Young Researcher Vision Camp

An international Career building Symposium

2016
See & Hear

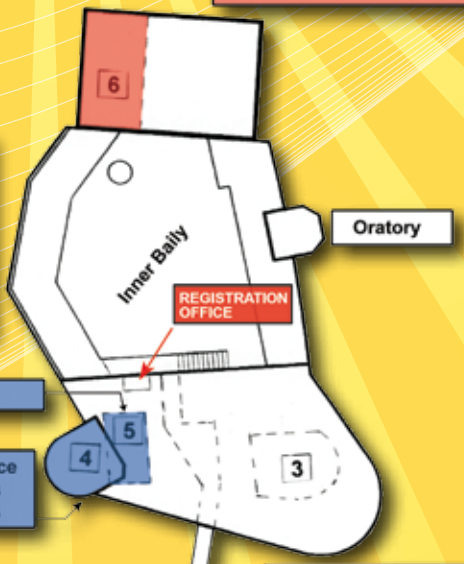
Castle Wildenstein
Leibertingen
Germany

www.vision-camp.eu

Jugendherberge
88637 Burg Wildenstein

Lounges:

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



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Preamble

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

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

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

ENJOY THE
YOUNG RESEARCHER VISION CAMP

Thomas Wheeler-Schilling

on behalf of the organising committee (in alphabetical order)

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

FRIDAY, JUNE 10TH, 2016

until 16:00	Arrival (for details see 'How to get there')
17:30 – 17:35	Welcome
17:35 - 18:35	**KEYNOTE LECTURE I** **„PERSONALIZED MEDICINE FOR HEARING LOSS"** Prof. Hubert Löwenheim Oldenburg, Germany
19:30 - open end	Open-air Barbecue
21:00 – 22:00	Adventure Tour with Wild Life Rangers in the Valley Danube (optional) – **Attention:** separate reservation necessary

SATURDAY, JUNE 11TH, 2016

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

07:00 - 08:00	Early morning exercises
08:00 – 9:00	Breakfast
09:00 - 10:00	**SCIENTIFIC SESSION I: ‚GAIN IN THE BRAIN - CAN SENSORY LOSS IN THE AUDITORY SYSTEM BE CENTRALLY COMPENSATED?'** Chair: Lukas Ruettiger, Department of Otolaryngology, University of Tuebingen, Germany • "Central Auditory Gain after Cochlear Trauma" Daniel Polley • "Examination of the Human Auditory Brain" Oliver Profant • "Functional Brain Imaging in Hearing Impaired Patients with and without Tinnitus - a Pilot Study" Ebrahim Saad Aldamer • "Age Related Inner Ear Synaptopathy and Central Compensation" Dorit Möhrle
10:00 - 10:30	Coffee Break
10:30 - 11:30	**SCIENTIFIC SESSION II: ‚PHOTORECEPTOR CELL DEATH MECHANISMS'** Chair: Francois Paquet-Durand, Department of Ophthalmology, University of Tuebingen, Germany • "Simulating diabetic retinopathy in retinal explant cultures" Laura Trachsel • "Activity of histone deacetylase in secondary cone photoreceptor degeneration" Eleni Petridou • "Identification and Characterization of Novel Cone Photoreceptor Enriched Factors that are Conserved in Zebrafish, Mouse and Human" Andrew Smith • " Ca^{2+} imaging in degenerating cone photoreceptors" Michael Power

11:30 - 12:30	**SCIENTIFIC SESSION III** Chair: Thomas Wheeler-Schilling, Department of Ophthalmology, University of Tuebingen, Germany • "Investigating new therapeutic targets for inherited photoreceptor degeneration: Secondary medical indications for PARP inhibitors used in cancer treatment" Ayse Sahaboglu • "Role of BDNF for adaptive and non-adaptive central circuit formation" Wibke Singer • "Neurodegeneration in glaucomatous PTP-Meg2 deficient mice" Jacqueline Reinhard • "Mechanisms and effects of neuroprosthetic intervention" Juan Ordonez
12: 30 - 14:00	Lunch
14:00 - 15:00	**SCIENTIFIC SESSION IV** Chair: Sigrid Diether, Department of Ophthalmology, University of Tuebingen, Germany • "Ongoing Activation of the Deaf Auditory System via Cochlear Implant Augments GABAergic Neuronal Networks and Glia Hypertrophy" Nicole Roßkothen-Kuhl • "Comparative real-time analysis of cGMP signals linked to degeneration and regeneration of the eye and ear" Markus Wolters • "Restoration of visual function following cell transplantation into a mouse model of complete blindness" Tiago Ferreira • "Development of endocytosis in cochlear inner hair cells" Stephanie Eckrich
15:00 - 16:00	**SCIENTIFIC SESSION V'** Chair: Michaela Bitzer, Department of Ophthalmology, University of Tuebingen, Germany • "Layout of an Actuator Array for Local Inner Ear Stimulation" Daniel Schurzig • "Improving visual function in neurodegenerative diseases by a battery of mobile Apps" Iliya Ivanov • "Exploring the neurosensory perception formation: Pupillary response as window for understanding gating mechanisms" Krunoslav Stingl • "Concept for fabricating direct contacts between neuroprostheses electrode surfaces and neurites by means of artificial adhesive and synaptic structures in cochlea implants (CI)" Pooyan Aliuos
16:00 - 16:30	Coffee Break

16:30 - 17:30	**SCIENTIFIC SESSION VI** Chair: Hasan Avci, Department of Otolaryngology, University of Tuebingen, Germany • "Cochlear Implant with Gapless Interface to Auditory Neurons" Masaaki Ishikawa • "Generation of hair-cell-like cells from IPS cells" Aurélie Dos Santos • "Could spontaneous Ca2+ activity of developing inner hair cells hold a key to trigger regenerative processes or differentiation of stem cells in the mammalian inner ear?" Tobias Eckrich • "The importance of the tubulin turnover for the correct function of the cochlear sensory epithelium" Kristen Rak
17:45 - 18:45	**KEYNOTE LECTURE II** **'RESTORING VISION IN BLIND PEOPLE'** Prof. Eberhart Zrenner Institute for Ophthalmic Research, Tuebingen, Germany
18:45 - 19:00	Group Photo
19:00 - open end	Poster Session
20:30 - open end	Buffet in the inner bailey

SUNDAY, JUNE 12TH, 2016

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

7:00 - 7:45	Early morning exercises
8:00 – 9:00	Breakfast
9:00 - 10:00	**SCIENTIFIC SESSION VII: ‚ADVANCES IN RETINAL IMAGING'** Chair: Arne Ohlendorf, ZEISS Vision Science Lab, Tuebingen, Germany • "Functional imaging of single photoreceptor cells in the living human eye" Niklas Domdei • "Parallel line scanning ophthalmoscope for retinal imaging" Kari Vienola • "Aberration-free functional imaging of human retina using full-field swept-source optical coherence tomography" Hendrik Spahr
10:00-11:00	**SCIENTIFIC SESSION VIII: ‚INVERTEBRATE VISION IN MOTION'** Chair: Gavin Taylor, Lund University, Sweden "Disentangling the origins of vision by investigating marine zooplankton" Alberto Valero Gracia • "Facilitatory receptive field effects in dragonfly target motion detecting neurons" Joseph Fabian • "Honeybee visual cognition: a miniature brain's simple solutions to complex problems" Mark Roper • "What type of route should we learn and follow" Olivier Bertrand
11:00 – 12:00	**POSTER AWARDS & TALKS OF THE AWARDEES** • Carl Zeiss Award Winners Short presentations (each 5 minutes)
12:00 - 13:30	FAREWELL Lunch (optional)


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An international Career building Symposium
The Power to Shape The Future in Vision Research and Ophthalmology
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Content

Abdullaeva, Oliya Sadrillaevna

Photoelectrical Stimulation of Neuronal Cells by an Organic Semiconductor-Electrolyte Interface

Oliya S. Abdullaeva (1), Matthias Schulz (2), Frank Balzer (3), Jürgen Parisi (1), Arne Lützen (2), Karin Dedek (4), Manuela Schiek (1)*

(1) Energy and Semiconductor Research Laboratory, Institute of Physics, University of Oldenburg, D-26111 Oldenburg, Germany - (2) Kekulé Institute of Organic Chemistry and Biochemistry, Rheinische-Friedrich-Wilhelms-University of Bonn, Gerhard-Domagk-Str. 1, D-53121 Bonn, Germany - (3) Mads Clausen Institute, University of Southern Denmark, Alsion 2, DK-6400 SØnderborg, Denmark - (4) Neurosensorics, Institute of Biology and Environmental Sciences, University of Oldenburg, D-26111 Oldenburg, Germany

Purpose
The prosthetic treatment of visual impairment has focused so far on the development of retinal implants based on inorganic electrode arrays and photodiodes. Alpha IMS (Retina Implant AG, Germany) and ARGUS II (Second Sight, US), which were approved for clinical trials, show promising results. Nevertheless there is a strong demand for alternative materials, as for instance good biocompatibility and effective electrical signaling are challenging issues. Especially due to their high biocompatibility, organic semiconductors are being considered as potential material for retinal prosthesis. This study focuses on squaraine dyes, in particular 2,4-bis[4-(N,N-diisobutylamino)-2,6-dihydroxyphenyl]squaraine (SQIB), a small molecular model organic semiconductor blended with PC60BM. We grow N2A cells, a model neuronal cell line, on the SQIB:PC60BM-based artificial photoreceptor. Above mentioned retinal implants have already shown that prosthetic treatment is possible by electrical stimulation of biological cells. Now we want to perform proof-of-principle investigations if our model organic semiconductor is capable of inducing ionic currents in N2A cells by means of photoelectrical stimulation. Our study focuses strongly on investigating the signaling pathway of the photostimulation.

