Doc Ophthalmol (2015) 130:11–58
DOI 10.1007/s10633-015-9500-3

ABSTRACTS

53rd Symposium of International Society for Clinical Electrophysiology of Vision (ISCEV), 23–27 June 2015, Ljubljana, Slovenia

Published online: 24 May 2015

Wednesday, 24.06.2015

William Dawson Memorial Lecture: 11:00–11:50

The role of visual electrophysiology in neuro-ophthalmology

Graham E. Holder

Moorfields Eye Hospital and UCL Institute of Ophthalmology, London UK

The objective data provided by electrophysiological investigation can be fundamental to the diagnosis and management of patients in the neuro-ophthalmology clinic. Medically unexplained visual loss, for example, in which the patient has symptoms that are not explained by the findings on clinical examination, is not uncommon and presents particular challenges to the clinician: there could be post-retinal disease; occult maculopathy/retinopathy; or non-organic visual loss. Further, there are retinal masquerades of optic nerve disease and optic nerve masquerades of retinal dysfunction.

After an overview of the tests (full-field, pattern and multifocal ERGs; VEP) and their uses, the presentation will use a case-based approach to demonstrate the value of electrophysiological examination. A variety of disorders will be addressed, affecting both retinal and optic nerve. The role of the PERG, "driven" by the macular photoreceptors but much of which arises in the retinal ganglion cells, will be discussed: macular dysfunction affects the P50 component whereas optic nerve/retinal ganglion cell disease can selectively involve the N95 component. Pattern VEP abnormalities are usually non-specific and the PERG can thus play a pivotal role in the differentiation between optic neuropathy and maculopathy.

Poster session 1: 14:30–15:30

Neuro-ophthalmology

1. Longitudinal study of retinal and optic nerve function in patients with multiple sclerosis: a baseline report

Christina Gerth-Kahlert, Klara Landau, James V. M. Hanson

University Hospital Zurich, Department of Ophthalmology, Zurich, Switzerland

Purpose: To assess in detail retinal and optic nerve function using electrophysiological methods as an afferent measure in patients with newly diagnosed relapsing-remitting multiple sclerosis.
Methods: Patients diagnosed with MS with or without a history of optic neuritis (ON) or other ocular manifestation are included in this prospective longitudinal study. Each patient received a comprehensive eye examination. The following functional tests were performed: scotopic and photopic fullfield (ff) ERG according to ISCEV standards, photopic negative response (PhNR) of the flash ERG, photopic mfERG (61 and 103 hexagons), pattern-reversal VEP. Data were compared with age-matched normal controls.
Results: Data will be presented from the baseline examination of patients recruited at the time of the meeting. So far, 19 patients (15 female, 4 male) ages 23–51 years have been tested. 12/19 had a history of ON in one (9/12) or both (3/12) eyes. Abnormal responses were found: scotopic and photopic ffERG: 1/19, photopic ffERG: 6/19, reduced PhNR through 3/17, unilateral VEP (9/18), bilateral VEP 6/19), delayed mfERG responses 6/18.
Conclusions: Patients with MS demonstrate retinal and optic nerve dysfunction related or unrelated to the history of optic neuritis. Extended electrophysiological testing allows detailed

functional assessment to possibly characterize distinct phenotypes of MS.

2. Visual evoked potentials in ON-pathway disorders

Dennis Fritsch[1,2], Valeria Ricotti[3], Francesco Muntoni[3], Stephanie Grunewald[2,4], Jane Sowden[2], Dorothy Thompson[1]

[1]Clinical and Academic Department of Ophthalmology, Great Ormond Street Hospital, London, UK; [2]Stem Cells and Regenerative Medicine Section, UCL Institute of Child Health, London, UK; [3]Dubowitz Neuromuscular Centre, UCL Institute of Child Health and Great Ormond St, London, UK; [4]Department of Metabolic Medicine, Great Ormond Street Hospital for Children, London, UK

Purpose: Some animal models of ON-pathway dysfunction are reported to show disorganised ganglion cell firing rates. The purpose of this study is to examine whether patients manifesting ERG evidence of ON-pathway dysfunction—associated with conditions such as Duchenne Muscular Dystrophy (DMD), congenital disorders of glycosylation (PMM2-CDG), or Congenital Stationary Night Blindness (CSNB)—show evidence of altered VEPs, which may indirectly reflect ganglion cell firing.
Methods: Pattern reversal VEPs (pVEPs) from patients identified with 'negative' ERGs were analysed. The amplitude and latency of pVEPs elicited to checks of 50' side length, which phase reversed 3/s in a field subtending 30 degrees viewed at 1 m recorded from Oz referred to Fz were collated. These were compared with normative data. The study group comprised patients with DMD (n = 15, seven with mutations pre exon 30 and eight with post exon 30), PMM2-CDG (n = 10) and CSNB (n = 32). The CSNB cohort was further subdivided into 19 individuals with incomplete CSNB (iCSNB) and 13 with complete CSNB (cCSNB), of whom seven were female and six male.
Results: The normative range of pVEP latency for a 50' check size in the age range of 8–15 years is 90–116 ms. The following patient groups fell within normal range: (1) All DMD patients had clinically normal pVEP latencies (median 98 ms, range 92–114 ms). The groups were stratified according to mutation position, before exon 30 (median 100 ms, range 92–114 ms) and after exon 30 (median 98 ms, range 95–107 ms). None of these patients showed nystagmus. (2) All PMM2-CDG patients had normal pVEP latencies (median 107 ms, range 95–115 ms), except for one patient with a non-detectable response to 50'checks. Four of these patients had nystagmus. (3) All males with cCSNB had pVEP latencies just around normal range (median 112 ms, range 101–118 ms). One patient had nystagmus. Abnormal pVEPs were noted in three out of seven female cCSNB patients; two of these patients had nystagmus. Additionally, two of the three patients with abnormal pVEP had mutations in GRM6. Mutational analysis is pending on the third patient. The overall group pVEP median latency was 112 ms (range 103–131 ms). A majority of patients with iCSNB (16/19) showed abnormal pVEPs. Most showed delayed latencies (10/16), including four patients who did not have nystagmus. One patient had an abnormally small amplitude pVEP with normal latency. The remaining five patients had no detectable response to 50' checks. None of the three iCSNB patients with normal pVEPs had nystagmus. The median latency of iCSNB patients with measurable responses was 121 ms (range 103–133 ms).
Conclusions: In summary, we found patients with ON-pathway disorders who manifested abnormalities in pVEP latency and amplitude consistent with altered ganglion cell firing. These abnormalities occurred in the absence of nystagmus in males with iCSNB and females with cCSNB.
Funding: The Ulverscroft Foundation National Institute of Health Research Biomedical Research Centres, Great Ormond Street Hospital and University College London Institute of Child Health.

3. A BOLD venture: comparison of VEP with functional near-infrared spectroscopy and functional magnetic resonance imaging for assessment of visual acuity

Torsten Straßer[1], Dominic Hillerkuss[1], Lukasz Lisowski[1,2], Sabrina Schneider[3], Florian Häußinger[3], Michael Erb[4], Andreas J. Fallgatter[3,5], Klaus Scheffler[4,5,6], Eberhart Zrenner[1,5], Ditta Zobor[1]

[1]Institute for Ophthalmic Research, University of Tuebingen, Tuebingen, Germany; [2]Department of Pediatrics Ophthalmology, Medical University of Białystok, Białystok, Poland; [3]Department of Psychiatry and Psychotherapy, University of Tuebingen, Tuebingen, Germany; [4]Department of Biomedical Magnetic Resonance Imaging, University of Tuebingen, Tuebingen, Germany; [5] Werner Reichart Centre for Integrative Neuroscience (CIN), Tuebingen, Germany; [6]Max Plank Institute for Biological Cybernetics, Tuebingen, Germany

Purpose: To compare sweep-VEP with functional near-infrared spectroscopy (fNIRS) and functional magnetic resonance imaging (fMRI) for objective assessment of visual acuity.
Methods: After obtaining approval from the local ethics committee and written informed consents, 10 healthy volunteers with best corrected visual acuity were examined for changes of the blood oxygenation level-dependent (BOLD) response to visual stimuli with different spatial frequencies using fNIRS (ETG-4000, Hitachi). An optode cap consisting of 3 × 11 probes, resulting in 52 channels, was placed over the back of the head to measure oxyhemoglobin (oxy-Hb) in the visual cortex. Additionally, the BOLD response was examined in five volunteers using 3T fMRI (MAGNETOM Prisma, Siemens). Both examinations, fNIRS and fMRI, used a randomized block stimulus design: five blocks of ∼20 s duration, preceded and followed by a 10 s rest period, were presented three times in random order. Each block consisted of 58 iso-luminant pattern-onsets of checkerboards (50 ms, contrast ∼80 %), with one out of five spatial frequencies (fNIRS: 0.6, 1.6, 4.1, 8.2, 11.4 cpd, fMRI: 0.5, 1.4, 3.2, 7.0, 9.8 cpd), followed by a 300 ms pause. Within one block, the checkerboard was rotated by ∼6° after each onset. Checkerboard stimuli were presented using LCD monitors in a distance of 160 cm (≈10°, central visual field). Only the right eye was stimulated. EEG was recorded simultaneously to fNIRS and

fMRI. In a second session, the test was repeated with reduced contrast sensitivity by covering the eye with a Bangerter occlusion foil (0.1).

Results: In all volunteers, the change of hemoglobin concentration (oxy-Hb) in the visual cortex corresponded to the change of the VEP amplitudes in response to checkerboard stimuli with specific spatial frequencies. The same effect could be observed in the level of activation in V1 monitored with fMRI. Applying a Bangerter occlusion foil resulted in reduced VEP amplitudes and reduced BOLD responses. Additionally, the maximum BOLD response was shifted to lower spatial frequencies.

Conclusions: To our best knowledge, this study is the first to demonstrate the application of fNIRS for assessing visual acuity. Changes in the BOLD response reflect the spatial frequencies of the presented checkerboards and could be observed in fNIRS as well as in fMRI recordings. fNIRS has the potential to be a valuable clinical tool in assessing the hemodynamics of the visual system in a quantitative and localized manner and useful in detecting visual dysfunction objectively and non-invasively in patients with visual disturbance. We would like to thank Ramona Täglich and Edyta Charyasz for their support in conducting fNIRS and fMRI recordings, respectively, as well as Ashish Sahib for providing his expertise in recording EEG simultaneously to fMRI. This study was funded by the fortüne-Programme, Faculty of Medicine Tuebingen, Grant Number: 2188-0-0.

4. A case of optic atrophy and retinal degeneration with low mitochondrial respiratory enzyme V activity

Hiroko Yamazaki[1,2], Yuzhu Chai[1], Yasushi Ooya[2]

[1]Department of Ophthalmology, Kohnodai Hospital, National Center for Global Health and Medicine, Ichikawa, Japan; [2]Department of Neurology, National Center Hospital, National Center of Neurology, Kodaira, Japan

Purpose: We present detailed neurological and ophthalmological findings in a case of optic atrophy and retinal degeneration with low mitochondrial respiratory enzyme V activity.

Methods: Neurological examinations including muscle biopsy, mitochondrial respiratory enzyme assay and analysis of mitochondrial DNA were performed. Ophthalmic examinations including full-field ERG and optical coherence tomography (OCT) were performed.

Results: A 24-year-old woman had developed epilepsy at the age of 14 years. She noticed difficulty in hearing and decreased visual acuity at the age of 20. These symptoms gradually progressed. She was referred to the neurology clinic of National Center of Neurology and Psychiatry Hospital for neurological evaluation. She was referred to the ophthalmology clinic for ophthalmological evaluation. Family history was unremarkable. Neurological examinations and laboratory tests revealed weakness of the proximal muscle of the lower limb. Audiometry showed sensorineural hearing loss. Brain MRI demonstrated bilateral calcification in the basal ganglia. Electroencephalography showed spike and wave complexes. Serum and CSF lactate and pyruvate level were elevated. Muscle biopsy revealed no ragged-red fibers. The mitochondrial DNA analysis in the skeletal muscle demonstrated a T>C point mutation at m.9173. Low activity of the mitochondrial respiratory chain complex V was revealed in a muscle biopsy. On ophthalmic examinations, corrected visual acuity was 0.6 in both eyes with mild myopia. Slit lamp examination revealed mild punctata cataract. Fundus examination revealed optic atrophy and salt and pepper like retinopathy in the peripheral retina in both eyes. The patient had normal ocular movement without ptosis. Goldmann kinetic perimetry was almost normal. OCT showed thinning of the retina, prominent in the inner layer of the retina. ISCEV protocol full-field ERG with an intense white light stimulus after 30 min dark adaptation showed subnormal a- and b-wave peak amplitudes. Peak amplitudes of the b-wave of the cone ERGs, 30-Hz flicker ERGs, and on–off responses for 150 ms duration stimuli were reduced.

Conclusions: The patient was diagnosed with definite mitochondrial disease because of muscle weakness, epilepsy, high lactate and pyruvate levels, and low activity of the respiratory chain complex V. Many mitochondrial diseases have ophthalmologic involvement. Optic atrophy occurs in LHON. Pigmentary retinopathy can also occur in Leigh syndrome, MELAS, MERRF, LHON, Kearns-Sayre Syndrome and NARP. Ophthalmological findings in our case differ from those commonly found in known mitochondrial diseases. Low activity of the respiratory chain complex V seems to be the cause of the ophthalmologic involvement of this case. Clinical manifestations of mitochondrial diseases are heterogeneous. This case suggests that the ophthalmic examination is important in cases with mitochondrial diseases in which ophthalmologic involvement is not known.

5. Electrophysiologic analysis of visual complications, specifically optic atrophy, from graft vs host disease

Stephanie Lao[2], Margaret C. Hubbell[2], Amanda Selchau[2], Terry Wood[1]

[1]Loma Linda University Eye Institute, Loma Linda, United States; [2]Loma Linda University, School of Medicine, Loma Linda, United States

Purpose: To analyze the electrophysiological changes in an acute myeloid leukemia patient with vision loss presumed to be due to graft vs host disease.

Methods: Electrophysiologic testing, retinal imaging, and ophthalmologic examinations were performed on a 41 year old female patient with a history of acute myeloid leukemia, status post bone marrow transplant, with bilateral vision loss. Intraocular changes were investigated using visual evoked potentials (VEP), optical coherence tomography (OCT), multifocal electroretinogram (mfERG), and electro-oculogram (EOG).

Results: After receiving a bone marrow transplant, the patient's vision loss rapidly progressed over 2 days and persisted for 5 months. On clinical examination, she was noted to have optic disc pallor bilaterally prompting an extensive diagnostic work up. The VEP revealed normal latency with reduced amplitude OU. The OCT demonstrated generalized retinal thinning OU and diffuse thinning of the optic nerve OU.

Conclusions: The results suggested an optic atrophy with an etiology of either leukemia or graft versus host disease.

Because of the onset of symptoms following bone marrow therapy and the concurrent presence of intestinal manifestations, a diagnosis of graft vs host disease was favored.

6. Comparison of electroretinographic responses in albinism, idiopathic infantile nystagmus and healthy controls

Zhanhan Tu[1], Christopher Degg[2], Viral Sheth[1], Irene Gottlob[1], Frank Proudlock[1]

[1]Ophthalmology Group, University of Leicester, Leicester, United Kingdom; [2]Medical Physics, University Hospitals of Leicester, Leicester, United Kingdom

Purpose: Previous studies using small sample sizes and skin electrodes describe children with albinism showing significantly larger ERG responses compared to controls, while children with idiopathic infantile nystagmus (IIN) had normal ERGs. We have compared ERG responses between a large sample of adults with albinism, IIN and controls also investigating whether nystagmus can affect ERG responses by comparing ERG measurements near and away from the null region. The correlation between ERG measurements with visual fields (VF) and retinal structure measured using optical coherence tomography (OCT) was also explored.
Methods: Sixty-seven albinism, 43 IIN and 24 controls were recruited for ERG comparison. Dilated Ganzfeld flash ERG testing was performed using DTL^{TM} corneal electrodes. Three IIN and 13 albinism were recruited for the paired comparison of ERG measurements tested at gaze angles near and away from the null region. Every test was repeated 5 times. The InertiaCube head posture device was used to locate the null region accurately. The thicknesses of retinal layers in the foveal area were obtained using the OCT. Humphrey VF tests were performed.
Results: The IIN group demonstrated significantly smaller photopic a- and b-wave amplitudes compared to controls ($P < 0.001$). The albinism group had smaller b-wave amplitudes compared to controls ($P < 0.05$). The IIN group also showed significantly longer photopic b-wave latencies compared to the albinism group ($P < 0.01$). The success rates of ERG measurements were higher when tested at the null region compared to away from the null region. Under dark-adaptation conditions with standard flash, a- ($P < 0.05$) and b-wave ($P < 0.05$) amplitudes testing at null region were significantly larger than the responses obtained away from the null region. The b-wave latency under photopic condition with standard flash also was statistically different between the two testing positions ($P < 0.05$). The thickness of photoreceptor layers was correlated with a-wave amplitudes and latencies. The VF was strongly correlated with scotopic b-wave latency with dim flash.
Conclusion: Our findings of reduced photopic a- and b-wave amplitudes and longer b-wave latencies in IIN indicate a subclinical reduction of retinal function in IIN which has not been previously detected. Interestingly, participants with albinism did not show the same changes despite having nystagmus, possibly because hypo-pigmented retinae can cause increased ERG responses. Our results also suggested that the ERG tested at null region in individuals with nystagmus can improve the success rate of recording and achieve better quality waveforms.

7. Vitamin A deficiency retinopathy: one year follow up

Evelyn Longhin[1], Elisabetta Pilotto[1], Enrica Convento[1], Alma Patrizia Tormene[1], Edoardo Midena[1,2]

[1]Department of Ophthalmology, University of Padova, Padova, Italy; [2]G. B. Bietti Foundation, IRCCS, Roma, Italy

Purpose: To report a 1-year follow up case of vitamin A deficiency treated with vitamin A supplementation, assessed by means of a comprehensive approach including electrophysiological, psychophysical and imaging investigation.
Methods: A 66-year-old woman with a history of gastric bypass surgery, complaining of visual impairment and night blindness, was assessed by means of a complete ophthalmic examination, Flash ERG, mfERG, microperimetry, spectral domain optical coherence tomography (SD-OCT), blue and near infrared-wavelength fundus autofluorescence (B- and NIR-FAF). Colour fundus photography was also performed before and during 1-year follow up of vitamin A treatment.
Results: At baseline, best corrected visual acuity (BCVA) was 0.9 in right eye (RE) and 0.6 in left eye (LE). Dark-adapted (DA) 0.01 ERG b-wave was undetectable, DA 3.0 ERG b-wave amplitude was reduced, light-adapted (LA) 3.0 ERG and LA 30 Hz flicker ERG were slightly reduced. MfERG showed diffuse marked reduction of the P1 wave amplitude and slightly delayed P1 implicit time, mainly in the LE. On microperimetry mean retinal sensitivity was 9.6 dB in RE and 9.5 dB in LE. The SD-OCT linear scans showed changes in the outer retinal layers: defragmentation of the external limiting membrane, and of the inner segment/outer segment junction; absence of the outer photoreceptor tips in the subfoveal and perifoveal areas. B-FAF revealed loss of the normal foveal hypo-FAF with a ring of mild hyper-FAF and diffuse irregular hyper-FAF at the posterior pole and mild periphery; NIR-FAF appeared normal. After 1-month of oral vitamin A treatment, symptoms subjectively regressed. BCVA improved to 1.0 and 0.9 in RE and LE respectively. DA 0.01 and DA 3.0 ERG b-wave amplitude increased to normal values, while LA responses remained unchanged. Mean retinal sensitivity was 14.3 dB in both eyes. The mfERG P1 wave changes slightly improved. SD-OCT, B- and NIR-FAF remained unchanged. At the 1-year follow up visit, BCVA was 1.0 in both eyes. DA and LA ERGs didn't change. MfERG improved only in the central 5°. Mean retinal sensitivity further increased to normal values (18.6 dB in RE, 18.8 dB in LE). The outer retinal layers appeared normal at the SD-OCT. Mild changes were present at the B-FAF.
Conclusions: In this clinical case the restoration of visual acuity, rod function, and retinal sensitivity was observed gradually during follow up visits and was well correlated with SD-OCT outer retinal layer recovery. These findings demonstrate the importance of a comprehensive assessment, which includes different investigation modalities.

8. Clinical case: multifocal ERG demonstrates residual abnormality years after traumatic macular injury in an eye with 20/20 vision

Linden Doss, Terry Wood

Loma Linda University School of Medicine, Loma Linda, USA

Purpose: To report the clinical utility of ERG to detect subtle macular functional changes following blunt ocular injury.
Methods: We evaluated a 16 year old female who had suffered a blunt injury by a soccer ball 18 months earlier. The patient's initial symptoms included floaters and photopsias which resolved over time. Complete ophthalmic examination was performed, including visual acuity testing, fundus examination, Heidelberg spectral-domain optical coherence tomography (SD-OCT), fundus photography, ultrasound B-scan, Humphrey visual field (HVF) and multifocal electroretinogram (mfERG).
Results: Initial examination revealed mildly decreased acuity (20/25) and a subtle but discrete area of macular hypopigmentation consistent with a partial thickness retinal injury. Early visual field testing (Humphrey 10-2) and optical coherence tomography confirmed an infranasal curvilinear tear penetrating through the neurosensory retina subtending between 1 and 2 degrees of parafoveal macula. Ultrasonic B-scan showed a partial posterior vitreous detachment in the right eye. Initial fundus examination showed a retinal tear confirmed by early Heidelberg OCT, surrounded by mild intraretinal edema. Symptoms gradually improved over the following 18 months. Late OCT imaging revealed partial healing of the tear resolving to a 60 micron partial thickness macular scar with photoreceptor disruption. MfERG performed a year and a half following the trauma demonstrated a well demarcated reduction in retinal function precisely at the site of the original injury, with approximately a 30 ms increase in latency and a 2 standard deviation reduced response density in the involved hexagon.
Conclusion: We report a very unusual case of a small macular tear without a macular hole or retinal detachment following blunt ocular trauma in a patient with a noticeable scotoma but 20/20 vision.

9. High-resolution imaging of patients with Bietti crystalline dystrophy with a CYP4V2 mutation

Kiyoko Gocho[1], Shuhei Kameya[1], Keiichiro Akeo[1], Sachiko Kikuchi[1], Ayumi Usui[2], Kunihiko Yamaki[1], Takaaki Hayashi[3], Hiroshi Tsuneoka[3], Atsushi Mizota[2,4], Hiroshi Takahashi[5]

[1]Department of Ophthalmology, Nippon Medical School Chiba Hokusoh Hospital, Inzai, Japan; [2]Department of Ophthalmology, Juntendo University Urayasu Hospital, Urayasu, Japan; [3]Department of Ophthalmology, The Jikei University School of Medicine, Tokyo, Japan; [4]Department of Ophthalmology, Teikyo University School of Medicine, Tokyo, Japan; [5]Department of Ophthalmology, Nippon Medical School, Tokyo, Japan

Purpose: To determine the retinal morphology of eyes with Bietti crystalline dystrophy (BCD) associated with a CYP4V2 mutation using high-resolution imaging techniques.
Methods: Three subjects with BCD underwent detailed ophthalmic examinations. High resolution fundus images were obtained with an adaptive optics (AO) fundus camera.
Results: A common homozygous mutation was detected in the three patients. Funduscopic examination of the three patients revealed the presence of crystalline deposits in the retina, and all of the crystalline deposits were also detected in the infrared (IR) images. The crystals observed in the IR images were seen as bright reflective plaques located on the RPE layer in the SD-OCT images. The clusters of hyperreflective signals in the AO images corresponded to the crystals in the IR images. High magnification AO images revealed that the clusters of hyperreflective signals consisted of circular spots that are similar to the signals of cone photoreceptors. Most of these circular spots were detected in healthy areas in the fundus autofluorescence images.
Conclusions: There is a possibility that circular spots observed by AO are residual cone photoreceptors located over the crystals.

Inner retinal function and the optic nerve

10. Functional and structural characteristics of early glaucoma

Maja Šuštar[1], Darko Perovšek[1], Barbara Cvenkel[1]

[1]Eye Hospital, University Medical Centre Ljubljana, Ljubljana, Slovenia

Purpose: To investigate the diagnostic value of the photopic negative response (PhNR) and pattern electroretinography (PERG) in early glaucoma and their correlation with the peripapillary retinal nerve fibre layer (RNFL) and macular thickness, using spectral-domain optical coherence tomography (SD-OCT).
Methods: This cross-sectional study included 34 patients (68 eyes): early glaucoma (20 eyes), glaucoma suspects (37 eyes), and ocular hypertension (11 eyes). Twenty-four aged-matched controls (44 eyes) were also tested. Glaucoma suspect eyes were defined as a glaucomatous appearing optic disc with normal standard automated perimetry results. ERGs were recorded with an Espion visual testing system. PhNRs were elicited with red stimuli on a blue background, and PERGs were recorded on a $21.6° \times 27.8°$ screen using a 0.8° checkerboard pattern that reversed 1.8 times per second. RNFL, macular nerve fibre layer (mNFL) and ganglion cell complex (GCC) thickness measurements were performed with a Topcon 3D OCT-1000 mark2 instrument and Fastmap v6.21 viewing and analysis software. Descriptive statistics, ANOVA, receiver operating characteristic curves, and correlation tests were used for analysis of the variables.
Results: The mean PhNR amplitude was significantly reduced in the early glaucoma (-14.3 ± 7.8 μV), glaucoma suspect (-14.1 ± 7.4 μV), and ocular hypertension (-15.6 ± 8.1 μV) groups, compared to responses of the normal controls (-26.3 ± 8.3 μV). Similarly, the PhNR/b-wave amplitude ratio was reduced in all three groups (0.25 ± 0.1), in

comparison to the controls (0.40 ± 0.15). The mean amplitudes of PhNR and PhNR/b-wave amplitude ratios were reduced to a similar extent, and thus did not differ among the early glaucoma, glaucoma suspect, and ocular hypertension groups. In PERG, the P50 amplitude differed only between controls (5.1 ± 1.6 μV) and early glaucoma (3.9 ± 1.1 μV), and the N95 amplitude was significantly decreased in early glaucoma (5.1 ± 1.3 μV) and glaucoma suspect (5.7 ± 1.1 μV) eyes, compared to the controls (6.8 ± 1.8 μV). Significant differences between early glaucoma, glaucoma suspect and ocular hypertensive eyes were found for RNFL (83 ± 12, 93 ± 10, 99 ± 8 μm, respectively), mNFL (29 ± 8, 34 ± 6, 42 ± 4 μm), and GCC thickness (89 ± 12, 96 ± 8, 109 ± 6 μm). Among the ERG parameters, PhNR amplitude distinguished best between glaucoma and the control group, with an area under the ROC curve of 0.85 for early glaucoma and 0.87 for glaucoma suspect eyes. A significant correlation between ERG parameters and RNFL/GCC thickness was found for all of the eyes, which was strongest between PERG amplitude and mNFL (r = 0.51) and GCC thickness (r = 0.49). In the subgroup analysis, the highest correlations were found in the glaucoma suspect eyes between PhNR/b wave ratio and RNFL thickness (r = 0.53), and mNFL thickness (r = 0.54).

Conclusions: ERG and OCT measurements are useful for assessing early glaucoma. In eyes with suspected glaucoma, small changes in mNFL and RNFL thickness were associated with important changes in the amplitude of PhNR. These findings suggest that PhNR is a useful, sensitive test in eyes that present a diagnostic dilemma, although further follow up of these eyes is required for definitive diagnosis.

11. Can variability of pattern ERG signal help to detect retinal ganglion cell dysfunction in glaucomatous eyes?

Alberto Mavilio[1], Francesca Scrimieri[2], Donato Errico[2]

[1]Social Health District, Glaucoma Center, ASL, Brindisi, Italy; [2]Ophthalmology Unit, Glaucoma Service, G. Panico, Tricase, Italy

Purpose: To evaluate variability of the steady-state pattern electroretinogram (SS-PERG) signal in normal, suspected, and glaucomatous eyes.

Methods: Twenty-one subjects with suspected glaucoma due to disc abnormalities (GS), 37 patients with early glaucoma (EG), and 24 normal control (NC) subjects were tested with spectral-domain optical coherence tomography (SD-OCT), standard automated perimetry (SAP), and SS-PERG. SAP Mean deviation (MD), SAP pattern standard deviation (PSD), SD-OCT retinal nerve fiber layer (RNFL) thickness, and ganglion complex cell (GCC) thickness were evaluated. The SS-PERG was recorded five consecutive times and the amplitude and phase of second harmonic were measured. PERG amplitude and coefficient of variation of phase (CVphase) were recorded, and relation to structural and functional parameters of disease was analysed by means of one-way ANOVA and Pearson's correlation.

