity at both lower and higher frequencies, whereas the late characteristics were low-pass, falling-off in sensitivity at higher frequencies with a slope consistent with a one-stage low-pass filter.

Conclusions
The early temporal characteristics, which can be related to photoreceptor responses, suggest the nonlinearities are relatively early in the visual pathways. Potentially, these nonlinearities can be used to localize clinical losses in visual function.

Statement on proprietary interests
Mainly, contrast sensitivity and temporal processing. Also, colour vision mechanisms; brightness, colour and saturation perception; nonlinear and linear system analysis; ON and OFF pathway asymmetries; and slew rate limiters.

Acknowledgement
Supported by BBSRC.

Toward a MEA-based assay of retinal function in the mouse.

Daniel L. Rathbun, Thomas Euler

University of Tuebingen, Institute for Ophthalmic Research

Purpose
As advances are made in molecular biology, the quest to treat or cure many diseases of the retina (e.g. retinitis pigmentosa and macular degeneration) intensifies. Because they are easy to manipulate genetically, mice have become the model animal of choice for many of these studies. Currently, histological and molecular techniques are frequently used both to characterize various disease models in the mouse and to assess the effectiveness of potential therapeutic interventions. However, for human patients, the gold standard by which any treatment will be measured is its ability to preserve, or restore, visual function. With this criterion in mind, many investigators have begun to expand their skill-set to include electrophysiological measures of retinal function. Unfortunately, the complexity of retinal electrophysiology poses a significant barrier for many, making a partnership between molecular biologist and electrophysiologist more attractive than ever. Here we propose the development of a robust multi-electrode array (MEA)-based assay of retinal function. The proposed assay will provide a high-throughput, turnkey system to supplement molecular investigations of retinal disease mechanisms and treatments.

Methods
Retina Preparation For acute recordings, eyes will be collected immediately following euthanasia. Retinas will be dissected from the RPE and sclera, cut into halves or quarters, and subsequently perfused in carbogen-saturated artificial medium. Prepared retinas will be mounted on a transparent multielectrode array (MEA; Multichannel Systems; Reutlingen, Germany) that is then secured to a microscope stage. Electrical signals (primarily extracellular RGC spikes) from the 60 electrodes of the array will be amplified and recorded on a personal computer using Multichannel Systems hardware and software. Temperature, oxygenation, and nutrient requirements of the tissue will be regulated via a continuously-refreshed perfusion medium.
For recordings from cultured tissue, retinas will initially be mounted photoreceptor side down on a membrane. These membrane-mounted retinas will then be transferred, RGC side down, onto the MEA. Thus, light stimuli will pass through the transparent MEA and retina to stimulate photoreceptors, while the MEA records extracellular signals from the RGC side of the retina.
Visual Stimuli Visual stimuli will be presented from below to the MEA-mounted retina via a custom apparatus consisting of a commercial DLP-based projector (K10; Acer Inc.) and a number of optical elements. The visual stimuli to be presented are diverse, but will include full-field flash stimuli to assess ON and OFF pathway responses as well as a white noise stimulus to assess the spatial and temporal sensitivity profiles of retinal ganglion cell receptive fields. Additional stimuli will be generated and optimized as needed to reveal disease- and treatment-induced changes of the encoded visual signal.

Results
A wide range of methods has been used to characterize the spiking responses of retinal ganglion cells (RGCs) in the mouse. This variety makes it difficult to draw meaningful comparisons between the limited number of existent studies. Therefore, our first step in developing the MEA-based assay will be to establish a baseline characterization of the distribution of extracellular RGC responses found in the wild type mouse.
Once a baseline of normal responses has been established, a pilot study will be conducted investigating the functional effects of trichostatin A (TSA) treatment in the rd1 mouse in order to evaluate the utility of MEA approach as a useful functional assay. Previous research has demonstrated that overactivation of certain histone deacetylases (HDACs) is associated with retinal degeneration in the rd1 mouse; and that pharmacological inhibition of these HDACs with TSA can reduce photoreceptor cell death (Sancho-Pelluz et al. 2010). In the proposed study, we will evaluate whether TSA-induced photoreceptor preservation results in preservation of visual function.
Functional examination of rd1 retina using electroretinography (ERG) has revealed that the b-wave was significantly attenuated and altered temporally at postnatal day 14 (P14) (Strettoi et al. 2003). This suggests that cone ON-bipolar cell function is impaired at this stage of degeneration. Therefore, we expect to record altered photopic ON responses with the MEA. Furthermore, we will be able to quantify any changes in ON latencies and ON/OFF antagonism that may exist. Since ERG recordings are most sensitive to changes in ON responses, it is unclear whether the OFF pathway exhibits a parallel pattern of dysfunction. Through MEA recordings, we will be able to simultaneously probe the OFF pathway for matching deficits. Following characterization of visual deficits in the rd1 mouse as they develop from P10 to P16, we will evaluate how TSA treatment alters this degeneration. It is hoped that TSA treatment will delay the onset of cone-mediated deficits and that rod function may be preserved long enough to produce visual responses around P10 when the retinal circuitry is sufficiently developed to yield light-driven RGC responses.

Conclusions
MEA-based measurements of retinal function will be shown to provide useful characterization of mouse models of retinal disease. Furthermore, these measurements will be shown to be useful in evaluating the effectiveness of potential therapeutic interventions in preserving or even restoring visual function.