Methods
The samples were prepared by spincoating from solution on glass substrates coated with a transparent conductive oxide, and were subsequently annealed. We employed patch clamp experiments (voltage and current clamp mode) to study the electrical response of the N2A cells during illumination and conducted transient photocurrent measurements on floating samples under physiological conditions. The stability of the samples and their surface morphology was further tested using atomic force microscopy and optical spectroscopy.

Results
Due to the high annealing temperature the SQIB:PC60BM blend film showed a textured crystalline morphology which was beneficial for cell growth, even without an adhesion layer. Furthermore, the stability of the blend film was not impaired during patch clamp recordings. By illuminating the SQIB:PC60BM-based photoreceptor with short light pulses we were able to induce a change in the membrane potential of the N2A cells. This resulted in rapid capacitive transmembrane currents, which we could record in voltage clamp mode. Further transient photocurrent measurements revealed fast capacitive displacement currents in the Ringer's solution upon illumination, indicating an electrical coupling mechanism. However, in spite of the change in the membrane potential we did not observe any ionic conductance through voltage-gated channels.

Conclusions
Our study demonstrates the biocompatibility of our SQIB:PC60BM device. We did not trigger ionic currents across the cell membrane, but achieved direct capacitive coupling between the N2A cells and the SQIB:PC60BM blend by photoelectrical stimulation. To finally induce ionic currents in neurons, we need a deeper understanding of the coupling mechanism at the semiconductor-neuron junction. This understanding is necessary to develop retinal implants that meet the requirements for prosthetic treatment.

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

Acknowledgement of funding, if applicable
OSA thanks the DFG for a scholarship within the research training group Molecular Basis of Sensory Biology GRK 1885/1.

Aldamer, Ebrahim

Functional Brain Imaging In Hearing Impaired Patients with and without Tinnitus – Pilot Study.

Ebrahim Aldamer (MD), Dr.med. Stephan Wolpert (investigator), Prof. Dr. med. Uwe Klose (fMRI imaging), Prof. Dr. rer. nat. Marlies Knipper (coordination), Moritz Walter (Medical ph.D)

Primary: University of Alexandria – Department of Otolaryngology / Neuroscience (Faculty of Science) , Moharam Bek - Masged El Hadary St.49, 21515 Alexandria – Egypt | Currently : University of Tübingen, Department of Otolaryngology Hearing Research Centre Tübingen, Molecular Physiology of Hearing, Elfriede-Aulhorn-Straße 5, D-72076 Tübingen, Germany

Purpose
Tinnitus is a non-curable stress-related brain disorder, that is mostly noise-induced and whose origin is unknown. The overall aim of the study is to gain a better understanding of the pathological mechanisms of tinnitus and the importance of stress coupled distress.

Methods
In this clinical pilot study, 9 pairs of tinnitus patients and hearing impaired matched controls were Examined audiologically (Audiometry), Resting state functional connectivity and evoked activation of auditory pathway (fMRI) were investigated in defined regions of interest. Correlation of Tinnitus Intensity with different aspects of Tinnitus questionnaire scores was also done.

Results
Results of the audiological investigations, resting state fmri functional connectivity between defined regions of interest, stimulus evoked fmri and correlations of tinnitus Intensity with different emotional and cognitive aspects will be presented.

Conclusions
Data will be presented that illustrate the differential central auditory responsiveness of defined regions of interest in selected brain structures that may lead to the option for objective diagnosis of tinnitus using imaging techniques.

Barraza-Bernal, Maria

Positions in the visual field with good attentional capabilities are candidates for a new preferred retinal locus of fixation

Maria Barraza-Bernal1, Iliya Ivanov1, Svenja Nill1, Katharina Rifai1, Susanne Trauzettel-Klosinski2, Siegfried Wahl1

1 Institute for Ophthalmic Research, Eberhard Karls University Tübingen.
2 University Eye Clinic, Tübingen, Germany

Purpose
Sustained attention enhances perception of stimulus located at eccentric positions of the visual field. Perception is not equal for all eccentric directions, leading to variations in attentional performance. Similar variations found in normally sighted subjects were found in patients with maculopathies. Patients with maculopathies fixate an object eccentrically at a preferred retinal locus of fixation (PRL). The chosen PRL location might be influenced by these attentional variations. In this study, the relationship between the attentional variations and the location of the developed PRL was investigated.

Methods
Thirteen normally sighted subjects participated in the study. The sustained attention was measured in eccentric locations of the visual field. Subsequently, a 6° macular scotoma was simulated and PRL training was performed in a set of visual tasks during two two-hour sessions. The sustained attention measurement and the two simulations of macular scotoma were separated by at least 24 hours.

Results
The attentional capabilities at different positions of the visual field and the chosen PRL location showed similarities.

Conclusions
Positions with good local attentional capabilities are candidates for a new PRL location.

Bertrand, Olivier

Choosing the type of route to learn and follow in cluttered environments

Olivier J.N. Bertrand

University Bielefeld & CITEC

Purpose
Central-place foragers, such as bees and ants, travel back and forth between food locations and their nest. To repeatedly visit the food location, they tend to follow idiosyncratic routes. In cluttered environments the animal may attempt to learn the shortest route, but – assuming that it should avoid collisions with obstacles – it may also strive for the safest route, i.e. the route minimizing the risk of collision. Moreover, the animals may be displaced from their learned course, e.g. by a gust of wind, and then need to relocate it by some search strategy. Therefore certain search strategy will be better to be used than other, and may depend on characteristics of the route to be found.

Methods
The route to learn, and the search strategy to follow, have been addressed by computational modeling. The position of the objects, e.g. tree trunks, in cluttered environments have been used to create a network of path followed by a simulated agent. A series of path, i.e. a route, is assumed to be known by the agent. Then the agent has been displaced from its known route, at different distance from the route. The agent, guided by a search strategy, had a certain probability to find back to the route.

Results
The search strategy and the route known by the agent can share common characteristics, e.g. avoiding narrow gaps. We found that close to the route, the agent has a higher probability to find the route with a search strategy sharing a common characteristic with the route to be found than without sharing a common one.

Conclusions
In a new city, learn and follow paths according to a rule (e.g. avoiding narrow streets), except if you are lost for a long time.

Acknowledgement
The project is funded by the Deutsche Forschungsgemeinschaft (DFG)

Bertelli, Pietro Maria

Distribution of Amyloid-beta and phosphorylated-Tau in eyes from patients with Alzheimer's disease

Pietro Maria Bertelli1; Roz Whitaker1; Lloyd Warren1; Lajos Csincsik1 and Imre Lengyel1

UCL Institute of Ophthalmology, Ocular Biology and Therapeutics, University College London, London, United Kingdom

Purpose
Alzheimer's disease (AD) is associated with Amyloid-? (A?) deposition and hyperphosphorylation of Tau in the brain. Reports suggest retinal changes associated with AD, but the distribution of A? and phosphorylated-Tau in the retina is not well understood. We used a mouse model (APP/PS1; eyes and brains from University of Tübingen) and human eyes (UCL Institute of Ophthalmology Eye Tissues Depositary) with AD to explore this issue.

Methods
Frozen and paraffin embedded sections from brain and eye were immunolabeled with several antibodies against A? (6E10 and 4G8, Covance; ab2539, Abcam; CN6, from University of Tübingen), Tau (A0024, Dako) and phosphorylated-Tau (AT8, Thermo Scientific), and analysed by confocal microscopy.

Results
We were unable to detect A? in the neurosensory retina with any of the A? antibodies, despite the clear and specific labelling of mouse and human brain tissues with all of these. Interestingly, in human tissues A? immunoreactivity was clearly identified in extracellular deposits, called drusen, in the same eyes. A significant increase in Tau phosphorylation was detected in specific neuronal cells in the retina of AD patients.

Conclusions
Based on these results we suggest that phosphorylated-Tau, rather than A?, has the potential to become a biomarker for AD in the eye.

Csincsik, Lajos

Multimodal retinal imaging in dementia

Lajos Csincsik 1; Timothy Shakespeare 2; Sebastian Crutch 2; Tunde Peto 1,3; Imre Lengyel 1;

1 UCL Institute of Ophthalmology, London, United Kingdom; 2 UCL Dementia Research Centre, London, United Kingdom;3 NIHR Biomedical Research Centre at Moorfields Eye Hospital NHS Foundation Trust, London, United Kingdom;

Purpose
Pathological changes in the eye have been reported in a range of neurodegenerative diseases. Changes of the retinal nerve fiber layer (RNFL) due to ganglion cell loss, retinal vessel caliber as well as increased accumulation of extracellular drusen deposits had been associated with dementia related changes in the brain. Here we report on the baseline characteristics of patients with Alzheimer`s disease (AD) and Posterior Cortical Atrophy (PCA).

Methods
Ultra-wide-field colour, autofluorescence images (UWFI) and optical coherence tomography (OCT) images were acquired of 72 healthy controls (HC; MMSE(bigger than)28), 22 AD (MMSE(smaller than)20) and 27 PCA (MMSE(smaller than)24) patients using OPTOS TX 200 scanning laser ophthalmoscope and OPTOS SD-OCT/SLO. The studies had full local Ethical Committee approval. UWFI were analyzed for presence/absence of pathologies like drusen, pigmentary changes or hypo and hyper autofluorescence. OCT images were analysed for macular volume (MV) and thickness (MT), peripapillary (pp) RNFL, RPE-Photoreceptor, ONL, OPL-INL and whole retinal (WR) thickness as well as overall peripapillary vessel caliber (ppVC) using OPTOS OCT SLO viewer and the OCTseg software. Statistical analysis was carried out using STATA and SPSS. Images with poor quality were removed from analysis.