Results: PERG amplitude was reduced, indicating of retinal ganglion cell (RGC) dysfunction, in EG patients and GS subjects compared to NC patients ($P < 0.0001$). CVphase was significantly increased in EG patients and GS subjects, compared to NC ($P < 0.0001$), and it was correlated with PSD ($P = 0.0009$), GCC ($P = 0.028$), and RNFL ($P = 0.0078$) only in EG patients.

Conclusions: Increased intrasession variability of phase in suspected glaucomatous eyes may be a sign of RGC dysfunction.

12. Comparison of the pattern ERG and focal macular PhNR to evaluate ganglion cell function

Souichi Matsumoto[1,2], Kazuma Yagura[1], Kei Shinoda[1], Harue Matsumoto[1,2], Atsushi Mizota[1]

[1]Teikyo University, Department of Ophthalmology, Tokyo, Japan; [2]Matsumoto Eye Clinic, Awa, Japan

Purpose: Inner retinal integrity is crucial for good visual function. The pattern ERG (PERG) and photopic negative response (PhNR) of the focal macular ERG (fmERG) have been used to assess retinal ganglion cell function in the macular area. The purpose of this study is to compare the sensitivity and the diagnostic usefulness of the PERG and macular PhNR in evaluating macular ganglion cell function.

Methods: Nine patients with unilateral optic nerve disease, three males and six females, were included. The mean (±SD) age was 55 (±24) years. The ocular disease was anterior ischemic optic neuropathy (AION) in four eyes, optic nerve atrophy after optic neuritis in three eyes, normal tension glaucoma in one eye, and superior segmental optic hypoplasia (SSOH) in one eye. We recorded fmERG by projecting a 20° visual angle circular spotlight onto the macula using a modified infrared fundus camera (ER-80, KOWA Co., LTD, Tokyo). The stimulus frequency was 5 Hz, and the duration of ON and OFF periods was 100 ms. The amplifier band-pass filter was 1–200 Hz with average rate of 300×. The steady state PERG was recorded with a stimulation frequency of 11.9 rev/s; the check-size was 1°; stimulated area was 20°; shape was round; and amplifier filter was 5–200 Hz with average rate of 300×. The results were compared with the retinal nerve fiber layer (RNFL) thickness of the spectral domain optical coherence tomography (SD-OCT) measurements.

Results: The mean PERG amplitude in the affected eye was significantly reduced (0.84 μV) compared with the contralateral healthy eye (1.44 μV). The mean amplitude of the macular PhNR in the affected eye was reduced (1.24 μV) compared with the contralateral eyes (1.66 μV), but this effect was not statistically significant. There was no significant correlation between the RNFL thickness and either PhNR amplitude or steady-state PERG amplitude.

Conclusions: In this pilot study for optic nerve diseases, the amplitude of PERG was useful to detect functional change of the macular ganglion cells.

13. Functional assessment of pre-ganglion and ganglion cell levels in autosomal dominant optic atrophy by PERGLA, PERG and multifocal ERG

Gabriella Cammarata[1], Gemma Tremolada[1], Paola Ciasca[1], Chiara Manclossi[1], Costanza Lamperti[2], Eleonora Lamantea[2], Stefania Bianchi Marzoli[1]

[1]Neuro-Ophthalmology Unit, Department of Ophthalmology, IRCCS Italian Auxologica Institute, Milan, Italy; [2]Unit of Molecular Neurogenetics, Foundation "C. Besta" Neurological Institute, Milan, Italy

Purpose: Considering that the primary abnormality of autosomal dominant optic atrophy (ADOA) lies within the retinal ganglion cells, functional studies are usually focused on ganglion cell (GC) and post GC analysis by means of PERG and VEP. Although GC function can be analyzed by the PERGLA protocol, of Porciatti V, and Ventura LM, (Ophthalmology (2004) 111:161–168) to detect early damage in glaucoma, it has never been tested in ADOA patients. Reduced pre-ganglion response has also been shown in ADOA using mfERG by Reis A et al., ([a]Graefes Arch Clin Exp Ophthalmol (2013) 251:221–234) suggesting that functional impairment also occurs at a preganglion level. Our aim is to assess ganglion cell dysfunction using PERG and PERGLA protocols and to investigate functional impairment at the preganglion level with mfERG in ADOA patients in order to compare functional results with the structural results obtained by SD-OCT analysis.
Methods: Ten consecutive ADOA patients (median age 29.5; range 12–58 years; M:F = 7:3) with genetically confirmed mutations in OPA1 gene and ten age and sex matched healthy controls were included in the study. All patients underwent PERG, PERGLA, mfERG and SD-OCT measurements of peripapillary retinal nerve fiber layer (PRNFL) and macular ganglion cell complex (GCC) thickness. Statistical analysis was performed using the mean value obtained in both eyes of each patient (40 eyes) evaluating PERG and PERGLA P50 and N95 amplitude, mfERG P1 amplitude in five concentric rings (R1-5), PRNFL and GCC mean thickness. Data were analyzed using non-parametric tests for all the variables (Wilcoxon/Kruskal–Wallis Tests) with Jump 7.0.
Results: PERG and PERGLA amplitudes were significantly reduced in ADOA patients when compared with the control group (PERG P50: $p = 0.05$, N95: $p = 0.004$; PERGLA P50: $p = 0.0022$, N95: $p = 0.0010$). Significantly decreased mfERG amplitudes were found in all rings (R1: $p = 0.0008$; R2: $p = 0.0013$; R3: $p = 0.0007$; R4: $p = 0.0005$; R5: $p = 0.0005$). Although PRNFL and GCC thicknesses were significantly different between patients and controls, they were not significantly correlated with PERG, PERGLA and mfERG in ADOA patients.
Conclusions: These data show that the PERGLA protocol is effective in detecting retinal ganglion cell dysfunction in ADOA patients. Multifocal ERG results confirm that functional impairment at the pre-ganglion level also occurs in ADOA patients suggesting retrograde damage mechanisms within the retina layers. PERGLA can be used as a valid alternative to PERG which can be methodologically difficult to perform with consistently reliable results and mfERG may be useful to detect pre-ganglionic dysfunction in hereditary optic neuropathies. The lack of significant structure–function correlations might be related to differences in the disease course within the small study group.

14. Red–blue pupil reaction and the melanotic ganglion cell response

Audrey Chia[1,2], Daniel Kai Peng Chua[1], Li Yu Chen[1], Rena Png[1]

[1]Singapore National Eye Centre; [2]Singapore Eye Research Institue, Singapore

Aim: There has recently been interest in how the melanotic ganglion cells may affect the pupil reaction. In this study, we wanted to determine if we could measure the pupil reaction to red and blue light stimulation in the visual electro-diagnostic lab using our existing Espion E^2 system with the ColorDome (Diagnosys LLC).
Method: Twelve normal individuals were tested using a protocol in which after 10 min of dark adaptation, red and blue stimuli were presented at 0.001, 0.01, 200 and 398 cd/m^2. Subjects were then light adapted for 5 min, and the red and blue stimuli were presented at 200 and 396 cd/m^2 against a 6.0 cd/m^2 blue background. Each flash was presented for 1 s and the subsequent pupil responses video-taped for 30 s.
Results: We noted that the pupil response to red stimuli was rapid with pupil size returning to baseline within 10–14 s. The pupil reaction increased with the increase in intensity until it plateaued at 200–398 cd/m^{-2}. The pupil response to blue stimuli was also rapid with 0.001–0.01 cd/m^{-2} stimuli but became progressively more prolonged with the brighter blue stimuli.
Conclusion: The pupil light responses to red and blue stimulation could be assessed using the Espion E^2 system with the ColorDome.

Pediatric electrophysiology

15. Paediatric electrodiagnosis in Hong Kong

Carol Po-Sang Lam, Winnie Wai Ying Lau, Carmen Kar Mun Chan, Andrew Chung Tin Fok

Hong Kong Eye Hospital, Hong Kong, Hong Kong SAR

Purpose: To evaluate the indications, test strategies, techniques, results and management implications of patients, ages 18 years or younger, who underwent electrodiagnostic testing.
Methods: A retrospective case series of 56 patients, 18 years or younger, who underwent electrodiagnostic testing over a 4-year period (2011–2014) at Hong Kong Eye Hospital.
Results: The series included 23 girls and 23 boys. 37 of the 56 patients were 10 years or younger, including six babies under 6 months of age. The most common indication for performing electrodiagnosis in the paediatric age group was for evaluation of unexplained visual loss (n = 21). Other common indications included suspected retinitis pigmentosa (n = 11), cone dystrophy (n = 6), suspected maculopathy (n = 6), pre-operative evaluation of visual function (n = 3) and evaluation of nyctalopia (n = 3). Tests performed included PERG (n = 9), full field ERG (n = 37), pattern VEP (n = 13), flash VEP

(n = 10), fERG (n = 10), fERG (n = 4). Test strategies, the various techniques used, and the clinical implications of the results will be described.
Conclusions: Electrodiagnostic testing in the paediatric age group may be challenging and labour-intensive. Various testing strategies and techniques may need to be adopted and altered depending on the age and degree of cooperation of the child. Successful testing may yield valuable information with clinical and management implications.

16. Electroretinogram changes in retinopathy of prematurity after photocoagulation

Hongbo Cheng, Guoming Zhang, Feng Wen, Shizhou Huang, Dezheng Wu

[1]Shenzhen Eye Hospital, Second Clinical Medical College of Jinan University, Shenzhen, China; [2]Zhongshan Ophthalmic Center, Sun Yat-sen University, Guangzhou, China

Purpose: The effects of photocoagulation on visual function in eyes with retinopathy of prematurity (ROP) were studied.
Methods: ERG was examined using the Espion electrophysiological instrument (Diagnosys, LLC, USA) in 24 ROP cases (48 eyes) after photocoagulation and 20 non-ROP cases (40 eyes). B wave amplitude and latency of the rod response and a and b wave amplitude and latency of the mixed reaction and the rod and cone responses were compared between the two groups.
Results: The b wave amplitude of the rod response was 95.76 ± 45.18 μV in the photocoagulation group and 111.70 ± 48.12 μV in the control group. There was a significant difference between the two groups ($t = 2.52$, $p = 0.029$). B wave latency was 107.36 ± 8.27 ms in the photocoagulation group and 100.81 ± 11.79 ms in the control group. There was a significant difference between the two groups ($t = 2.69$, $p = 0.021$). The amplitude and latency of a and b wave in the mixed reaction and cone responses showed no significant difference between the two groups ($p > 0.05$).
Conclusions: Photocoagulation for retinopathy of prematurity impaired rod cell function, but had little effect on cone function.

17. Evaluation of visual function by visual evoked potentials prior to surgery for congenital cataract

Hande Taylan Sekeroglu, Kadriye Erkan Turan, Ali Sefik Sanac

Hacettepe University Faculty of Medicine, Department of Ophthalmology, Ankara, Turkey

Purpose: To evaluate the clinical role of visual evoked potential (VEPs) in the assessment of preoperative visual function in cases with congenital cataract.
Methods: The medical records of patients with uni-/bilateral congenital cataract who had reliable preoperative VEP recordings were reviewed. The flash VEP (RETIport21, Roland Consult, Brandenburg, Germany) was recorded in a dimly illuminated room with monocular stimulation and undilated pupils. prior to lensectomy and anterior vitrectomy. The N2 and P2 latencies and P2 amplitude were evaluated. The flash stimulus was given by a hand held stroboscopic light. Manual correction was applied to the automatic cursor placement when necessary. The presence of any systemic diseases and nystagmus was recorded.
Results: 31 eyes of 17 children (seven boys, ten girls) were enrolled in the study. Three patients underwent unilateral surgery. The mean age at surgery was 6.76 ± 5.69 months (2–24). Preoperative ophthalmological evaluation of the patients revealed no fixational ocular movement. The mean preoperative N2 latency was 92.46 ± 19.91 ms, P2 latency was 123.72 ± 25.69 ms and P2 amplitude was 7.49 ± 4.98 mV, respectively. Seven patients had systemic diseases including microcephaly, seizures, myopathy, thin corpus callosum and rhizomelic chondrodysplasia punctata. Of the 17 patients, six patients had nystagmus preoperatively. There was no significant difference of N2 and P2 latencies and P2 amplitudes among patients with and without systemic diseases ($p > 0.05$ for all). Postoperatively all patients experienced fixational eye movements, recognition of their mothers' faces and voluntarily directed saccades.
Conclusions: Preoperative visual acuity is not measurable in children with congenital cataract. The patients often have nystagmus and uncertain ocular movements. The decision to operate may be challenging particularly in children who present at older ages. Even though the age of presentation is critical in the determination of the final visual acuity and the presence of severe nystagmus and accompanying systemic/neurological problems warrants poor prognosis, the majority of the surgeons would prefer to operate. Since VEP is a well-established technique in the evaluation of the integrity of the visual pathways, in the present study flash VEP was carried out as an auxiliary tool for predicting visual performance. It was demonstrated that the preoperative VEP disclosed delayed P2 latencies and reduced P2 amplitudes. However, all patients underwent surgery and all of them had evident fixational eye movements postoperatively. In conclusion, preoperative VEP recording, albeit prone to recording difficulties, may be a valuable test in the assessment of visual function particularly in patients with mental-motor retardation and who have a low probability of good visual prognosis. It is also possible that the electrophysiological differences between the patients might be explained by the accompanying developmental problems, laterality of the cataract and the age of the infants. It is worthy of note that low amplitudes and delayed latencies do not predict worse visual outcome and should not affect the decision for surgery.
Funding: None.

18. Comparison between ERG after photocoagulation and ERG after intravitreal injection of ranibizumab for treating retinopathy of prematurity

Guoming Zhang, Hongbo Cheng, Jun Mei, Linli Wang, Honghui He

Shenzhen Eye Hospital of Second Clinical Medical College of Jinan University, Shenzhen, China

Purpose: To determine the ERG changes after photocoagulation and after intravitreal injection of ranibizumab for treating retinopathy of prematurity (ROP).

Methods: From Jan. 2014 to Dec. 2014, twenty cases were collected in Shenzhen eye hospital. They were all diagnosed threshold or type 1 prethreshold ROP and were randomly divided into two groups. Laser photocoagulation was performed in the first group (Laser group) and intravitreal injection of ranibizumab was performed in the second group (IVR group). The ERG was examined using the Espion electrophysiological instrument (Diagnosys LLC, USA), and the rod and cone responses were compared between the two groups.
Results: B wave amplitude of the rod response was 85.17 ± 34.81 μV in the laser group and 108.07 ± 54.21 μV in the IVR group. There was a significant difference between the two groups ($t = 3.25$, $p < 0.05$). B wave latency was 91.63 ± 7.82 ms in the laser group and 99.18 ± 10.97 ms in the IVR group. There was a significant difference between the two groups ($t = 3.96$, $p < 0.05$). The amplitude and latency of a and b wave in the mixed and the cone responses showed no significant difference between the two groups ($p > 0.05$).
Conclusions: Photocoagulation for retinopathy of prematurity damaged rod cell function but IVR had little effect on rod cell function.

19. Electroretinograms in idiopathic infantile nystagmus, optic nerve hypoplasia, and albinism, in comparison to healthy controls and early-onset retinal dystrophies

Alma Kurent, Branka Stirn-Kranjc, Jelka Brecelj

Eye Hospital, University Medical Centre Ljubljana, Ljubljana, Slovenia

Purpose: We have previously studied electroretinogram (ERG) characteristics in children with infantile nystagmus syndrome and early-onset retinal dystrophies [1]. Here, we investigated children with idiopathic infantile nystagmus (IIN), optic nerve hypoplasia (ONH) and albinism, to differentiate their ERGs from those of early-onset retinal dystrophies, and to study possible effects of nystagmus on ERGs.
Methods: Children who showed nystagmus from the first few months after birth were included, 30 with IIN, 18 with severe bilateral ONH, and 18 with albinism. Three ERG protocols were applied according to the ages of the children and their ability to cooperate: 58 children (mean age, 2.0 years) were recorded with skin electrode to flash stimulation (Great Ormond Street Hospital protocol), 11 (mean age, 5.3 years) with skin electrode to full-field stimulation (ISCEV protocol), and 7 (mean age, 12.2 years) with HK electrode to full-field stimulation (ISCEV protocol). The ERG amplitudes and latencies were analysed and compared statistically with those of 82 age-matched controls and 37 children with early-onset retinal dystrophies (Mann–Whitney U test), with significant differences for $p < 0.05$.
Results: The IIN group compared to the controls showed no significantly reduced amplitudes, under any of the protocols, although they showed increased amplitudes for white scotopic, white photopic (skin flash stimulation), and rod responses (HK full-field stimulation). They showed significantly shorter latencies for white scotopic (skin flash stimulation), combined rod-cone, rod, and cone responses (skin full-field stimulation), and prolonged latencies for combined rod-cone responses (HK full-field stimulation). For the ONH group compared to the controls, they showed significantly reduced amplitudes for combined rod-cone, cone, and 30-Hz responses (skin full-field stimulation), and increased amplitudes for white scotopic, white photopic, blue (skin flash stimulation), and 30-Hz responses (HK full-field stimulation). They showed significantly shorter latencies for cone (skin, HK full-field stimulation), and prolonged latencies for 30-Hz responses (skin full-field stimulation). In comparison to the early-onset retinal dystrophies, the ONH group showed significantly increased amplitudes. For the albinism group compared to the controls, they showed significantly reduced amplitudes for cone and 30-Hz responses (skin full-field stimulation), and increased amplitudes for white scotopic, white photopic, 30-Hz (skin flash stimulation), and 30-Hz responses (HK full-field stimulation). They showed significantly shorter latencies for white scotopic, blue (skin flash stimulation), combined rod-cone, rod, and 30-Hz responses (skin full-field stimulation), but no prolonged latencies under any of the protocols. In comparison to the early-onset retinal dystrophies, the albinism group showed significantly increased amplitudes.
Conclusion: These findings show no reduced amplitudes for the children with IIN, which indicates normal retinal function, while the increased amplitudes for these children indicate possible effects of nystagmus. For the children with albinism, the increased ERGs are possibly due to hypo-pigmented retina. In these children with IIN, ONH and albinism, their ERG amplitudes differ statistically from those of early-onset retinal dystrophies. 1 Kurent et al. Eur J Ophthalmol 2015.

Toxicology and treatment

20. Aripiprazole induced chorioretinopathy

Matthieu Robert[1], Isabelle Audo[2], Christina Zeitz[3], Jean-Bernard Letessier[4], Celine Faure[5]

[1]Hopital Necker-Enfants Malades, Paris, France; [2]Centre Hospitalier National des XV-XX, Paris, France; [3]INSERM, U968, Paris, France; [4]Hôpital Côte de Nacre, Caen, France; [5]Hôpital Privé Saint Martin, Caen, France

Purpose: To report the first documented case of aripiprazole induced chorioretinopathy.
Methods: A 47-year-old schizophrenic patient, who had been treated with aripiprazole for 8 years, complained of a visual loss. An atypical retinopathy was found and investigated by multimodal imaging and electrophysiological examination.
Results: Imaging of the right eye (RE) showed a large area of retinal atrophy predominating in the outer retina, including the posterior pole up to the upper temporal periphery; imaging of the left eye (LE) showed a serous retinal detachment. The electroretinogram exhibited decreased and delayed responses of both the rod and cone systems; the electrooculogram showed no light peak.
Conclusions: Aripiprazole, an atypical antipsychotic, was introduced more recently than the antipsychotics classically incriminated in chorioretinopathies, such as thioridazine. Although pathophysiological mechanisms are only partially understood, imaging and electrophysiological findings in the present case point towards an involvement of the retinal pigmentary epithelium. Clinicians should be aware of the potential chorioretinal toxicity of new atypical antipsychotics.

21. Photopic response before and after vitreous removal during vitreous surgery

Kei Shinoda[1], Soiti Matsumoto[1,2], Gaku Terauchi[1], Goichi Akiyama[1,3], Harue Matsumoto[1,2], Atsushi Mizota[1], Yozo Miyake[4]

[1]Department of Ophthalmology, Teikyo University school of medicine, Tokyo, Japan; [2]Matsumoto Eye Clinic, Tokushima, Japan; [3]Department of Ophthalmology, The Jikei University School of Medicine, Tokyo, Japan; [4]Aichi Medical University, Aichi, Japan

Purpose: Electroretinographic alterations during vitrectomy in human eyes have previously been reported by Miyake et al. They observed delayed peak times at first and then reduced amplitudes in 30 Hz flicker ERG after removal of the vitreous body. The changes were reversible and the temperature played a considerable role in those changes. We measured photopic ERG with long duration stimulus and analyzed several components before and after core vitrectomy.
Methods: Intraoperative ERG recording and vitreous temperature measurement were performed on 23 eyes of 23 patients (mean age 63.1 ± 12.6, male 16). The vitreoretinal pathology was macular hole (n = 5), macular edema (n = 1), epiretinal membrane (n = 5), proliferative diabetic retinopathy (n = 4), macular telangiectasia (n = 2), IOL dislocation (n = 2), and localized rhegmatogeneous retinal detachment (n = 4). A contact lens with a built-in light-emitting diode (LS-100, Mayo Co, Inazawa, Japan) was sterilized and used as both a stimulus source and a recording electrode for photopic ERG. The amplitude and implicit time of the b-, and d-waves, and photopic negative responses (PhNRs) following b-, and d-waves (on-PhNR and off-PhNR, respectively) were analyzed.
Results: The mean amplitude (pre/post vitrectomy μV) of each component was 39.1/32.1 (b-wave), 39.8/28.1 (on-PhNR), 32.4/28.4 (d-wave), and 26.6/26.2 (off-PhNR). The mean implicit time (pre/post vitrectomy ms) of each component was 41.6/52.0 (b-wave), 61.3/76.5 (on-PhNR), 25.4/31.3 (d-wave), and 44.7/53.0 (off-PhNR). The temperature (pre/post vitrectomy centigrade) was 33.6/31.8. These changes were significant except the amplitude of the off-PhNR.
Conclusions: Most components in the photopic ERG showed reduced amplitude and delayed implicit time following core vitrectomy. Only the off-PhNR amplitude was preserved—the mechanism and its meaning are unknown.

22. MfERG assessment of hydroxychloroquine toxicity

Enid Chelva, Monika Dolliver, Sarina Laurin, Terri Mclaren, John De Roach

Department of Medical Technology and Physics, Sir Charles Gairdner Hospital, Perth, Australia

Purpose: To evaluate the prevalence of retinal toxicity as measured by mfERG abnormality in a large cohort of patients on hydroxychloroquine (HCQ) therapy, and further to determine the pattern of mfERG abnormality and relate these findings to the dose regimen and clinical indications in this group.
Methods: A retrospective consecutive case series of patients referred to this tertiary referral centre for HCQ toxicity screening during the period July 2006 to January 2015 was undertaken. Records were extracted from a database of medical, ocular and drug history of patients for whom either mfERG R1-R4 response amplitudes, latencies or ring ratios were abnormal at the 1–99 % confidence level and the pattern of abnormality classified as generalised, paracentral, annular, peripheral or foveal.
Results: From 817 patients, 140 patients (17 %) (114 F, 26 M, age = 65 ± 13.5 years) were found to have abnormal mfERG. In this group, 36, 29, 3 and 32 % were being treated for rheumatoid arthritis, systemic lupus erythematosus, Sjogren's syndrome and other connective tissue disorders, respectively. The mean cumulative dose and duration of therapy was 1087 ± 1025 g (range 77–5121) and 9.6 ± 8.13 years (range 1.25–37.9), respectively. Retinal toxicity was confirmed on at least one other standard diagnostic test in 23 cases (2.8 %), pre-existing retinal disease was present in 28 (3.4 %), no other sign of toxicity was found in 52 (6.4 %) and clinical records were not up to date for the remainder. In the group with confirmed toxicity, there was no dominant pattern of abnormality between generalised, paracentral or annular reduction in amplitude, with rings 2–4 being most commonly affected, but frequently extending out to ring 6. Ring ratios were normal when amplitude in the outer (reference) ring was reduced. Delayed latencies were evident in six of the cases with confirmed toxicity (26 %).
Conclusions: This study demonstrates that retinal toxicity is a significant concern in long term HCQ therapy. Both mfERG ring amplitudes and ratios provide an effective means of detecting toxicity; however, the latter should only be used when the reference ring amplitude is still normal.

23. Multifocal ERG analysis of primary CNS lymphoma with ocular manifestations

Margaret Hubbell[1], Amanda Selchau[1], Stephanie Lao[1], John Hamilton[2], Mark Pennesi[3], Terry Wood[1]

[1]Loma Linda University Eye Institute, Loma Linda, CA, USA; [2]University of Ottawa Eye Institute, Ottawa ON, Canada; [3]Casey Eye Institute, Oregon Health and Science University, Portland, OR, USA

Purpose: To demonstrate eletrophysiologic changes in the visual pathway secondary to metastasis of primary CNS lymphoma.
Methods: A 37 year old previously healthy female presented with floaters of 2 year duration. Initial workup was positive for vitreitis and choroiditis in the absence of blurred vision. Toxoplasmosis titers were found to be positive, and she was treated for this without improvement. Despite therapy, symptoms worsened, and 2 years after the initial encounter she developed headaches, prompting neuro-ophthalmic consultation. MRI of the brain was ordered and showed multicentric enhancing masses with surrounding vasogenic edema seen most prominently on the left occipital horn and left splenium as well as the bilateral frontal lobes, left parietooccipital lobe, and right paramedian parietal lobe. Biopsy of the lesions revealed diffuse large B cell lymphoma with plasmacytoid differentiated and meningothelial meningioma (WHO grade I). Following 21

rounds of intravenous Methotrexate and Rituximab, marked decrease in the enhanced intracranial lesions and vasogenic edema was seen on MRI. Following apparent stabilization of intracranial masses, several electrophysiological assays were performed to assess and describe intraocular changes including: multifocal electroretinogram (mfERG), electro-oculogram (EOG), visual evoked potentials (VEP), and optical coherence tomography (OCT) in addition to a standard ophthalmic examination.

Results: The mfERG showed bilateral areas of depressed activity, corresponding to areas of macular and perimacular scarring. EOG showed an Arden ration within normal limits for the right eye and moderately subnormal findings for the left eye. The VEP showed a moderately prolonged P100 peak latency in the left eye. The OCT demonstrated general thickening of the retina, more prominent in the left eye. Following the final 6 months of Methotrexate therapy, OCT demonstrated extensive resolution of retinal inflammation with resolution of floaters and additional symptoms improved.

Conclusions: A previously healthy female patient presented with floaters that progressed to headaches and decreased vision. Initial workup found vitreitis and choroiditis with positive toxoplasmosis titers that was treated without improvement. Neuro-ophthalmic consultation prompted MRI of the brain and showed multicentric enhancing masses with surrounding vasogenic edema. Biopsy of the lesions revealed diffuse large B-cell lymphoma with plasmacytoid differentiated and meningothelial meningioma (WHO grade I) which regressed following 21 rounds of intravenous Methotrexate and Rituximab and then stabilized. This uncommon manifestation of primary CNS lymphoma was analysed with several electrophysiological assays including novel application of multifocal ERG.

24. Retinal functional changes associated with anti-VEGF treatment in subjects with wet ARMD

Güliz Fatma Yavas[1], Tuncay Kusbeci[2], Umit Ubeyt Inan[1]

[1]Afyon Kocatepe University School of Medicine, Department of Ophthalmology, Afyonkarahisar, Turkey; [2]Bozyaka Education and Research Hospital, Department of Ophthalmology, Izmir, Turkey

Purpose: To evaluate retinal functional changes in subjects who underwent intravitreal ranibizumab injection 3 times because of choroidal neovascularisation.

Methods: 28 subjects with the diagnosis of wet age related macular degeneration (ARMD) who underwent 3 intravitreal ranibizumab injections (one injection per month) were included in this prospective study. After routine ophthalmic examination, visual acuity was recorded. A multifocal ERG was performed on all subjects using Metrovision Monpack 3 before treatment, and again 3–4 weeks after the last injection. Concentric ring analysis was performed and mean amplitudes and implicit times were recorded. N/Np, P/Pp and P/N indices were calculated for the central 2 degrees and central 10 degrees. Results were evaluated by paired samples *t*-test.

Results: Before treatment, mean visual acuity was found to be 0.70 ± 0.4 logMAR, and 1 month after ranibizumab injection mean visual acuity was found to be 0.60 ± 0.3 logMAR ($p = 0.17$). Multifocal ERG amplitudes showed a decrease 1 month after the last injection compared to baseline measurements whereas latencies did not show a significant change. P/N ratio showed an increase in the central 2 degrees 1 month after the last injection. Other parameters did not show a significant change.

Conclusion: The multifocal ERG is useful in evaluating macular functional changes related to intravitreal ranibizumab injection. Long term prospective studies are needed to evaluate macular functional changes related to intravitreal ranibizumab.