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Expression profiling of the Cpfl1 mouse – a mouse model of cone dystrophies

Karin Schaeferhoff1, S. Michalakis2, N. Rieger3, B. Wissinger3, M. Biel2, O.Riess1, M. Bonin1.

1University of Tuebingen, Dept. of Human Genetics, Germany,
2Munich Center of Integrated Protein Science and Dept. of Pharmacy – Center for Drug Research, Ludwig-Maximilians-University Muenchen, Germany,3Molecular Genetics Laboratory, University Eye Hospital, Dept II, Tuebingen, Germany.

Purpose
The cpfl1 mutant (cone photoreceptor function loss 1) is a mouse model carrying mutations in the cone specific phosphodiesterase 6 (pde6c). The phenotype is characterized by a loss of cone photoreceptor function and a fast, progressive degeneration of the cones. To investigate the biological events leading to the loss of photoreceptors we combined mRNA expression analysis with whole genome miRNA expression profiling.

Methods
Expression analysis of Cpfl1 and wildtype retinas was performed using Affymetrix MOE 430 2.0 microarrays. miRNA expression profiling was conducted on an Illumina whole genome Universal Bead Array. Differential regulated transcripts with a minimum change in expression level of 1.5 fold (p-value ≤0.05) were obtained and gene regulation networks were generated by the Ingenuity Pathways Analysis software. Data was verified via qRT PCR and immunohistochemistry.

Results
338 transcripts were differentially regulated in the retinas of 4 week old Cpfl1 animals. A large number of genes encoding proteins involved in phototransduction were down regulated. A strong up regulation of Stat3 signaling was detected that could be verified by qRT PCR and immunohistochemistry. miRNA expression analysis of 4 week old mice revealed two significant regulated miRNAs which have potential target genes included in the differential transcript list of our microarray analysis

Conclusions
Expression analysis of the Cpfl1 mouse highlighted a misregulation of the phototransduction cascade in accordance with the loss of visual function that characterizes the phenotype. Up regulation of Stat3 signaling indicates a rescue attempt of the degenerating photoreceptors. The combination of mRNA and miRNA expression profiling permits a closer monitoring of the neurodegenerative events in the retina occurring during the course of degeneration.

Statement on proprietary interests
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Analysis of the allelic frequency of common mutations in CNGB3 and ABCA4

Alexandra Sauer1, Bernd Wissinger,1 Susanne Kohl1

1Molecular Genetics Laboratory, Institute for Ophthalmic Research, Centre for Ophthalmology, University Tübingen

Purpose
Mutations in four genes CNGA3 (20-30%), CNGB3 (40-50%), GNAT2 (<2%) and PDE6C (>2%) have been associated with achromatopsia, a congenital, autosomal recessively inherited disorder. This retinal disease is characterized by lack of color discrimination, low visual acuity, photophobia and nystagmus. The most prevalent mutation, accounting for ~75% of all CNGB3 mutant alleles, is the 1bp-deletion c.1148delC.
Mutations in ABCA4 are the cause of autosomal recessive Stargardt disease, but also autosomal recessive cone-rod dystrophy and autosomal recessive retinitis pigmentosa. Several mutations are observed recurrently in different populations. In a study performed in Tübingen, the most frequent mutation was c.5461-10T>C, a mutation with unclear pathogenicity. In contrast to that, the most common mutation found in other studies was c.2588G>C, which was found with an overall frequency of up to 1:54 in the general population.
Almost no data on the prevalence of genes and frequency of certain common mutations for inherited retinal dystrophies are available at the moment. Yet this information is necessary for adequate genetic counseling and estimation of recurrence risk.

Methods
A control panel of 500 healthy probands were analyzed by PCR/RFLP (restriction fragment length polymorphism) for the mutation c.1148delC in CNGB3 and c.5461-10T>C in ABCA4. The mutations c.1622T>C and c.2588G>C in ABCA4 were analyzed using high resolution melting (HRM). To confirm found sequence variants, a sequence approach was performed.

Results
In our panel of 500 controls we identified two subjects that were heterozygous for the mutation c.1148delC in the CNGB3 gene. Therefore the prevalence of this common mutation in the general population is estimated to be 1:250. For ABCA4, no proband carried the mutation c.1622T>C, nor c.5461-10T>C, while the mutation c.2588G>C was seen heterozygously in three probands, estimating a prevalence of this mutation of 1:166.

Conclusions
According to the Hardy-Weinberg Equilibrium, the data for the mutation c.1148delC in CNGB3 match with a calculated frequency of 1:250. The data for ABCA4 show that allele frequencies vary substantially between different populations, and confirm that c.2588G>C is commonly found in the general population. Additionally, the exclusion of c.5461-10T>C in 500 controls supports an impact of this unclear mutation for ABCA4-related retinal disease.