Results
Difficulties in imaging PCA patients were noted, and a greater proportion of images were discarded in this group. There was no significant age difference between HC, AD and PCA patients (66.2±7.4 vs 64.9±6.5 vs 65.5±7.8; p(bigger than)0.1). We found significant reduction in ppOPL-INL thickness (p=0.006) in patients with PCA compared to HC and AD, and significant increase in ppVC in AD (p(smaller than)0.001) and PCA (p=0.032) compared to HC. While we found no significant differences (p(bigger than)0.05) between PCA, AD and HC in any other OCT measures overall there was a significant age related decrease on all of those parameters.

Conclusions
Earlier studies on dementia concentrated on the changes in RNFL thickness. Our results suggest that analysing changes in all retinal layers might be necessary to obtain a full picture of the pathology in the retina associated with dementia. In addition, retinal vessel caliber segmentation might hold relevant information in regards to dementia associated pathologies in the eye. While there are challenges in imaging patients with AD and PCA new imaging modalities have the potential to reveal previously unrecognized association between retinal manifestations of dementia.

Acknowledgement
We would like to thank the participants of this study; Professor Alan Bird for his helpful contributions; and the ESRC, the Bill Brown Charitable Trust, the Mercer fund from Fight for Sight and the Eye Charity of Moorfields Eye Hospital for financial support. We also thank OPTOS plc for an unrestricted grant to carry out this work. Tim Shakespeare is funded by a fellowship from Alzheimer's Research UK

Dellaa, Ahmed

Characterizing the retinal function of Psammomys obesus: A diurnal rodent model to study Human retinal function

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Purpose
To compare the retinal function of a diurnal murid rodent, Psammomys obesus, with that of Wistar albino rat and human subjects.

Methods
Adult Psammomys obesus were captured and transferred to the animal facilities where they were maintained at 25°C with standard light/dark cycles and natural halophilic plants, rich in water and mineral salts. Standard full-field photopic and scotopic electroretinogram were obtained.

Results
The right eye of all animals displayed well detectable and reproducible scotopic and photopic ERG responses. Results were compared with those obtained from human subjects and Wistar rats. ERG measurement showed that the amplitudes of scotopic responses in Psammomys obesus are quite similar to those of human subjects. The amplitude of the photopic a-wave was comparable to that of human and 6 times higher than that of the albino rat. The amplitudes of photopic b-wave, photopic oscillatory potentials and 30 Hz flicker were all markedly larger in Psammomys obesus compared to those obtained from human subjects and Wistar rats. Furthermore, like the human photopic ERG, the photopic ERG of Psammomys obesus, also includes prominent post b-wave components (i.e. i- and d-waves) while the ERG of Wistar rats does not.

Conclusions
Our results suggest that the retinal function of Psammomys obesus, especially the cone-mediated function, share several features with that of human subject. We believe that Psammomys obesus represents an interesting alternative to study the structure and function of the normal and diseased retina in a human-like rodent model of retinal function.

Acknowledgement
This study was supported by EU within the framework of PASRI program from The Ministry of Higher Education and Scientific Research in partnership with UNIMED Laboratories.

Dockery, Adrian

Target 5000: Genetic characterization of a cohort of inherited retinal degeneration (IRD) patients.

Adrian Dockery, Matthew Carrigan, Conor Malone, John McCourt, David Keegan, Julie Silvestri, Kirk Stevenson, Andrew Green, Pete Humphries, Paul F. Kenna, G. Jane Farrar

The School of Genetics & Microbiology,Trinity College Dublin, Dublin 2, Ireland. | Royal Victoria Eye and Ear Hospital, Dublin 2, Ireland. | The Mater Misericordiae Hospital , Dublin 7, Ireland. | The Royal Victoria Hospital, Belfast, Northern Ireland.

Purpose
The Target 5000 research project aims to provide genetic testing for the estimated 5,000 people in Ireland who have an inherited retinal condition. Many clinical trials are available for patients with sight loss, however, many such trials require patients to have their causative mutation identified in order to enter the trial. The objective of the study is to genetically characterise patients with inherited retinal degenerations (IRDs) in Ireland and in principle to make clinical trials more accessible to some Irish people suffering from sight loss. The study also seeks to identify previously undiscovered pathological mutations in a panel of known retinopathy genes evaluated utilizing target capture next generation sequencing (NGS).

Methods
NGS was employed to find the causative gene mutation responsible for a patient's condition. It is currently estimated that by screening a panel of approximately 220 genes that about 60% of causative mutations can be established in IRD patients. If the responsible gene mutations are not apparent using this strategy, then further analysis will be carried out on this subset of 'unresolved' IRD patients.

Results
The results of this study so far, with approximately 600 patients sequenced to date, have provided an overview of the frequency of the different genetic forms of IRDs in the Irish population. Additionally the study has helped to resolve the genetic diagnoses of members of a pedigree where more than one disease gene may be segregating. Furthermore new phenotypes have been linked to already known disease causing genes, which in turn, aids in the future diagnoses of patients at clinical presentation.

Conclusions
Thus far in the study, as part of Target 5000 roughly 10% of the Irish IRD population has been sequenced and the results obtained are encouraging. Target 5000 offers not only a chance to discover new causative mutations, but is vital in giving patients access to information regarding the pathogenesis of their disease. Over 50 novel mutations have been discovered, as well as some previously ambiguous phenotypes resolved. More precise matching of genotype with phenotype from this study and similar studies globally should start to enable clinicians to better formulate accurate future diagnoses and at times prognoses.

Acknowledgement
Fighting Blindness Ireland
The Health Research Board of Ireland
Science Foundation Ireland

Domdei, Niklas

Rate variability analysis of auditory pathway units in barn owls

Niklas Domdei1, Philipp Tellers2, Hermann Wagner3

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2) Department of Vision Sciences, University of Alabama at Birmingham, AL, USA
3) Department of Zoology and Animal Physiology, RWTH Aachen, Aachen, Germany

Purpose
Variability is inherent to every neuronal network and may affect the behavior of an animal. Since the barn owl's auditory pathway is very well understood, this organism offers a good opportunity to study in vivo how a neuronal system deals with variability. Like mammals, barn owls use the interaural time difference (ITD) to determine the azimuthal direction of an acoustic stimulus. In the barn owls external nucleus of the inferior colliculus (ICX), ITD is represented by the firing-rate of single neurons. The firing-rate of most ICX neurons is influenced by additional external (e.g. multiple stimuli, background noise) and internal (e.g. spontaneous discharge) factors. For high stimulus intensities, the effect of the stimulus dependent inputs is expected to increase, leading to a decreased influence of stimulus independent inputs on the response of a neuron. To assess the impact of stimulus intensity on the representation of ITD, we analyzed the firing-rate variability of ICX neurons in stimulation with different sound levels.

Methods
We recorded extracellular form isolated single units in the ICX of anesthetized barn owls (Tyto furcata pratincola) with tungsten electrodes. Stimuli were bursts of 100 ms long broad band noise (5 ms onset/offset ramps), covering the audible spectrum of barn owls, presented via earphones. The targeted recording site (ICX) was defined by the specific tuning behavior of the neurons towards stimulus bandwidth, frequency, interaural level differences and ITD. Following this basic characterization, ITD response functions (± 330 µs) were recorded with a minimum 10 trials per ITD at three different sound levels. The variability in the ITD response functions was measured by the mean correlation between the 10 single ITD response functions (r1-10) and the averaged ITD response function (rAll). In addition, the index of dispersion (DI) at the neuron's best ITD (ITD that elicits the highest firing-rate) was calculated. The DI was given by the ratio of the variance in spike count and the mean spike count. For each measured ITD response functions, 10000 simulated functions were generated using a Monte-Carlo approach. By determining the ambiguity and precision in the distribution of the best ITDs of the simulated functions, we estimated the reliability of the ITD representation.

Results
We included 51 units recorded from 4 owls in our analysis. The median value for the mean correlation of r1 to rAll increased from about 0.5 for a near threshold stimulus to about 0.75 for the highest stimulus sound level. With decreasing sound level, the variability of the spike rate increased, indicated by a small but significant increase in the DI from about 6 spk/s (high sound level) to about 8 spk/s (low sound level). Even though the best ITD was generally robust across varying sound levels, our Monte-Carlo analysis showed that the ambiguity (miss rate: ~10% to ~40%) and precision (Standard deviation: ~8.5 µs to ~15.0 µs) in the representation of the best ITD drops with decreasing sound level.

Conclusions
The representation of ITD in the barn owl's ICX is sound level dependent. Since ITD representation is ambiguous at the level of single ICX neurons while sound-localization behavior is not, these findings suggest that either a population code or further processing underlies the output of ICX before it reaches the centers generating motor responses.

Acknowledgement
DFG grant WA606/21

Domdei, Niklas

Functional imaging of single photoreceptor cells in the living human eye

Niklas Domdei, Frank Holz, Wolf Harmening

Department of Ophthalmology, University of Bonn, Germany

Purpose
Adaptive optics scanning laser ophthalmoscopy (AOSLO) can image single photoreceptors in vivo. Combined with real-time eye tracking and correction for chromatic aberrations the AOSLO can be used as a microscopy platform to make single cells optically accessible for functional testing. Due to its scanning nature, visual stimuli can be encoded into the imaging beam with high-speed acousto-optic modulation (AOM), thereby creating an acutely focused visual display directly on the retina. We here characterize the spatial properties of stimulation.

Methods
For imaging we use near infrared light with 840 ± 25 nm wavelength with light power below 200 µW at the cornea. Reflected light from the subject's retina is detected via photo multiplier tubes and rendered into a stream of 512x512 pixel image frames at 30 Hz video rate. Closed-loop wavefront correction with a deformable mirror enables near diffraction limited imaging on a cellular resolution scale. Because the imaging light is visible to the subject (seen as dimly red), visual stimuli can be presented by modulating the imaging beam with a high-speed AOM. For other psychophysical tasks where a reversed luminance contrast is needed (bright stimulus against a darker background for e.g. microperimetry) without losing the AOSLO's imaging benefits, an additional stimulus channel (543 ± 12 nm in our system) is utilized. Online eye-tracking, monitoring the subject's fixational eye movements, enables the presentation of retina stabilized stimuli and therefore repeated stimulation of the same cone.