25. Insidious hydroxychloroquine macular toxicity: a case report

Livia Brandao, Anja Palmowski-Wolfe

University of Basel, Eye Hospital

Purpose: Hydroxychloroquine (HCQ) has a low risk for retinal toxicity which increases dramatically with a cumulative dose of >1000 g. Here we report a case of HCQ macular toxicity presentation in a young patient with a cumulative dose of 438 g.

Methods: A female patient, 19 years, started attending annual consultations for retinal toxicity screening in our clinic after 3 years of HCQ treatment for juvenile idiopathic dermatomyositis, diagnosed at age 12. Regular treatment included hydroxychloroquine 200 mg/day, cyclosporin 150 mg/day and vitamin D3. Screening consultations included: complete ophthalmologic examination, automated perimetry (M Standard, Octopus 101, Haag-Streit), multifocal eletroretinogram (VERIS 6.06TM, FMSIII), optical coherence tomography (fast macular protocol, Cirrus SD-OCT, Carl Zeiss), fundus autofluorescence imaging (Spectralis OCT, Heidelberg Engineering Inc.) and color vision testing (Farnsworth-Panel-D-15).

Results: After 5 years of treatment automated perimetry demonstrated reduced sensitivity in only one extra-foveal point in each eye ($p \leq 0.2$). Even though other exams showed no alteration (cumulative dose around 353 g), consultations were changed to every 6 months. After 2 years follow-up, despite the apparent intact retinal pigmented epithelium and completely normal automated perimetry in both eyes, bilateral central macula thinning was evident at the OCT, sparing the foveolar area, suggestive of bull's-eye maculopathy. Further evaluation with ganglion cell analysis (GCA = ganglion cell + inner plexiform layer, Cirrus SD-OCT, Carl Zeiss) showed also a concentric thinning in the same area. Although daily and cumulative doses were still under the nominal threshold for retinal toxicity, HCQ was suspended. At consultation 1 year later, visual acuity was 20/16 without any further changes in OCT or any other exam.

Conclusions: After literature search we found reports of no changes in inner retinal thickness in patients with only 6 months exposure to HCQ (400 mg/day) and exclusive inner retinal thinning in patients with an average 10 years treatment and total cumulative doses ranging from 792 to 2628 g. This may be the first case report of insidious bull's-eye maculopathy in a patient with long term treatment in which both cumulative and daily dosages were under the higher risk thresholds for screening and the average toxic exposures reported in studies. As already demonstrated in monkeys, it is probable that chloroquine toxicity disrupts inner retinal layer integrity before

changes in RPE are evident. As ganglion cell analysis has only recently become available, further studies are needed to understand toxicity mechanisms, and may lead to a reconsideration of screening recommendations.
Acknowledgements: AMP: SNF (Swiss national Science Foundation) NMS 1823, LHW Stiftung Lichtenstein.

Paper session 1: 16:00–18:00

Neuro-ophthalmology

16:00 Introductory lecture: Paraneoplastic and autoimmune retinopathy

Richard Weleber

Casey Eye Institute, Oregon Health and Science University, Portland, OR, USA

This presentation will review the history of cancer-associated and autoimmune retinopathy, beginning with the report in 1976 by Sawyer, Selhorst, Zimmerman, and Hoyt of the phenomenon of "blindness from photoreceptor degeneration as a remote effect of cancer." Examples will be presented and discussed of retinopathy from anti-recoverin and anti-enolase as well as autoimmunity to less common antigens, for example, anti-arrestin. The evaluation and management of these disorders will be reviewed, emphasizing the need for coordination of care among the specialties of ophthalmology, internal medicine, oncology, and immunology.

16:30 Retrospective analysis of the progression of electrophysiological changes after the first visual symptoms in juvenile onset optic neuritis

Márta Janáky[1], Imre Fejes[1], Rita Alács[3], György Benedek[2], Gábor Braunitzer[2]

[1]Department of Ophthalmology, University of Szeged, Szeged, Hungary; [2]Department of Physiology, University of Szeged, Szeged, Hungary; [3]Markusovszky Teaching Hospital, Szombathely, Hungary

Purpose: The aim of our retrospective analysis of VEP and PERG changes was to determine the progression of optic nerve damage during the course of optic neuritis from the first ophthalmological symptoms onward.
Methods: Among the 250 patients with suspected multiple sclerosis, 17 had repeated electrophysiological tests because of acute optic nerve function loss at a young age. The mean age was 20 years (range: 12–25 years). Two patients had binocular progressive visual loss during pregnancy. Repeated electrophysiological tests (VEPs and PERGs) were performed according to the ISCEV standards (RETIport program of Roland) immediately after monocular visual loss, and 1 month, 2 months, 6 months and 1 year thereafter. For detecting PERG, a DTL electrode was used.
Results: At the time of acute visual loss the VEP had decreased amplitude (it was almost at noise level) in the involved eye. After remission of the visual loss, the peak time of the VEPs was delayed, and the PERG ratio (P50/N95) reflected axon damage in the involved eye. Within this 3 month period we detected the development of electrophysiological alterations in the asymptomatic fellow eye as well. Nine of these 17 cases with early onset optic neuritis exhibited rapid progression of optic nerve dysfunction, and had bilateral involvement. The progression of bilateral visual loss was extremely rapid in 2 patients during pregnancy. Among the patients with rapid progression, 5 had the final diagnosis of NMO (Neuromyelitis Optica, Devic disease).
Conclusions: Young patients with acute visual loss need frequent electrophysiological testing not only for the diagnosis, but for the detection of subclinical axon damage in the fellow eye. It can also contribute to the early detection of the axon damage for the immediate treatment aimed at nerve protection in MS patients.
Acknowledgment: Supported by OTKA/Hungary Grant: 83810.

16:45 Visual pathways in humans with ephrin-B1 deficiency

Michael Hoffmann[1,2], Hagen Thieme[1], Karin Liedecke[1], Synke Meltendorf[1], Juliane Reupsch[1], Martin Zenker[3], Ilse Wieland[3]

[1]Department of Ophthalmology, Otto-von-Guericke-University, Magdeburg, Germany; [2]Center for Behavioral Brain Sciences, Magdeburg, Germany; [3]Institute for Human Genetics, Otto-von-Guericke-University, Magdeburg, Germany

Purpose: Numerous animal studies demonstrated the importance of the Ephrin/Eph system for correct visual system development, i.e., normal retinotopic mapping and optic nerve crossing [1]. Analogous investigations of the human visual system are missing. In this study we examined the visual system in humans with ephrin-B1 deficiency, which is X-linked and, counter-intuitively, associated with the condition cranio-fronto-nasal syndrome in heterozygous females. Disease manifestation has been shown to be due, in part, to cellular interference of mutant and wildtype ephrin-B1 expressing cells in females while hemizygous males are usually only mildly affected [2].
Methods: For one male with ephrin-B1 deficiency and three affected females from two independent families with molecular-genetically confirmed mutations, the integrity of the partial decussation of the optic nerves was tested with VEPs [3] and compared to albinotic, achiasmic and control participants with normal vision. In addition, retinal morphology and function and the retinotopic representation of the primary visual cortex were examined with SD-OCT, ERG/mfERG, and mfVEPs [4] for the male and some of the carriers. The strabismic status was determined in all participants.
Results: Strabismus and lack of stereo-vision was evident in the male and two of the females, the latter with associated amblyopia. Other characteristics of the visual system organisation and function were normal: (i) Retina: SD-OCT and funduscopy indicate normal foveal and optic nerve head morphology. ERGs and mfERGs indicate normal retinal

function. (ii) Optic chiasm: VEPs demonstrate normal routing of the optic nerves. (iii) Visual cortex: mfVEP characteristics indicate normal retinotopic representation of the contralateral visual hemifield in each hemisphere.
Conclusions: While ephrin-B1 deficiency leads to abnormal visual pathways in mice [1,5], it leaves the human visual system, apart from deficiencies in binocular vision, largely normal. We presume that other components of the ephrin-system can substitute for the lack of ephrin-B1 in humans.
Funding: German Research Foundation (HO 2002/10-2).

References

1. McLaughlin T and O'Leary DD (2005) Annu Rev Neurosci 28:327–55.
2. Wieland I et al. (2004) Am J Hum Genet 74:1209–1215.
3. Hoffmann MB and Demoulin SO (2015) Trends Neurosci 38:55–65.
4. Hoffmann MB et al. (2006) Invest Ophthalmol Vis Sci 47:3195–3201.
5. Chenaux G and Henkemeyer M (2011) Eur J Neurosci 34:1620–1633.

17:00 Genetic associations to the ERG in autism spectrum disorder

Paul Constable[1,2], Sebastian Gaigg[2], Dermot Bowler[2], Dorothy Thompson[3]

[1]Applied Vision Research, Centre, City University London, London, UK; [2]Autism Research Group, City University London, London, UK; [3]Clinical and Academic Department of Ophthalmology, Great Ormond Street Hospital, London, UK

Purpose: To investigate if differences in the ERG waveform in autism spectrum disorder (ASD) can be associated with known markers for ASD.
Methods: Genotypes of 9 ASD (8M:1F) individuals and 22 control participants (16M:6F) with no neurodevelopmental history for case–control analysis were included in this study. Fourteen Single Nucleotide Polymorphisms (SNPs) were genotyped using competitive allele specific PCR (Kbiosciences) with a call rate of >91 % in the control sample and 100 % in the ASD case sample. SNPs that have been associated with the ASD phenotype were specifically selected. These were SNPs in genes that co-ordinate neural development and synaptic integrity (NRCAM, rs2300045, CDH 9/10 rs4307059, DISC1 rs1322784, ROBO4 rs6590109) or ion channel receptors GRIK2 rs2235076 with two haplotypes relating to the GABA receptor (r22280073, rs1912960 and rs2351297) as well as the glutamate solute linked carrier SLC1A1 (rs301430, rs3933331 and rs7858819). Quantitative single locus and haplotype association were explored for the b-wave amplitudes of the DA 0.01 and the LA 10 ERG. All SNPs were in Hardy–Weinberg Equilibrium ($p > 0.01$) except GRIK2 in which a single genotype G/G was present in 98.4 % of the sample and therefore was not used as a marker in the study.
Results: Single marker association between case and controls was not significantly different at the $p = 0.01$ level with a modest false discovery rate (FDR) of 0.240. DISC1 ($p = 0.054$) and CDH 9/10 ($p = 0.127$) showed the strongest differences between groups. Consequently these two SNPs were used as the most likely candidates to show a quantitative trait difference within the ASD group. The b-wave amplitude of the LA 10 ERG and the C/C genotype of CDH 9/10 showed a significant ($p = 0.03$) interaction: mean (sem) [N = 3 T/T 100 (3.5); N = 3 T/C 93.0 (17.8); N = 3 C/C 136.3 (11.9)] but not the DA 0.01 ERG ($p = 0.50$). DISC1 was not significant ($p = 0.17$) for either response. Two main GABA haplotypes were identified in the groups CGG/GCG with frequencies of 0.559/0.180 in the total sample and showed no case–control association at the $p = 0.01$ level ($p > 0.34$; FD = 0.42). Similarly the two main SLC1A haplotypes (TGC/TCC) with frequencies of 0.431/0.263 in the total sample were not significantly different in cases and controls at the $p = 0.01$ level. TGC; $p = 0.11$; FDR = 0.54 and TCC; $p = 0.49$; FDR = 0.76.
Conclusions: The Cadherin CDH 9/10 polymorphism was associated with a reduced LA b-wave in the ASD group suggesting synaptic connectivity may be responsible for the different ERG responses observed in the ASD group. The finding that the ionotropic glutamate receptor polymorphisms were identical in both groups suggests the OFF pathway is not involved in the ERG differences. Further large scale studies may enable a greater understanding of the genes involved in ASD and ERG markers. This follow-up genetic study was supported by the Marmor award.

17:15 Structural and functional effects of dysmyelination in a large animal model

James Verhoeve[1], Michael Nork[1], Leandro Teixeira[2], Richard Dubielzig[2], Abigail Radcliff[3], Ian Duncan[3]

[1]Department Ophthalmology and Visual Science, University of Wisconsin School of Medicine, Madison, USA; [2]Department of Pathobiological Science, University of Wisconsin School of Veterinary Medicine, Madison, USA; [3]Department Medical Science, University of Wisconsin School of Veterinary Medicine, Madison, USA

Purpose: Mutation in proteolipid protein gene plp1, allelic to the mutation causing Pelizaeus-Merzbacher disease in humans, results in a primary failure of myelin development. Recent studies have shown loss of retinal nerve fiber layer thickness (RNFLT) in numerous demyelination syndromes, all of which have been associated with inflammation. Unlike demyelination syndromes, including multiple sclerosis (MS), the dysmyelination of plp1 is not associated with inflammation. To determine whether axonal loss occurs in the absence of myelin without the presence of inflammation, we performed structural and functional assessments in canines with the plp1 mutation.
Methods: Four dogs (2 with plp1 mutation and 2 littler mate controls) underwent spectral-domain OCTs (sdOCTs) and photopic ERGs and flash visual evoked potentials (VEPs) assessments at quarterly intervals over a period of 2 years. Eight additional dogs were examined once. At the termination of the study, axon numbers in the optic nerve and in the retina were quantitated histologically.

Results: sdOCT RNFL thickness was reduced 18 % (15 microns) in the affected dogs' retinae with substantial loss of neural rim thickness and increased cupping. Axon loss was greatest in the superior quadrant. RNFLT loss estimated by sdOCT correlated well with quantitative histology ($\sim$20 micron loss in the superior quadrant), which also found reduced axon numbers in plp1 dogs. Photopic ERGs in affected dogs showed a reduced late photopic negative response with normal a- and b-waves, consistent with decreased ganglion cell function. Flash VEPs were markedly abnormal in affected dogs.
Conclusions: Congenital absence of myelin in plp1 results in loss of RNFLT consistent with reported studies of patients with chronic progressive MS (5–40 microns) and is accompanied by loss of function. This suggests that lack of myelin may be sufficient to result in loss of axons in progressive demyelinating diseases.

17:30 Naso-temporal thickness of the retinal ganglion cell layer by OCT-segmentation in lesions of the visual pathway

Hannes G. H. Wildberger[1], Werner Wichmann[2], Klara Landau[1], Luca Regli[3], Christina Gerth-Kahlert[1]

[1]Department of Ophthalmology, University Hospital Zurich USZ, Zurich, Switzerland; [2]Department of Neuroradiology, University Hospital Zurich USZ, Zurich, Switzerland; [3] Department of Neurosurgery, University Hospital Zurich USZ, Zurich, Switzerland

Purpose: To measure the thickness of nasal and temporal halves of the retinal ganglion cell layer (GCL) with optical coherence tomography (OCT) in patients with chiasmal, retrochiasmal and retrogeniculate disorders and to correlate GCL thickness with visual field defects.
Methods: Thirty patients were divided into three groups based on their MRI findings: group A: compression of the optic chiasm; group B: disorders of the optic tract (OT) and lateral geniculate nucleus (LGN) and group C: posterior lesions in the optic radiation and occipital lobe. The thickness of GCL+ (including the inner plexiform layer) was manually measured (in microns) in both eyes at 15 positions along two parallel vertical lines situated on the nasal side and on the temporal retinal side respectively, within the inner ETDRS-ring at 1250 microns horizontal distance from a vertical coordinate passing through the foveola. In addition, the papillomacular bundle (PMB) and the circumpapillary retinal nerve fiber layer (RNFL) were measured automatically. The thickness values were compared with age-matched normative data.
Results: Patients in group A demonstrated a chiasmal pattern with a binasal GCL loss, but the findings pointed, in most pairs of eyes, toward an asymmetric compression of the chiasm. Structural loss within the GCL was variable depending on the location and severity of the lesion. Group B demonstrated deep homonymous GCL-thinning of the affected hemiretinas. In Group C, no structural loss of GCL was observed. Thus, structural variability of GCL from mild to severe could be easily identified in group A and were clearly distinct from those in group B. Homonymous lesions of group B seemed to follow an absolute rule, i.e., GCL was regularly below a 2 SD limit. Group B confirmed earlier observations of a specific topographic RNFL loss in OT and LGN lesions (Hoyt-Kommerell paradigm) in a nearly pathognomonic appearance.
Conclusions: Naso-temporal analysis of retinal GCL provides unique information about both location and severity of a visual pathway lesion, which may be complementary or even superior to information obtained by MR-imaging or perimetry. GCL hemiatrophy may appear before quadrantic VF-defects progress to hemifield defects.

17:45 Evidence for compartment syndrome damage in a primate model of non-arteritic anterior ischemic optic neuropathy (pNAION)

Mary Johnson[1], Neil Miller[2], Steven Bernstein[1]

[1]Department of Ophthalmology and Visual Science, University of Maryland School of Medicine, Baltimore, USA; [2]Wilmer Eye Institute, Johns Hopkins University School of Medicine, Baltimore, USA

Purpose: To evaluate the anatomic and physiologic sequelae of early peripapillary edema in a primate model of NAION (pNAION).
Methods: We induced pNAION in six rhesus macaques by subthermal laser irradiation of the optic nerve head following an intravenous injection of rose bengal, a dye that when photoactivated creates oxygen free radicals that damage vascular endothelial cells, in our case in optic nerve capillaries (Chen, Johnson et al., Invest. Ophthalmol. Vis. Sci. 2008; 49:2985–2992). We performed spectral domain-optical coherence tomography (SD-OCT), simultaneous pattern VEPs and pattern ERGs, color photography and fluorescein angiography prior to induction and at 1 day, 1 week, 2 weeks, 4 weeks and 8 weeks post-induction. In addition, we recorded ganzfeld ERGs prior to and at 1 day, 4 weeks and 8 weeks post-induction.
Results: The greater the initial peripapillary nerve fiber layer (PRNFL) edema on SD-OCT, the greater the subsequent atrophy ($r = 0.63$). Furthermore, changes in PRNFL thickness were significantly correlated with changes in VEP amplitude ($r = 0.89$, $p = 0.017$).
Conclusions: Early edema in the PRNFL in pNAION leads to atrophy of these retinal ganglion cell axons and results in permanent loss in VEP amplitude. These data are consistent with predictions from compartment syndrome, and they establish the importance of controlling edema at NAION onset.

Thursday, 25.06.2015

Paper session 2: 09:00–10:45

Retinal and optic nerve function and treatment

9:00 Detecting glaucoma with photopic negative response

Marta Kiszkielis[1], Wojciech Lubiński[1], Krzysztof Penkala[2]

[1]Ophthalmology Department, Pomeranian Medical University, Szczecin, Poland; [2]Department of Electronics, West Pomeranian University of Technology, Szczecin, Poland

Purpose: To evaluate photopic negative response (PhNR) sensitivity and specificity in detecting different stages of glaucomatous visual field defect.
Methods: Ninety eyes of 50 patients with primary open angle glaucoma [POAG, diagnosis based on European Glaucoma Society (EGS) criteria] and 45 eyes of 23 healthy age- and sex-matched controls were investigated. PhNR was elicited with red stimuli, flash strength of 3.2 cd s/m^2 on blue background of 25 cd/m^2. PhNR amplitude (from baseline to trough) and PhNR/b-wave ratio were recorded and analyzed. Sensitivity and specificity were calculated with standard formulas. Receiver operating characteristic (ROC) curves were used to determine optimal cut-off values. The area under the curve (AUC) was used to compare the ROC curves between PhNR amplitude and PhNR/b-wave ratio.
Results: The sensitivity and specificity when optimal cut-off values were used were 53.3 and 90 % for PhNR amplitude and 60 and 70 % for PhNR/b-wave ratio in early POAG, 63.3 and 80 % for PhNR amplitude and 60 and 86.7 % for PhNR/b-wave in moderate POAG and 76.6 and 80 % for PhNR amplitude, 90 and 73.3 % for PhNR/b-wave in advanced POAG. There were no significant differences between AUCs for PhNR amplitude (0.76–0.86) and PhNR/b-wave ratio (0.78–0.86), $p > 0.05$.
Conclusions: Test sensitivity and specificity were high and comparable with previous published data. PhNR amplitude and PhNR/b-wave are equal in glaucomatous visual field defect detection. PhNR might be useful in glaucoma diagnosis.

9:15 Exploring methods of data analysis in multifocal visual evoked potentials (mfVEP)

Lasse Malmqvist[1], Clare Fraser[2], Luis De Santiago[3], Alexander Klistorner[2], Steffen Hamann[1]

[1]Department of Ophthalmology, University of Copenhagen, Glostrup Hosital/Rigshospitalet, Copenhagen, Denmark;
[2]Sydney Eye Hospital, Save Sight Institute, Sydney, Australia;
[3]Electronics Department, University of Alcala, Alcala de Henares, Spain

Purpose: The multifocal visual evoked potentials (mfVEP) is a topographical technique that has been investigated for use predominantly in glaucoma and multiple sclerosis patients. The mfVEP has not been adopted into regular clinical practice as there is no gold standard regarding analysis of the data. Peak-to-peak values and signal to noise ratio (SNR) are methods currently being used for analysis of mfVEP amplitude. The purpose of this study was to compare these two methods by examining the within-subject and intersubject variability of 23 normals. The logarithm of SNR (logSNR) has been used in previous studies due to its compatibility to visual field analysis. Therefore, we also assessed the variability of this method and compared it to the peak-to-peak and SNR methods. We also compared the cross-correlation method versus the second peak method to describe interocular latency variation.
Methods: MfVEP (VisionSearch1, Sydney, Australia) was performed on 46 normal eyes. Averaged amplitude from all sectors was examined using peak-to-peak values, SNR and logSNR. Within-subject and intersubject coefficients of variability were used for the comparison. Latency asymmetry was examined using second peak values and cross-correlation.
Results: The mean mfVEP amplitude for normals using peak-to-peak values was 169.1 nV (CI 95 % 152.9–185.3). Mean SNR amplitude was 4.47 (CI 95 % 4.1–4.85). There was a significant correlation of mfVEP amplitude using peak-to-peak method and SNR method ($R^2 = 0.83$, $p < 0.001$). A statistically significant difference was obtained when comparing within-subject variability using peak-to-peak method (30 %) and SNR method (37.2 %) ($p = 0.043$). Within-subject variability using logSNR was 31.4 %.This value was significantly different from the SNR method ($p = 0.017$) while no difference was seen between the peak-to-peak and logSNR methods. The intersubject variability was 19.9 % in the peak-to-peak method and 16.9 % in the SNR method. The intersubject variability using logSNR was 11.2 %. Latency asymmetry values for the cross-correlation analysis was 1.73 ms (CI 95 % 1.19–2.27) and for the second peak analysis 2.18 ms (CI 95 % 1.35–3.03 ms). No significant difference between cross-correlation analysis and second peak analysis was found. When comparing within-subject variability of cross-correlation (96.9 %) and second peak analysis (108.1 %), no significant difference was found. Intersubject variability was 71.7 % when using cross-correlation and 88.5 % when using second peak analysis.
Conclusions: A significant correlation between peak-to-peak values and SNR values was found when assessing normative mfVEP amplitude. When comparing within-subject variability of the two methods, a significant difference was found. However, the intersubject variability of the two methods was similar. These results indicate that the peak-to-peak method is the superior technique in analyzing within-subject mfVEP amplitude variability. The logSNR method, useful for its comparability to the log scale of the Humphrey visual field analysis, proved to be similar to the peak-to-peak method in within-subject variability with less intersubject variability.

9:30 Interocular difference of retinal ganglion cell function measured by objective and subjective methods

Imre Fejes, Péter Balázs Kocsis, Márta Janáky

Department of Ophthalmology, University of Szeged, Szeged, Hungary

Purpose: Interocular differences in photoreceptor and ganglion cell density of the central retina has been proved by histological examinations. The interocular differences of visual evoked potential (VEP), pattern electroretinogram (PERG), full field ERG and multifocal electroretinograms (mfERG) presented interocular difference up to 30 % even at 1.0 visual acuity. These methods used high intensity and/or high contrast stimuli. The aim of our study was to compare the interocular difference of parvo- and magnocellular retinal ganglion cell function with objective and subjective methods.
Methods: A total of 28 subjects (age 33.8 ± 15.2 years), free from any ophthalmological and systemic disease, were examined. No static visual field defect was present and best corrected visual acuity was 1.0 in both eyes. VEP, PERG according to ISCEV standards and glaucoma PERG according to the Freiburg paradigm were performed as objective methods.

The latter should detect the difference between parvocellular and magnocellular pathways, using 16° and 0.8° check size with 8 Hz and also with 4 Hz pattern reversal frequency. A subjective method for assessment of ganglion cell function measured static and dynamic contrast sensitivity with the computerized Venus system. 0.48–14.34 cycle/deg spatial frequency stimuli were used, and, in case of dynamic contrast sensitivity, the temporal frequency was 8.56 Hz. Differences between eyes were calculated as interocular difference divided by the value of the higher side. Results are expressed in mean (95) %.

Results: Interocular difference of VEP and PERG values were similar as previously reported. Both glaucoma PERG and contrast sensitivity showed side differences. In glaucoma, the PERG the difference of 0.8°/16° ratio was 15.3 (31.1) % at 8 Hz stimulation, and 9.1(20.6) % at 4 Hz stimulation. The subjective method showed somewhat higher interocular differences: static contrast sensitivity 15.5 (35.4) %–27.5 (51.9) % depending on spatial frequency and dynamic contrast sensitivity 13.4 (34.3) %–22.7 (49.6) % depending on the spatial frequency with the peak difference at the high, 14.34 cycle/deg, and the lowest difference at 1,91 cycle/deg in case of both static and dynamic contrast sensitivity.

Conclusions: Interocular difference of ganglion cell function is similar to other left versus right differences detected by electophysiological methods. With measurement of contrast sensitivity, as a subjective test, the difference is comparable, but somewhat higher. These findings suggest that psychological factors may interfere with visual function. Therefore, for testing the function of retinal ganglion cells, objective electrophysiological tests are superior. Awareness of the left versus right differences may have importance in evaluation of monocular pathologies affecting ganglion cells.

Supported by OTKA Grant, Hungary 83810.

9:45 Electrophysiology and colour: a comparison of methods to evaluate inner retinal function

Anne Kurtenbach[1], Christoph Kernstock[1], Eberhart Zrenner[2,1], Hana Langrová[3]

[1]Centre for Ophthalmology, University of Tübingen, Tübingen, Germany; [2]Werner Reichardt Centre for Integrative Neuroscience, University of Tübingen, Tübingen, Germany; [3]University Eye Hospital, Hradec Králové, Czech Republic

Purpose: Functional testing plays an important role in the early detection and monitoring of retinal disease. The aim of this study was to compare the results of four methods that are routinely used for the evaluation of inner retinal function: PERG, mfPERG, chromatic contrast thresholds and blue-on yellow (SWAP) perimetry. We correlate the results of control subjects with those of patients with diabetes or glaucoma and assess the sensitivity/specificity of each method.

Methods: 20 control subjects, 12 patients with early glaucoma and eight patients with diabetes mellitus (DM) were enrolled in the study. The mean age of the control subjects was 41.2 years ± 13.3 SD. The DM patients were type 1 (four patients) or 2 (four patients) and were aged 44.8 years ± 15.8 SD. The mean duration of diabetes was 24.3 years (±15.3 years). Six patients had no retinopathy, two a background retinopathy. The glaucoma patients (three primary open-angle, four normal tension, four open angle and one angle closure) were aged 57.8 years ± 15.3 SD. All underwent four examinations: transient full-field PERG (Espion E2, Diagnosys LLC), mfPERG (VERIS 4.9.1, Electro-Diagnostic Imaging Inc) and protan and tritan contrast threshold measurements (Chromatest, Breakpoint) and SWAP (HFA II, Zeiss). Before analysis, all parameters were adjusted for age, based on linear regression modelling of the results of the control group. The results were corrected for multiple testing with the Bonferroni-Holm correction factor. A receiver-operating-characteristic (ROC) curve was generated for each test and its diagnostic accuracy assessed by the area under the curve (AUC).

Results: When all 40 results are considered together, there is a significant correlation between the amplitudes of the PERG and those of the mfPERG, as well between the tritan contrast thresholds and the SWAP MD (Mean Deviation). No significant correlations were found between electrophysiological parameters and SWAP MDs or colour contrast thresholds. Furthermore, ROC analyses show that both tritan and protan contrast thresholds could significantly distinguish between the patient and the control group [AUC = 0.79 ($p = 0.25$) and 0.80 ($p = 0.014$) respectively]. None of the other parameters could significantly differentiate between the two groups. Protan contrast thresholds could also significantly identify glaucoma patients alone (AUC 0.86: $p = 0.017$).

Conclusions: The comparison of four methods used for testing inner retinal function shows only a small agreement in the results between tests, indicating that the methods do not rely on the same cells and/or pathways. Colour contrast thresholds show the highest diagnostic accuracy and, despite the small sample size studied, were sensitive enough to significantly distinguish between the control and patient groups and between controls and glaucoma patients.