Ocurrance and importance of RGMA in the degeneration and regeneration of the crushed rat optic nerve

Sven Schnichels, Peter Heiduschka & Sylvie Julien

Centre for Ophthalmology, University Eye Hospital Tuebingen, Institute for Ophthalmic Research Tuebingen, Schleichstr. 12/1, D-72076 Tuebingen, Germany

Purpose
The death of retinal ganglion cells represents the final common pathway of virtually all diseases of the optic nerve. The injured neurons have the genetic program to regenerate their axons, however without success. Several repulsive proteins governing developmental axon guidance are reported to contribute to this failure. One chemorepulsive factor is the Repulsive Guidance Molecule A (RGMA), which has been functionally characterized as a molecular determinant for retinotectal map formation. The presence of RGMA in the glial barrier after SCI and brain trauma and its inhibitory activity in vitro suggest that it might exert inhibitory effects on regenerating axons in the glial scar. The precise temporal and spatial pattern of RGMA and its receptor neogenin in the optic nerve and retina after optic nerve crush is essential to understand the role of RGMA and other repulsive molecules in the regenerative failure of central nervous system neurons.

Methods
We used an adult rat model of regenerating axons (by performing lens injury (LI)) and non-regenerating axons after optic nerve crush. Sham, sham plus LI operated and untreated animals were also analyzed. Nine points of time (from six hours to twenty days after crush) were investigated to see potential differences between the five conditions. To identify the cells or tissue which were RGMA positive we double-labelled optic nerve and retina sections with cell marker antibodies against: macrophages, microglia, astrocytes, oligodendrocytes, intact axons, retinal ganglion cells, basal laminae of the glia limitans externa and blood vessels. Western-Blot analyses of the optic nerves and retinae from animals treated under the five previously described conditions were performed.

Results
We found RGMA at the crush site (CS) and in the maturing and matured scar of ONC rats at every time point investigated whereas it was absent in the CS of ONC+LI rats. Western blots of ON tissue displayed a significantly lowered amount of RGMA in ONC+LI animals at time points 2, 4 and 6 days after crush compared to ONC rats. Independent of the model, many cells were RGMA+ in the retina and ON: RGCs, nerve fibres, astrocytes, oligodendrocytes, some microglia and Müller cells, blood vessels and the sheath of the optic nerve. Moreover, RGMA was found in the nerve fiber layer, the inner and outer plexiform layers and in the inner and outer nuclear layers in all investigated retinae independent of the treatment. The neogenin pattern in the retina was congruent to the RGMA pattern except for the RGCs which were not neogenin+. Western blots of the retina showed cleavage products of RGMA only in LI animals. Furthermore, a higher amount of RGMA was found in the retinae of ONC+LI rats compared to ONC rats.

Conclusions
In conclusion, we showed that RGMA is part of the inhibitory environment at and around the CS after ONC. Our results suggest that RGMA has to be removed out of the CS to assure a regenerative environment at the CS. Furthermore, we have shown that RGMA expression is not only negative for axons, hence LI seems to increase RGMA expression in several cells.

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Blue Light-Induced Damage in Photoreceptors

Ulrike Schumann 1, R.H.W. Funk 1, M. Ader 2, C. Roehlecke 1

1 Institute of Anatomy, Technical University Dresden, Germany
2 Center of Regenerative Therapies, Technical University Dresden, Germany

Purpose
Age-related macular degeneration (AMD) -the most common cause of blindness- is characterized by progressive degeneration of photoreceptors, retinal pigment epithelium and choroid. Currently, there is no efficient cure or prevention method. Visible light, especially short wavelengths, is a significant factor in the production of radical oxygen species, thus contributing to the formation of AMD.
In this study, we have investigated the in vitro effect of visible blue light on photoreceptors to determine the molecular events leading to the death of cells. We hypothesize that membrane disks of the outer segments are the primary target of the light damage.

Methods
Murine retina explant culture was irradiated with blue light (405 nm) with an output power of 1 mW/cm2. The retinal layer was checked for apoptosis by TUNEL assay method. Morphological alterations in photoreceptors were determined by live imaging microscopy and on ultrastructural level by SEM and TEM. Images of living retina tissue were achieved by staining of the cell membranes of the outer segments with the mitochondrial dye JC-1.

Results
The examined vibratome sections of the retina showed apoptosis after 6 h especially in the outer nuclear layer and it accumulated during an extended blue light treatment. Although you can detect some apoptosis in the controls there is a rising in the blue light damaged retina observable. Using confocal microscopy we observed that blue light damage caused a misalignment of the normally neatly stacked outer segments in the retina. Ultrastructural analysis revealed changes in the morphology of outer segments of irradiated retinas compared to the control. The outer segments appeared tortuous after 24 h, and the lamellar structures became disrupted. We observed breaks in the cell membrane of outer segments already after 6 h as well, most of them occurred in the middle segment of the outer segments. The breaks or decomposition of the cell membrane lead to the disorganization of the stacked disks in the outer segments and their proper function during the visual cycle is endangered. There were no differences in the mitochondria noted.

Conclusions
These results show that visible blue light induces photoreceptor cell death in an in vitro model. The first alterations occur in the outer segments. The alignment of photoreceptors is affected by visible blue light which may explain the known dysmorphia in the central visual field of AMD patients. Constant light exposure can detrimentally affect photoreceptors. This may have implications for the pathogenesis of AMD.