Results
The smallest full contrast stimuli presentable were on the order of 3 pixels across in raster scanning coordinates. This corresponds to about 2 µm on the retina (raster scanning excursion of 1.2 deg of visual angle). Optical modelling confirms that this size would place about 75% of stimulus light within the dimensions of a single cone inner segment diameter at 2 deg eccentricity. Currently, residual light leak (~4.3 cd/m2 at 543 nm, around 4100 isomerizations per second) through the AOMs - inherent to this technique - likely saturates any rod photoreceptor contribution, thereby limiting functional testing to cone photoreceptors only.

Conclusions
AOSLO-based micro-stimulation promises to be useful for fundamental and clinical vision research applications. Testing of single photoreceptor cells close to and in the fovea (< 1° eccentricity) remains challenging and invites further technical innovation. Nevertheless, early experimental results prove that single cone psychophysics is indeed possible. This creates the unique opportunity to probe the relationship between retinal structure and visual function on single cell level in vivo.

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Emri, Eszter

The effects of zinc supplementation on autophagy in primary human foetal retinal pigment epithelial cells

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Purpose
The Age related macular degeneration (AMD) is linked to oxidative stress, inflammation and decline in autophagy in the retinal pigment epithelium (RPE). These all contribute to the progression AMD especially at the early stages that are characterised by accumulation of extracellular proteins, lipids and trace elements rich deposits. It is suggested that supplementation with zinc is beneficial to slow the progression of AMD clinically. In this study we investigated whether zinc supplementation could directly influence autophagy.

Methods
We characterised the autophagosome formation in primary human foetal RPE cells in culture using LC3I/II antibody staining and confocal microscopy. Results using different autophagy modulators, such as Chloroquine and Bafilomycin were quantified using quantitative high-throughput image screening before and after zinc supplementation.

Results
We found that zinc supplementation had no effect on autophagosome formation at basal conditions. However, it significantly decreased the autophagosome number, induced by Chloroquine or Bafilomycin (p < 0.05).

Conclusions
Based on these results we propose that one of the ways zinc supplementation affects the progression of AMD is by directly affecting autophagy in RPE cells.

Acknowledgement
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FABIAN, JOSEPH

Facilitatory receptive field effects in dragonfly 'small target motion detector' neurons.

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Purpose
For over 50 years the invertebrate nervous system has been a valuable model system for investigating visual processing. Most study focused on describing how brains process the optic flow generated by ego-motion, however much less is known about how invertebrate brains detect moving features. Dragonflies excel at pursuing small moving prey or conspecifics when competing for food or territory. This complex task requires the detection of tiny signals superimposed on highly cluttered backgrounds. Previously we showed that maximal responses in dragonfly Small Target Motion Detecting (STMD) neurons require facilitation by a target drifting on a continuous trajectory for several hundred milliseconds. Here we show that this facilitation elicits local changes in receptive field properties that simultaneously enhance sensitivity to a target whilst decreasing sensitivity in the surround.

Methods
Dragonflies were immobilised with wax, in front of a LCD monitor. We presented target trajectories on the display whilst recording the intracellular, electrophysiological responses from STMD neurons. Data was analysed offline with Matlab.

Results
During a target trajectory, a small region directly ahead of a targets current position shows enhanced sensitivity. Furthermore, the location of this region allows a drifting target to generate strong local direction selectivity, such that only targets that continue to move in a similar direction will produce facilitated responses. This enhancement in sensitivity was quantified by comparing contrast-sensitivity functions for primed and unprimed targets, revealing a 9-fold improvement in detectability. In addition, a drifting target produces a significant motion after effect (MAE). This produces a global inhibitory drive that competes with local facilitation, resulting in the suppression of false positives located in the surround.

Conclusions
Dragonfly target-detecting neurons employ a facilitation mechanism to enhance target discriminability in cluttered environments. This work demonstrates how a nervous system can use previous stimulus history and context to improve performance in complex tasks. These findings also present opportunities for bio-inspired developments in neuronal prosthetics, computer vision and robotics.

Acknowledgement
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Gimeno-Hernández, Roberto

Oxidative stress and autophagy in a retinitis pigmentosa animal model

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Purpose
Oxidative stress has been associated with retinitis pigmentosa (RP). Increased oxidative stress can induce lipid, protein and DNA changes which may lead to retinal cell death. These damaged cellular components should be degraded by different systems, including autophagy, which can be triggered by oxidative stress. Autophagy is crucial for maintaining cellular homeostasis and cell survival under stressful conditions. Recently, accumulating data has pointed to an essential role of reactive oxygen species (ROS) in the activation of autophagy. Therefore, oxidative stress and autophagy may be important factors in the pathogenesis of RP. The aim of this work was to study changes in different markers of oxidative stress and autophagy in the retina of a RP animal model, the rd10 mouse.

Methods
Animals were treated in accordance to the statements for the use of animals in ophthalmic and vision research. We obtained retinas from C57BL and rd10 mice at different postnatal days (PN 13, 21, and 28). Malondialdehyde (MDA) and glutathione was determined by HPLC. Western blot analysis was performed to assess microtubule-associated protein 1 light chain 3 II/I (LC3II/I) ratio, Beclin and lysosomal associated membrane protein-2 (LAMP2A).

Results
At PN21 there was a significant increase in MDA and a significant decrease in GSH in the retina of rd10 mice compared to control ones (p

Conclusions
Further studies are needed to clarify autophagy role in RP. However, drugs that could target at the same time oxidative stress and autophagy may be new strategies in RP treatment.

Acknowledgement
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Gross, Janine

Ultraviolet radiation-B exposure of one eye triggers substance P receptor expression in a mouse model

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Purpose
Investigation of UVR-B exposure effects to the exposed eye in comparison to the contralateral eye in vivo in a mouse model. Additionally the involvement of substance P receptor (NKR-1) in a signaling process to the non-exposed, contralateral eye has been analyzed.

Methods
Six-week-old C57Bl/6 mice were unilaterally exposed in vivo to a triple threshold dose (9,4 kJ/m²) of UVR-B in anaesthesia. The other eye was covered with aluminium foil. UVR-B irradiation in the 300-nm wavelength region (UVR-B peak at 312nm) was performed in mydriasis using a Bio-Spectra system (Vilber Lourmat, Germany). After a latency period of 3 and 7 days following UVR-B exposure, morphological changes in the lenses were photographed with a Leica dark-field microscope. Exposed and contralateral eyes were fixed in 4 % paraformaldehyde, embedded in paraffin, sectioned and stained with fluorescence coupled antibodies for substance P receptor, NKR-1 and DAPI for cell nuclei.

Results
UVR-B exposure induced corneal edema and anterior subcapsular cataract in the exposed eye after latency periods of 3 and 7 days post exposure. Unexposed lenses developed microscopically visible lens haze but no cataract on the epithelial level. Histological examination of control eyes as well as exposed eyes revealed positive staining for substance P receptor in the nuclear bow epithelium of the lens, the ciliary body epithelium, the iris and the inner plexiform layer of the retina higher than in control eyes. The exposed eyes also showed positive NKR-1 staining in the corneal epithelium and endothelium after a latency period of 7 days.

Conclusions
Unilateral UVR-B exposure of the eye affects the contralateral eye in a sympathetic reaction. Substance P receptor expression is upregulated in the exposed and contralateral, non-exposed eye following in vivo UVR-B exposure. The role of substance P, and other neuroinflammatory peptides in UVR-B –induced cataractogenesis needs further investigation.

Leube, Alexander

Wavefront-based 3-dimensional Refraction from Objective Visual Strehl Metrics

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Purpose
To evaluate the best sphero-cylindrical refraction of the human eye using 3-dimensional optimization algorithms regarding wavefront-based visual Strehl metrics and to investigate their correlation to subjective measurements of refractive errors.

Methods
462 eyes of 231 participants with a mean age of 33.1 ± 11.5 years (range: 18 to 78 years) were analyzed. Non-cycloplegic, monocular subjective refraction was assessed using a digital phoropter (ZEISS Visuphor) and a high luminance screen (ZEISS, Visuscreen) displaying SLOAN letters. The wavefront errors of the eye were measured using a commercial Shack-Hartmann sensor (ZEISS, i.Profiler plus) and scaled to a pupil diameter of 4.0 mm. The visual Strehl metrics from the optical and, the modulation transfer function (VSOTF and VSMTF, respectively) as well as from the point spread function (VSPSF) were calculated for a range of spherical and cylindrical refractive errors in the wavefront domain. To minimize computational effort, range of spherical search was pooled around the wavefront error of lowest RMS value. Cylindrical range of optimization was set from -2.50 to +2.375 D within steps of 0.125 D. To evaluate the difference between the wavefront based refraction and the subjective measures, correlation analysis and Bland-Altmann plots were calculated for each type of visual Strehl metric.

Results
The spherical value of the refraction showed high correlation between wavefront-based and subjective assessed results (VSPSF: r = 0:972; VSOTF: r = 0.969; VSMTF: r = 0.971; pall < 0.001). Correlations for the cylindrical refraction (r > 0.7) and its axis (r > 0.3) were lower but still statistically significant (p < 0.001). The mean squared error was lower 0.25 D in case of the spherical refractive error and below 0.15D for the astigmatism, which is in the order of the precision of a subjective refraction. Limits of agreement (LoA), calculated as 1.96 * standard deviation, of the sphere were ± 0.811 D, ± 0.878 D and ± 0.815 D for the refraction from the VSPSF, VSOTF and VSMTF metric, respectively. LoA for cylinder were lower and resulted in ± 0.538 D, ± 0.550 D and ± 0.604 D for the VSPSF, VSOTF and VSMTF, respectively.