10:00 Electroretinographic evidence supportive of an organic cause in some forms of functional amblyopia

Raquel Beneish, Allison Dorfman, Ayesha Khan, Robert Polomeno, Robert Koenekoop, Pierre Lachapelle

Department of Ophthalmology, Neurology-Neurosurgery McGill University/MCH, Montreal, Canada

Purpose: 1. To uncover the presence of an underlying organic component, undetectable by standard ophthalmic evaluation in approximately 10 % of amblyopic children who failed to achieve optimal vision in the amblyopic eye with treatment. 2. To determine whether the anomaly originates in the retina.

Methods: To test this hypothesis, in addition to complete ophthalmic and orthoptic evaluations, kinetic Goldman Visual Fields (VF; smallest target = 03 e: 1/16 mm^2, luminance .315), flash electroretinograms [fERG; photopic: 5 cd s/m^2, background 30 cd/m^2; scotopic .005 cd s/m^2 (pure rod) and 1 cd s/m^2 (mixed rod/cone)] and multifocal electroretinograms (mfERG: 61 hexagons) and Spectral Domain Optical Coherence Tomography (SD-OCT) were included in the investigation.

Results: Twenty-four children with functional amblyopia treated with occlusion or optical and/or pharmacologic penalization of the non-amblyopic eye were recruited for this study (9 prospectively and 15 retrospectively). The mean age was 5.8 years (5–10.5). Amblyopia was anisometropic in 5, strabismic in 7 combined in 11. There were 21 hyperopes and 3 high myopes. VFs showed a central scotoma of varying size (3°–10°) and depth with increasing target size in 15/24 patients whose best post-treatment vision in the amblyopic eye ranged from 20/40 to 20/100. The scotoma decreased in size and depth with improving vision until a plateau of recovery was reached. The remaining 8/24 patients with acuity better than 20/25 showed no scotoma. fERGs and mfERGs were obtained in 14/24 patients. The fERG a and b wave amplitude ratios of the amblyopic eye to the dominant eye (ae/de) were abnormal in 4/14. Three of these patients had large scotomas on VF. The mfERG ae/de ratio was significantly attenuated for ring 1 in 9/14 patients (64.3 %), even though only 6/14 (43 %) showed a central scotoma on VF and a vision ranging from 20/40 to 20/100. However, there was no significant difference in ring 1 amplitude ratio between those who achieved 20/50 to 20/40 (.81 ± .26) and those with ≥20/25 (.86 ± .25) (*t* test; $p = .72$). The mfERG trace array showed an abnormal retinotopic distribution in the amblyopic eye of some patients. SD-OCT of the macula done in 16/24 patients showed a thicker 1 mm diameter central subfield (CSF) in 7, a thinner CSF in 7 and a normal CSF in 2. SD-OCT of the optic nerve done in 14/24, showed a Retinal Nerve Fiber Layer (RNFL) deficit in 7 and a normal RNFL in 7.
Conclusions: The combined finding of ERG abnormalities in 64.3 % of the patients who did not achieve optimal vision with treatment, a central field defect on kinetic perimetry in 100 %, and regional abnormalities around the optic nerve on SD-OCT, suggests an underlying organic component limited to macular function. One wonders whether the anomaly might lie in the retina, since the cortical deficit improves with patching as reflected by improved vision and scotoma size. Future studies aimed at elucidating the latter are currently underway.

10:15 Quality and quantity of rescue in retinal gene therapy: implications for vision

Mathias Seeliger[1], Vithiyanjali Sothilingam[1], Naoyuki Tanimoto[1], Marina Garcia Garrido[1], Susanne C. Beck[1], Christian Schoen[2], Martin Biel[2], Stylianos Michalakis[2], Regine Mühlfriedel[1]

[1]Ocular Neurodegeneration, Institute for Ophthalmic Research, Eberhard Karls University, Tübingen, Germany; [2]Department of Pharmacy, Center for Integrated Protein Science Munich (CIPSM), LMU, Munich, Germany

Purpose: Gene therapy appears particularly promising in retinal diseases. Here, we assess the influence on the degree of visual performance produced by the physical extension of the successfully treated region and the rescue quality within this area. Based on a simple model of outer retinal signal processing, we demonstrate further that a limited degree of rescue at the level of retinal photoreceptors may be sufficient to obtain quite substantial visual improvements.
Methods: Our work is based on the extensive preclinical dataset obtained in the murine counterpart of Achromatopsia type 2 (ACHM2), an inherited disease leading to a complete lack of cone photoreceptor function. Gene replacement therapy was done via injections of rAAV8 vector particles into the subretinal space of 2 week-old knockout mice. The functional status was assessed with electroretinography (ERG), a proven biomarker for the sensory performance of the eye. We have developed a model of the outer retina where randomly distributed stimuli ('photons') within a 2-dimensional matrix ('photoreceptors') activate a downstream layer ('bipolar cells'). Stimulation may be limited to certain areas as is the case after therapy. Since the ERG is dominated by bipolar cell responses, the model outcome can be correlated with real life data.
Results: The maximal ERG amplitude in the treated mouse mutants was well correlated to the size of the rescue area. Astonishingly the rescue was almost 'binary': either we found a substantial ERG response or we did not; there were practically no intermediate responses. Our model was able to explain this finding by the nonlinearity at the amplification step from photoreceptors to bipolar cells. In post mortem studies, we were further able to visualize protein level match or mismatch by the presence or absence of surplus protein in the cell body, and to relate it to treatment dosage.
Conclusions: This work gives a number of novel insights relating gene therapy to visual outcome. Most important, we found that a limited degree of rescue may be sufficient to regain substantial vision. Our findings will be important to better direct and titrate the amount of therapy needed in neurosensory tissues in general.

10:30 Treatment with transcorneal electric stimulation (TES) in hereditary retinal degeneration

Eberhart Zrenner[1], Florian Gekeler[1,2], Mariya Gosheva[1], Andreas Schatz[1,2]

[1]Centre for Ophthalmology, University of Tuebingen, Tuebingen, Germany; [2]Klinikum Stuttgart, Stuttgart, Germany

Purpose: Several animal studies in rat retinas have supported the beneficial effects of transcorneal electrical stimulation (TES) in vivo and in vitro on the survival of various retinal cell populations. Neuroprotective effects have been ascribed to the stimulation of the endogenous insulin-like growth factor (IGF)-1 system (Tagami Y, et al. IOVS 2008) and enhanced release of Fgf2, Bcl-2—protein, ciliary neurotrophic factor (CNTF), and brain derived neurotrophic factor (BDNF) as described by Ni et al. (Exp. Neurol. 2009).
Methods: In order to assess the safety of TES and explore its efficacy, 24 patients with retinitis pigmentosa (RP) underwent TES (5-ms biphasic pulses; 20 Hz; DTL electrodes) 30 min per week for 6 consecutive weeks in a prospective, randomized, partially blinded, good-clinical-practice study (Schatz et al. IOVS 2011). The patients were randomly assigned to one of three groups: sham, 66 %, or 150 % of individual electrical phosphene threshold (EPT). Visual acuity (VA), visual field (VF), electroretinography, dark-adaptation (DA), color discrimination, and EPTs were assessed at all visits four times.
Results: TES using DTL electrodes was tolerated well; all patients finished the study. No serious adverse events were encountered. There was a tendency for most functional

parameters to improve (8/18) or to remain constant (8/18) in the 150 % group. VF area and scotopic b-wave amplitude reached statistical significance for improvement ($P < 0.027$ and $P < 0.001$, respectively. Due to the short duration and small group size of the initial study a further prospective sham-controlled follow-up TES-study was performed. The study was performed using a commercial device: OkuStim® (by Okuvision GmbH, Germany) which is available in post-marketing surveillance studies for RP patients in several countries. It was carried out over a period of 1 year in 63 RP patients to assess efficacy of treatment with this device. Patients were randomly assigned to sham, 150 %, or 200 % stimulation current of their individual electrical phosphene threshold. The primary outcome measure was visual field area (VFA; Goldmann III/4e), the secondary outcome measures were ganzfeld and mfERG values, BCVA, and threshold to full field stimuli (FST; Diagnosys), assessed in weeks 1, 10, 16, 22, 28, 40, 46, 52. Fifty-two patients (27 m, 25 w; TES = sham: n = 20, TES = 150 %: n = 15 and TES = 200 %: n = 17) completed the study. As described by Gekeler et al. adverse events (AEs) were dry eye symptoms (82 % of all patients, significantly more in stimulated eyes), ocular missensations, corneal erosion, macular edema (all unrelated to treatment, sham/partner eyes). No serious AEs related to the treatment were observed. VFA decreased 9 % less in the 200 % group, showing a tendency but not reaching significance (REML, $P = 0.19$). In the initial study. BCVA, FST also showed only tendencies but no statistical significance (REML, $P = 0.38$, $=0.40$, $=0.70$, resp.).

Conclusions: TES was found to be safe, AEs were mainly dry eye feelings always treatable by artificial tears. Primary and secondary outcome measure showed positive tendencies but did not reach levels of statistical significance, showing results similar to those seen in the first study by Schatz et al. (IOVS 2011). Exploratory analyses on high responders were promising to identify patients who might benefit from TES.

Paper session 3: 11:15–13:00

Pediatric electrophysiology

11:15 Introductory lecture: Electrophysiologic exploration of early visual development in human infants

Daphne L. McCulloch[1,2]

[1]School of Optometry and Vision Sciences, University of Waterloo, Waterloo, ON, Canada; [2]Vision Sciences, Glasgow Caledonian University, Glasgow, Scotland, UK

Purpose: The human visual system undergoes remarkable development in infancy. Early in infancy this development comprises concurrent growth, and maturation of neural connectivity, and efficiency. Data from electrophysiologic studies are central to our understanding of visual development in typical human infants and are essential for diagnosis of paediatric disorders. This introductory lecture will provide a selective outline of early ERG and VEP development highlighting some of the major clinical research findings.

Methods and Results: In young infants, early development of full-field ERG amplitudes are principally determined by changes in ocular dimensions and growth of the photoreceptor outer segments. An overview of retinal sensitivity and responsiveness, calculated from full-field ERG amplitudes provide an example of early development based primarily on ocular and cellular growth. Visual resolution reflects not only the optical quality and density of the foveal mosaic but also the precision of connections throughout the visual pathways. VEP techniques, such as sweep VEPs and step VEPs, demonstrate the early rapid development of visual resolution, which is concurrent with synaptic proliferation and selective pruning back of connections. Neural efficiency is reflected in the conduction time through the visual pathways. Gains in neural efficiency are reflected in flash and pattern VEP waveforms. The most widely reported VEP waveform, the P100 peak of the pattern VEP, will be reviewed to demonstrate the time course of the early development of neural efficiency as reflected by conduction time.

Conclusions: Rapid growth and the development of neural connectivity and efficiency underpin extensive changes in standard ERGs and VEPs within the first 6 months of life, whereas maturation of neural complexity has a much longer time course (i.e. responses to visual stimuli involving motion, recognition or memory).

11:45 Retinal dystrophy in young patients presenting as refractory intermediate uveitis

Ymkje Marije Hettinga[1,2], Maria van Genderen[2], Wietse Wieringa[3], Annette Ossewaarde[1], Joke De Boer[1]

[1]Department of Ophthalmology, University Medical Center Utrecht, Utrecht, Netherlands; [2]Ophthalmology Diagnostic Center of Bartiméus, Zeist, Netherlands; [3]Department of Ophthalmology, University Medical Center Groningen, Groningen, Netherlands

Purpose: Intermediate uveitis (IU) is the second most common form of uveitis in childhood and often no underlying disease can be found. We report the characteristics of seven young patients with idiopathic IU complicated by/associated with cystoid macular edema (CME) who were finally diagnosed as an inherited retinal dystrophy.

Methods: Description of seven young patients with treatment-resistant intermediate uveitis with CME who were seen at the University Medical Center and/or the ophthalmologic diagnostic center of Bartiméus in the Netherlands.

Results: The young patients (between 5 and 22 years old) were diagnosed with idiopathic IU by an ophthalmologist who specializes in uveitis. All patients had a reduced visual acuity between 0.12 and 0.5. They all have severe CME on optical coherence tomography. They all were treated with oral (immunosuppressive) medication without success. With additional research we found relative concentric limited visual fields. Dark adaptation was performed in three patients, and was decreased by more than 2.0 Log in all of them. The electroretinogram was severely reduced in all seven patients under scotopic and photopic conditions. Finally all were diagnosed with retinal dystrophy. DNA testing showed an

abnormality in 3 patients in the CRB1 gene. One patient had a mutation in the ABCC6 gene.
Conclusions: In young patients with idiopathic IU, which is accompanied by CME, a retinal dystrophy belongs in the differential diagnosis. We recommend testing of visual fields, dark adaptation test and ERG before starting immunosuppressive medication. Patients with retinal dystrophy with mutations in the CRB1 gene may be at increased risk of intermediate uveitis and CME.

12:00 Decreased foveal function causing faster myopic progression in children

Serena Zhe Chuang Li, Allen Ming Yan Cheong, Chea Su Kee, Henry Ho Lung Chan

Laboratory of Experimental Optometry (Neuroscience), School of Optometry, The Ho, Hong Kong, China

Purpose: Myopia is one of the results of emmetropization, which is a process to regulate the growth of the eyeball to compensate the refractive power in order to achieve clear vision. Although previous studies report that peripheral defocus is the main factor in myopic progression, it is not clear how the fovea contributes in this process. This study investigates foveal retinal function related to myopic progression over a 1-year period.
Methods: The global flash multifocal ERG (mfERG) with both high (96 %) and low contrast (49 %) protocol is used to measure foveal activity. The global flash mfERG response contains a direct component (DC) and an induced component (IC) which represents the outer and inner retinal activity, respectively. Eighteen children (7–11 years old), with spherical equivalent refractive errors ranging from +0.75 D to −1.00 D at their first visit, were recruited. All subjects had best-corrected visual acuity of logMAR 0.00 or better, normal color vision and good ocular health. All subjects received two eye examinations 12 months apart that included cycloplegic refraction, axial length and global flash mfERG measurements.
Results: These 18 subjects showed an average of −0.45 D myopic progression over the 1-year period. Nine children with less than −0.45 D myopic progression were classified as the slow progression group, while 9 children with equal or more than −0.45 D myopic progression were classified as having relatively fast myopic progression. With 49 % contrast global flash mfERG measured at the first visit, the fast myopic progression group showed significantly lower central IC amplitude compared to the slow progression group ($p = 0.003$), which indicated that fast myopic progression was associated with initial subclinical reduced central inner retinal activity. The DC amplitudes under both high and low contrast conditions and IC amplitude under high contrast condition were not significantly different between slow and fast myopic progression groups.
Conclusions: Children with subclinical decreased foveal inner retinal function showed relatively fast myopic progression. The fovea is likely to be involved in the regulation of eye growth and may play an important role in emmetropization.
Acknowledgement: This study was supported by the General Research Fund (PolyU 5605/13 M) from Research Grants Council, HKSAR, and Internal Research Grants (G-YBBS, Z0GF) from The Hong Kong Polytechnic University.

12:15 Electrophysiological evaluation of superior and inferior visual fields in healthy school children

Manca Tekavcic Pompe[1], Maja Šuštar[1], Branka Stirn Kranjc[1], Jelka Brecelj[1], Katja Groleger Sršen[2]

[1]University Eye Clinic, Ljubljana, Slovenia; [2]University Rehabilitation Institute of Republic Slovenia, Ljubljana, Slovenia

Purpose: To evaluate VEP response to superior and inferior visual field stimulation in healthy school children.
Methods: Fifteen healthy children (age 6–17 years) were included in this study. Pattern reversal VEPs to standard full-field stimulus were recorded monocularly. In addition, superior (SUP) and inferior (INF) pattern reversal visual field stimuli were also used; the other half of the stimulus field was isoluminant homogenous background. P100 amplitude and peak latency were recorded and the amplitude ratio was calculated as INF P100 amplitude/SUP P100 amplitude.
Results: All eyes of healthy children showed significantly larger ($p < 0.0001$) P100 amplitude to INF field stimulation (21.2 ± 12.5) compared to the P100 amplitude to SUP field stimulation (15.3 ± 7.5). The P100 latency was similar to INF and SUP field stimulation (99.4 ± 2.7 and 102.7 ± 3.4, respectively; $p = 0.22$). The amplitude ratio for the group of healthy children was larger than 1 (average 1.40 ± 0.35) in all eyes. The study will be extended to a larger group of healthy children, as well as children with white matter damage (WMD) of immaturity.
Conclusions: The study showed that in healthy children, INF pattern stimulation elicits a larger response than the response to SUP stimulation. These findings might enable detection of inferior visual field abnormality in children with WMD in whom abnormal inferior visual field function is suspected.

12:30 Flash VEP in children with meningitis

Raman Prasad Sah, Jyoti Baba Shrestha

B.P. Koirala Lions Centre for Ophthalmic Studies, Kathmandu, Nepal

Purpose: The flash VEP is commonly used to evaluate visual pathway integrity in non-verbal children and infants. Many studies have demonstrated that VEP measurements at infancy are reliable predictors of long-term visual outcome. The VEP may be affected in any disorder that affects the visual pathway beyond retina. Meningitis (inflammation of the brain meninges) is a common disorder in infancy and usually occurs due to either bacterial or viral infection. It is an important cause of cortical visual impairment in infants. Depending upon the severity and location of the inflammation, damage to the visual system in these children may vary. The aim of this study was to assess the flash VEP in children who were under treatment for meningitis and compare their parameters with age matched normal children.
Methods: VEP was measured in 14 children who were under treatment for meningitis and nine age-matched normal children. The mean age of the cases was 5.21 ± 7.58 months (range: 1–24 months) and 6.21 ± 4.2 months for controls.

Visual function was assessed using a preferential looking technique (Keeler cards), fixation behavior, or response to a light source, depending upon the child's cooperation. All children underwent flash VEP evaluation with Roland Retiscan (Roland Consult, Stasche and Finger Gmbh-Germany) using program Retiport 4.8.1.12 following the ISCEV clinical flash VEP protocol (Odom et al. Doc Ophthalmol, 2010; 120:111–119). The flash VEP was performed with flash goggles at 2 Hz and 12 Hz for each eye separately. Measurement included N1, P1, N2, P2 peak latencies and N1P1 and N2P2 amplitudes. The values were compared with the age matched normal control group.

Results: There was a significant difference in latency and amplitude between VEP values in cases and controls. In cases, the mean peak latency of N1, P1, N2 and P2 on stimulation with 2 Hz was 40.5 ± 15.37, 67.42 ± 17.68, 89.14 ± 18.65, 131.78 ± 22.10 ms, respectively. The mean N1 and P1 latency at 12 Hz was 36.78 ± 8.78 and 69.35 ± 12.44 ms, respectively. The mean amplitude of N1P1 and N2P2 at 2 Hz was 1.92 ± 1.73 and 3.83 ± 3.43 μV, respectively. The mean amplitude of N1P1 at 12 Hz was 2.17 ± 1.96 μV. The mean latency of N1, P1, N2 and P2 in the control group with 2 Hz stimulation was 43.66 ± 1.52, 56.0 ± 2, 66.33 ± 0.57, and 106 ± 3.60 ms, respectively. The mean N1 and P1 latency at 12 Hz was 43.78 ± 2.78 and 55.35 ± 1.44 ms, respectively. The mean N1P1 and N2 P2 amplitude at 2 Hz was 2.25 ± 0.25 and 14.96 ± 1.73 μV, respectively. The mean N1P1 amplitude at 12 Hz was 3.57 ± 1.26 μV.

Conclusions: Abnormal flash VEP compared to age matched controls suggests that there is abnormal visual processing in children suffering from meningitis. However, long-term follow up is required to evaluate the predictive value of VEP measured at young age for long-term visual outcome.

12:45 Follow-up of electroretinogram and visual evoked potentials as markers of retinal dystrophy and phenotype–genotype relationship in a large sample of children affected by Joubert syndrome

Alice Tarantola[1], Giulio Ruberto[1], Enza Maria Valente[2], Chiara Bertone[1], Mauro Antonini[3], Sabrina Signorini[3], Carmine Tinelli[4], Umberto Balottin[3], Paolo Emilio Bianchi[1]

[1]Department of Ophthalmology, IRCCS Polyclinic San Matteo, Pavia, Italy; [2]Salerno university and C.S.S. Mendel Roma, Salerno, Roma, Italy; [3]Department of Child Neurology and Psychiatry, IRCCS C. Mondino, Pavia, Italy; [4]Biometric Service, IRCCS San Matteo Hospital, Pavia, Italy

Purpose: Joubert syndrome (JS) is a rare autosomal recessive congenital malformation of the brainstem and cerebellar vermis. It can be associated with visual system anomalies and oculomotor system defects. Congenital anomalies including retinal dystrophies are discovered as the child ages. The aim of our study was to test the ability of electrophysiological testing to identify the JS subjects with associated retinal dystrophy.

Methods: 44 JS affected children, (mean age 8.52 ± 5.06 years) with a long ophthalmological and neurological follow-up, were genetically tested for mutations. In 37 subjects ERGs were recorded, as well as pattern-evoked visual evoked potentials (VEP) done at five spatial frequencies (from 300 to 15 min of arc) in awake subjects. In 27 of the subjects the functional examinations were repeated two or three times. ERG and VEP latencies and amplitudes were compared to genetic analyses and clinical outcomes. Linear regression models for repeated measures were used to analyze change over time of electrophysiologic parameters and genetic and clinical outcomes.

Results: Mutations were found in 29 out of 44 subjects. Mutation in CEP290 was found in 9 children, mutations in INPP5, TMEM216, RPGRIP1L, AHI1, C5orf42, KIF7, TCTN1, NPHP1(EX2), in 4,4,3, 2,2,1,1,1,1,1 children respectively. Multiple statistically significant differences were seen in the ERG and VEP amplitudes and latencies. The trend was most regular in the VEP, with steady latency delays and amplitude reductions in JS dystrophic children compared to the non-dystrophic. Moreover, fewer spatial frequencies elicited VEPs in the dystrophic eyes. In the ERG, the amplitudes in JS-affected subjects seemed to show a recovery at the second ERG measurement in comparison with the baseline measurement, and a statistically significant reduction at the third measurement compared to children not affected by retinal dystrophy.

Conclusions: Electrophysiology may distinguish the JS affected subjects with retinal dystrophy alone or in association with genetic analysis. The significance of recording follow-up, multiple ERGs and VEPs is that these two tests show different trends, which may help to manage these impaired children. The electrophysiologic data suggest that different retinal layers and visual pathways are affected over the course of the patient's life. Diagnoses and prognoses in patients with variable involvement of other organs like the liver and kidney should be aided by the discovery or exclusion of associated retinal disease.

Poster session 2: 14:30–15:30

Methodological aspects of electrophysiology

26. Four decades of visual electrophysiology in Slovenia

Jelka Brecelj

Eye Hospital, University Medical Centre Ljubljana, Ljubljana, Slovenia

Purpose: To present some aspects of the development of clinical visual electrophysiology in Slovenia.

Methods: Visual electrophysiological diagnostics and research started at the Institute of Clinical Neurophysiology (ICN) of the University Medical Centre, Ljubljana with the introduction of: (i) corneal ERG in the 1970s by neurologist Martin Janko, which was replaced by ERG according to ISCEV standards in the 1990s; (ii) VEP and PERG in the 1980s by neurologist Tine S. Prevec and physiologist Martin Štrucl, also with VEP to half-field stimulation (according to Martin Halliday); (iii) paediatric electrophysiology (according to Anthony Kriss) in the 1990s by biologist Jelka Brecelj and ophthalmologist Branka Stirn-Kranjc; and (iv) the invention of the HK (Hawlina-Konec)-loop ERG electrode in the 1990s by

ophthalmologist Marko Hawlina and engineer Blaž Konec, and its introduction into clinical ERG practice. In 2002, clinical visual electrophysiology was also established at the Eye Hospital in Ljubljana, and the range of examinations was extended (including EOG, colour VEP, mfERG, on–off ERG, S-cone ERG, photopic negative response—PhNR). This period coincided with the third generation of PhD students.

Results: For two million people living in an area of 20,273 km^2 there are three visual electrophysiology units for clinical diagnostics. Two of these (ICN, Ljubljana, General Hospital, Celje) are neurologically oriented, while the third (Eye Hospital, Ljubljana) serves for ophthalmologic diagnostics and paediatric patients. In the Eye Hospital, 8513 visual electrophysiological examinations were performed on 6206 patients from 2002 to 2014; with 30 articles published in the field (as indexed in Medline) and two chapters in the book 'The Challenge of Nystagmus'. Now, the fourth generation of PhD students is studying ERG function in correlation with optical coherence tomography, autofluorescence morphology, and genetics. Since the beginning of visual electrophysiology in Slovenia, all generations of our professionals have been trained abroad. Many have attended ISCEV symposia, from the first one in Budapest in 1983, while this year, we are honored to host the 2015 ISCEV Symposium in Slovenia. To date, two international symposia on visual electrophysiology have been organized in Slovenia, the first in 1993 on 'Neurophysiological Evaluation of the Visual System' (with the main lecture by Hisako Ikeda, entitled 'Mammalian retinal neurotransmitters—as seen through the eyes of a neurophysiologist'), and the second in 2010 as a 'Symposium on Clinical Neurophysiology of Vision and Eye Movements' (with the main lecture by Ryusuke Kakigi, entitled 'Face recognition-related potentials: EEG, MEG and NIRS studies').

Conclusions: Forty years and four generations of collaboration between neurologists, ophthalmologists, doctors, engineers, biologists, assistants and nursing staff now offer our patients a range of ISCEV standardized (EOG, full-field ERG, mfERG, PERG, VEP) and non-standardized (ERG with skin electrodes in infants, on–off ERG, S-cone ERG, PhNR, colour VEP) examinations. We continue to work on clinical research for the future of clinical visual electrophysiology, and we now also have a growing number of paediatric patients from abroad.

27. LED-based tunable light source for visual electrophysiology

Gregor Belušič[1], Marko Ilić[1], Katja Horvat[1], Primož Pirih[2]

[1]Department of Biology, Biotechnical Faculty, University of Ljubljana, Ljubljana, Slovenia; [2]Sokendai (The Graduate University of Advanced Studies), Hayama, Japan

Purpose: We aimed to construct a flexible, robust and tunable light source for fast and complex stimulation in experiments in the visual system of animal models and humans.

Methods: We assembled several arrays of 24 or more light emitting diodes with narrowband emission peaking at wavelengths between 350 and 720 nm. Their light was synthesized with plane reflective diffraction gratings. This allowed us to fill in the spectral gaps with broadband ("white") LED. The array was driven with a multichannel, pulse width-modulating current source switching in the kilohertz range. The stimulator was controlled with Arduino microcontrollers and custom written software. Light fluxes were adjusted with a calibrated spectroradiometer. The system was tested in insects (ERG, single cell recordings) and in humans (ERG).

Results: The system proved to be very easy to tune. Light intensity at any wavelength could be precisely adjusted so that the stimulator delivered equiquantal light stimuli within the whole visible spectral range. Excitation patterns could be exchanged at several Hz. The light source allowed us to measure the spectral sensitivity in several different ways: with spectral sweeps, with pseudorandom binary spectral sequences, and with simultaneous selective chromatic adaptation. Recordings with sweeps could be completed in seconds, hence multiple repetitions yielded good signal to noise ratio. Stimulation with pseudorandom spectral sequences yielded high stimulus intensities and consequently high signal from the visual system, while the light adaptation that is otherwise elicited by spectral sweeps could be kept minimal. Chromatic adaptation was achieved by driving selected spectral channels at high intensities and simultaneously sweeping at nonadapting wavelengths. The stimulator could produce light flicker with 50 % duty cycle without the implementation of a light chopper. Thus, stimulation with flickering light allowed recording of low amplitude ERGs with a lock-in amplifier.

Conclusions: We constructed a low cost, robust and versatile prototype of a light source for experimentation in animal models and in humans. Its current implementation makes possible high speed electrophysiological assays of colour vision with limited spectral resolution (ca. 20 nm). The general design allows for a much higher resolution, intensity and speed, albeit requiring substantial effort to enhance the mechanical and optical construction.

Funding: Slovenian Research Agency (P3-0333).

28. Scotopic red ERG findings

Li Yu Chen[1], Rena Png[1], Ranjana Mathur[1,2], Audrey Chia[1,2]

[1]Singapore National Eye Centre, Singapore; [2]Singapore Eye Research Institute, Singapore

Purpose: In a poster presented last year, we explored the characteristics and appearance of the scotopic response to red stimulation intensities of 1.5 and 2.5 cd s/m^{-2}. However, based on feedback, we have lowered our stimulation intensities to see if we could better isolate the x wave (or cone-derived response).