Characterization of Mouse Mutants of α2δ-3, an Accessory Subunit of Voltage-gated Calcium Channels

Hartwig Seitter, Antonella Pirone, Jutta Engel, Marlies Knipper, Thomas Münch

Hartwig Seitter, Thomas Münch: Centre for Integrative Neuroscience, Tübingen
Antonella Pirone, Marlies Knipper: Tübingen Hearing Research Centre
Jutta Engel: Uniklinikum Saarland, Homburg

Purpose
Voltage-gated Calcium channels (VGCC) mediate synaptic vesicle fusion, regulate gene transcription, cause changes to signaling cascades and are the starting point to other crucial intracellular signaling functions. That is why VGCC have to be tightly controlled by modulatory and auxiliary factors, one of which are the α2δ subunits. In a mutant mouse model we want to characterize the impact of a loss of the α2δ-3 isoform of these subunits on the retina on several levels:

- Find out the expression pattern of α2δ-3 in the mouse retina
- Investigate the single cell physiology of the retinal cell types that express α2δ-3
- Study the system properties of retinal circuits that contain the affected cells
- Characterize the overall phenotype, including confirmation of multi-sensory phenotypes

Methods
In our mouse model, α2δ-3 is knocked out by insertion of the LacZ gene, which allows to readily determine where the α2δ-3 gene is expressed by co-localization with cell markers. In future studies, the physiological impact of the α2δ-3 mutation on the identified cells will then be characterized by patch-clamp recordings. In a second step the changes to overall retinal circuitry will be determined by micro-electrode array (MEA) measurements on the ganglion cell level. Finally the visual phenotype is to be investigated in the electroretinogram (ERG) and behavioural tests.

Results
In a tricky combination of immunohistochemistry and LacZ staining procedures, the cell type that expresses the LacZ under the α2δ-3 promotor could be determined to be almost exclusively horizontal cells. Further experiments confirm this observation and underline the specificity of this mutation.

Conclusions
The unique expression of α2δ-3 in one retinal cell type points to a highly specific function of this isoform of the α2δ Calcium channel subunit. Investigating into the mechanics of this mutation could reveal not only novel properties of the auxiliary α2δ subunits but also give further insights into the peculiar horizontal cell physiology. Finally we hope to see the sphere of influence of an altered Calcium signal in horizontal cells on downstream progression through the retinal circuitry.

To evaluate the effect of CYP450 modulators in diabetic cataractogenic rats

Akshaykumar Shah, Patel KV,Gandhi TR
Dharmaj Degree Pharmacy College Anand Pharmacy College

Purpose
Evaluation of the role of CYP450 modulators in diabetic cataract

Methods
Male SD suckling rats (18 days; 40-50 gms.) were randomly allocated to four groups (n=15). Group I animals were fed with normal laboratory chow whereas the animals of Group II, III and IV were fed with galactose (50 %) diet for 18 days starting from day 21 after parturition. Three days prior to the galactose feeding treatment with Pioglitazone (3.8 mg/kg; P.O; once daily) and Verapamil (40 mg/kg; P.O; once daily) was administered to Group III and IV respectively and continued till the end of study. Gross examination (daily), Microscopical examination (every 3rd day) was done. Three animals from each group were sacrificed on 4th, 9th, and 12th day of galactose feeding and the remaining animals were sacrificed on 18th day to estimate various biochemical parameters.

Results
Gross examination, microscopical examination and SEM photographs revealed that Pioglitazone treatment induced whereas Verapamil treatment delayed the appearance of cataract as compared to model control. Pioglitazone treatment decreased soluble & total protein, GSH, -SH and increased AR, insoluble protein, intracellular calcium and MDA on day 4, 9, 12, 18 corresponding to stages (I-IV) of cataract in model control. Verapamil treatment increased soluble & total protein GSH,-SH and decreased AR, insoluble protein, intracellular calcium and MDA on day 4, 9, 12, 18 corresponding to stages (I-IV) of cataract in model control

Conclusions
Pioglitazone treatment induces the cataract progression whereas Verapamil treatment delayed the cataract development.

Statement on proprietary interests
The authors reserve the right on the contents of the experiments

Identification of chemical compounds suitable to improve surface expression of trafficking deficient mutant CNGA3 channels using a calcium imaging based bioassay

Joachim Taeger1, Peggy Reuter1, Katja Koeppen2 and Bernd Wissinger1

1 Molecular Genetics Laboratory, Institute for Ophthalmic Research, Centre for Ophthalmology, University Clinics Tuebingen, Tuebingen, Germany 2 Department of Physiology, Dartmouth Medical School, Hanover, NH, USA

Purpose
CNGA3 and CNGB3 encode for the A3 and B3 subunit of the cone cyclic nucleotide-gated (CNG) channel, which is a crucial component of the phototransduction cascade. CNG channels enable the cGMP-dependent influx of sodium and calcium into the cone photoreceptor outer segment. Mutations in CNGA3 and CNGB3 are the major cause for achromatopsia, an autosomal recessively inherited retinal disorder characterized by a strongly reduced visual acuity, lack of color discrimination, photophobia and a nystagmus. Several CNGA3 mutations have been shown to affect protein folding and/or trafficking thus lowering the channel density in the plasma membrane in heterologous expression systems. Following the establishment of a bioassay, we will perform a medium-throughput screening for chemical and pharmacological chaperones, which may help to overcome the trafficking/folding deficits of mutant CNG channels.

Methods
HEK293 cells were co-transfected with plasmids encoding for the wild type or mutant (E228K, R427C and R563C) CNGA3 channel and pCAeq encoding for apoaequorin. Apoaequorin is the protein component of aequorin, a calcium sensitive photoprotein. After reconstitution of aequorin with coelenterazine, calcium binding leads to the oxidation of coelenterazine to coelenteramide, resulting in the emission of light. Following transfection, cells were treated for 24 hours with chaperones and the emitted light was recorded before and after the activation of the CNG channels with 8-Br-cGMP.