Conclusions
Wavefront-based 3-dimensional refraction from visual Strehl metrics resulted in high correlations to subjective measures and moderate limits of agreements between the methods. Objective refraction based on lower order as well as on higher order aberrations will give more insights into subjective perception of blur and can lead to novel methods in optometry and ophthalmology to assess the refractive errors of the human eye.

Acknowledgement
None

Leyk, Janina

Specific inhibition of histone deacetylase 6 by tubastatin A protects cone-like 661W cells against oxidative stress

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Purpose
Progressive degeneration of photoreceptors is the main cause for vision loss in retinal diseases, such as retinitis pigmentosa (RP) and age-related macular degeneration (AMD). The underlying molecular mechanisms are still unresolved, but impaired proteolytic systems and oxidative stress have been implicated to be involved in the pathogenesis of RP and AMD. Recent studies suggest a role for histone deacetylases (HDACs) in neuro- and retinal degeneration. In a mouse model for RP HDAC activity was increased and its inhibition prevented photoreceptor cell death. The HDAC family comprises 18 members of which HDAC6 is unique since it mainly deacetylates non-histone proteins, such as ?-tubulin, HSP90 and peroxiredoxin 1 (Prx1). HDAC6 is involved in cellular stress response mechanisms and its inhibition has been implicated to be protective in models for neurodegeneration. The present study aimed to elucidate the effect of specific HDAC6 inhibition by tubastatin A (TST) in 661W cells, a cell line with cone photoreceptor characteristics, during oxidative stress. The presence of HDAC6 was investigated in retina lysates and cryosections of C57BL/6 mice.

Results
Immunoblot analysis and immunohistochemistry reveal that HDAC6 is present in the retina of C57BL/6 mice and that it is prominently expressed in photoreceptor inner segments as well as in the outer plexiform layer. It is expressed in 661W cells, as demonstrated by RT-PCR, and its inhibition by TST results in hyperacetylation of ?-tubulin. Oxidative stress, exerted by H_2O_2, leads to cytotoxic responses of 661W cells. Preincubation with TST promotes cell survival and increases heat shock protein (HSP) 25 and HSP70 levels by activating heat shock factor 1 (HSF1). However, the protective effect is not causally related to the increased HSP expression as demonstrated by incubation with the specific HSF1 inhibitor KRIBB11. Treatment with H_2O_2 elicits the overoxidation and thereby inhibition of Prx1, a redox regulatory protein involved in the reduction of H_2O_2. This effect was significantly reduced by preincubation with TST suggesting that HDAC6 inhibition by TST regulates the activity of Prx1.

Conclusions
HDAC6 inhibition by TST provides a protective means against a stress situation which occurs in retinal degenerative diseases.

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Möhrle, Dorit

Age-Related Inner Ear Synaptopathy and Central Compensation

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Purpose
Poorer performance in supra-threshold speech understanding and temporal processing with age has been previously linked with progressing cochlear synaptopathy that precedes age-dependent elevation of auditory thresholds (Bharadwaj et al., 2014; Bramhall et al., 2015, Sergeyenko et al., 2013), but has also been thought to reflect auditory processing deficits at the level of the central auditory pathway (Herman et al., 1977; Konkle et al., 1977). To counteract speech in noise deficits with age in the future, it is crucial to know whether the primary target site is the cochlear or the central nervous system. Therefore, we here aimed to follow central changes in activation by auditory stimulation (central responsiveness) to age-induced cochlear synaptopathy.

Methods
We compared young, middle aged, and old rats for differences in central responsiveness to age-related cochlear synaptopathy by auditory brainstem response wave amplitudes and temporal sensitivity linked to sound in noise responsiveness.

Results
Middle aged animals with age-related cochlear neuropathy showed a surprisingly robust increase in neuronal gain that maintained normal auditory brainstem responses and responses to fast amplitude modulated stimuli, while in old animals response strength and temporal sensitivity were reduced.

Conclusions
The present study suggests that age-dependent cochlear synaptopathy may not per se lead to the loss of central responsiveness and impaired temporal coding.

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Patel, Aara

Investigating Molecular Pathways causing Microphthalmia, Anophthalmia and Ocular Coloboma

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Purpose
Eye morphogenesis begins with the bilateral evagination of optic vesicles, from the neuroepithelium of the developing brain. As each optic vesicle approaches the surface ectoderm, it folds asymmetrically to form a double layered optic cup with a fissure running down its ventral aspect, which subsequently closes. Complete or partial failure of these morphogenetic processes leads to microphthalmia, anophthalmia or coloboma, which cause childhood blindness. This study aims to elucidate the underlying genetic pathways by studying human patients with eye malformations.

Methods
We recruited a cohort of 84 children with coloboma, microphthalmia and/or anophthalmia. Patient DNA was screened using a custom designed Agilent SureSelect QXT capture Oculome panel followed by next generation sequencing using Illumina HiSeq2500 to identify potentially pathogenic variants that could explain their phenotypes. The panel included 86 MAC-associated genes. Whole exome and genome sequencing is being applied to resolve cases without identified mutations.

Results
We achieved over 30 X read depth for 99.5% of our targeted region. Bioinformatics analysis of the HiSeq data was performed using an in house NE Thames Regional Genetics Laboratory pipeline. Pathogenic single gene coding mutations could be identified in 7% of cases.

Several more cases contained variants in phenotypically relevant genes whose pathogenicity could not be proven. Cases in which pathogenic single gene mutations could be identified included unilateral and bilateral defects with additional non-ocular phenotypes.

Although some MAC cases could be explained by coding mutations in known genes, these form a small percentage of the total. The rest of the cases may be caused by mutations in genes not yet associated with eye development, regulatory mutations or multigenic effects. Exome sequencing is currently being applied to a set of unexplained cases to identify novel genes. These analyses are being combined with RNAseq analysis of developing human eyes to elucidate the pathways critical for early eye morphogenesis and optic fissure closure.

Conclusions
The Oculome panel test enabled the molecular diagnosis of six cases in our cohort of MAC cases. Each case was due to a mutation in a different gene. Two mutations were previously reported and 6 were novel. Both dominant and recessive patterns of inheritance were observed. These data indicate the need to simultaneously screen patients for mutations in multiple genes for genetic diagnostic testing.

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Petridou, Eleni

Progression of histone deacetylase activity and secondary cone degeneration in rd10 mouse model

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Pilgrim, Matthew

A primary retinal pigment epithelial cell (RPE) culture model produces lipid- and hydroxyapatite-rich extracellular deposits characteristic of early stage age-related macular degeneration?

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Purpose
Accumulation of lipid- and protein-rich drusen and basal linear deposits between the inner collagenous layer of the Bruch's membrane and the basal lamina of the RPE is a hallmark of early age-related macular degeneration (AMD). Hollow spherules composed of lipids and inorganic hydroxyapatite (HAP) have recently been described within these deposits (PMID 25605911). We report a primary RPE cell culture model capable of producing sub-RPE deposits with these major molecular components.

Methods
RPE cells were isolated from freshly enucleated porcine eyes and cultured on laminin-coated porous Transwell membranes (Millipore). Cells were cultured in Miller medium (PMID 16877436) without addition of photoreceptor outer segments for up to 6 months. Histochemical studies were performed and cultures were examined by light and fluorescence microscopy as well as by scanning (SEM) and transmission (TEM) electron microscopy. Mineral deposition was detected by micro-CT and synchrotron x-ray diffraction (?XRD), trace metal composition was determined using synchrotron x-ray fluorescence (?XRF), and protein and lipid components were identified using secondary ion mass spectrometry (SIMS).

Results
TEM analysis of differentiated and polarised RPE cell monolayers revealed an age-dependent accumulation of diffuse and focal deposits between the RPE cell monolayer and the underlying Transwell membrane. Deposits were highly mineralised and produced diffraction patterns characteristic of the inorganic calcium phosphate hydroxyapatite. Zinc and iron co-localized with hydroxyapatite, but copper signal could not be verified. SIMS analysis confirmed the presence of proteins and lipids in focal deposits.?

Conclusions
Healthy primary RPE cell cultures produced focal and diffuse deposits containing lipids, proteins, trace elements and hydroxyapatite, all components of human drusen and basal linear deposits. These deposits formed without the addition of photoreceptor outer segments suggesting that RPE cells are capable of generating nucleation sites for sub-RPE deposit formation, this is consistent with previous in vitro (PMID 21969589) and pathology (PMID 21890786) studies. This cell culture model of early stage AMD provides a novel system for which new therapeutic interventions against early stages of AMD could be trialled.?

Polley, Daniel

Central Gain Restores Auditory Processing Following Near-Complete Cochlear Denervation

Daniel B Polley

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Results

Cochlear degeneration induces a host of cellular and physiological changes in the periphery as well as the brain. At higher stages of the central auditory pathway, where physiological processing enables the perception of sound, this plasticity must contribute to the perceptual sequelae of cochlear hearing impairment. However, disambiguating the peripheral and central determinants of hearing loss has proven difficult. Here, we show that many aspects of auditory processing recover after profound cochlear denervation due to a progressive, compensatory plasticity at higher stages of the central auditory pathway. Lesioning >95% of cochlear nerve afferent synapses, while sparing hair cells, in adult mice virtually eliminated the auditory brainstem response and acoustic startle reflex, yet tone detection behavior was nearly normal. As sound-evoked responses from the auditory nerve grew progressively weaker following denervation, sound-evoked activity in the cortex – and to a lesser extent the midbrain – rebounded or even surpassed control levels. Increased central gain supported the recovery of rudimentary sound features encoded by firing rate, but not features encoded by precise spike timing such as modulated noise or speech tokens. What mechanisms might be employed by a cortical amplifier to change input gain? To address this question, we used a transgenic strategy to express channelrhodopsin in parvalbumin+ (PV) fast-spiking cortical interneurons. By implanting an optetrode assembly in the auditory cortex, we were able to track day-by-day changes in local inhibitory tone and sound-evoked single unit activity over an extended period surrounding cochlear afferent denervation. We found that PV-mediated inhibition and sound responsiveness were tightly coupled. Cortical inhibitory tone plummeted following cochlear denervation. Inhibition partially returned in mice that spontaneously recovered auditory sensitivity but remained at the noise floor in mice that never recovered. In all cases, changes in PV-mediated inhibitory tone lagged changes in sound responsiveness by 2-3 days, suggesting that disinhibition is an important feature of cortical gain changes but is not a determinant. Collectively, these findings highlight the default homeostatic response of higher auditory circuits deprived of bottom-up afferent drive. This plasticity enables the restoration of firing rate codes to support basic audibility and rudimentary feature selectivity. However, it does not support (and might even impede) recovery of fine temporal processing required for perception of complex signals such as speech or music.