Methods: Scotopic responses were recorded using red stimulation, with light intensities of 0.25, 0.5, 0.75, 1.0 and 1.5-cd s/m^2. Recordings were obtained from 37 healthy individuals aged 10–78 years, and 12 patients aged 12–67 diagnosed with various ophthalmic diseases. All tests were done at the Visual Electrodiagnostic Laboratory at the Singapore National Eye Centre between October and December 2014, by using the Espion v6 and Ganzfeld color dome. The a, x and b waves were identified and analysed.

Results: In normal subjects (mean age 44.8 years), with increasing light intensity, there was a slight increase in a-wave amplitude (from 17.6 μv to 22.4 μV), and more significant

increases in the x-(from 64.0 to 128.4 μv) and b-waves (from 68.0 to 136.5 μv) amplitudes. Additionally, the x-wave was best demonstrated using 0.25 cd s/m^2 red stimulation. Changes in implicit time were less marked (a-wave 19.8–17.9 ms × wave 50.7–53.3 ms, b wave 72.9–71.8 ms). Patients with CRD showed a decrease in amplitude along with increased implicit time in their a- as well as x-waves, as opposed to patients with RCD who showed a decrease in only the b wave amplitude, and increased implicit time.
Conclusion: Overall, we confirm that using lower red intensity stimulation, the scotopic red responses were better elicited and that the x wave was best demonstrated using the 0.25 cd s/m^2 red stimulation.

29. Multi-stage ANC filter applied to mfVEP recordings

Miguel Ortiz, Luis De Santiago, Luciano Boquete, Román Blanco

Department of Electronics, University of Alcala, Alcalá de Henares, Spain

Purpose: Noise due to several sources (EEG, muscles activity, electrical interference,…) is a typical issue in mfVEP recordings. The mfVEP signals are thus often difficult to measure reliably. Adaptive Noise Cancelation (ANC) is an optimal filter that minimizes the mean square error (MSE) of the corrupted signal (primary input) and the output signal. The ANC output is a function of the two inputs of the system: the corrupted signal and a noise reference. The success of the ANC depends on the noise reference input, which must be uncorrelated with the signal and highly correlated with the additive noise. The purpose of this study is to examine a new filtering method based on the interconnection of several stages, each implemented by the ANC method.
Methods: Three sets of mfVEP data were obtained from 48 healthy eyes. Set 1 is the unfiltered data (RAW data). Set 2 is data filtered with the most-common method used in clinical practice (band pass filter 1–20 Hz based on Fast Fourier transform, FFT). Set 3 is the data filtered by using 14 ANC stages. Each ANC stage processes the signal with the additive noise input and an estimation of the noise. The output of one stage is the primary input of the following stage. The noise reference is estimated using the detail coefficients (high-pass components) and approximation coefficients (low-pass components) of the discrete Wavelet decomposition of the primary input. Only levels 6, 7 and 8 of the decomposition were used. Signal to noise ratio is calculated before and after each stage of ANC. If one stage shows worsening of the SNR, this stage is eliminated. Results with different type of Wavelet decomposition were compared. A receiver operating characteristic (ROC) curve was obtained comparing the signal and noise of the recordings for raw data, FFT data and best Wavelet decomposition data. Area Under the ROC Curve (AUC) was also calculated.
Results: The SNR was 4.29 ± 1.26 for unfiltered data and after filtered with FFT was 5.37 ± 1.42 (33,38 % improvement compared to the raw values). Using the multi-stage filter, the values according to the Wavelet used are: Bior6.8 (6.77 ± 1.54), Coif5 (6.96 ± 1.53), Daubechies10 (6.37 ± 1.38) and Daubechies8 (6.88 ± 1.48) respectively. The best Wavelet is Coif5 (gain over raw 62.15 %) and a significant difference was found between Coif5 and all the methods. The area under the curve (AUC) was greater in the signals filtered using ANC (AUCCOIF5 = 0.96) than in the raw signals (AUCRAW = 0.92) or in those filtered using the FFT (AUCFFT = 0.92).
Conclusions: The multi-stage-ANC filter designed in this work, using the wavelet decomposition as a noise reference of the primary input significantly improved the SNR compared to raw signals or those signals filtered with the standard method. This technique may also be useful in separating the signal distribution from the noise distribution.

30. Using a novel electrode based on a super absorbent polymer for preparation-free recording of visual evoked potentials

Susanne Kramer[1], Torsten Strasser[2], Tobias Peters[2], Eberhart Zrenner[1,2,3]

[1]University Eye Hospital Tuebingen, Tuebingen, Germany; [2]Institute for Ophthalmic Research, University of Tuebingen, Tuebingen, Germany; [3]Werner Reichart Centre for Integrative Neuroscience (CIN), Tuebingen, Germany

Purpose: Visual evoked potentials (VEPs) are usually recorded using silver–silver chloride or gold disc skin electrodes, which require time consuming preparation, e.g. scalp abrasion. Instead of skin electrodes, modern dense array EEG systems use arrays of small sponges soaked in saline solution to contact the scalp, eliminating the need for scalp abrasion. Here we present a similar approach using a novel electrode made of a super absorbent polymer (SAP), the marble electrode (ME), for preparation-free recording of visual evoked potentials and compare results with conventional skin electrodes.
Methods: Small cup-like holders, which mount marble electrodes at the scalp, were manufactured by the workshop of the University Eye Hospital Tuebingen. VEPs were recorded according to the ISCEV standard using a Diagnosys e^2 system in five healthy volunteers with best corrected visual acuity. Recordings were performed using marble electrodes and conventional skin electrodes, subsequently after scalp abrasion. Impedances for each electrode were measured before each recording. Peak latencies and amplitudes for N75 and P100 were obtained for two checkerboard patterns (0.84 cpd and 0.25 cpd).
Results: Measured impedances for the skin electrodes in each test were well below 5 kΩ, whereas impedances of the marble electrodes were between 40 kΩ to 80 kΩ. Peak amplitudes and latencies of N75 and P100 for each condition were compared using paired-samples *t* tests. No significant differences of mean amplitudes and latencies between skin and marble electrodes were present except for P100 peak amplitude at 0.84 cpd. This analysis revealed significant lower mean amplitudes using marble electrodes (mean 12.3 μV) compared to skin electrodes (mean = 14.4 μV) [$t(4) = 3.75$, $p = 0.02$]. The observed difference between the amplitudes was 2.1 μV, and the 95 % confidence interval for the difference extended from 0.5 to 3.6 μV.
Conclusions: The marble electrode has been proven as a suitable alternative to conventional electrodes for recording

VEPs. It reduces the required time for preparation of subjects by avoiding uncomfortable scalp abrasion. Despite the high impedances of the marble electrode, a reliable recording and evaluation of VEPs was possible. Most of the noise can be cancelled out by averaging at a recording interval that is not a multiple of the periodicity of the line noise.. The small, but significant difference found for the peak amplitude of P100 is below the intra-individual amplitude variability and may be caused by the small sample size. Additional techniques for line noise removal and lowering amplitudes by particular soaking solutions should further improve recording quality.

31. Recording steady-state pattern ERG responses using a lock-in amplifier

Kazuma Yagura[1], Soiti Matsumoto[1,2], Kei Shinoda[1], Harue Matsumoto[1,2], Atsushi Mizota[1]

[1]Department of Ophthalmology, Teikyo University school of medicine, Tokyo, Japan; [2]Matsumoto Eye Clinic, Tokushima, Japan

Purpose: The pattern ERG (PERG) has been used for evaluating the function of the retinal ganglion cells in the macular area. However, the electrical responses obtained are very low in amplitude and thereby susceptible to noise and artifacts. The Lock-in Amplifier is a powerful tool to extract a signal with a known carrier wave and a constant frequency from an extremely noisy electrical signal. The purpose of this study is to evaluate the usefulness of a Lock-in Amplifier to record the amplitude of steady-state PERG.
Methods: The steady state PERG was recorded from nine patients with unilateral optic nerve disease. Three males and 6 females were included. The mean(±SD) age was 55(±24) years. The ocular disease was anterior ischemic optic neuropathy (AION) in 4 eyes, optic nerve atrophy after optic neuritis in 3 eyes, normal tension glaucoma in one eye, and superior segmental optic hypoplasia (SSOH) in one eye. The stimulation frequency was 11.9 rev/sec; the check-size was 1°; stimulated area was 20°; shape was round and amplifier filter was 5–200 Hz with average rate of 300×. The Lock-in Amp (LI5640, NF Co Tokyo) received the electrical signals from the bio amplifier and exported the responses to a data recording system (Powerlab 16/35, CO, USA).
Results: The mean amplitude of the PERG in the affected eye was significantly reduced compared with that of the contralateral eye both by conventional recording method and by the Lock-in Amp method. The amplitude of the PERG recorded using Lock-in Amp was lower compared with that obtained using conventional method.
Conclusions: The Lock-in Amplifier may be useful in recording the amplitude of steady state PERG in eyes with optic nerve disease.

32. Grouped responses of multifocal visual evoked potentials and their sensitivity in the detection of simulated visual field defects

Maja Šuštar, Jelka Brecelj

[1]Eye Hospital, University Medical Centre Ljubljana, Ljubljana, Slovenia

Purpose: The use of multifocal visual evoked potentials (mfVEP) in routine clinical testing is challenging, since the numerous multifocal responses obtained can be difficult to evaluate, because they can show large variability between the segments tested. To minimise this variability and increase the sensitivity, the responses can be grouped into larger areas of interest. Our aim was to explore three different methods of grouping these responses, and to evaluate the sensitivity for detection of various simulated visual field defects.
Methods: The mfVEPs were obtained using RETIscan (Roland, Germany) with three-channel recording of 15 normal subjects. The stimulus consisted of sixty segments, presented on a display with a diameter of 54°. Peak-to-peak amplitude values of the best VEP responses were analysed for each segment stimulated. The data were exported to Excel 2003 (Microsoft), and the following grouping methods were evaluated: (a) software predefined sectors (rings, quadrants, global response), which were calculated as the means of the signals within each sector; (b) means of the peak-to-peak amplitudes of the responses using the same sectors (rings, quadrants, global response); and (c) the means of peak-to peak amplitudes within nine sectors, grouped according to waveform similarity (central 3° and two ring eccentricities, divided by quadrants into eight segments). For each of the grouping methods, receiver-operating characteristics analysis was performed to evaluate the sensitivity for the detection of various simulated visual field defects: central scotoma (1°, 3°), peripheral visual-field loss (>3°, >7°, >12°) and hemi-field loss (superior, inferior, right, left). The means of the areas under the curves (AUC) were calculated for each of the methods used.
Results: The software predefined sectors (rings, quadrants, global response) grouped by the means of the signals of the responses provided a mean AUC of 0.77 ± 0.09. When the same sectors were grouped by the means of the peak-to-peak amplitudes of those responses, the mean AUC was higher (0.83 ± 0.09). However, the highest mean AUC (0.87 ± 0.06) was achieved for the means of the peak-to peak amplitudes inside the nine sectors, grouped according to waveform similarity. The sensitivity of the grouping methods will be further tested with other types of simulated visual-field defects in a larger group of control subjects.
Conclusions: Grouping of the localised responses into larger sectors according to waveform similarity appears to increase the sensitivity of mfVEP testing and to simplify the detection of visual field defects.

Retinal diseases

33. A case of peripheral cone dystrophy with different cone dysfunction in each eye

Rei Arai[1], Yujin Mochizuki[1], Nobuo Ishida[2], Atsushi Mizota[3], Nobuyuki Ebihara[1]

[1]Department of Ophthalmology, Juntendo Urayasu Hospital, Chiba, Japan; [2]Ishida Eye Clinic, Nigata, Japan; [3]Department of Ophthalmology, Teikyo University School of Medicine, Tokyo, Japan

Purpose: Peripheral cone dystrophy was first described in 2004 as an unusual form of cone dystrophy. Normally it occurs

bilaterally, and several cases have been reported to date. We present a case of atypical unilateral peripheral cone dystrophy and report electrophysiological findings and ultrastructure objective data.
Methods: A 34-year-old woman came to the clinic for prescription of contact lenses. She complained of blurred vision of both eyes and central field loss of the right eye. Her symptoms have continued without progression for 17 years. At initial visit, her corrected visual acuity was 0.02 OD and 1.2 OS. The slit-lamp examination and ophthalmoscopy showed no abnormal findings. SD-OCT showed that the boarder of the IS and OS line and the COST line in the macula area were absent in the right eye. Humphrey perimetery indicated that a central scotoma was present in the left eye. The photopic full-field electroretinograms (ERGs) were reduced but scotopic ERGs were normal in both eyes. The multifocal ERGs were severely reduced throughout the visual field except in the central area of the left eye.
Results: These result showed cone function was decreased throughout the whole retina in the right eye, but only peripherally in the left eye. It was suggested that peripheral cone dysfunction occurred asymmetrically.
Conclusions: In the present case, one eye showed findings of typical cone dysfunction but in the other eye central cone function was maintained. The mechanism of pathogenesis is unknown. Subjective symptoms remained stabile for more than 17 years. Electrophysiology and SD-OCT are valuable tests to determine the pathogenesis of unusual ocular findings objectively.

34. Electroretinographic follow-up in cone and cone–rod dystrophies

Cornelia Andreea Corici[1], Maja Sustar[2], Martina Jarc-Vidmar[2], Branka Stirn-Kranjc[2], Marko Hawlina[2], Jelka Brecelj[2]

[1]University of Medicine and Pharmacy of Craiova, Craiova, Romania; [2]Eye Hospital, University Medical Centre Ljubljana, Ljubljana, Slovenia

Purpose: To study the progression of changes in the electroretinogram (ERG) in patients with cone (CD) and cone–rod (CRD) dystrophies.
Methods: This retrospective study included three patients with CD and five patients with CRD. All of the patients underwent ophthalmological examination and standard full-field ERG at first presentation and after a follow-up period [mean, 6.4 years (CD), 5.2 years (CRD)]. The ERGs were recorded with Roland Consult or Espion Diagnosys systems, which have the same stimulus parameters, in compliance with the ISCEV standard. The amplitudes and implicit times were analysed for dark-adapted 0.01 ERG (rod ERG), dark-adapted 3 ERG (combined rod cone standard flash ERG), light-adapted 3 ERG (standard flash 'cone' ERG), light-adapted 30-Hz flicker ERG, and these responses were compared to normative data. Non-parametric paired-sample Wilcoxon signed rank tests were used for the comparisons between the first and second examinations for both groups.
Results: In patients with CD, only the light-adapted responses showed reduced amplitudes compared to the normative data. Comparisons between the first and follow-up examinations showed significant decreases in mean amplitudes of the light-adapted responses. The light-adapted 3 ERG a-wave decreased from 12.0 ± 4.1 to 6.9 ± 3.8 μV ($p = 0.03$), and the light-adapted 30-Hz flicker response from 26.4 ± 9.8 to 12.5 ± 15.7 μV ($p = 0.03$). The mean amplitudes of the dark-adapted responses remained unchanged between these examinations. The mean implicit times stayed within normal values for the first and second examinations. In patients with CRD, the amplitudes of the dark-adapted and light-adapted responses were reduced, compared to the normative data. Comparisons between the first and second examinations showed significant decreases in mean amplitude of the light-adapted 30-Hz flicker response, from 29.4 ± 19.1 to 22.1 ± 15.8 μV ($p = 0.02$), and significant prolongation of its implicit time (33.6 ± 5.6 vs. 36.3 ± 8.0 ms, $p = 0.03$).
Conclusions: Our preliminary findings on this small group of patients show that in CD there was progressive cone dysfunction, while rod function stayed within the normal limits. In the CRD group, the disease is seen mostly as a reduction in cone function, while the altered rod function remained relatively stable.
Grant: European Social Found, Human Resources Development Operational Programme 2007–2013, Project No. POSDRU/159/1.5/S/136893.

35. Retina-wide disease and foveal sparing in ABCA4-related retinopathy

Ana Fakin[1,2], Anthony G. Robson[1,2], Kaoru Fujinami[3,4], Anthony T. Moore[1,2], Andrew R. Webster[1,2], Michel Michaelides[1,2], Graham E. Holder[1,2]

[1]UCL Institute of Ophthalmology, London, United Kingdom; [2]Moorfields Eye Hospital, London, United Kingdom; [3]National Institute of Sensory Organs, Tokyo Medical Center, Tokyo, Japan; [4]Department of Ophthalmology, Keio University, School of Medicine, Tokyo, Japan

Purpose: To determine the contribution of common missense mutations to retina-wide disease processes and foveal sparing in ABCA4-related retinopathy.
Methods: A retrospective analysis of ERG recordings and optical coherence tomography (OCT) images was undertaken in 62 molecularly confirmed patients with ABCA4 retinopathy (Stargardt disease). Studied missense mutations were c.2588G>C (G863A or 863delG; N = 14), G1961E (N = 12), L541P/A1038V (N = 1), P1380L (N = 3), R2030Q (N = 2) or L2027F (N = 8). Only those in trans with a likely-null mutation (stop, frame-shifting indel or splice-site mutation) were included, in order to distinguish the precise effect of missense mutations. Twenty-two patients had a combination of two likely-null mutations. Right eyes were chosen for analysis in all patients. Foveal sparing was determined using OCT images.
Results: Mean age was 29 ± 16 years (range 6–69). All patients carrying two likely-null mutations had evidence of generalized retinal dysfunction with abnormal cone or rod and cone responses on full-field ERG, with none having foveal sparing. Patients with stop and frame-shifting mutations (N = 9; mean age 20 ± 12 years, range 6–40) showed a significant correlation between log DA 10.0 a-wave amplitude and age (Pearson's correlation -0.7, $p < 0.05$). Using this regression line, the age-related response for patients with splice

mutations was examined, with most (11/13) being within the 95 % confidence interval of this line including: c.5461-10T>C, c.2160 + 1G>C, c.4253 + 4C>T, c.6479 + 1G>A, c.859-9T>C, c.6729 + 5del15 and p.V256V. Two patients carrying splice mutation c.5714 + 5G>A had near normal ERG responses falling outside the 95 % confidence interval. Evidence of peripheral retinal dysfunction was observed in 14 % of patients with c.2588G>C, 8 % of patients with G1961E, 100 % of patients with L541P/A1038 V, 67 % of patients with P1380L, 62 % of patients with L2027F and 0 % of patients with R2030Q mutation. Foveal sparing was seen in 0 % (c.2588G>C), 7 % (G1961E), 0 % (L541P/A1038 V), 0 % (P1380L), 22 % (L2027F) and 80 % (R2030Q) of patients harboring these missense variants.

Conclusions: Stop, frame-shifting and most splice mutations (with the exception of c.5714 + 5G>A) were associated with severe retinal dysfunction affecting central and peripheral retina. Missense mutations L541P/A1038V and P1380L produced a similarly severe phenotype. The most common mutations, c.2588G>C and G1961E, usually spared the peripheral retina. The R2030 variant was the mildest, usually only affecting the perifoveal macula. The L2027F mutation often presented with retina-wide disease but there was also a relatively high incidence of foveal sparing, suggesting two separate disease mechanisms.

36. Clinical and genetic characteristics of a Japanese family with autosomal dominant retinitis pigments

Miki Hata[1,3], Kaoru Fujinami[1,4], Takeshi Iwata[2], Toru Noda[1,2], Kazushige Tsunoda[1,2]

[1]Department of Ophthalmology, Tokyo Medical Center, Tokyo, Japan; [2]National Institute of Sensory Organs, Tokyo Medical Center, Tokyo, Japan; [3]Department of Ophthalmology, Keio University, School of Medicine, Tokyo, Japan; [4]UCL Institute of Ophthalmology, London, UK

Purpose: To describe the clinical and genetic characteristics of a Japanese family with autosomal dominant retinitis pigmentosa (adRP).

Methods: Five subjects, including three affected patients (Cases 1–3) and two unaffected subjects (Cases 4, 5), were investigated in a Japanese family with RP. The proband was a 35 year-old female (Case 1). The proband's father (73 years; Case 2) and paternal aunt (70 years; Case 3) were affected and the proband's brother (37 years; Case 4) and mother (63 years; Case 5) were unaffected. The proband's paternal grandmother and uncle were reported to be also affected. Comprehensive examinations were performed, including visual acuity (VA), visual field (VF), optical coherence tomography (OCT) and fundus autofluorescence imaging (AF). Full-field ERGs (FFERGs) incorporating to the ISCEV standard were recorded. Focal macular ERGs (FMERGs) were obtained for assessing macular function. Targeted mutation detection with next generation sequencing was performed and pipeline filtration with customized annotation was conducted according to the National Institute of Sensory Organs (NISO) bioinformatics protocol.

Results: Night blindness was reported in Cases 1 and 2. The VA of three affected patients (Cases 1–3) was (0.5/0.7), (0.15/0.02), and (1.2/1.2), respectively. VF showed severe constriction in Case 2, and blind spot enlargement with arcuate scotoma in Cases 1 and 3. Diffuse retinal atrophy with macular involvement, vessel attenuation, and bone-spicule pigmentation were observed in Case 2, while peripheral retinal atrophy was less apparent in Cases 1 and 3. Cases 1 and 2 had cystoid macular edema (CME) which spontaneously resolved and led to macular atrophy. The OCT demonstrated well-preserved macular structures in Case 3. AF revealed ring enhancement of high density in Cases 1 and 3, and diffuse low density of the atrophic region in Case 2. In FFERG, bright-flash response (DA 10.0) was severely reduced with electronegative waveform in Case 1, but mildly reduced in Cases 1 and 3. In Cases 1 and 3, rod responses (DA 0.01) showed slightly delayed and decreased b-wave, but cone-derived responses (LA 3.0 and LA 30 Hz) were normal. FMERG in Case 1 showed delayed and decreased a- and b-waves with 15 degree stimulation and extinguished responses with 10 or 5 degree stimulation. Genetic screening revealed candidate variants including a possibly disease-causing variant in the IMPDH1 gene.

Conclusions: A Japanese family with adRP was documented. The proband had mild retinal dysfunction accompanied by CME. The proband's father showed severe retinal degeneration with macular involvement, while the aunt in the same generation was mildly affected. The phenotypic findings in this family were compatible to the spectrum of the previously reported IMPDH1-retinopathy, although two affected members had relatively mild clinical features.

37. Characteristics of global flash mfERG in patients with retinitis pigmentosa

Christie Hang I. Lam, Serena Z. C. Li, Man Pan Chin, Henry Ho Lung Chan

School of Optometry, The Hong Kong Polytechnic University, Hong Kong SAR, Hong Kong

Purpose: Previous ERG studies have found abnormal post-receptoral activities in animals with retinitis pigmentosa (RP) [1, 2]; however, the post-receptoral response in human RP patients is not well understood. The aim of this pilot study was to investigate the characteristics of outer and inner retinal activity in RP patients using the global flash mfERG.

Methods: Eight clinically diagnosed RP adults with tunnel vision and five healthy adults were recruited. Global flash mfERG with 103 scaled high-contrast (96 %) hexagons was performed. The prescription at 33 cm working distance was optically corrected. The resultant wavefront consists of a direct component (DC) and an induced component (IC), which is mainly contributed by the outer and inner retinal responses, respectively. The derived focal responses were divided into two regions: the central (ring one to two: about central five degrees of visual field) and peripheral region (ring four to six: about peripheral nine to 24 degrees eccentricity) for the comparison of the retinal activity in vision preserved and non-preserved retinal region.

Results: The RP subjects showed decreases in the outer (DC amplitude) and inner (IC amplitude) retinal responses; the DC and IC amplitudes at both the central and peripheral regions were significantly lower in the RP subjects than that of the healthy adults (Mann–Whitney U Test, all $p \leq 0.03$).

However, the central inner retinal response (drop in IC amplitude: 45.2 %) was less affected when compared to the peripheral inner retinal response (drop in IC amplitude: 94.5 %), and the central (drop in DC amplitude: 63.5 %) and peripheral outer retinal responses (drop in DC amplitude: 88.9 %). This may imply that inner retinal function is relatively preserved at the central retinal region of the RP subjects.
Conclusions: Retinitis pigmentosa appeared to reduce both the inner and outer retinal responses at the central and peripheral regions, but the drop was less significant in the central inner retina. The relatively preserved central inner retinal activity in the human eye is consistent with our previous finding using the Rhodopsin P347L Transgenic (Tg) porcine model [1], which suggested an ectopic synaptogenesis between the cone photoreceptors and rod bipolar cells.

References

1. Ng YF et al., Invest Ophthalmol Vis Sci 2008;49:2208–2215.
2. Hirota R et al., Invest Ophthalmol Vis Sci 2012;53:1467–1472.

38. Foveal hypoplasia and atypical retinitis pigmentosa in a family: clinical phenotype and genetic investigations

Ajoy Vincent[1,2], Heather Macdonald[1], Jeffrey Locke[1], Anupreet Tumber[1], Carol Westall[1,2], Elise Heon[1,2]

[1]Department of Ophthalmology, Hospital for Sick Children, Toronto, Canada; [2]Department of Ophthalmology, University of Toronto, Toronto, Canada

Purpose: To report the clinical phenotype and genetic results in an autosomal dominant pedigree with a peculiar form of retinitis pigmentosa (RP) and foveal hypoplasia.
Methods: The proband (12 years) and affected mother (33 years) underwent detailed clinical evaluation including best corrected visual acuity (BCVA) and color vision testing, contrast sensitivity measurement and fundus photography. Spectral-domain optical coherence tomography (SD-OCT), fundus autofluorescence (FAF) testing and full-field electroretinogram (ERG) were performed in both cases. Molecular genetic testing was performed.
Results: BCVA in the proband was 0.6 and 0.2 logMar in the right and left eyes, respectively. BCVA in the mother was 0.7 and 0.8 logMar in the right and left eyes, respectively. Compound myopic astigmatism was observed in both. Color vision was normal in both cases, but contrast sensitivity was reduced. Fundus evaluation in the mother showed inferior sectoral RP with pigmented para-venous chorio-retinal atrophy (PPRCA) in the other quadrants; fovea was hypoplastic. Fundus evaluation of the proband revealed similar changes, although the pigmentary changes were milder. FAF confirmed areas of retinal pigment epithelial atrophy in both cases. Foveal hypoplasia was confirmed on SD-OCT in both the proband (Grade-2 hypoplasia) and the mother (Grade-1 hypoplasia). SD-OCT also showed focal areas of disruption of the external limiting membrane and photoreceptor layers within the macula in both cases. ERG showed normal rod responses and borderline cone responses. No mutations were detected in RHO or RP1; a large panel of retinal dystrophy genes (160 genes) tested negative in the proband. Array comparative genomic hybridization testing of numerous eye genes (130 genes) did not reveal any insertions/deletions in the mother.
Conclusions: The retinal phenotype in this family appears to be unique with both degenerative (RP and PPRCA) and developmental (foveal hypoplasia) features. The normal rod responses and borderline cone responses suggest limited involvement of the photoreceptors in these cases. The underlying genetic cause remains to be determined.

39. Clinical and electrophysiological findings in two siblings with mutations in the USH2A gene

Eva Lenassi[1,2], Maria Bitner-Glindzicz[3], Linda M. Luxon[4], Graham E. Holder[1], Andrew R. Webster[1], Anthony G. Robson[1]

[1]UCL Institute of Ophthalmology and Moorfields Eye Hospital, London, UK; [2]Eye Clinic, University Medical Centre, Ljubljana, Slovenia; [3]UCL Institute of Child Health, London, UK; [4]UCL Ear Institute and National Hospital for Neurology and Neurosurgery, London, UK

Purpose: To present the clinical, electrophysiological and genetic findings in a family demonstrating extreme intrafamilial phenotypic variability.
Methods: Two siblings were examined. Clinical assessment included a complete medical history, fundus photography, fundus autofluorescence imaging (FAF), optical coherence tomography (OCT), electrodiagnostic testing and pure-tone audiometry. Genetic testing was performed in both study subjects using Sanger and/or exome sequencing.
Results: Both patients were found to harbour biallelic mutations in the USH2A gene—the most commonly mutated gene in both Usher syndrome and non syndromic autosomal recessive retinitis pigmentosa (RP). The male sibling had a diagnosis of Usher syndrome and had been using hearing aids since age 3 years. At age 30, he was noted to have an abnormal fundus appearance during a routine eye test. He had night vision problems from his late 20 s and peripheral vision problems from his early 30 s. Fundus examination, FAF, OCT and ERGs at age 47 were consistent with a diagnosis of RP. His sister, a 43-year-old woman, had no visual complaints. Hearing problems were noted at age 18 months and she had been using hearing aids since age 4 years. Ocular examination and retinal imaging were unremarkable. The bright-flash (dark-adapted 10.0) ERG a-wave on the right was marginally reduced, but electroretinographic findings were otherwise normal bilaterally and were not typical of RP.
Conclusions: This is the first report of USH2A mutations causing nonsyndromic hearing impairment, and the non-penetrance of the ocular phenotype in one of the two affected siblings is highly unusual. Our findings have important implications for counselling, and the normal retinal structure and function in the female sibling could suggest protective environmental, genetic or epigenetic factors.