Results
Comparison of measurements using cells expressing wild type and mutant CNG channels revealed a significant reduction in the luminescence signal in channel mutants. The luminescence signal was elevated after the treatment of the transfected cells with glycerol. Three additional chemical chaperones (6-aminohexanoic acid, TMAO and Sorbitol), that have been tested so far, had no effect onto protein trafficking/folding.

Conclusions
The assay is suitable for the screening of substances, which could improve the trafficking/protein folding of mutant CNG channels. Glycerol, a known chemical chaperone, increased the luminescence of cells expressing the three analyzed mutant CNG channels indicating that the assay is sensitive and effective for a compound screening strategy.

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Rotenone is similar but not identical to light-induced apoptosis in RGC-5 cells, and is attenuated by two green tea flavonoids.

Tengku Ain Kamalden1,2, A. Bron1 & N.N. Osborne1

1Nuffield laboratory of Ophthalmology, Oxford University, Headley Way, Oxford OX3 9DU, UK
2Department of Ophthalmology, University of Malaya, Kuala Lumpur, Malaysia

Purpose
To understand the mechanism by which rotenone kills a transformed line of cells that exhibit some ganglion cell properties (RGC-5 cells) and to compare the potential neuroprotective properties of two naturally occurring flavonoids, epicatechin gallate (ECG) and epigallocatechin gallate (EGCG).

Methods
Equivalent amounts of RGC-5 cells were transferred to 96 or 12-well plates and exposed to different concentrations of rotenone alone or in combination with defined concentrations of various substances which given 60 minutes earlier. After 24 hours generally the cells were analysed for their viability (MTT), reactive oxygen species (using the DCF-DA) and apoptosis (TUNEL staining). Proteins were extracted and subjected to electrophoresis and western blotting for the analysis of various proteins involved in apoptosis.

Results
Rotenone dose-dependently increased ROS production and reduced the viability of RGC-5 cells in culture. In order to reduce viability by about 30%, 10μM of rotenone was used in subsequently studies. Rotenone-induced death was not affected by caspase inhibitors or necroapoptosis inhibitor (necrostatin). Western blot analysis showed rotenone stimulated p-c-Jun and heme-oxygenase-1 while downregulating Bcl-2. However, AIF was unaffected. Rotenone-induced apoptosis was counteracted by both green tea extracts (ECG and EGCG).

Conclusions
Rotenone is known to act on complex 1 of mitochondria. Like light-induced cell death mediated by action on mitochondria ROS is elevated. However, the apoptotic mechanisms for the two types of cell death are in detail different. Rotenone and light both stimulated p-c-jun, heme oxygenase-1, but only light activated AIF. The present studies also show that the both green tea flavonoids are effective at attenuating rotenone-induced cell death in a dose-dependent manner.

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A model of the mouse cone photoreceptor

Alexandra Tikidji-Hamburyan, Thomas Münch
Center for Integrative Neuroscience, Tübingen University

Purpose
The purpose of this work was to construct a model of cone photoreceptor, suitable for subsequent using in the network models of the retina. Biologically plausible modeling is becoming a powerful tool for research. However, large-scale modeling requires a huge computational power to proceed, and one way to diminish it is to simplify all single cell models. On the other side, excessive simplification leads to a loss of reliability of model and restrict possible using of the model. This work was dedicated to developing computationally effective and still capturing the main properties of as well phototransduction as ionic currents model, which would be able to reproduce the dynamics of the photoresponses.

Methods
Two published models have been taken. One model (van Hateren&Lamb, 2006) implements the main steps of phototransduction cascade. However, the ionic currents of the cone inner segment are simulated as one ionic channel with properties, fitted to each single pattern of light stimulus. Another model (Kourennyi at al, 2004), simulates the photocurrent of the outer segment through the sigmoid function, whereas the inner segment includes 5 Hodjkin-Huxley-styled ionic currents. To avoid the necessity of fitting parameters of ionic channel/sigmoid function each time when parameters of stimulus are changed, I have combined the model of phototransduction cascade and the model of the ionic currents from these two works. To fit and verify the model, I used the records of mouse cone photoresponses, presented in the work of Heikkinen at al., 2007.

Results
Overall, the model responses to the simulated light stimulation show a good correspondence to the photovoltages recorded in the mouse cone in a wide range of intensities. Only at the very bright illumination the model's response shows much faster recovery than the real data.
Analysis of the parameters after fitting has shown, that slight changes of chloride channel conductance lead to changes of the response shape, evoking small depolarization after returning to the baseline level (-40 mV), what have been observed in some species (Schneeweis&Schnapf, 1999).

Conclusions
The reason for faster recovery of the model response is that that the model of phototransduction is not complete and supposes an infinite amount of rhodopsin in the cone. It allows reduction of computational costs. If simulation of high light intensity conditions is needed, the necessary equations could be easily integrated into the model.
Adjusting of some parameters (e.g. Cl--conductance) allows to capture the features of photovoltage in different species. Overall, computational efficacy and response properties of the model make it suitable for using in network models.