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Power, Michael

Calcium imaging in degenerating cone photoreceptors

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

Methods
Here, we use the transgenic HR2.1:TN-XL mouse line that expresses a calcium biosensor in cone photoreceptors to study calcium signals and concentrations in an acute retinal slice preparation. We use two-photon microscopy to measure the calcium dynamics in the retinas of HR2.1:TN-XL mice as our wild type (wt) control, as well as in the retinas of models for primary cone degeneration (cpfl1-mouse) and secondary cone degeneration (rd1-mouse) which have been stably crossbred with the HR2.1:TN-XL mice. In parallel with the capturing of calcium dynamics upon light stimulation, we can also detect enzymatic activities thought to be associated with intracellular calcium. By adapting an approach used commonly on thin retinal sections we have developed a protocol for the combination of enzymatic activity (i.e. calpain activity) and live cell calcium dynamics.

Results
Our preliminary data suggests that the activity of the calcium-dependent protease calpain is markedly increased in both disease models, when compared to the wt control.

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

Acknowledgement
DFG

Rak, Kristen

DFG- Einzelantrag/Sachbeihilfe - Proposal - The importance of microtubule dynamics and turnover for the correct function of the cochlear sensory epithelium

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Purpose
Microtubules (MT) are composed of tubulin filaments and are essential cytoskeletal components. The distribution of different isotypes and posttranslational modified isoforms of tubulin in the cochlea is cell type specific. Tyrosinated tubulin is the main form expressed in hair cells. In contrast MT in the supporting cells are composed of detyrosinated, acetylated and polyglutamylated tubulin. In addition modulating proteins of MT dynamics, like the TBCE protein, are cell type specifically expressed in the organ of Corti. There are some relationships between MT dysfunction and hearing loss. The specific function of MT in the cells of organ of Corti is not completely understood, but the divergent dynamic and stability of them could be linked to the maintenance of the sensory epitheliums function. Especially in traumatic situation, like sound overexposure, the correct distribution and stability of MT could be of importance. From these observations and hypotheses the main objective of the project was derived, which is the further investigation of MT dynamics and Tubulin turnover in the sensory epithelium in normal hearing situation and after sound overexposure.

For elaboration different questions should be investigated;

1) Does the modification of microtubule dynamics alter the function of outer hair cells and supporting cells after sound overexposure?

2) Does the application of a microtubule stabilising drug (Taxol) to the cochlea affect the function of the sensory epithelium?

3) Does the complete loss of the TBCE protein modify the development and structure of outer hair cells and result in dysfunction of the cochlear sensory epithelium?

Methods
For investigation different animal models and techniques shall be applied. The pmn animal line has high levels of active tyrosinated Tubulin and will allow the investigation of high rates of MT polymerization in the outer hair cells. A model of destabilized MT with low levels of polymerization in the outer hair cells will be achieved by crossbreeding a Prestin CreERT2 mouse line with a Stat3 loxP line. Altered microtubule dynamics in the supporting cells will be investigated in the offspring of the Fgfr3-iCreERT2 with the Stat3 loxP line. In addition MT dynamics can be altered by intracochlear application of the drug Taxol, which will lead to high levels of stable acetylated and detyrosinated MT. The specific function of the TBCE Protein in the cochlear hair cells will be investigated in the crossbreeds of different hair cell specific Cre lines (Brn3.1 Cre and Prestin CreERT2) and a TBCE loxP line. The investigations will include electrophysiological measurements, histological preparations and atomic force lever measurements.

Conclusions
In conclusions the results shall bring more insight into the function of MT and their modulating partners in the development and the integrity of the organ of Corti and a possible role of these cytoskeletal components on protection against hearing loss after acoustic overstimulation.

Acknowledgement
DFG- Einzelantrag/Sachbeihilfe - Proposal -

Reinhard, Jacqueline

Neurodegeneration in glaucomatous PTP-Meg2 deficient mice

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Purpose
Glaucoma is one of the leading causes of irreversible vision loss worldwide, yet the molecular mechanisms of optic nerve and retinal ganglion cell (RGC) degeneration are not fully understood. Persistent high intraocular pressure (IOP) is one of the major risk factor for glaucoma progression. In addition, several studies indicate that glaucomatous neurodegeneration is accompanied with immune activation and extracellular matrix (ECM) remodeling. Heterozygous (HET) protein tyrosine phosphatase Meg2 (PTP-Meg2) mice show progressive IOP elevation, optic nerve degeneration, RGC loss and retinal dysfunction. In the present study, we evaluate microglia reactivity, complement activation and remodeling of the ECM component Tenascin-C (TNC) in glaucomatous PTP-Meg2 HET mice.

Methods
Numbers of Iba1+/NOS2+ reactive microglia (MG) and membrane attack complex (MAC)+ cells as well as TNC immunoreactivity were assessed by immunohistochemistry in the optic nerve and retina (n=3-5/group) of PTP-Meg2 HET and wildtype (WT) littermates at 28 weeks. Expression levels of the complement factors C1qa, C1qb, C1qc and C5 were evaluated in the HET and WT retina at 10 and 28 weeks (n=3-4/group) by quantitative real-time-PCR analyses.

Results
PTP-Meg2 HET mice showed a significantly elevated number of Iba1+ and Iba1+/NOS2+ MG in the optic nerve ($p<0.05$) and retina ($p<0.05$) at 28 weeks. Expression of the complement components C1qa, C1qc ($p<0.001$) and C5 ($p<0.01$) was significantly up-regulated in the HET retina at 10 weeks. At 28 weeks C1qa, C1qb and C1qc expression levels were down-regulated ($p<0.001$), although C5 expression was still up-regulated ($p<0.001$) in the retina. Increased numbers of MAC immunoreactive cells were found in the HET optic nerve and retina at 28 weeks ($p<0.01$). TNC immunoreactivity was significantly increased in the retina ($p<0.001$) of glaucomatous PTP-Meg2 HET animals in comparison to WT mice at 28 weeks.

Conclusions
In summary, we demonstrated that glaucomatous neurodegeneration in PTP-Meg2 HET mice is accompanied with microglial reactivity, complement activation and remodeling of the ECM component TNC. Interestingly, our data suggest that the complement system is activated via the classical pathway in this glaucoma model. Our findings indicate that immune activation as well as ECM remodeling could contribute to glaucomatous neurodegeneration. In conclusion, PTP-Meg2 HET mice may serve as a useful animal model to further characterize the molecular mechanisms of glaucomatous neurodegeneration.

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

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Roper, Mark

Honeybee visual cognition: a miniature brain's simple solutions to complex problems

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Purpose
In recent decades we have seen a string of remarkable discoveries detailing the impressive cognitive abilities of bees (social learning, concept learning and even counting). But should these discoveries be regarded as spectacular because bees manage to achieve human-like computations of visual image analysis and reasoning, or could it be that the small brains of bees (and other animals) have found unique and elegant solutions of how to deal with complex visual challenges? Here we offer a radically different explanation using theoretical bee brain models to counter the widespread view that complex image analysis and classification requires animals to not only store representations of the images, but also perform advanced computations on them.

Methods
Using a bottom-up approach we created theoretical models of the bee brain and assessed how much neural complexity is required to accomplish behaviourally relevant tasks. We produced several simple models inspired by the known anatomical structures and neuronal responses within the bee brain and subsequently compared their ability to generalize achromatic patterns to the observed behavioural performance of honeybees on these same cues.

Results
Model simulations of just eight large-field orientation-sensitive input neurons from the optic ganglia and a single layer of simple neuronal connectivity within the mushroom bodies (learning centres) show performances remarkably similar to the empirical results without requiring any form of fine-tuning of neuronal parameters to replicate these results. Indeed, a model simply combining sensory input from both eyes onto single mushroom body neurons returned correct discriminations even when parts of the patterns were obscured, as well as an impressive invariance to the location of the test patterns on the eyes. This model also replicated surprising failures of bees to discriminate certain seemingly highly different patterns, providing novel and useful insights into the inner workings facilitating and limiting the utilisation of visual cues in honeybees.

Conclusions
We find that a wide variety of published behavioural results on bee cognition can be explained by the output of fewer than 10 known – very basic feature detector - optic ganglion interneurons. Remarkably, our model simulations revealed both discrimination and generalization results very similar to the empirical data without requiring any storage of an actual image, no further analysis of features contained within it, and no feature binding. The true impact of our research is therefore, not merely showing a model that can solve a particular set of generalization problems, but in providing a fundamental shift in how we should perceive visual recognition problems. Across animals, equally simple neuronal architectures may well underlie the cognitive affordances that we currently assume to be required for more complex conceptual and discrimination tasks.