40. Analysis of patients with electronegative b-waves (2008–2014): a case series

Ranjana Mathur[1,2], Li Yu Chen[1], Rena Png[1], Gemmy Cheun Cm[1,2], Audrey Chia[1,2]

[1]Singapore National Eye Centre, Singapore; [2]Singapore Eye Research Centre, Singapore

Purpose: To evaluate characteristics of patients who showed electronegative b-waveforms on full-field ERG.
Methods: We performed retrospective analyses of all cases referred to our ERG lab from 2008 to 2014 who demonstrated an electronegative maximal full-field ERG b-wave. All ERGs were performed with ISCEV standard protocol.
Results: Ninety-four patients were found to have an electronegative ERG. The majority of them were male (90 %) with a mean age of 43.4 years. (range 6–74 years). The clinical diagnosis was CSNB in 33 %; X-linked retinoschisis in 10 %, retinal dysfunction dystrophy 17.6 %, central retinal artery occlusion (CRAO) in 8.6 %, myopia (either electronegative response or reduced b/a ratio) in 9.2 % and other 13 %. A myopic refraction was seen in approximately one third of the cases. The main presenting symptom was either night vision problems or decreased vision. The severity of the full-field ERG electronegative response varied with minimal changes seen in myopic patients and maximum changes in CSNB patients. The severity however did not show consistent correlation with visual acuity. Multifocal ERG P1 and N2 responses showed various patterns: normal waveform, abnormal N2 components, reduced P1 responses and increased implicit time.
Conclusions: Congenital stationary night blindness was the most common etiology of an electronegative ERG in our case series.

41. Geographic atrophy secondary to age-related macular degeneration: functional and morphological evaluation

Elisabetta Pilotto[1], Evelyn Longhin[1], Francesca Guidolin[1], Enrica Convento[1], Alma Patrizia Tormene[1], Edoardo Midena[1,2]

[1]Department of Ophthalmology, University of Padova, Padova, Italy; [2]G.B. Bietti Foundation, IRCCS, Roma, Italy

Purpose: To compare morphological and functional differences in patients with geographic atrophy (GA) secondary to age-related macular degeneration, with or without choroidal neovascularization (CNV) in the fellow eye.
Methods: Consecutive patients with GA and foveal sparing, in at least one eye were enrolled. All eyes were studied by means of: enhanced depth imaging spectral-domain optical coherence tomography to measure choroidal thickness; microperimetry to assess retinal sensitivity; blue (B-FAF) and near infrared-wavelength fundus autofluorescence (NIR-FAF) to measure GA; and mfERG. Patients were divided into two groups: (1) patients with bilateral GA without evidence of CNV (B-GA group); and (2) patients with GA in one eye and CNV or disciform scar in the fellow eye (unilateral GA group, U-GA group). Only one eye from each patient in the B-GA group was enrolled in the study.
Results: Ten patients [five in the B-GA group (five eyes) and five in the U-GA group (five eyes)] were studied. Mean GA area was not significantly different in the two groups in NIR-FAF nor in B-FAF. Choroid was significantly thicker in the B-GA group eyes compared to the U-GA group ($p = 0.042$). Mean retinal sensitivity was not significantly different between the groups. MfERG P1 amplitude was reduced not only in the GA areas but also in the spared macular areas in both groups with no significant difference. P1 implicit time delay was also not significantly different between groups.
Conclusions: Patients with GA with or without choroidal neovascularization (CNV) in the fellow eye do not seem to differ from a functional point of view. However, a thinner choroid detected in the U-GA group may reflect a different pathophysiologic mechanism.

42. Autoimmune retinopathy in the fellow eye 10 years after initial presentation

Takehito Iwase[1], Yosuhe Nakamura[1], Gen Miura[1], Hisao Ode[1], Atsuhiro Kanda[3], Wataru Saito[3], Shuichi Yamamoto[4]

[1]Department of Ophthalmology Chiba University; [2]Makuhari Ode Eye Clinic, [3]Department of Ophthalmology Hokkaido University; [4]Department of Ophthalmology and Visual Science, Chiba University

Purpose: Autoimmune retinopathy (AIR) is an autoimmune disorder caused by the antiretinal antibodies which are produced by the body. The disease generally progresses rapidly, develops in both eyes, and the prognosis of visual function is poor. We describe a case of AIR which occurred in the fellow eye 10 years after initial presentation in one eye.
Methods: Case report.
Results: A 51-year-old male patient visited the Chiba University Hospital with a progressive visual field defect in the right eye on April in 2005. Best-correlated visual acuity (BCVA) was 0.9 OD and 1.2 OS. In the right eye the retinal blood vessels were narrowed, and peripheral retina was degenerated. Fluorescein angiography (FA) demonstrated window defects corresponding to the retinal degeneration. Electroretinography (ERG) responses were unrecordable in the right eye and normal in the left. Goldman perimetry showed concentric constriction of the visual field. Antirecoverin antibodies measured in the previous examination indicated as positive up to 2/6 dilutions. AIR was diagnosed from clinical findings. Systemic examination was performed, but there were no abnormalities found. Because steroid therapy was ineffective, a calcium antagonist (Nivadil®) was prescribed. Nevertheless, the right eye eventually progressed to blindness. Although the left eye had been followed with photophobia from the time of the first visit, electrophysiological abnormal findings were not initially observed. The patient became aware of a superior defect in the visual field of the left eye on August in 2014. The BCVA was 1.2 OS. Formal perimetry demonstrated a ring scotoma. The fundus findings were similar to the previous findings in the right eye. Optical coherence tomography demonstrated the

damage of the outer layer of retina corresponding to ophthalmoscopically abnormal sectors. The ERG response was unrecordable. AIR was diagnosed in the left eye, and 2 courses of steroid pulse therapy and oral immunosuppressant (cyclosporine®) were started. Antirecoverin antibodies measured during the course in our hospital were consistently negative on three occasions. Anti-α-enolase antibody was only positive in antiretinal antibodies serum. We could not find a primary systemic disease. At the present time, BCVA as been maintained 1.2 OS, and the visual symptoms and the visual field were without changes.

Conclusion: We experienced a case that developed autoimmune retinopathy in both eyes, separated by 10 years between the initial presentation in one eye, and subsequent presentation in the fellow eye. Although the visual acuity and the visual field are maintained by the combination therapy of steroids and immunosuppressive agents in the left eye, photophobia is still present, and strict observation and examination are necessary in the future.

43. Maculopathy following exposure to visible and infra-red laser radiation

James Hanson[1], Julian Sromicki[1], Mario Mangold[2], Matthias Golling[2], Christina Gerth-Kahlert[1]

[1]Ophthalmology, University Hospital Zurich, 8091 Zurich, Switzerland; [2]Institute for Quantum Electronics, ETH Zurich, 8093 Zurich, Switzerland

Purpose: In recent years, hand-held laser devices have become popular as aids to presentation ("laser pointers"). Online trade has led to the proliferation of unregulated devices which may be considerably more powerful than those allowed by local law, and which have the potential to cause ocular damage. An underappreciated risk presented by such devices is that typically an infra-red laser source is used, which is then frequency-doubled to a visible wavelength[1]. Without subsequent filtering (which cannot be assumed in unregulated devices), this infra-red radiation remains present in the output beam and presents an additional risk to observers. We present the case of a patient with visual loss and a history of intentional exposure to the output of a powerful laser pointer (green beam, marked as <100 mW 532 nm output) some months previously. The purpose of the investigation was to exclude a retinal dystrophy through electrophysiology and to characterise the precise nature of the laser radiation to which she had been exposed.

Methods: A complete ophthalmological examination was performed including SD-OCT and autofluorescence. Additionally, ERG, MF-ERG (61 and 103 Hexagons) and EOG were performed according to ISCEV standards. The output power spectrum of the laser pointer was measured. The ophthalmological examination, together with SD-OCT and MF-ERG, was repeated after 7 months.

Results: Best corrected visual acuity (BCVA) was 0.3 OD, 0.6 OS (Snellen). Ocular examination revealed central hyper- and hypopigmented atrophic areas in both eyes, whilst OCT showed highly circumscribed damage to the outer retina and retinal pigment epithelium at the foveola (OD > OS). The fundus appearance was consistent with thermal retinal damage[2]. All electrophysiological measures were normal, thereby excluding retinal dysfunction beyond the central atrophic area. The laser pointer output was measured as containing 20 mW energy at 532 nm and 22 mW at 1064 nm. MF-ERG and OCT remained unchanged 7 months after the initial examination.

Conclusion: Exposure to combined visible and infra-red laser radiation was responsible for visual loss and lasting structural damage to the foveal region despite the absence of electrophysiologically measurable functional abnormality. Given the size and discrete nature of the lesion, it is likely that standard MF-ERG stimulus arrays were too coarse to capture functional disturbance at the fovea. The case highlights an underappreciated aspect of laser pointer injuries, namely the risk of exposure to radiation outside the visible spectrum.

References

1. Galang et al. NIST Technical Note 1668 (2010).
2. Ham et al. Vision Research 20 1105–1111 (1980).

44. Clinical and electrodiagnostic findings in a family with autosomal dominant RGR mutation

Monique Leys[1], J. Vernon Odom[1], Xinjing Wang[2]

[1]WVU Eye Institute, Morgantown, WV, USA; [2]National Eye Institute, Bethesda, MD, USA

Purpose: To describe clinical and electrodiagnostic findings in a family with RGR mutation causative of Autosomal Dominant Choroidal Peripapillary Atrophy.

Methods: To date we examined 4 affected and 3 unaffected members. Molecular diagnosis was established with the EyeGENE network. We collected clinical data in this family over a time span of 20 years including Goldmann Visual fields, electrodiagnostic tests and serial imaging.

Results: We identified a disease causing RGR mutation; c.824dupC using Next Generation Sequencing and validated results with Sanger Sequencing. The four affected patients (M 77, F81, F 60, M 51) were mildly hyperopic or emmetropic. The 51 yr old affected male (M51) was asymptomatic, had 20/20 acuity, no cataract and had a normal EOG and normal ganzfeld ERG. His father M77, a former smoker with advanced cardiovascular disease, had at age 59 already a very reduced ERG and pronounced atrophy around optic nerve and arcades approaching the fovea in the left eye. Serial Goldmann visual fields showed progressive enlarging of the blind spot with preservation of the peripheral field.

Conclusions: We identified a family with Peripapillary Choroidal Atrophy resulting from a RGR mutation, reviewed the literature and provide documentation of the clinical and electrodiagnostic findings in this rare disease in 2 generations.

Supported by Foundation Fighting Blindness and EyeGENE.

45. Why am I losing my vision? LORD or something else: a case report

Rena Png[1], Ranjana Mathur[1,2], Edmund Y. M. Wong[1,2]

[1]Singapore National Eye Centre, Singapore; [2]Singapore Eye Research Institute, Singapore

Purpose: We report an unusual case of a 72-year-old Chinese male who had been regularly followed up at our hospital since 2003 but developed bilateral progressive blurred vision.
Methods: A Case report.
Results: Our patient had undergone bilateral cataract surgery in 2003 with good visual outcome and had bilateral dry age-related macular degeneration. He was referred to the Vitreo-Retinal department in 2008 for LE metamorphopsia, VA 6/7.5. Two years later he noted difficulty in night vision. Acuity measured RE 6/60 and LE remained stable at 6/7.5. OCT showed hyper-reflective material at the IS-OS junction in both eyes (L > R). Fundus angiography could not explain cause for asymmetrical loss of vision. Neuro-Ophthalmic consult confirmed presence of bilateral central scotoma, RE RAPD, abnormal colour vision (R > L), normal optic disc, bilateral maculopathy; worse in LE rather than RE and no optic neuropathy. Autofluorescent imaging confirmed larger hypofluorescent area at RE macula compared to the LE. PERG responses were undetectable in both eyes. Full-field ERG rod system responses were borderline reduced in amplitude but not delayed. The cone system response was reduced and delayed. The multifocal ERG confirmed bilateral macular dysfunction.
Conclusions: This case is of unusual macular dystrophy and we have considered a differential diagnosis including late onset retinal dystrophy (LORD), autoimmune retinopathy (due to rapid progression), Sorsby macular dystrophy (phenotype). However, phenotype does not fit any one of the differentials considered in this elderly patient with rapid onset loss of vision.

Animal electrophysiology

46. Comparison of waveforms of the scotopic threshold response elicited by different methods of stimulation

Takako Hidaka, Hideki Chuman, Nobuhisa Nao-I

Department of Ophthalmology, Miyazaki University, Miyazaki, Japan

Purpose: To evaluate the scotopic threshold responses (STRs) of rats using our conventional stimulus system and Mayo's newly developed system.
Methods: Subjects were male Sprague–Dawley rats (200–240 g; Kyudou, Kumamoto, Japan). Animals were dark-adapted overnight (= 12 h) and prepared for recording under dim red light. Anesthesia was induced with intramuscular injection of ketamine (100 mg/kg) and xylazine (10 mg/kg). Pupils were fully dilated with tropicamide. STRs were recorded using a PuRec system (Mayo Co., Nagoya, Japan). Retinal STRs were recorded from a cornea using contact lens electrodes. Active electrodes were placed at the corneal apex and an indifferent electrode was placed on the skin. A needle electrode placed in the tail served as a ground. We compared two types of stimulus systems: our conventional system consisted of a Ganzfeld integrating sphere and a xenon lamp (PS33PLUS photic stimulator). Mayo's new stimulus system consisted of an acrylic hemisphere and a LED photic stimulator (LS-100). STR responses were obtained for stimulus intensities ranging from -7.00 to 1.50 log cd-s m^{-2} in 0.5 log unit increments by averaging 20 responses per intensity, with an interstimulus interval of 3–5 s. Latencies and amplitudes of both groups (our conventional system (n = 10), and Mayo's new system (n = 9)) were evaluated using a two-tail Mann–Whitney U-test.
Results: There was no significant difference in peak latency between the two groups (our system: 122.02 ± 8.68 ms; Mayo's system: 115.7 ± 9.39 ms; $p = 0.154$). We chose 120 ms as the criterion time to measure the amplitude of the positive STR (-4 log cd-s m^{-2}). The amplitudes using Mayo's system were significantly higher than those using our conventional system (Mayo system: 126.80 ± 29.38 μV; Our system: 57.11 ± 20.53 μV; $p = 0.00238$).
Conclusion: Although both stimulus systems were useful for recording STRs, Mayo's newly developed stimulus system generated significantly larger STRs. This difference may be due to the longer stimulus duration of the LED stimulus compared with that of xenon flash.

47. Pivotal roles of Fezf2 in differentiation of cone OFF-bipolar cells and functional maturation of cone ON-bipolar cells in retina

Yujin Mochizuki[1], Atsushi Mizota[2], Sumiko Watanabe[3], Nobuyuki Ebihara[1]

[1]Department of Ophthalmology, Juntendo University School of Medicine, Tokyo, Japan; [2]Department of Ophthalmology, Teikyo University School of Medicine, Tokyo, Japan; [3]Division of Molecular and Developmental Biology, University of Tokyo, Tokyo, Japan

Purpose: During development of the retina, common retinal progenitor cells give rise to six classes of neurons that subsequently further diversify into more than 55 neuronal subtypes. Here, we investigated the expression and function of Fezf2, Fez zinc finger family of proteins, in the developing mouse retina.
Methods: We demonstrate that Fezf2 is expressed in, and is required for, the generation of a subpopulation of cone OFF-bipolar cells (BCs). We further reveal the dysfunction and abnormal synaptic formation of cone ON-BCs in Fezf2 knockout mice, which is suggestive of an interaction between cone OFF- and ON-BCs during their maturation. We also examined the light response of Fezf2 knockout retinas using single flash electroretinograms (ERGs).
Results: Expression of Fezf2 was strongly observed in the embryonic retinal progenitors at E14 and declined quickly in subsequent development of the retina. In the postnatal stage, at around post natal day 8, Fezf2 was transiently expressed and then declined again. Loss-of-function analysis, using retinas from mice in which the Fezf2 coding region was substituted with -D-galactosidase, showed that Fezf2 is expressed in a subset of cone OFF-BCs and is required for their differentiation. Using the ERG, we found that Fezf2 knockout retinas exhibited significantly reduced photopic b-wave peak

amplitudes, suggesting functional abnormality of cone ON-BCs. Furthermore, both reduced immunohistochemical expression of synaptic protein Trpm1 and structural alteration of ON-BC invagination, identified by transmission electron microscopy, affected cone photoreceptor terminal synaptic activity.
Conclusions: Taken together, our results show that Fezf2 is indispensable in differentiation of bipolar precursors into cone OFF-bipolar cells and in functional maturation of cone ON-bipolar cells during development of mouse retina. These results contribute to our understanding of how diversity of neuronal subtypes, and hence specificity of neuronal connections, are established in the retina by intrinsic cues.

48. Assessment of the pupillary light reflex in monkeys using a new portable device designed for clinical use

Serge Rosolen[1], Nadège Sarrazin[2], Youssef Lourhzal[3], Pierre Moissonnier[4], Sophie Gilardeau[2], Cedric Lamirel[5], Pierre Pouget[2], Jean Lorenceau[6], Anne Baron-Van Evercooren[2], Serge Picaud[1]

[1]Institut de la Vision, INSERM UMR_S 968, UPMC Univ Paris 06, CNRS UMR 7210, Paris, France; [2]Institut du cerveau et de la moelle épinière, ICM-A-IHU, UPMC 06 UM 75,INSERM U, Paris, France; [3]SIEM Bio-médicale, Nimes, France; [4]Ecole Nationale Vétérinaire d'Alfort, Maisons-Alfort, France; [5]Fondation Ophtalmologique Adolphe de Rothschild, Paris, France; [6]Lab. des systèmes perceptifs, Départ. d'Etude Cognitive, ENS CNRS ULMR8248, Paris, France

Purpose: To assess the pupillary light reflex (PLR) in monkey under varying chromatic stimulus conditions
Methods: Two cynomolgus male monkeys used for operant conditioning were assessed in awake condition in accord with the ARVO statement. An induced-optic nerve demyelination (IOPD) was created in the left eye of each monkey. VEPs and PLR were recorded at pretest and 1 week after IOPD. A portable SIEM Bio-Medicale device including an infrared-sensitive camera delivering continuous infrared illumination and enabling concurrent pupil images at 60 frames per second was used. The direct PLR was evaluated for each eye and each condition using a series of 1-second illuminations elicited with a blue-light (BL) (480 nm) and a red-light (RL) (630 nm) of increasing intensities (min: −0.02 and max: 2.96 log cd/m^2). PLR responses were recorded for 9 s after stimulation and the following parameters were calculated: PLR latency (PLRL), PLR constriction amplitude (PLRca), PLR constriction time (PLRct), PLR redilation amplitude (PLRrda), PLR redilation time (PLRrdt), and PostIllumination Pupil Response (PIPR). Amplitude measurements were converted into a percentage of baseline pupil area. VEPs were recorded in monocular and binocular conditions. Black and white checkerboards of 15, 30 and 60 degrees were displayed on a screen placed at a constant distance of 60 cm from the monkey's eyes.
Results: For control eyes (RE = 4), PLRL were similar regardless of the color or intensity stimulation. PLRca increased with intensities of 65 % and of 42 % for RL and BL, respectively. PLRct increased with intensities of 25 % for RL and of 42 % for BL. PLRrda decreased with intensities of 12 % for RL and of 20 % of BL. PLRrdt increased with intensities of 20 % for RL and of 180 % for BL. PIPR increased with intensities of 40 % in BL exclusively. Pattern-reversal VEP waveforms consisted of N1, P1 and N2 peaks. For the IOPD-eye, for one monkey neither VEPs nor PLR were discernible. For the other animal, PLRL increased 50 % in comparison with the control eye. PLRca increased with intensities of 6 % and 15 % for RL and BL, respectively. PLRrda decreased with intensities of 14 % for RL only. PIPR was not discernible. For the same animal, VEP waveforms were strongly altered in all the pattern-reversal stimuli.
Conclusions: Our data suggest that our portable device enables reliable, quantitative assessment of the PLR in monkeys affected with optic nerve disease. SUPPORT: "Investissements d'Avenir" ANR-10-IAIHU-06".

Retinal function and structure

49. "En face" spectral domain optical coherence tomography compared with functional tests in patients treated with anti-malarial drugs

Carl Arndt, Mathieu Costantini, Sylvie Berthemy, Alain Ducasse, Christophe Chiquet, Martine Mauget

[1]Reims University Hospital, Reims, France; [2]Grenoble University Hospital, Grenoble, France; [3]Rothschild Ophthalmologic Foundation, Paris, France

Purpose: The aims of this retrospective study were to compare the results of functional tests, central visual field and multifocal ERG, with the results of «en face» optical coherence tomography and to analyze disparities between the structural and functional findings.
Methods: In consecutive patients screened for anti-malarial toxicity between July 1st 2014 and December 31st 2014, the following tests were performed: central visual field, multifocal electroretinogram (mfERG) recording, "en face" spectral domain-optical coherence tomography (SD-OCT) and short-wavelength fundus autofluorescent imaging (SW-FAF).
Results: Among 16 patients screened, only one patient had abnormal results in all tests and three presented with abnormalities at least in one test: one patient had abnormal visual fields and mfERG changes with no SD-OCT changes; one patient had abnormal visual fields, a normal mfERG, and slight hyporeflective dots on the "en face" scans at the level of the ellipsoid (IS-OS junction); and one patient only had abnormal mERG with no other changes.
Conclusions: The results of this pilot study highlight the difficulty of decision making in patients treated with anti-malarial drugs for chronic inflammatory disease. Although "en face" technology provides a new approach in analyzing focal abnormalities in the photoreceptor-retinal pigment epithelium interface, it probably cannot replace functional testing to screen for anti-malarial toxicity.

50. Multimodal imaging of a case of peripheral cone dystrophy

Shuhei Kameya[1], Naoko Ito[2], Kiyoko Gocho[1], Takaaki Hayashi[2], Sachiko Kikuchi[1], Satoshi Katagiri[2], Tamaki Gekka[2], Kunihiko Yamaki[1], Hiroshi Takahashi[3], Hiroshi Tsuneoka[2]

[1]Department of Ophthalmology, Nippon Medical School Chiba Hokusoh Hospital, Inzai, Japan; [2]Department of Ophthalmology, The Jikei University School of Medicine, Minato-ku, Japan; [3]Department of Ophthalmology, Nippon Medical School, Bunkyo-ku, Japan

Purpose: To characterize the peripheral cones in a patient with peripheral cone dystrophy from images obtained by spectral domain optical coherence tomography (OCT), swept source OCT, and adaptive optics fundus camera.
Methods: A 28-year-old Japanese man underwent detailed ophthalmic evaluations including high resolution imaging of the fundus of both eyes.
Results: The decimal best-corrected visual acuity (BCVA) was 1.2 in both eyes. The results of slit-lamp biomicroscopy and ophthalmoscopy were essentially normal. Fluorescein and indocyanine green angiographies did not show any hyper- or hypofluorescent areas of the retina. Goldmann perimetry showed full peripheral visual fields but relative central scotomas within the central 20 degrees. The results of the Humphrey Field Analyzer showed limited preservation of central sensitivity. Color vision tests showed no errors in either eye. Spectral domain OCT showed attenuation of both the ellipsoid and interdigitation zones throughout the macular region with the exception of the center of the fovea. The scotopic full-field ERGs were normal but the photopic ERGs were markedly reduced in amplitude. Regular cone mosaics were not observed especially more than 450 μm from the fovea in the adaptive optics retinal images. The parafoveal cone densities were severely decreased in both eyes.
Conclusions: Our findings indicate that the peripheral cone dystrophy diagnosed by full-field ERGs and perimetry is due to a reduction in the density of parafoveal and peripheral cones.

51. Simultaneous recording of electroretinography and visual evoked potential in normal subjects and patients with vitreous hemorrhage

Young-Hoon Ohn, Tae Kwann Park, Hoon Dong Kim, Yoon Kyung Kim, Jung Woo Han

Department of Ophthalmology, Soonchunhyang University College of Medicine, Bucheon, Korea

Purpose: To determine the normal data in simultaneous recording of photopic electroretinography (ERG) and flash visual evoked potential (VEP) and investigate the usefulness of the test on patients with vitreous hemorrhage.
Methods: The photopic ERG and flash VEP were recorded simultaneously on 20 eyes of 10 normal subjects, and 34 eyes of 17 patients who were diagnosed with diabetes mellitus. Of the 34 eyes, 18 eyes had vitreous hemorrhage caused by proliferative diabetic retinopathy and underwent pars plana vitrectomy after the test. The participants were classified as; normal eyes (Group 1), eyes with vitreous hemorrhage (Group 2), and fellow eyes of patients without vitreous hemorrhage (Group 3). Simultaneous ERGs and VEPs were recorded again after vitrectomy on 5 eyes of Group 2. Intensity of light stimulus was gradually increased from 1.0 to 10 cd s/m^2 on each eye to analyze the responses of ERG and VEP. All responses were analyzed to find differences between each groups at each stimulus intensity and evaluate changes after operation. Best corrected visual acuity (BCVA) was also checked before and after the surgery.
Results: When compared with Group 1, amplitudes of ERGs were decreased significantly ($P < 0.05$) and implicit times of ERG were increased significantly ($P < 0.05$) with Group 2 and Group 3 at all stimulus intensities, but VEP amplitudes and latencies were not statistically different. Preoperative simultaneous ERG and VEP revealed that implicit times of ERG were increased in Group 2 when compared with Group 3. Postoperative recordings demonstrated no significant changes compared to preoperative recordings. There was no correlation between postoperative BCVA and preoperative recordings of simultaneous ERG and VEP.
Conclusions: Simultaneous recordings of ERG and VEP showed that the function of retinal and other visual pathways was reduced not only in diabetic eyes with vitreous hemorrhage but also in the fellow eyes of these patients. However, the postoperative findings suggest that diabetic eyes with vitreous hemorrhage may have irreversible damage.

52. The effect of sustained bedrest and hypoxia on electroretinographic responses

Maja Potrč[1], Maja Šuštar[1], Polona Jaki-Mekjavić[1], Marko Hawlina[1], Ola Eiken[2], Igor Mekjavić[3]

[1]Eye Hospital, University Medical Centre Ljubljana, Ljubljana, Slovenia; [2]Department of Environmental Physiology, Swedish Aerospace Physiology Centre, Stockholm, Sweden; [3]Department of Automation, Biocybernetics and Robotics, Jozef Stefan Institute, Ljubljana, Slovenia

Purpose: In view of the recently reported structural and functional changes in the eyes of astronauts following short and long duration spaceflight, the present study investigated the effect of sustained bedrest and hypoxia on visual function; the rationale was that reduced gravity and hypoxia are anticipated conditions within future Lunar and Martian habitats.
Methods: The study was conducted in the Planica Olympic Sport Centre's Hypoxic Facility, which is located at an altitude of 940 m in Rateče, Slovenia. Twelve healthy females participated in three 10-day experimental campaigns in randomised order: normoxic bedrest (NBR; FiO2 = 0.209; PiO2 = 133.1 ± 0.3 Pa; 7 subjects), hypoxic bedrest (HBR, 9 subjects) and hypoxic ambulatory confinement (HAMB; FiO2 = 0.141; PiO2 = 89.6 ± 0.4 Pa; ~4000 m simulated altitude; 4 subjects). Before, and on the last day of each intervention, their visual function was measured by electroretinography (ERG): full-field light adapted 3.0 ERG (LA

3.0 ERG), pattern ERG (PERG) and multifocal ERG (mfERG) using CSO Retimax System with HK-loop electrodes.
Results: None of the three experimental conditions (NBR, HBR, HAMB) influenced the amplitude or implicit times of the a- or b-waves of LA 3.0 ERG. However, there was a significant increase of the PERG P50 amplitude after 10 days of HBR (baseline: 7.9 ± 1.5 μV; HBR: 8.7 ± 1.6 μV; $p = 0.03$), while the N95 component was not significantly affected. Similarly, there was also an increase of the central mfERG response in the group exposed to HBR (baseline: 100.9 ± 23.0 μV; HBR: 135.7 ± 37.8 μV; $p = 0.01$). The described changes of PERG and mfERG amplitude were not detected in the other two experimental conditions (NBR or HAMB).
Conclusions: A transient increase in the macular response was recorded after hypoxic bedrest only, which most likely reflects the combined influence of increased perfusion due to hypoxic vasodilatation and postural-induced hydrostatic pressure loading. However no effect on the cone system of the peripheral retina was observed. This finding might indicate that central and peripheral cones might differ in the oxygen supply or that they are exposed to different microenvironmental conditions.
Acknowledgements: This study was supported, in part, by the European Space Agency Programme for European Cooperating States (Contract No. 4000111808/14/NL/Nde), and the Swedish National Space Board (Project No. 2014-109/11).