Optical and electrophysiological recordings of synaptic input to retinal gangilion cells of transgenic mice expressing the genetically encoded calcium sensor TN-L15

Benjamin Usai, L. Dai, A. Brombas, D. Kaschuba, M. Mank*, O. Griesbeck*, F. Müller

Institute of Structural Biology and Biophysics, Cellular Biophysics (ISB-1), Forschungszentrum Jülich, Germany
* MPI for Neurobiology, München, Germany

Purpose
Recently optical imaging methods became a useful approach to record neuronal activity and study neuronal processing. Here we characterise a trangenic mouse line that expresses the genetically encoded calcium sensor TN L-15 in retinal ganglion cells (RGCs). The retina provides a neuronal network that performs the first steps in visual information processing. The RGCs serve as output neurons that relay the visual information to the brain. We employed a pharmacological approach to measure neuronal activity in RGCs in vitro. Calcium signals were compared with electrophysiological recordings of the same cell type. In particular we tested, whether the calcium imaging would visualise on the soma level changes in the excitatory synaptic input of RGCs.

Methods
Transgenic mice that express the calcium sensor protein TN L15 under the control of the thy 1 promoter were used. TN-L15 consists of the calcium-binding protein troponin C and a pair of fluorescent proteins (donor and acceptor) engineered for Förster resonance energy transfer (FRET).Calcium signal were imaged on flat-mounted retinae using dual emission CCD camera based microscopy. Patch-Clamp recordings of RGCs in the whole cell mode were performed to measure voltage as well as current responses in RGCs. RGC types that express TN-L15 were identified using immunohistochemical markers.

Results
Glutamate is the main excitatory transmitter in the retina. Compared to all other cells in the retina that mainly express ionotropic glutamate receptor types, ON-bipolar cells express the metabotropic glutamate receptor 6 (mGluR6). We used the mGluR6 agonist L-APB and the antagonist CPPG to specifically stimulate or inhibit ON-bipolar cells and measured postsynaptic activity in RGCs. ON-GCs receive direct synaptic input from ON-bipolar cells.In the whole-cell patch-clamp mode superfusion with L-APB lead to hyperpolarisation of the membrane voltage and a decrease in the action potential frequency in ON-RGCs. CPPG had the opposite effect. As the OFF-pathway is inhibited by the ON-pathway, OFF-RGCs showed responses opposite to those found in ON-RGCs.
In calcium-Imaging experiments roughly 30% of the cells show a decrease in the intracellular calcium concentration upon L-APB superfusion, while CPPG increased the calcium concentration. Those cells are most likely ON-RGCs. Cells showing the opposite effects, most likely OFF-RGCs, were frequently observed.

Conclusions
Transgenic mouse lines that express genetically encoded calcium sensor proteins will be helpful to study calcium signals and synaptic input in RGCs in vitro. We showed that the changes in intracellular calcium concentration reflect the electrical activity as well as synaptic input of RGCs. Therefore, the transgenic TN-L15 mouse line is a convenient tool to investigate calcium signals and synaptic excitation in ganglion cell populations in vitro.

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Molecular analysis of the PTEN GENE in a CHOROIDAL SCHWANNOMA in the context of a HAMARTOMATOUS SYNDROME

Giulia Venturini1, S. Uffer2 , L. Zografos2 , C. Rivolta1 , A.P. Moulin2

1 Department of Medical Genetics, University of Lausanne, Lausanne, Switzerland;
2 Ophthalmology, Jules-Gonin Eye Hospital, Lausanne, Switzerland

Purpose
To investigate the molecular involvement of PTEN, a gene acting as a tumor suppressor gene in many cancers, in a rare case of cellular pigmented choroidal Schwannoma in a patient affected by a hamartomatous syndrome due to a heterozygous PTEN germline missense mutation (c.406T>C).

Methods
The enucleated eye from the patient was formalin fixed and paraffin embedded.Histopathological, immunoistochemical, and electron microscopy analyses were performed to confirm the diagnosis of choroidal schwannoma. DNA and RNA were extracted from 3 x 10 µM paraffin slices each. Sequencing analysis was performed by Sanger in order to investigate the allelic status of PTEN and the presence of somatic intragenic mutations. Promoter methylation analysis was performed by pyrosequencing technique to evaluate the possibility of PTEN epigenetic silencing. Finally expression of both PTEN mRNA and protein was tested in the tumor compared to a panel of healthy and cancerous tissues.

Results
Sequencing analysis showed that in the tumor there is no loss of heterozygosity of the germline mutation. Moreover no other intragenic somatic mutations were detected within all PTEN exons and flanking introns. Methylation analysis of 5 CpG islands within the PTEN promoter region showed that there is no epigenetic silencing of the gene in the tumor.
Finally both PTEN mRNA and protein expression levels showed a reduction of around 40% in our tumor compared to another schwannoma without PTEN germline mutation.

Conclusions
This is the first time that a cellular pigmented choroidal Schwannoma was developed in the context of a hamartomatous syndrome caused by germline PTEN missense mutation.
No other mechanisms of PTEN inactivation that could act as a second hit in the process of tumorigenesis were found, however, a 40% reduction of both mRNA and protein expression level was observed within the tumor, suggesting a role for this gene in the molecular etiology of this malignancy.

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Searching for mutations in OPA1 gene in Polish patients with Autosomal Dominat Optic Atrophy.