Acknowledgement
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Rosskothen-Kuhl, Nicole

Ongoing Activation of the Deaf Auditory System via Cochlear Implant Augments GABAergic Neuronal Networks and Glia Hypertrophy

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Purpose
Does the activation of an adult deaf brain induce structural plasticity in the ascending auditory pathway? To answer this question we studied neurons and glia cells in the mature midbrain of hearing and neonatally-deafened rats 1 and 7 days after monaural cochlear implant stimulation.

Methods
Structural plasticity of the adult brain was analyzed by focusing on, first, expression of GAD65, GAD67, GABA, and VGLuT1, markers for inhibitory (GABAergic) and excitatory (glutamatergic) circuitries, respectively. Second, glial fibrillary acidic protein (GFAP), and ionized calcium-binding adapter molecule 1 (IBA1), markers for astrocytes and microglia, respectively, were observed histochemically. Recent studies have implicated neuron-glia interactions in synaptic plasticity associated with learning as well as the involvement of glia in regulating neuronal activity in vitro. Here, stimulation induced changes in excitatory and inhibitory circuitries, and of glia morphology were evaluated in brain sections of the central inferior colliculus (CIC) using immunohistochemistry.

Results
Compared to hearing rats, sustained activation of CIC neurons in deaf rats resulted in a strong hypertrophy of GFAP positive astrocytes and IBA1 positive microglia. This region-specific glia response was accompanied by a massive increase of size and intensity of GAD65 and GAD67 positive synaptic terminals and an apparent increase of the number of GAD65, GAD67, and GABA stained neuronal somata, while the level of VGLuT1 positive synaptic terminals remained unchanged following stimulation.

Conclusions
Thus, the initial question must be answered affirmatively: stimulation-induced remodeling of a deaf, but not of a hearing, system involves a massive neuron-glia co-modification accompanied by an augmentation of the GABAergic neuronal network.

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Schlüter, Tina

Essential role of miR-96 in the auditory brainstem

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Purpose
Deafness is the most common sensory disorder. 5 out of 1000 infants are born with or develop hearing loss in early childhood. In many cases, cochlear implants will restore hearing, but about one third of cochlear implant carriers do not benefit, yet. One reason for this shortcoming may be deficits in the central auditory system due to retrocochlear functions of deafness genes. microRNA-96 (miR-96) potentially fits into this category, as mutations therein cause deafness in man and mice. MicroRNAs are small-noncoding RNAs that play important regulatory roles by binding to specific sites in the 3' UTR of target mRNAs. They are important for multiple cellular processes including development, differentiation and maintenance of cellular functions in the mature system. miR-96 is part of the miR-183 cluster, consisting of miR-183, -96 and -182 and is highly expressed in sensory cells. The mouse mutant Dmdo harbors a point mutation in miR-96 which causes deafness due to arrested cochlear hair cell development. Since miR-96 is also expressed in the brainstem, our aim was to analyze its expression and function in the auditory hindbrain. To this end, young-adult wild-type and Dmdo were analyzed. Furthermore, we included Claudin14-/- mice since they display a cochlear phenotype similar to Dmdo mice. These mice will thus inform about the contribution of peripheral deafness to alterations in the auditory brainstem of Dmdo mice.

Methods
To determine the precise expression pattern of miR-96 in the developing brainstem, in situ hybridization was done in wild-type mice. Possible retrocochlear functions of the microRNA were investigated by anatomical studies of Nissl-stained brain slices of homozygote Dmdo and Claudin14-/- animals. To narrow down potential target mRNAs of the mutated miR-96, immunohistochemical analyses of bioinformatically predicted targets were performed in Dmdo mice.

Results
Prominent miR-96 expression was observed in the auditory brainstem throughout development. Anatomical studies revealed a significant 25-35% volume reduction in various auditory brainstem nuclei of young-adult animals. This reduction was due to both cell loss and reduced cell size. In contrast, neonatal Dmdo mice showed no significant volume reduction, while an intermediate reduction was observed at P4. Non-auditory nuclei of Dmdo and auditory nuclei of Claudin14-/- mice showed only minor volume changes. Immunohistochemistry using antibodies against the potassium channel proteins BK beta subunit 2 and Kv1.6 revealed significantly reduced expression of these proteins in Dmdo mice.

Conclusions
Volume reductions in Dmdo mice likely reflect an on-site effect of the mutated miR-96 and not merely a general degeneration of auditory structures in deaf mice, as they were not present in deaf Claudin14-/- mice. Altogether the data demonstrate that mutations in miR-96 affect postnatal development of the auditory brainstem of mouse, implying an important role of this microRNA throughout the auditory system. This adds miR-96 to the list of deafness genes with a function in both, the cochlea and central auditory pathways.

Acknowledgement
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Schneider, Magdalena

Decorin deficiency leads to glaucomatous changes in mice

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Purpose
An elevated intraocular pressure (IOP), caused by an increased outflow resistance to the aqueous humor in the trabecular meshwork (TM), is the major risk factor to develop glaucoma. TGF-?2 and CTGF are involved in the molecular mechanisms causing an increased outflow resistance by stimulating the extracellular matrix (ECM) synthesis and TM cell contractility. In this study we investigate the influence of Decorin (DCN), a potential endogenous antagonist of TGF-?2 and CTGF, on the TM in vitro and in vivo.

Methods
Anterior chamber angles of eyes from human glaucomatous and healthy donors were stained for DCN. DCN-deficient mice and their wild-type littermates were analyzed at different ages. IOP was measured by tonometry. Total number of optic nerve axons was counted. Amounts of TGF-?2 and CTGF were analyzed in the region of the TM of DCN-deficient mice and their wild-type littermates. Human TM cells were treated with DCN, TGF-?2 and CTGF to analyze reciprocal effects and effects on ECM production. In vivo and in vitro samples were analyzed by real-time-RT-PCR, immunoblotting and immunohistochemistry.

Results
Staining intensity for DCN is reduced in the anterior chamber angle of glaucomatous donors compared to healthy donors. In mice, DCN deficiency causes an induction of CTGF, TGF-?2 and ECM proteins in the TM, leading to an increased IOP and a loss of axons in the optic nerve. DCN treatment leads to a decreased synthesis of ECM proteins, CTGF and TGF-? in TM cells, while treatments with TGF-?2 and CTGF reduce DCN expression.

Conclusions
Our results strongly indicate that DCN is a negative modulator of TGF-?2 and CTGF and is able to reduce ECM production in the TM. The deficiency of DCN causes a glaucoma-like phenotype, showing in an increased IOP and a severe a loss of ON axons. Therefore therapeutic modulation of DCN expression might be a feasible approach to treat glaucoma.

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Smith, Andrew

Identification and Characterisation of Novel Cone Photoreceptor-Enriched Factors Conserved in Zebrafish, Mouse and Human

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The inability of the retina to detect/transmit light-triggered signals due to dysfunction or death of photoreceptor cells is manifested in incurable blinding conditions. The purpose of this research is to identify novel cone-enriched factors conserved in zebrafish, mouse and human macula and characterise their involvement in cone development, function and survival.

Microarray analysis of cone photoreceptors of Tg(3.2TαCP:EGFP) zebrafish and RNAseq data of photoreceptors of the cone dominant Nrl -/- mouse and rod dominant Nrl-GFP mouse were compared to identify conserved cone-enriched factors. Human retina and macular RNAseq data was analysed to confirm evolutionary conservation. Genes were ranked on cone enrichment. Fluorescent in situ hybridisation (FISH) of high-ranking factors was performed on developing and adult Tg(3.2TαCP:EGFP) zebrafish. Gene knockdown of clul1, one of these high-ranking genes was performed using morpholino technology. Morphants visual behaviour was assessed using the optokinetic response, and retinal integrity examined using light microscopy. We are currently developing CRISPRCas9 mediated gene knockout for our 5 highest-ranking genes.

Upon ranking based on enrichment in zebrafish and mice, twenty-seven novel, conserved, cone-enriched genes were selected for further analysis. These factors were conserved and enriched in human retina. FISH revealed the gene clul1 was specifically expressed in adult zebrafish cone photoreceptors. Knockdown of the gene clul1 resulted in a significant impairment of visual behaviour without substantial morphological differences in the retina.

Twenty-seven novel, conserved cone photoreceptor-enriched factors were identified and spatiotemporal expression elucidated. Knockdown of clul1 indicates it is not required for normal cone photoreceptor morphogenesis but is required for cone photoreceptor mediated visual function. Cone photoreceptor specific expression is aiding the development of CRISPR knockout models to elucidate the role these factors play in cone morphogenesis and function.

Spahr, Hendrik

Aberration-free functional imaging of human retina using full-field swept-source optical coherence tomography

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Purpose
Non-invasive imaging of molecular and cellular processes of vision is expected to have immense impact on research and clinical diagnostics. Different fast intrinsic optical signals after light stimulus were reported. In humans, however, optical signals related to light stimulation were weak and hardly observable. Aim of this study was to evaluate OCT for measuring changes in the photoreceptor outer segments after light stimulation.

Methods
Measurements were done with a full-field swept-source optical coherence tomography (FF-SS OCT) system in combination with an LCD based projection system to stimulate the retina. A mirror coupled the light stimulus into the sample illumination arm of the OCT system which consisted of an tuneable light source (Superlum Broadsweeper BS-840-1) and an ultrafast CMOS camera (Photron FastCAM SA-Z). The light backscattered by the retina was imaged onto the camera, where it was superimposed with the reference light. A single volume was acquired by obtaining multiple images while tuning the wavelength of the laser.

Results
In contrast to other state-of-the-art OCT systems, FF-SS-OCT allowed significantly faster data acquisition of up to 180 volumes per second. We were able to register series of volumes over up to 3 seconds with sub-speckle and sub-photoreceptor accuracy. This allowed for the first time to measure changes of the length of the photoreceptor outer segments upon optical stimulation in humans. The phase stability in the recorded volumes also enabled computational correction of aberrations of the eye, which increased the lateral resolution significantly. In aberration corrected volumes we were able to assign the observed length changes to single cones.