53. Screening of cone dysfunction by the RETeval (TM) handheld ERG device

Soichiro Tamaki[1,2], Natsuko Nakamura[1,2], Kaoru Fujinami[1–4], Yoshinobu Mizuno[1,2], Toru Noda[1,2], Yozo Miyake[5], Kazushige Tsunoda[1,2]

[1]Department of Ophthalmology, Tokyo Medical Center, Tokyo, Japan; [2]National Institute of Sensory Organs, Tokyo Medical Center, Tokyo, Japan; [3]Department of Ophthalmology, Keio University, School of Medicine, Tokyo, Japan; [4]UCL Institute of Ophthalmology, London, UK; [5]Department of Ophthalmology, Aichi Medical University, Aichi, Japan

Purpose: To evaluate the usefulness of a handheld flicker ERG device, RETeval™ (LKC Technologies, Inc., Gaithersburg, MD) for the screening of patients with cone dysfunction.
Methods: Thirty-three eyes of 33 patients (8–61 years, mean 32.3 ± 18.0) who showed reduced cone responses detected by the conventional Ganzfeld-ERG system using contact lens electrodes were recruited. All of these patients demonstrated reduced amplitudes of 30-Hz flicker ERG, which were lower than the minimum of the normative range (mean value—2.0 SD). Causative diseases of the patients included achromatopsia (5 eyes), cone dystrophy (5 eyes), cone-rod dystrophy (7 eyes), retinitis pigmentosa (12 eyes), choroidal dystrophy (1 eye), autoimmune retinopathy (2 eyes) and Stargardt disease (1 eye). RETeval™ was used to record flicker responses under undilated condition with skin electrodes, according to the preset protocol (stimulus intensity; 3.0 $cdsm^{-2}$, frequency; 28.3 Hz, background light; none). In order to investigate the difference between the cohort with cone dysfunction and controls, flicker responses were recorded from 30 healthy eyes (age; 22–37 years, mean 27.9 ± 4.0 years). Comparison analysis between these two groups was performed with Mann–Whitney U test, in terms of the amplitude and implicit time of RETeval™ flicker response.
Results: The mean flicker amplitude of 33 eyes with cone dysfunction and 30 control eyes were 2.4 ± 3.4 and 22.8 ± 5.4 μV, respectively. The mean implicit time of 20 available eyes with cone dysfunction and 30 control eyes were 34.4 ± 3.2 and 30.9 ± 1.5 ms, respectively. The implicit time was not measurable due to extremely low amplitude in 13 eyes with cone dysfunction. Comparison analysis revealed significant differences between the two groups both in the amplitude ($p < 0.01$) and the implicit time ($p < 0.01$). In addition, all the patients in the cone dysfunction group showed markedly reduced RETeval™ flicker amplitudes lower than the minimum of normative range (mean value—2.0SD) except for one subject with retinitis pigmentosa.
Conclusions: Reduction of flicker responses was confirmed in patients with cone dysfunction using the noninvasive RETeval™ handheld ERG recording system with skin electrodes. This simplified system may be useful as a screening tool for cone dysfunction, especially in young patients in whom recoding ERGs can occasionally be difficult with a conventional Ganzfeld-ERG system and those who cannot tolerate contact lens electrodes.

Paper session 4: 16:00–18:15

Methodological aspects of electrophysiology

16:00 Plagiarism dangers lurking in scientific publishing

Michael Bach

Section Visual Function/Electrophysiology, Eye Center, Freiburg University, Freiburg, Germany

Purpose: I recently submitted a paper to our journal Documenta Ophthalmologica and was alerted that I had plagiarised 39 % of the manuscript content from other sources. This turned out to be an error by the plagiarism scanning software, iThenticate® (iParadigms, LLC, USA), triggered by my use of the term "Literature" to head the bibliography. On this background, I looked into the field of plagiarism, self-citation ("word recycling") and the dangers of automated tools with their typical narrow horizon of artificial intelligence.
Methods: I tried to understand the mechanisms used by the iThenticate® software (to the degree that is disclosed), consulted available sources on scientific conduct (e.g. < http://ori.hhs.gov>) and analysed my own publications for "word recycling". Finally, legal issues were considered.
Results: Artificial intelligence has always suffered from generalizability: the "real world problem". It is obvious (and considered by editors) that plagiarism scan reports have to be read with deeper understanding than proffered by the software itself. Examples of software 'misunderstandings' include flagging the acknowledgement section or the term "evoked potential" as plagiarism. Leaving aside the obvious point that all sources should be appropriately acknowledged,

the issue of self-citation is more difficult. Typically, in a paper there are two regions where one tends to use one's own words from previous publications: Introduction (state of the art) and Methods. I hold that there is a case to use exactly the same words as in a previous paper when the state of the art has not changed, or when identical methods are applied to a new population or with a major twist. Once good words have been found (a challenge for a non-native speaker), a brief repetition is the best approach when nothing substantial has changed. The German Research Council (DFG) also supported this view in a recent statement. A complication can arise with copyright, where permission should be sought if the re-used text extends to several paragraphs. For further education, the site <http://ori.hhs.gov > gives excellent advice on good research conduct and detailed rephrasing examples.

Conclusions: As a scientist, one should approach open research questions with the naïvety of a curious child—but with respect to possible plagiarism, my naïvety needed to be educated. This is the case even when one has the best of intentions; or when 'source agnosia' has occurred. Understanding some aspects of this treacherous field, as touched upon in this presentation, can help to avoid unnecessary complications.

Funding: None.

16:15 Effect of shorter dark-adaptation on ISCEV standard ERGs and an exploration of the dark-adapted red flash ERG

Ruth Hamilton[1,2], Kirsten Graham[1,2]

[1]Department of Clinical Physics and Bioengineering, NHS Greater Glasgow and Clyde, Glasgow, UK; [2]College of Medical, Veterinary and Life Sciences, University of Glasgow, Glasgow, UK

Purpose: The ISCEV ERG standard stipulates a 20 min minimum period of dark adaptation (DA). In the interests of greater patient acceptability, we aimed to quantify the effect of shorter DA on ISCEV standard ERGs. We also investigated the dark-adapted ERG to a red flash, whose initial positive peak is thought to reflect dark-adapted cones, and which has been reported as useful for clinical diagnoses. We aimed to define the red flash strength which scotopically matches the ISCEV 0.01 cd s m^{-2} flash and to characterise the red flash ERG changes during DA.

Methods: Sixteen healthy adult subjects were dark-adapted for 20 min during which red flash ERGs and white 0.01 cd s m^{-2} ERGs were recorded at 1, 2, 3, 4, 5, 10, 15 and 20 min using LED stimuli. White 3.0 cd s m^{-2} ERGs were also recorded at 2, 5, 10, 15 and 20 min. ERGs were recorded from both eyes with skin electrodes and natural pupils.

Results: Relative to ERG amplitude and peak time after 20 min DA, ISCEV standard white 0.01 ERGs were 60 % and 90 %, respectively, after only 1 min of DA, and were 90 % and 100 % after 10 min. ISCEV standard 3.0 ERGs recorded after 2 min of DA did not differ from those recorded after 20 min. ERG variability after 10 min DA did not differ from variability after 20 min DA. Red flashes with strength 1.5 (phot) cd s m^{-2} were scotopically matched to ISCEV standard white 0.01 cd s m^{-2} flashes, based on b-wave amplitudes after 20 min DA. This red flash strength fitted the predicted match given the stated photopic:scotopic ratio of the red stimulus. The red 1.5 cd s m^{-2} ERG showed a triple-peaked a-wave and an x-wave. A-waves and x-waves showed little amplitude change and no peak time change over 20 min DA while the red flash b-wave changed in a very similar manner to the b-wave of the ISCEV standard white 0.01 cd s m^{-2} b-wave. Subtraction of white 0.01 cd s m^{-2} ERGs from red 1.5 cd s m^{-2} flash ERGs isolated the DA cone waveform, which did not substantially change throughout DA, but which was more clearly defined at 1–5 min DA than at 10–20 min DA.

Conclusions: Shortening DA, for example to 10 min, had no measureable effect on the DA 3 ERG or the DA 0.01 ERG peak time and caused only a 10 % amplitude loss of the DA 0.01 ERG amplitude. There was no increase in variability. In the light of improved efficiency and patient acceptability, it might be argued that minor loss of amplitude of the DA 0.01 ERG was an acceptable compromise. The red flash ERG was best recorded to a 1.5 cd s m^{-2} flash as this allowed isolation of the cone response by subtraction of the white 0.01 cd s m^{-2} ERG. Red flash ERGs recorded after 5 min DA had the clearest waveform.

16:30 Comparison between two types of skin electrode ERG recording system

Atsuhiro Tanikawa, Iyo Ueda, Ryota Sakurai, Yoshiaki Shimada, Masayuki Horiguchi

Department of Ophthalmology, Fujita Health University School of Medicine, Toyoake, Japan

Purpose: To compare two types of skin electrode ERG recording system; PuREC with a ganzfeld dome (Mayo, Nagoya, Japan) and RETeval™ complete (LKC Technologies, Maryland, USA).

Methods: The ERGs were elicited from 11 eyes of 11 normal subjects. The pupils were maximally dilated with 0.5 % tropicamide and 0.5 % phenylephrine hydrochloride. After 20 min of dark adaptation, dark-adapted (DA) ERGs were elicited by PuREC system, using a Sensor strip of RETeval as skin electrode. Then light-adapted ERGs were recorded by the same system. After that, another set of ISCEV standard ERGs was recorded from the same subject by the RETeval system, using the same Sensor strip. Both systems had the same luminance, and frequency bandwidth ranged from 0.3 to 300 Hz in accordance with ISCEV standards. The amplitudes and the peak times of each component were compared by the paired *t* test.

Results: The mean amplitudes of each component were as follows (the former; PuREC and the latter; RETeval, respectively): DA 0.01 ERG; 63.6 ± 4.2 and 59.0 ± 5.0 μV ($p = 0.256$), DA 3.0 ERG a-wave; 63.8 ± 4.0 and 57.9 ± 4.4 μV ($p = 0.239$), DA 3.0 ERG b-wave; 99.6 ± 7.2 and 95.5 ± 7.2 μV ($p = 0.966$), LA 3.0 ERG a-wave; 9.1 ± 0.6 and 9.6 ± 1.0 μV ($p = 0.457$), LA 3.0 ERG b-wave; 40.4 ± 3.1 and 40.7 ± 2.4 μV ($p = 0.905$), flicker ERG; 33.7 ± 2.0 and 31.4 ± 2.3 μV ($p = 0.398$). The mean peak times of each component were as follows: DA 0.01 ERG; 83.1 ± 3.8 and 92.3 ± 3.9 ms ($p = 0.006$), DA 3.0 ERG a-wave; 18.2 ± 0.2 and 14.5 ± 0.5 ms ($p = 0.002$), DA 3.0 ERG b-wave; 45.3 ± 1.9 and 48.2 ± 1.6 ms ($p = 0.107$), LA 3.0 ERG

a-wave; 16.8 ± 0.2 and 13.3 ± 0.2 ms ($p < 0.001$), LA 3.0 ERG b-wave; 30.2 ± 0.4 and 27.1 ± 0.5 ms ($p < 0.001$), flicker ERG; 27.6 ± 0.4 and 24.4 ± 0.3 ms.
Conclusions: Both skin electrode ERG recording systems revealed almost the same amplitudes. However, RETeval system showed significantly faster peak times in some components than PuREC system.

16:45 Effect of cataract on flicker electroretinogram (ERG) recorded with RETeval

Takayuki Koike, Yosuke Nakamura, Junki Azuma, Yumi Tanaka, Gen Miura, Takeshi Sugawara, Shuichi Yamamoto

Departments of Ophthalmology and Visual Science, Chiba university, Chiba, Japan

Purpose: A noninvasive and mydriatic free flicker electroretinogram (ERG) device, RETeval (LKC Technologies), utilizing skin electrodes, has been commercialized and is expected to be applied for clinical use in infants and bedridden patients that have difficulty with traditional ERG measurement. We evaluated the effect of lens opacification (cataract) on ERGs recorded with RETeval.
Methods: In this study, we evaluated 82 eyes in 60 subjects, 76.2 ± 6.4 years of age, who had nuclear cataract without any other abnormalities, and 35 eyes in 26 subjects, 74.6 ± 5.9 years of age, that had intraocular lens (IOL) without any abnormalities in anterior segment, media, or fundus. Flicker ERG for each subject was recorded under mydriatic free condition. The subjects with cataract were divided into 2 groups (Grade 2 and Grade 3 cataract groups). A total of 3 groups, including the IOL subject group, were compared for latency and amplitude. The ERG was recorded using a white light stimulus with a frequency of 28.3 Hz and intensity of 8 Td-s.
Results: Averages of amplitude and implicit time were 9.2 ± 3.7 μv and 35.5 ± 1.8 ms for Grade 2 cataract group, 7.1 ± 2.1 μv and 37.0 ± 2.1 ms for Grade 3 cataract group and 13.4 ± 4.9 μv and 34.9 ± 1.7 ms for IOL group, respectively. Amplitude was significantly decreased with lens opacification (Grade 2 cataract vs IOL: $p = 0.0002$, Grade 3 cataract vs IOL: $p < 0.0001$, Grade 2 cataract vs. Grade 3 cataract: $p = 0.014$). Implicit time of Grade 3 was significantly delayed (Grade 3 cataract vs IOL: $p = 0.0004$, Grade 2 cataract vs. Grade 3 cataract: $p = 0.0084$). However, there was no statistical difference between Grade 2 cataract and IOL ($p = 0.29$).
Conclusions: Amplitude obtained from flicker ERG recorded with RETeval at 8 Td-s is decreased by the more severe cataract stage. Implicit time of RETeval is also influenced by lens opacity, especially with grade 3 cataract. RETeval is expected to be a clinically useful tool to provide adequate flicker ERG results under the conditions where traditional ERG recording is difficult. However, careful evaluation is needed in the analysis of RETeval flicker ERG in patients with moderate cataract.

17:00 Functional imaging of the retina with multi-angular electroretinography (maERG)

Antoine Brassard Simard[1], Mathieu Gauvin[2], Bing Yu Chen[2], Pierre Lachapelle[2], Jean-Marc Lina[1–3]

[1]Département de génie électrique, École de Technologie Supérieure, Montréal, Québec, Canada;
[2]Department of Ophthalmology and Neurology-Neurosurgery, McGill University - Montreal Children's Hospital Research Institute, Montreal, Quebec, Canada;
[3]Centre de Recherches Mathématiques, Montréal, Québec, Canada

Purpose: ERG recording from a single active electrode is a well-known method to objectively assess retinal function. However, this method cannot detect local variations in retinal response unless a specific stimulus is used, as is done in mfERG. This study had two purposes. The first purpose was to propose a method for recording an ERG with multiple spatial derivations (as is done in electrocardiography). The second purpose was to solve the electromagnetic problem of computing retinal activity from corneal potentials.
Methods: We proposed a novel method that uses the rotating capability of the human eye in order to record corneal potentials from various positions (i.e., the maERG). In this method, three skin electrodes are positioned on the subject's lower lid, internal canthus and external canthus. We recorded the maERG from two healthy subjects using 11 different gaze positions for a total of 33 virtual electrodes per subject. Based on realistic eye and skin conductivities and geometries, we built a Boundary Elements Model (BEM) of the human eye. In order to determine if the maERG method can be used for retinal imaging, we compared the performance of a maERG model with a model containing an electrode array in direct contact with the cornea. We simulated three scenarios: an inactive patch, a central inactivity and a peripheral inactivity. We propagated the simulated retinal activity on the electrodes (i.e., the forward model). Based on these simulated electrode measurements, we reconstructed the retinal activity (i.e., the inverse problem) and compared the reconstructed image with the theoretical sources configuration using a balanced area under the ROC-curve (AUC) approach. We compared AUCs obtained with both models as a metric of the maERG reconstruction potential. We also reconstructed the retinal activity of the experimental data in order to visualize the reconstruction quality.
Results: The different skin electrodes detected different ERG signals for each gaze position, meaning that our recording method can give multiple ERG derivations and thus, the inverse problem is feasible. Our simulation results showed that the LORETA algorithm (i.e., obtaining a spatially smooth inverse problem solution) was not influenced by the maERG model for any scenario (highest AUC decrease of 0.00089), but the Minimum Norm algorithm (i.e., low energy solution) gave poor reconstruction quality for peripheral inactivity ($AUC < 0.1$) compared with the multi-electrode model ($AUC = 0.9998$). The reconstruction of retinal activity from recordings in healthy subjects was qualitatively similar to the healthy subject simulation meaning that our simulated and experimental data are concordant.
Conclusion: We propose a novel method for recording of multiple derivations of the ERG response by using the rotating capability of the human eye, a method that we name the maERG. We also propose a model of the human eye and a method to solve the inverse problem in order to represent local retinal activity, therefore generating a functional imaging of retinal responses. We believe that our novel technique of recording the ERG will

increase the sensitivity of the ERG, thus permitting earlier diagnosis and precise monitoring of retinopathies.
Funding: Funded by CIHR and the Réseau-Vision of the FRSQ.

17:15 Effect of pupil size on flicker ERGs recorded with RETeval system: experiments using mydriatics and artificial pupils

Mineo Kondo, Kumiko Kato, Masahiko Sugimoto, Kengo Ikesugi, Hisashi Matsubara

Ophthalmology, Mie University Graduate School of Medicine, Tsu, Japan

Purpose: At the last ISCEV meeting (Boston, 2014) we reported on the effect of pupil size on the 28.3-Hz flicker electroretinograms (ERGs) recorded by RETeval, a new mydriasis-free full-field flicker ERG system. In addition to our previous experiment using mydriatics, we also performed another experiment using artificial pupils.
Methods: Ten healthy subjects were studied. The RETeval system is designed to deliver a constant flash retinal illuminance by adjusting the flash luminance to compensate for changes in the pupil size. Two experiments were performed. First, RETeval flicker ERGs were recorded every 3 min after the instillation of mydriatics; second, RETeval flicker ERGs were recorded while the subjects wore soft contact lenses with two different artificial pupil sizes.
Results: After the instillation of the mydriatics, the amplitudes of the fundamental component of the RETeval flicker ERGs did not change significantly, but the implicit times of the fundamental component were significantly prolonged for larger pupil sizes. There was a significant positive correlation between the pupillary area and implicit times of the fundamental component ($r = 0.93$, $P < 0.001$). The results of the second experiment showed that the implicit times of the fundamental component in the RETeval flicker ERGs were significantly longer with larger artificial pupils.
Conclusions: These results indicated that the implicit times of the fundamental component in the RETeval flicker ERGs can be significantly influenced by the pupil size. Results also suggested that not only the equal flash retinal illuminance, but also other factors should be taken into consideration to obtain the same flicker ERGs with naturally constricting pupils.

17:30 Comparison of constant luminance (cd s/m^2) versus constant retinal illuminance (Td-s) stimulation in the flicker ERG

Quentin Davis[1], Olga Kraszewska[1], Colette Manning[2]

[1]LKC Technologies, Gaithersburg, MD, USA; [2]Wedgwood Optometry, Fort Worth, TX, USA

Purpose: This study measured the dependence of the flicker ERG on pupil size when using either constant luminance or constant retinal illuminance stimulation.
Methods: Flicker ERG (28.3 Hz, white light) recordings were made using one randomly chosen eye of each subject while the subject was dilating after instillation of 2 drops of 0.5 % Tropicamide. Every 3 min, a measurement was made using the ISCEV standard luminance flash energy (3 cd s/m^2) and background (30 cd/m^2) and another measurement was made using a constant Troland equivalent (assuming a 3.7 mm pupil) of 32 Td-s with 320 Td background. Measurement order was randomized. The first measurements were taken 3 min before pupil dilation and the second immediately prior to pupil dilation. The pupil diameter was measured at the stimulus frequency and was summarized as the trimmed mean of all diameters measured after the stimulus had been on for 2.5 s. Amplitudes and implicit times (IT) were evaluated for the fundamental of the waveform and for the waveform with its harmonics as a linear function of pupil size. A bisquare robust estimation of the linear slope was computed for each parameter as a function of pupil size. The RETeval device (LKC Technologies, Inc.) was used to perform all measurements using Sensor strip skin electrodes and its built-in pupil measurement capability.
Results: Preliminary findings ($n = 5$) suggest that constant Troland stimuli generate results significantly more independent of the pupil size when compared to those obtained with constant luminance flicker. Troland-based stimulation had a slight increase in the fundamental IT with pupil diameter (0.16 ms/mm) while candela-based stimulation had a 3× larger decrease in the fundamental IT with pupil diameter (−0.49 ms/mm). Harmonic-based IT showed no systematic dependence on pupil size for either stimulation type. Constant Troland stimulation showed no change in amplitude with pupil size. In contrast, constant luminance stimulation had amplitudes that increased as pupil size increased: fundamental amplitude increased on average 7 %/mm and harmonic-based peak-to-peak amplitudes increased on average 14 %/mm.
Conclusion: Constant Troland-based stimulation was shown to have less dependence on pupil size than constant luminance stimulation. With constant luminance and the more common harmonic-based analysis, implicit time was unaffected by pupil size while amplitude had a 14 % increase per mm, which may account for amplitude's greater variability, Subjects don't dilate to the same extent which in turn affects amplitudes. It is not clear why Troland-based stimulation showed effectively no dependence on three out of four flicker ERG parameters while showing a dependence on the fourth (IT of the fundamental). These results suggest that Troland-based stimulation may enable clinicians to forgo pupil dilation, making the recording of an ERG faster, easier, and more willingly performed.

17:45 The topography of spatial-frequency tuning of the steady-state pattern-onset VEP

Sven P. Heinrich, Lisa Herold, David J. Marhöfer, Michael Bach

Eye Center, University of Freiburg, Freiburg, Germany

Purpose: Steady-state VEPs are recorded to fast sequences of stimuli that are evenly spaced in time at intervals substantially shorter than the train of responses that follows a single stimulus. When recorded to checkerboard stimuli, the amplitude-versus-checksize tuning is often non-monotonic and exhibits a so-called 'notch' in many subjects, i.e. a reduced amplitude despite the pattern being easily visible. A potential explanation involves superposition of responses from separate cortical sources,

resulting in a spatially variable cancellation of the scalp potential. If true, recording the VEP with different combinations of reference and active electrodes might avoid the notch in the tuning curve, or a notch at one location might be counterpoised by an increased response at a different location ('anti-notch'). We tested this using a multi-channel EEG system.
Methods: Steady-state pattern-onset VEPs to seven different check sizes (0.011°–0.133°) were recorded from 32 active electrodes referenced to the FCz electrode. We computed all possible pairwise bipolar derivations and estimated the response corresponding to the stimulation rate through frequency-space analysis. Notches and anti-notches exceeding a certain threshold size were flagged by an automatic algorithm.
Results: Sizeable notches were found in 11 out of 41 subjects. Anti-notches mostly occurring outside the occipital half of the scalp (remote from the visual cortex) were small and did not cluster spatially. They were thus not able to individually or collectively serve as a compensation for the notches. In the cases where there was a sizable anti-notch, it occurred typically at a different checksize than the corresponding notch.
Conclusions: Re-referencing, or selecting specific bipolar derivations, does not provide a solution to the notch problem. We assume that further increasing the number of EEG channels will not substantially alter this finding. This does not rule out the superposition hypothesis, as certain local combinations of EEG sources may result in a global cancellation of surface responses, which would indicate close interweaving of function and morphology.

18:00 *iSim:* a smart ERG signal generator for calibration of instruments and alignment of recording regimes across clinical laboratories

Anthony Fisher, Rasam Teymouri, Matthew Elt

Department of Medical Physics and Clinical Engineering, Royal Liverpool University Hospital and Department of Physics, University of Liverpool

Purpose: Modern bio-amplifiers used in clinical visual electrodiagnostics are a hybrid of analogue hardware and digital processing software functions. However, the performance characteristics of these devices are specified only notionally by ISCEV, making it challenging to establish true reference norms across clinical laboratories using equipment for different manufacturers. The characterisation and calibration of bio-amplifiers is not trivial, requiring an informed understanding of the subtleties of signal-to-noise (SNR) enhancement and rejection of recording artefacts. The *iSim* smart ERG simulator has been developed to non-naively address this challenge.
Methods: An ERG reference library using deterministic and stochastic models is available (Fisher *et al.*, ISCEV Annual Symposium, Boston, 2014) which provides explicitly characterised recordings over a range of SNRs with autoregressive continuous noise and spontaneous eye movement and blink artefacts. These digital records are freely accessible over the Internet from the Liverpool MatSOAP server using an MS Excel-based toolset for characterising ERG signal processing algorithms. *iSim* is a hardware device which accesses these records as an explicitly defined panel of exemplar ERGs to generate a range of test signals with realistic continuous and discontinuous noise of precisely defined SNR, spectral content and bandwidth.
A program written in MS Excel VBA and MatLab (Mathworks) was developed which downloads ERG reference library records with user-specified frequency content, SNR (ERG *w.r.t.* ARMA autoregressive noise) and number and amplitude of spontaneous noise artefacts arising from eye movements and blinks. A 4-channel 16-bit D-to-A converter controlled by an Embed ARM microprocessor provides: i. & ii. reference ISCEV 'standard' ERG signals at low (e.g. photopic ERG) and high (e.g. PERG) gains; iii. ARMA continuous noise; iv. spontaneous discontinuous noise. These components are selectively summed and scaled in a bandpass amplifier. A graphical interface implemented on a LapTop PC over a USB connection provides the user control interface. The base bandwidth of the device spans 0.1 KHz to 1.0 KHz, and the balanced output impedance is 4.7 kohm. Trigger synchronisation is provided by either BNC hardwire connection or optical fibre. The output signals are available through standard sockets, compatible with current clinical instruments.

The comparative performance of a number of clinical instruments was assessed with a single panel representing a range of SNR's for 'standard' photopic, scotopic and pattern-reversal ERG's.
Results: All instruments tested were 'sensitive' in their recording of cardinal points of amplitude and implicit times to SNR. These sensitives differed across makes of instrument and were of magnitudes which would be interpreted as significance in a clinical scenario.
Conclusion: The *iSim* ERG signal generator provides highly realistic clinical test recordings from an Internet-based reference library for the characterisation of electrodiagnostic instruments. It is suggested that such a device might be of use in calibration within quality control systems and alignment of recording regimes across clinical laboratories. ISCEV might take the lead in developing a programme of clinical and technical governance in visual electrodiagnostic testing by recommending a standard test protocol using a standard panel of test signals delivered by *iSim*.

Friday 26.06.2015

Paper session 5: 09:00–10:45

Toxicology and treatment

09:00 Contribution of wide field imaging to the screening for vigabatrin-related retinopathy

Carl Arndt[1], Kim Vardi[1], Jacques Motte[2], Nathalie Bednarek[2], Joseph Bursztyn[3], Alain Ducasse[1], Catherine Brugniart[1]

[1]Ophthalmology, Reims University Hospital, Reims, France; [2]Paediatrics, American Memorial Hospital, Reims University Hospital, Reims, France; [3]Ophthalmology, Hôpital Necker-Enfants Malades, Paris, France

Purpose: Vigabatrin is an anti-epileptic drug widely prescribed in the treatment of infantile spasms (or West Syndrome). It inhibits GABA transaminase and therefore reduces the

occurence of seizures by increasing GABA concentration. Chronic administration of vigabatrin is known to induce retinal toxicity responsible for bilateral constriction of the visual field. Children are closely monitored by visual field testing and electroretinography (ERG) during their treatment. However, these investigations remain difficult to perform in young infants. The objective of the study was to evaluate the diagnostic yield of wide field imaging in vigabatrin retinal toxicity.
Methods: A retrospective study was performed in the department of ophthalmology at Reims university hospital, including all patients treated with vigabatrin. In each patient, modified confrontation visual field testing, a flicker electroretinogram (ERG) and ultra wide field imaging were performed. If the visual field testing was considered as abnormal or unreliable, flicker ERG was performed. A group of age matched patients with available ultra wide field imaging served as a control group. A masked interpretation of all the available wide field images (patients and controls) was performed by a trained ophthalmologist (KV).
Results: 17 vigabatrin treated patients were included; in 11 patients, flicker ERG was performed. In 12 patients, wide field imaging was available; however, the image quality was sufficient in only eight patients. 41 wide field images of vigabatrin treated patients and controls were analyzed. Among the four patients with abnormal ERG recordings, peripheral peri-vascular depigmentation was detected in three patients on wide field retinography; in one patient the wide field retinography was classified as normal. No retinal changes were detected in the control group.
Conclusions: In selected cases, wide field imaging could be useful in screening for vigabatrin-related retinopathy. However, ERG changes may be more sensitive in detecting patients at risk for visual field impairment.