Anna Wawrocka, Maciej R. Krawczyński

Department of Medical Genetics at Poznan University of Medical Sciences, Poland

Purpose
To determine whether mutations in the OPA1 gene are present in Polish patients with Autosomal Dominant Optic Atrophy (ADOA)

Methods
1. The first purpose of this study was to identify mutations in OPA1 gene in 48 individuals (26 sporadic cases, 22 patients from 11 pedigrees) with a clinical diagnosis of autosomal dominant optic atrophy, by using PCR-SSCP analysis and direct sequencing. A novel splicing mutation was characterized by RT-PCR of total RNA of leukocytes obtained from patient and one normal individual.
2. ADOA patients are also analysed for the presence of genomic rearrangments in OPA1 gene by means of multiplex ligation probe amplification (MLPA) and for the presence of mutations by using DNA microarray technology

Results
1.The mutations in OPA1 gene were detected in 28% of the affected families (3 of 11 families), which amounts to 17% of 48 examined people with ADOA.
The result of novel splicing mutation 873+2t□a in intron 8 will be present on poster.
2. We identified 3 different mutations in 6 patients with ADOA by using DNA microarray technology. Searching for large rearangments in OPA1 gene by using MLPA is in the course of analysis.

Conclusions
Screening studies based on sequencing of the coding region detected mutations in OPA1 gene in only 17% patients with ADOA. To improve mutation detection in OPA1 gene, molecular analysis is based on DNA microarray technology (to identify 118 known mutations in OPA1 gene) and multiplex ligation probe amplification(to discover larger genomic rearrangments deletions and duplications in OPA1 gene)

Modulation of cGMP synthesis in the mouse retina by endogenously released and by exogenously applied nitric oxide.

Zhijian Zhao, Frank Müller

Institute of Structural Biology and Biophysics, Cellular Biophysics, Research Center Jülich, Germany

Purpose
Cyclic guanosine monophosphate (cGMP) is a second messenger that plays an important role in vertebrate retinal phototransduction. In photoreceptors, cGMP is synthesized by particulate guanylate cyclases (pGC). In contrast, in cells of the inner retina cGMP seems to be synthesized mainly by soluble guanylate cyclases (sGC) upon the simulation by nitric oxide (NO). The mouse has become an important model system. Here we investigate the NO-cGMP pathway in the mouse retina. Using a specific antibody against cGMP, we visualize cGMP-like immunoreactivity (cGMP-IR) in retinal neurons upon application of an NO donor and after manipulation of release of NO from retinal amacrine cells.

Methods
Adult mice were deeply anaesthetized and quickly decapitated. Eyes were enucleated and opened by a encircling cut at the equator. The anterior parts of the eyes were discarded. The retinae were carefully isolated from the eyecups, flat mounted onto membrane filters and incubated in Ames' medium aerated with 95% O2 and 5% CO2, pH 7.4 at ~20 ? for 30 minutes. The following agents were added either alone or in combination: the phosphodiesterase inhibitor isobutyl-methy-xanthine (IBMX, 1 mM); the NO donor S-nitroso-N-acetylpenicillamine (SNAP, 300 µM); the nitric oxide synthase inhibitor L-nitroagrininemethyl ester (L-NAME, 200 µM). In some experiments, retinae were incubated in Ames' medium containing 20 mM K+ with either 1 mM IBMX and 200 µM L-NAME or 1 mM IBMX alone for 10 minutes. After incubation, the retinae were fixed, cryoprotected, and sectioned. The sections were immunostained for cGMP and celltype specific markers. Finally, the stained sections were examined using laser scanning confocal microscopy.

Results
Incubation with the NO donor SNAP strongly stimulated cGMP synthesis in mouse inner retinal neurons. Numerous bipolar cells as well as some amacrine cells and cells in the ganglion cell layer showed strong cGMP-IR. Using celltype specific markers, the cGMP-immunoreactive bipolar cells were identified as OFF-cone bipolar cell types 3a and 3b and ON-cone bipolar cell types 5 and 7. In retinae incubated in IBMX only, weak cGMP-staining was found that could be abolished by adding L-NAME to the incubation medium. In retinae incubated with high potassium, the number of cGMP-immunoreactive bipolar cells in the proximity of NOS-positive amacrine cells increased.

Conclusions
The NO-cGMP signaling pathway is functional in the mouse retina. We have identified four bipolar cell types whose cGMP levels are strongly modulated by NO. Moreover, our data indicated that NO is released endogenously from NOS-positive amacrine cells and modulates the cGMP level in the neurons in the inner retina.

Distribution of somatostatin-immunoreactive cells in the neural retina of selected mammalian species

Marina Zieger, P. Ahnelt

Medical University Vienna Dept. of Physiology
Schwarzspanierstr. 17
A 1090 Wien

Purpose
In the mammalian retina, a sparse population of displaced amacrine cells in the ganglion cell layer contains somatostatin (somatotropic release-inhibiting factor, SRIF). There is growing evidence that this neuropeptide could be added to the list of the natural factors that protect retina against ischemic effects occurring in a variety of retinal diseases.
In several mammalian retinas somatostatin-immunoreactive (SST-IR) amacrine cells show a peculiar deviation from the common topography of retinal neurons. Their somata are essentially confined to the inferior retina in the WT mouse, rabbit and cat. This coincides with the dorsoventral gradient of S-opsin expression in these species.
We aim to answer a question: Is there a consistent correlation between the distribution of somatostatin-immunoreactive neurons and S-opsin cone photoreceptors in the adult neural retina of mammalian species?