Conclusions
FF-SS-OCT was able to non-invasively detect the activity of single cones in living humans. It may provide new diagnostic options in ophthalmology and neurology and might also give new insights into visual phototransduction.

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Trachsel-Moncho, Laura

Simulating Diabetic Retinopathy In Retinal Explant Cultures

Trachsel-Moncho L, Valdés J, Sahaboglu S, Trifunovic D, Miranda M, Ueffing M, Schmachtenberg O, Paquet-Durand F .

Introduction
Diabetic retinopathy (DR) is a major cause of vision loss due to complications of diabetes. It is generally considered a microvascular disorder; however, evidence has been found of retinal neurodegeneration before the vascular alterations start taking place. Its study is particularly difficult because of the lack of experimental model systems that faithfully reproduce the pathology. In vitro studies of cell cultures that preserve the structure of the retina (organotypic retinal explant) may provide insight into the cellular processes occurring under diabetic conditions.

Aim
The aim of this study is to evaluate whether organotypical explant cultures may be an appropriate method to simulate pathologic conditions in the diabetic retina, and if with it, it is possible to study the cell death mechanisms displayed along the disease.

M&M
Retinal explants cultures were exposed to conditions emulating type 1 or type 2 diabetes. The cultures obtained at post-natal day 5 (P5), were incubated with R16 complete medium (CM) until P7. From P7 to P11, cultures were cultured with different treatments: 15mM D-mannitol (osmotic control), CM without insulin, CM plus 15mM glucose (reproducing type 2 diabetes), CM without insulin plus 15mM glucose (reproducing type 1 diabetes), and CM with 20 mM 2-deoxy-glucose (2-DG) which emulates alterations of glucose metabolism observed in certain forms of type 2 diabetes. Explants were sectioned and a histologic study was accomplished. TUNEL assay was used for cell death detection, and for immunohistochemistry, cleaved caspase-3 for studying whether apoptosis may have contributed to retinal cell death, PKCα (specific marker for bipolar cells), glycogen phosphorylase (a cone photoreceptor marker) and cone arrestin (an alternative cone marker) primary antibodies were used.

Results
The explants that were exposed to diabetic conditions showed high cell death rates among inner retinal neurons as well as photoreceptors. Removal of insulin from the culture medium caused a significant increase in cell death in both INL and ONL (particularly cones, which may explain the color vision abnormalities in DR patients), whereas just the photoreceptors in the ONL were sensitive to perturbation of intracellular glucose metabolism via 2-DG treatment. Deprivation of insulin as well as high glucose caused a dramatic loss of cones, which suggests that cones have a high insulin dependency and presence of insulin in the retina plays a key role in cell survival.

Discusion
The study shows that simulating DR conditions leads to neuronal cell death independently of vascular or immunologic alterations. Retinal organotypical explants appear to be a useful way of entirely control the retinal conditions and a great tool for studying the different cell death mechanisms that may be occurring in the retina. Moreover, with this method, mice population needed for experimentation is halved, thus, reducing their suffering.

Vagionitis, Stavros

Investigations on an in vitro model for diabetic retinopathy

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Abstract
Diabetic retinopathy is one of the most common complications of diabetes mellitus during the patients' life. It is ranked as a most prominent cause for blindness globally, with high impact on the health and productivity of the population. While there are a number of animal models for diabetic retinopathy, both genetic and chemically induced, their use is challenging since they require either long maturation times for the symptoms of diabetes to fully manifest, or they exhibit a high phenotypic variability, or both. In addition, the phenotype observed often only very poorly reflects the human situation. A way around this problem is the use of ex vivo retinal tissue cultured in defined, serum-free media, simulating, for instance, the changes in the levels of blood insulin and blood glucose levels. This novel technique was established recently in our lab (Valdes et al., 2016, doi: 10.14573/altex.1603111) and can be used for investigating the mechanisms of cell death in the diabetic retina as well as the expression of key-components of the insulin uptake cascade.

Here, we used different in vitro diabetic retinopathy paradigms to extend the previous observations also to the more mature post-natal retina. Since the pathogenesis of diabetic retinopathy critically depends on the expression of different glucose transporter proteins (Glut), we also investigated the retinal expression of class I glucose transporters in histological preparations, both in vivo and in vitro. Moreover, the in vitro simulation of diabetic retinopathy may be used for the screening of potential anti-diabetic drugs, with known anti-diabetic drugs serving as controls. In the future, these observations may contribute to a better understanding of the retinal pathology under diabetic conditions, ultimately providing the fine-tuning required for designing a most effective treatment.

Valero-Gracia, Alberto

Disentangling the origins of vision by investigating marine zooplankton

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Purpose
The most primitive kind of photoreceptor (the functional group Class I) comprise a cell expressing opsins: proteins which initiate the photoreception cascade in response to light. These receptors, although difficult to identify without molecular tools, can be considered the evolutionary precursors of all animal eyes, including our own. Class I photoreceptors cannot discriminate the directionality of light, but enable the animal to monitor the ambient light intensity and regulate feeding, movement, and reproduction. Using these photoreceptors marine plankton can mediate vertical migration: the largest movement of biomass on Earth. However, despite its ecological and evolutionary significance, the mechanism by which these simple receptor cells regulate phototaxis is not well understood. In this talk, the most important evolutionary steps needed for the formation of complex eyes will be considered. Furthermore, a custom made set-up to investigate the neuroethology of vertical migration in marine invertebrate larvae will be presented. In addition, the function and evolutionary antiquity of the opsin-positive cells discovered in the sea urchin larva of Strongylocentrotus purpuratus will be discussed in light of their neural and phylogenetic context. To conclude, a mechanistic model for understanding how simple photodetection works in marine larvae will be proposed.

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Vienola, Kari

Parallel line scanning ophthalmoscope for retinal imaging

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Purpose
To visualize retinal structures using a newly developed parallel line scanning ophthalmoscope (PLSO).

Methods
A PLSO was built using a digital micro-mirror device (DMD) instead of traditional scanning mirrors to scan lines over the field of view (FOV). The DMD consists of 912 × 1140 micro-mirrors which can be individually switched on/off based on a programmed binary pattern. By switching on multiple (parallel) two-element wide lines in the DMD, the corresponding lines on the retina are imaged on a CMOS camera. After acquisition of each frame, the micro-mirrors are turned off and the mirrors for the next set of adjacent lines are turned on. This is repeated until the whole FOV is imaged. Confocal images are generated from the data by subtracting the maximum and minimum intensity values for each pixel in the sequence. The fovea and optic nerve head (ONH) of a healthy subject were imaged using 10° × 10° FOV at 100 Hz with 7 parallel lines resulting in a full image frame rate of 1.4 fps. The images were acquired through a dark-adapted pupil without any dilatation. The acquired data were processed into confocal images as well as non-confocal images (by averaging all frames).

Results
The non-confocal images show a strong uniform background, which originates from the corneal scattering and the multiple-scattered light from the retinal tissue making the features of the retina almost undetectable. In the confocal images, confocality and contrast are drastically improved and the foveal avascular zone and smaller blood vessels are visible in the fovea image. Additionally the quality of the ONH image is improved and many of the main features can be distinguished such as the small blood vessels.

Conclusions
The PLSO provided high contrast images of the fovea and ONH and detailed retinal structures could be observed. The use of a DMD eliminates moving parts from the system and exposure time for each frame is shorter than in full-field imaging, which reduces intra-frame motion. In retinal imaging, such a setup will provide better images because higher imaging speeds reduce motion artifacts.

Statement on proprietary interests
Prof. Johannes F. de Boer holds a patent regarding the PLSO technology.

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Wolters, Markus

Comparative real-time analysis of cGMP signals linked to degeneration and regeneration of the eye and ear

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Purpose
Seeing and hearing are important for interactions with our environment. Individuals that have lost these senses experience severely reduced life quality. The intracellular second messenger cGMP is central to the biochemistry of phototransduction in the retina. Recent studies have implicated dysregulated cGMP signalling also in degenerative processes in the retina and inner ear. Interestingly, elevated cGMP concentrations lead to degeneration of photoreceptors, but protect cochlear hair cells from noise-induced damage. However, the molecular and cellular mechanisms of cGMP-dependent degeneration/protection of neurosensory tissues are not fully understood. This study aims to characterise cGMP signalling in real time in live cochlea and retina under baseline and pathophysiolological conditions.

Methods
cGMP was visualised in tissues of transgenic mice that express the FRET-based cGMP biosensor cGi500. Cellular cGMP levels were monitored in freshly isolated cochlea and retina in response to various endogenous hormones that stimulate cGMP synthesis (e.g., NO or natriuretic peptides).

Results
Application of the NO-releasing compound DEA/NO produced a robust cGMP increase in inner hair cells of the cochlea. Pillar cells also produced cGMP in response to DEA/NO application, but to a smaller extend than inner hair cells. In contrast, we did not detect cGMP changes in these cell types upon exposure to the natriuretic peptides, ANP or CNP. Outer hair cells did not generate cGMP upon application of NO, ANP, or CNP. In retinal whole-mounts an increase in cellular cGMP was observed after application of DEA/NO in ganglion cells and bipolar cells. Interestingly, CNP induced a long-lasting cGMP increase in ganglion cells, while ANP showed no effect.

Conclusions
NO stimulates cGMP generation in distinct cell types of both cochlea and retina under baseline conditions. While ANP was unable to induce cGMP elevation in these tissues, CNP led to cGMP production in retinal ganglion cells. Further cGMP imaging experiments are required to determine if the profile of cGMP signals is altered in degenerated retina and cochlea and if these signals can be restored pharmacologically with cGMP-modulating drugs (e.g. Sildenafil, also known as Viagra). Ultimately, these studies might identify potential new therapeutic options to treat neurosensory diseases.

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