Methods
For immunofluorescense, eyecups were fixed with 4% paraformaldehyde by immersion. The eyecups were saturated with sucrose, mounted in OCT solution, and cut frozen. Alternatively, the staining was performed on floating whole-mounts, which were mounted on slides. Here, we use rabbit antisera to somatostatin, and ganglion cell type-specific goat antisera to the POU-domain factor Brn3a.

Results
To begin to understand the possible implications of the unusual topography, we examined the distribution of SST-IR amacrine cells in one further species that show an inferior bias of peak S cone density (roe deer, Capreotus capreotus) and compare with the distribution of SST-IR neurons in species with the peak of S-cone density in the superior retina. Together, the current results indicate that the SST-IR amacrine cells are distributed in both amacrine and ganglion cell layers, irrelative of the S-cone distribution.

Conclusions
A further research is required to a better understanding of the possible correlation between the distribution of somatostatin-immunoreactive neurons and S-opsin cone photoreceptors in other than the WT mouse, cat and rabbit mammalian species.

IgG and IgM anti retinal antibody patterns in an animal model for spontaneous autoimmune uveitis

Johanna Zippliesı, S. Hirmerı, S.M. Hauck2, C.A. Deegı

1 Institute of Animal Physiology, Department of Veterinary Sciences, LMU Munich, Germany
2 Department of Protein Science, Helmholtz Center Munich, Neuherberg, Germany

Purpose
Equine recurrent uveitis (ERU) is a spontaneous animal model for autoimmune uveitis, in which autoreactive T cells cross the blood retinal barrier. The disease is characterized by an inflammation of the inner eye and alternating relapses leading to destruction of the retina and finally to blindness. It has been shown, that epitope spreading to different retinal antigens plays an important role in the pathogenesis of disease. The aim of this study was to investigate a comparison of IgG and IgM reactivities to retinal proteins, intra- and interindividually, in vitreous and sera of healthy and ERU diseased horses.

Methods
To test IgG and IgM antibody responses to retinal tissue, sera (n=40) and vitreous samples (n=40) of ERU diseased horses and controls were used for analysis. Retina was resolved by 12% SDS PAGE and blotted semidry on PVDF membranes. Membranes were blocked to unspecific binding with 1% PVP in PBS-T for 1h and incubated with sera (1:100) or vitreous samples (1:10) for 3h, washed and further incubated with POD conjugated anti-horse IgG (Fc) or IgM. Protein bands were detected on Hyperfilm with ECL.

Results
Overall we could detect complex IgM and IgG patterns in vitreous and sera samples. Interestingly 41,5 % of ERU diseased horses showed an IgM response in vitreous samples, whereas in vitreous of controls no signals could be detected. Some individuals even showed IgM reactions to various retinal proteins. Additionally most of the diseased horses showed an IgG response in vitreous samples, but no IgG response could be detected in healthy vitreous. In sera samples an IgM and IgG reactivity could be detected in ERU samples and healthy controls, but no specific pattern neither intra- nor interindividually could be noted.

Conclusions
The considerable IgM response to retinal proteins in vitreous of horses with uveitis is a new finding, which demonstrates an ongoing autoimmune response, which might contribute to the remitting relapsing character of this disease.

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Two pharmacological animal models of retinal degeneration: their impact on function, morphology and induction of cell death in the retina

Rahel Zulliger, Sylvie Eigeldinger-Berthou, Stéphanie Lecaudé, Ute E. Wolf-Schnurrbusch, Volker Enzmann
Universitätsklinik für Augenheilkunde, Inselspital, University of Bern, Bern, Switzerland

Purpose
To mimic retinal degeneration found for example in age-related macular degeneration or in retinitis pigmentosa, we used two pharmacological animal models: sodium iodate ($NaIO_3$), which induces dystrophy of the retinal pigment epithelium (RPE) followed by photoreceptor (PR) degeneration and N-Methyl-N-Nitrosourea (MNU), directly affecting PR. To compare the two models, visual function was analyzed and corresponding morphometric measurements performed.

Methods
For the experiments 6-8 weeks old male C75 BL/6 mice were used. $NaIO_3$ was injected i.v. at a dose of 15, 25 or 35 mg/ kg body weight (BW); whereas, MNU was applied i.p. at a dose of 30, 45 or 60 mg/ kg BW. Visual function was tested by optokinetic reflex (OKR) measurement and cued water maze behaviour at day 3, 7, 14 and 21 post injection (PI). At the same time points eyes were enucleated, fixed in 4% PFA and paraffin sections (7 µm) cut. Sections were then H&E stained or used for immunohistochemistry. Thereby, samples were labelled with TUNEL reagent or with rabbit anti-cleaved caspase-3 antibody

Results
After application of either compound, visual function decreased significantly and morphometry revealed a significantly reduced retina. The time course of the degeneration was comparable between the MNU and $NaIO_3$. However, $NaIO_3$ effects were time and concentration dependent, whereas MNU was concentration dependent only. Furthermore, immunohistochemical staining revealed involvement of different cell death pathways – caspase-3 dependent and independent.

Conclusions
Both investigated compounds induced retinal degeneration but through different mechanisms: $NaIO_3$ is affecting the PR via RPE through cleaved caspase-3 pathway, MNU directly induced caspase-3 independent cell death. Analysis of gene regulation and protein production will give further insight into the pathological processes in these animal models.

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