09:15 Diagnostic methods in ocular argyrosis

Monika Sarnat-Kucharczyk[1,2], Ewa Mrukwa-Kominek[1,2], Dorota Pojda-Wilczek[1,2]

[1]Department of Ophthalmology, University Center of Ophthalmology and Oncology, Katowice, Poland; [2]Silesian University of Medicine, Katowice, Poland

Purpose: To present retinal changes with the use of mfERG and to describe corneal lesions observed in a patient with ocular argyrosis.
Methods: A 61-year-old male was referred for ophthalmic examination because of blurred vision. Confocal microscopy, optical coherent tomography (OCT) of the macula and mfERG were performed in a patient who was exposed to industrial silver salts for over 20 years. He was retired for 5 years and during that time he was no longer exposed to sliver compounds. UCDVA, BCVA, UCNVA, BCNVA, visual field, intraocular pressure, slit lamp biomicroscopy examination, fundus examination, photography of anterior segment were also performed.
Results: Slit-lamp examination revealed dense confluent deposits in the central and peripheral cornea, from anterior to deep stroma extending to Descement's membrane. Fundus examination was remarkable for retinal pigment epithelium changes. Confocal microscopy showed hyperreflective karatocytes, scattered, small, highly reflective deposits from anterior to deep stroma and through Descement's membrane in the central and peripheral cornea. OCT of the macula revealed large drusenoid deposits and mfERG showed decrease in central retinal function.
Conclusions: Confocal microscopy, OCT of the macula and mfERG are non invasive tools which are extremely helpful to support the diagnosis of ocular argyrosis. Confocal microscopy may allow to obtain in vivo cross-sectional images of the corneal layer, while OCT and mfERG can reveal retinal changes in ocular argyrosis.

09:30 Multifocal ERG in eyes subjected to inverted ILM flap technique for treating macular hole

Masayuki Horiguchi, Yoshiaki Shimada, Mitsuo Sugimoto, Atsuhiro Tanikawa

Department of Ophthalmology, Fujita Health University School of Medicine, Toyoake, Japan

Purpose: A macular hole can be treated surgically by removing the vitreous and performing fluid-gas exchange in the vitreous cavity. Peeling of the internal limiting membrane (ILM) increases the success rate of macular hole-closure. However, large and old macular holes may be difficult to close using only these techniques. Recently, the inverted ILM flap technique for closing of macular holes (diameter >400 μm) was reported. In this technique, the vitreous is removed and the peeled ILM is left on the retina and inverted to cover the hole, after which, fluid-gas exchange is performed. We performed multifocal ERG to study the effect of the inverted ILM on retinal function.
Methods: The single-layered inverted ILM flap technique was performed in 16 eyes with large macular holes. The ILM inferior to the macular hole was peeled and removed, and a superior ILM flap of 2-disc diameters was made after vitreous removal. This was followed by inversion of the superior ILM flap for covering the hole, and fluid-gas exchange. Thus, the macular hole and a 2-disc diameter area inferior to the hole were covered by the ILM flap. The stimulus for the multifocal ERG was newly designed to record focal ERG values in the retinas without the ILM (superior area) and from those with the inverted ILM (inferior area). Multifocal ERG was performed in operated and normal (control) eyes using a skin electrode.
Results: In all eyes, macular holes were closed. The amplitudes of the superior and inferior focal ERG from the normal eyes were 0.62 ± 0.22 and 0.58 ± 0.19 μV, respectively. The peak times of the superior and inferior focal ERG from the normal eyes were 27.6 ± 1.1 and 27.4 ± 1.2 ms, respectively. The amplitudes of the superior and inferior focal ERG from the operated eyes were 0.54 ± 1.1 and 0.51 ± 1.1 μV, respectively. The peak times of the superior and inferior focal ERG from the operated eyes were 27.7 ± 1.4 and 27.2 ± 0.5 ms, respectively. We found no statistically significant difference in the amplitude ($p > 0.16$, paired t test) and peak time ($p > 0.8$) between the retinas without the ILM and those with the inverted ILM.
Conclusions: This electroretinographic study indicates that the inverted ILM flap technique does not affect retinal function.

09:45 ERG findings after one year intravitreal Ranibizumab and single or multiple spot panphotocoagulation treatment for proliferative diabetic retinopathy

Katharina Messias, Rafael De Montier Barroso, Fabiano Sakamoto, Vinicius Castro, Amanda Marega, Rodrigo Jorge, Andre Messias

Department of Ophthalmology, Ribeirão Preto School of Medicine, Ribeirão Preto, Brazil

Purpose: To compare the effects of intravitreal Ranibizumab (IVR) with or without panphotocoagulation (PRP) using single (EDTRS) or multiple (pattern scan laser—PASCAL) spot targeting laser on retinal function in proliferative diabetic retinopathy.
Methods: A total of 44 patients were enrolled in this randomized, prospective clinical trial, and assigned to treatment with only IVR, IVR-PASCAL or IVR-EDTRS. Comprehensive ophthalmological evaluations were performed at baseline and every 4 weeks after treatment, and included full-field electroretinography (ERG) using a recording protocol in accordance with the ISCEV standard. ERGs also were recorded using an LED full-field stimulator, which first used red (635–638 nm) then blue (465–470 nm) and then white (6500 K) stimuli, with a 5 min inter-session interval at baseline and at 12, 24 and 48 weeks after treatment (Espion E2—Diagnosys LLC, Lowell, MA). PRP was performed exclusively at baseline in 2 sessions. In eyes with macular edema, macular short pulse grid laser was added to IVR at baseline. IVR was repeated monthly if the central subfield macular thickness measured with SD-OCT was higher than 300 μm, or quarterly if neovascularization was detected by angiography.
Results: IVR = 13, PASCAL = 14, and ETDRS = 13 eyes finished the 48 weeks follow-up. No significant difference was observed between groups for any ERG parameters at baseline. A significant amplitude reduction was observed in the dark and light adapted ERG stimuli for EDTRS and PASCAL groups, but not for IVR, up to 48 weeks. No difference was observed between EDTRS and PASCAL groups regarding ERG amplitude reduction. There was no significant correlation between ERG amplitude or amplitude reduction and OCT macular thickness or visual acuity.
Conclusions: These data indicate that single spot or PASCAL laser PRP in conjunction with IVR cause similar ERG amplitude reductions, which is not observed with IVR alone, up to 1 year follow-up.

10:00 Functional outcome in prospective intravitreal bevacizumab treatment of macular edema secondary to central retinal vein occlusion

Ivana Gardašević Topčić[1], Maja Šuštar[2], Marko Hawlina[2], Polona Jaki Mekjavić[2]

[1]Department of Ophthalmology, General Hospital Novo mesto, Novo mesto, Slovenia; [2]Eye Hospital, University Medical Centre Ljubljana, Ljubljana, Slovenia

Purpose: To evaluate functional improvement after intravitreal bevacizumab (IVB) treatment in patients with central retinal vein occlusion.
Methods: Twenty-two CRVO patients were treated with IVB injections and followed for 1 year. Functional effect was followed with best corrected visual acuity (BCVA), mean sensitivity (MS) evaluated by microperimetry and ERG: dark-adapted ERG of the standard full-field ERG (DA 3.0 ERG) including b:a ratio, PERG to standard (12° × 16°) stimulus and photopic negative response (PhNR). Patients underwent fundus-monitored microperimetry using a Nidek MP1 Microperimeter. DA 3.0 ERG was elicited with Ganzfeld stimulator of the Roland Consult RETI port unit. PERG was recorded by the use of Roland Consult RETIscan. PhNR was elicited with Ganzfeld Espion Color Dome stimulator. The recording electrodes were HK loops.
Results: Parameters reflecting function of central retina and outer layers of the peripheral retina showed significant improvement already after 6 months of follow-up: BCVA improved by 18.2 letters after 6 months ($p \leq 0.001$) and additional 4.7 letters by the 12th month ($p \leq 0.001$); MS improved from 7.26 ± 4.98 to 12.4 ± 4.4 ($p \leq 0.001$) and 13.4 ± 5.3 dB ($p \leq 0.001$), the standard PERG P50 amplitude from 0.2 ± 0.3 to 0.9 ± 0.6 ($p \leq 0.001$) and 1.1 ± 0.6 μV ($p \leq 0.001$); and N95 amplitude from 0.4 ± 0.6 to 1.2 ± 0.9 ($p \leq 0.001$) and 1.6 ± 0.9 μV ($p \leq 0.001$); a-wave implicit time of DA 3.0 ERG from 25.6 ± 2.3 to 24.1 ± 2.1 ($p \leq 0.01$) and 24.1 ± 2.0 ms ($p \leq 0.01$). Parameters reflecting function of inner retinal layers of peripheral retina did not show any significant improvement after 6 months, but recovered significantly after 12 months of follow-up: PhNR improved from -5.9 ± 6.6 to -10.4 ± 4.6 μV ($p \leq 0.05$) and b:a ratio of DA 3.0 ERG from 1.8 ± 0.4 to 2.0 ± 0.3 ($p \leq 0.01$).
Conclusions: This study showed a complex functional response to treatment of macular edema due to CRVO with IVB. Improvement was first reflected in BCVA, MS, PERG and a-wave implicit time of DA 3.0 ERG and later in PhNR and b:a ratio. It appears that functional improvement of the retina was gradual from outer to inner layers and from central towards peripheral retina.

10:15 Neuroprotective effect of angiotensin receptor blockers and angiotensin converting enzyme inhibitors, used for high blood pressure, on the retina of diabetes mellitus type 2 patients studied by mfERG

Graciela Garcia Briones[1], Mario Alberto Rendon M[2], Bruno Estañol Vidal[3]

[1]Department of Neuro-ophthalmology, Hospital de Nuestra Señora de la Luz, México, México; [2]Departamento de Investigación en Pediatría, Instituto Mexicano del Seguro Social, México, México; [3]Departamento de Neurologia y Neurofisiología Instituto Nacional de Nutrición, México, México

Purpose: The mfERG is abnormal in diabetic patients even before they present any sign of retinopathy. We found that diabetic patients had better mfERG results when they also had HBP (High Blood Pressure). The purpose of this study is too show the effect of angiotensin receptor blocker (ARB) and angiotensin converting enzyme inhibitor (ACEI),used for high blood pressure, on the retina of diabetic type 2 patients before they present any sign of diabetic retinopathy.

Methods: MfERG's were recorded monocularly after pupil dilation in both eyes of diabetic patients without diabetic retinopathy. They were separated into two groups, one with high blood pressure and one with normal blood pressure. First order kernel multifocal ERGs were extracted at 61 visual field locations. Amplitude and implicit time (IT) were evaluated for the multifocal ERG components N1 (first negative deflection), and P1 (first positive deflection). Five rings of the mfERG were analyzed. Patients did not have either diabetic or hypertensive retinopathy when reviewed using fundus photographs.
Results: 44 patients (88 eyes) were evaluated. mfERG's were obtained from eyes both with (N = 21) and without HBP (N = 23). The hypertensive patients were on losartan or enalapril. The demographic characteristic of both groups were similar. The mfERG was abnormal in both groups of patients in comparison with 10 normal individuals. The amplitude of P1 in the ring 2 was 61.5(18.6) nvol/dg^2 in HBP patients compared to 51.9(18.8) nvol/dg^2 ($p = 0.04$) in non-hypertensive patients. Adjusted by age, patients with high blood pressure showed better responses on the mfERG than non-hypertensive patients. In a logistic regression model of all the variables studied, (age, gender, glycosylated HB, glycaemia, dyslipidemia, HBP and smoking;)the HBP group showed a protective effect on beta standardized result for IT (implicit time) 0.35 $p = 0.002$, and Amplitude 0.30, $p = 0.007$.
Conclusions: The retina of diabetic patients shows damage even when funduscopic examination is normal. Measurement of amplitude and IT were abnormal in all the rings of the mfERG in diabetics before they showed retinopathy. A statistically significant result, showing a protective effect on retinal function in the HBP group was demonstrated by logistic regression analysis of the variables studied in Type 2 diabetic patients. We believe the protective effect of the high blood pressure variable is due to the use of angiotensin receptor blockers (ARB) and angiotensin converting enzyme inhibitors (ACEI) used to control HBP. No financial or proprietary interest in any material or method mentioned.

10:30 Multifocal ERG before and after pars plana vitrectomy in ILM peeling: one-year follow-up

Andre Messias, Ana Claudia De Lucca Perches, Felipe Almeida, Katharina Messias, Rodrigo Jorge

Department of Ophthalmology, University of São Paulo, Ribeirão Preto, Brazil

Purpose: To evaluate retinal function in eyes before and after pars-plana vitrectomy with internal limiting membrane (ILM) peeling, to determine functional/structural interrelations and investigate values predictive of post-operative visual improvement.
Methods: 51 eyes of 50 patients with idiopathic macular hole (MH) or epiretinal membrane (ERM) were included. Comprehensive ophthalmologic evaluation including best-corrected visual acuity (BCVA) was performed at baseline, 1, 3, 8, 24 and 48 weeks after surgery. Optical coherence tomography (OCT) and multifocal ERG were performed before surgery, and at 2 and 12 months after surgery. mfERG results are shown using the ratio of the amplitude averages of the 2 central rings to the averages of the 3 peripheral rings (P1 ratio),. OCT was used to calculate the macular hole index (MHI) defined as the quotient between hole height/base.
Results: 45 patients (46 eyes; 30 MH and 16 ERM) completed the 1-year follow-up. Mean ± SE (logMAR) preoperative BCVA was 0.93 ± 0.22 and 0.58 ± 0.11 for MH and ERM respectively, and improved −0.25 ± 0.07 logMAR for MH and −0.29 ± 0.06 logMAR for ERM. The mfERG P1 ratio was reduced before surgery, and increased significantly after surgery. There was significant correlation between preoperative P1 ratio and BCVA gain at week 8 for the MH group ($r = -0.42$; $p = 0.033$), but not for the ERM group. There was no correlation between preoperative MHI and postoperative BCVA.
Conclusion: These data indicate that patients with MH and poor mfERG amplitudes have worse prognoses for BCVA improvement. However, this does not apply to ERM patients.

Paper session 6: 11:15–13:00

Retinal diseases

11:15 The clinical and electrophysiological features of unilateral pigmentary retinopathy

Anthony G. Robson[1,2], Marie-Helene Errera[1], Tracy Wong[1], Philip G. Hykin[1], Bishwanath Pal[1], Mandeep S. Sagoo[1], Carlos E. Pavesio[1], Anthony T. Moore[2,3], Andrew R Webster[2,3], Graham E Holder[1,2]

[1]Electrophysiology, Moorfields Eye Hospital, London, UK; [2]Institute of Ophthalmology, University College London, London, UK; [3]Department of Genetics, Moorfields Eye Hospital, London, UK

Purpose: To review the clinical and electrophysiological features of unilateral pigmentary retinopathy (UPR).
Methods: Forty two patients with UPR were identified from the Moorfields Eye Hospital electrophysiology database. All had undergone full field and pattern electroretinography (ERG; PERG), performed to incorporate the ISCEV Standards. The clinical findings, fundus photographs and autofluorescence (AF) images were reviewed.
Results: Five patients were asymptomatic and referred after routine optometric examination. Peripheral field loss and photopsia were common, but reduced visual acuity was often a later feature. Nyctalopia was reported in <10 % of cases. Fundoscopy revealed a range of abnormalities; there was intra-retinal pigment deposition in most, but five showed pigmentary changes without bone-spicules. There was a parafoveal ring of increased AF in seven of 16 affected eyes and AF was undetectable outside the vascular arcades in 13 eyes. The ERGs showed generalized photoreceptor dysfunction with a similar degree of rod and cone involvement in the majority of eyes with abnormal pigment. There were bilateral ERG or PERG abnormalities in 10 patients, with five showing dysfunction in the fellow eye confined to the macula. A relevant medical history was ascertained in 15 cases (36 %) including AZOOR (N = 4), direct

ocular trauma (N = 4), systemic carcinoma or autoimmune disease (N = 2), retinal vasculitis, pregnancy-related hypotension and meningitis. A definite heritable disorder could be identified in only two patients; one with a dominant family history of RP1 and the other a female carrier of X-linked RP.

Conclusions: UPR is most commonly associated with non-genetic aetiologies such as AZOOR and trauma. The majority of cases show unilateral generalised retinal dysfunction with a similar degree of rod and cone dysfunction. Chromosomal mosaicism is a possible cause of true unilateral RP in the rare cases where a genetic aetiology can be identified.

11:30 Electrodiagnostic testing facilitates the identification of two novel retinal dystrophy genes: a new paradigm for disease gene discovery

Panagiotis I. Sergouniotis[1,2], Anthony G. Robson[1], Uwe Wolfrum[3], Eva Lenassi[1], Ucl-Exomes Consortium, Martin Mckibbin[4], Manir Ali[5], Vincent Plagnol[6], Andrew R. Webster[1], Graham E. Holder[1]

[1]Moorfields Eye Hospital and UCL Institute of Ophthalmology, London, UK; [2]Manchester Royal Eye Hospital and University of Manchester, Manchester, UK; [3]Institute of Zoology, Johannes Gutenberg University of Mainz, Mainz, Germany; [4]St. James's University Hospital, Leeds, UK; [5]University of Leeds, Leeds, UK; [6]UCL Genetics Institute, London, UK

Purpose: To combine electrophysiology and detailed phenotyping [including fundus autofluorescence imaging (FAF)] with state of the art genetic testing in order to gain insights into the molecular pathology of retinal dystrophies and to discover novel disease-associated genes.

Methods: Exome sequencing (i.e. DNA sequencing of the interpretable, protein-coding part of the genome) was performed in 28 unrelated patients and 1750 controls (UCL-exomes controls). All patients had received electrophysiological testing and inclusion criteria were (1) a retinal dystrophy phenotype with early cone photoreceptor involvement; (2) no previous genetic testing or an unknown molecular diagnosis after previous genetic screening; and (3) an absence of features such as retinal flecks and peripapillary sparing on FAF that may have suggested ABCA4-retinopathy (Stargardt disease). Genetic data from cases and controls were analysed using the same bioinformatic methods and a gene-based case–control association study performed.

Results: The case–control association study revealed TTLL5 mutations as a novel cause of retinal dystrophy when focussing on presumed loss-of-function DNA variants. A second novel disease-associated gene was highlighted when investigating likely disease-causing changes acting in an autosomal recessive fashion. The role of these two genes in retinal dystrophies was confirmed by the identification of further affected families and through localisation studies of the associated proteins in the human retina. Overall, the molecular diagnosis was identified in 15/28 probands; changes in the PROM1, RPGR, ABCA4 and CDH3 genes were detected and a previously unreported autosomal dominant macular dystrophy phenotype caused by CRX mutations was identified.

Conclusions: Electrophysiology was used to identify a group of patients with a homogeneous clinical phenotype, predominantly affecting the cone system. The genetic diagnosis was identified in the majority of probands by exome sequencing, with important implications for the affected families in terms of counseling and access to future gene therapy trials. Genetic testing is becoming increasingly available in the clinical setting and approaches based on electrophysiological testing and careful phenotyping are expected to increase significantly the sensitivity and specificity of such tests. The value of electrophysiology as a diagnostic and monitoring tool in retinal dystrophies is currently well established; the present study suggests this is likely to continue in the post-genomic era.

11:45 Japan whole exome project for inherited retinal diseases 2014

Kaoru Fujinami[1,3], Takaaki Hayashi[4], Kazuki Kuniyoshi[5], Mineo Kondo[6], Shinji Ueno[7], Kei Shinoda[8], Kazuo Tsubota[2], Yozo Miyake[1], Kazushige Tsunoda[1], Takeshi Iwata[1]

[1]National Institute of Sensory Organs, National Tokyo Medical Center, Tokyo, Japan; [2]Department of Ophthalmology, Keio University, School of Medicine, Tokyo, Japan; [3]UCL Institute of Ophthalmology, London, UK; [4]Department of Ophthalmology, The Jikei University School of Medicine, Tokyo, Japan; [5]Department of Ophthalmology, Kinki University Faculty of Medicine, Osaka-Sayama City, Japan; [6]Department of Ophthalmology, Mie University Graduate School of Medicine, Tsu, Japan; [7]Department of Ophthalmology, Nagoya University Graduate School of Medicine, Nagoya, Japan; [8]Department of Ophthalmology, Teikyo University School of Medicine, Tokyo, Japan

Purpose: We describe the clinical and genetic characteristics of a large cohort with inherited retinal diseases in the Japanese population.

Methods: A cohort of 808 subjects from 496 Japanese families with inherited retinal diseases was ascertained between 2008 and 2013. The clinical diagnosis was determined with comprehensive ophthalmic examinations including fundus photography, autofluorescence imaging, optical coherence tomography, visual field test, and full-field, focal macular and multifocal ERGs at 12 institutes throughout the nation; then the clinical data were uploaded to the National Institute of Sensory Organs (NISO) databank. The cohort included retinitis pigmentosa (RP; 208 families), occult macular dystrophy (OMD; 64 families), Leber congenital amaurosis (LCA; 23 families), cone (-rod) dystrophy (42 families), macular dystrophy (34 families), Stargardt disease (34 families), congenital stationary night blindness (3 families), and others. Genetic screening and molecular analysis were performed in 387 subjects from 159 families; whole exome sequencing was applied in 147 families and targeted direct sequencing (for RP1L1 or CNGA1) in 12.

Results: Disease-associated mutations were detected in 71/159 (45 %) families, including previously reported mutations in 29/159 (18 %), novel mutations of previously reported genes in 32/159 (20 %), and mutations of putative new associated genes never reported in inherited retinal disease in 10/159 (6 %). No definitive disease-associated variants were found in 88/159 (55 %) families. Previously reported mutations were detected in 9/61 (15 %) of RP, 11/36 (31 %) of OMD, and 3/13 (23 %) of LCA. Novel mutations of previously reported genes were

identified in 13/61 (36 %) of RP, 6/36 (17 %) of OMD, and 5/13 (38 %) of LCA. Putative new genes were associated in 5/61 (8 %) of RP and 3/13 (23 %) of LCA.
Conclusions: Conclusive molecular genetic diagnosis was obtained in 45 % of this Japanese cohort, a value similar to other cohorts including autosomal dominant and X-linked disorders in European/American populations. Novel mutations of previously reported genes or putative new associated genes were frequently revealed compared to other populations, which suggests the distinctive genetic background of Japanese population in inherited retinal diseases.
Funding: This research was supported in part by research grants from the Ministry of Health, Labor and Welfare, Japan and Grant-in-Aid for Scientific Research from Japan Society for the Promotion of Science; Grant-in-Aid for Young Scientists (B) of the Ministry of Education, Culture, Sports, Science and Technology (Japan). The sponsor or funding organization had no role in the design or conduct of this research.

12:00 ERG in Slovenian patients with Usher syndrome

Ana Fakin, Martina Jarc-Vidmar, Branka Stirn Kranjc, Maja Šuštar, Jelka Brecelj, Marko Hawlina

Eye Hospital, University Medical Centre Ljubljana, Ljubljana, Slovenia

Purpose: To evaluate ERG responses in Slovenian patients with Usher syndrome.
Methods: Retrospective study included 23 Usher patients (10 male, 13 female; 9 USH1, 15 USH2; average age 31 ± 13 years, range 7–56; average onset of nyctalopia 15 ± 11 years) who were examined by full-field (N = 19), PERG (N = 14) and/or mfERG (N = 9) according to ISCEV protocols. Average values for both eyes were calculated. Examination included Snellen visual acuity, fundus autofluorescence imaging (Spectralis, Heidelberg) and Goldmann visual fields.
Results: Response above noise level to at least one of full-field ERG stimulus was detectable in 7/19 (37 %) patients and non-detectable in 12/19 (63 %) patients. Average maximal response b wave amplitude was 10 ± 3 μV (N = 6; normal limit 169 μV), average cone response b amplitude was 8 ± 4 μV (N = 4; normal limit 101 μV) and average 30 Hz cone response amplitude was 5 ± 3 μV (N = 4; normal limit 64 μV). None of the patients had detectable rod or oscillatory responses above noise level. Patients with detectable responses were younger (avg. 22 ± 5 vs. 35 ± 11; $p < 0.05$) and had shorter disease duration (avg. 7 ± 8 vs. 21 ± 11 years; $p < 0.05$) than patients without detectable responses on full-field ERG. In 4/8 (50 %) of the latter PERG and/or mfERG showed detectable central retinal responses. Those patients had significantly shorter disease duration (17 ± 4 vs. 29 ± 4 years; $p < 0.01$). All patients with detectable central retinal responses had a hyperautofluorescent ring on fundus autofluorescence (N = 15) whereas patients with undetectable central responses had either ring (N = 2) or patch pattern (N = 2). There was correlation between the amplitude of 30 Hz response and radius of II/4 Goldmann stimulus (Pearson's correlation R = 0.74; $p < 0.05$). Responses were consistently detectable only in the eyes with visual field radius larger than 20 degrees. There was a correlation between the first mfERG ring amplitude and visual acuity (Pearson's correlation R = 0.71; $p < 0.01$). Amplitude of mfERG responses were lower than normal in all eyes, including six with Snellen visual acuity of 1,0.
Conclusions: Patients with Usher syndrome aged 7–56 years had no detectable rod responses on full-field ERG whereas cone responses were either non-detectable or very low (<10 % of normal values) and correlated well to remaining visual field. Central retinal responses were detected in half of the patients with non-detectable full-field responses and were associated with hyperautofluorescent ring on fundus autofluorescence imaging. MfERG responses were abnormal in all patients and correlated well with visual acuity.

12:15 Interesting phenotype and electrophysiological findings in a Slovene family with ABCA4 mutation

Martina Jarc-Vidmar[1], Ana Fakin[1], Maja Sustar[1], Jelka Brecelj[1], Branka Stirn-Kranjc[1], Damjan Glavač[2], Marko Hawlina[1]

[1]Eye Hospital, University Medical Centre Ljubljana, Ljubljana, Slovenia; [2]Department of Molecular Genetics, Institute of Pathology, Faculty of Medicine, Ljubljana, Slovenia

Purpose: To report phenotype and electrophysiological results in a Slovene family with homozygotic mutation in exon 47 of the ABCA4 gene c.6445C>T, p.R2149X. Screening for mutation was done by next generation sequencing (NGS) technology and segregation of the mutation within the family was confirmed by Sanger sequencing.
Methods: Four affected members in family KR (41 year old sister, 38 year old brother and two daughters of the cousin—12 and 8 years old girls) with visual loss in the first year of primary school, impaired colour vision and central scotoma, were tested with pattern ERG, multifocal ERG and full field ERG. Autofluorescence imaging (AF) of the fundus and OCT was done by HRA.
Results: Visual acuity was severely affected in both older patients (0.01 bilaterally), whereas it was RE: 0.04, LE: 0.03 in the older girl, and RE: 0.5, LE: 0.25 in the younger girl. In the older two patients, extensive central atrophy with numerous bone spicule pigmentations (resembling those in RP) was found in the fundus, whereas central bull's eye atrophy with some yellowish pigmentations around in the periphery was found in the girls. AF showed extensive hypofluorescent areas centrally and in the periphery in the older patients and central hypoautofluorescence with scattered hyperautofluorescent spots in the girls. OCT showed atrophy of photoreceptor layers. Electrophysiology in the older two patients showed generalized cone and rod dysfunction and PERG was not detectable, whereas it showed dysfunction of central retina with reduced PERG and mfERG and generalized cone dysfunction in the younger girl and generalized cone and rod dysfunction in the older girl.
Conclusions: ABCA4 mutation was found in these all members of the family. Fundus in the younger two girls showed changes

typical for Stargardt dystrophy—fundus flavimaculatus, whereas extensive bone spicule pigmentation with central atrophy was seen in the older two patients, resembling fundus in patients with advanced AR cone-rod dystrophy. It is currently believed that homozygous ABCA4 mutations can cause different clinical phenotypes: autosomal recessive RP, Ar cone-rod dystrophy and Stargardt-fundus flavimaculatus.

12:30 Inner retinal macular dysfunction in patients with 'macula-on' rhegmatogenous retinal detachment

Kunihiko Akiyama[1,2], Kaoru Fujinami[1,2,3,4], Ken Watanabe[1,2], Natsuko Nakamura[1,2], Toru Noda[1,2], Yozo Miyake[2,5], Kazushige Tsunoda[1,2]

[1]Department of Ophthalmology, National Tokyo Medical Center, Tokyo, Japan; [2]National Institute of Sensory Organs, National Tokyo Medical Center, Tokyo, Japan; [3]Department of Ophthalmology, Keio University, School of Medicine, Tokyo, Japan; [4]UCL Institute of Ophthalmology, London, UK; [5]Aichi Medical University, Aichi, Japan

Purpose: To assess the macular function of patients with macular-on rhegmatogenous retinal detachment using the focal macular ERG (FMERG).
Methods: Seven patients with bullous rhegmatogenous retinal detachment, who did not show foveal detachment on optical coherence tomography, were recruited. The FMERG was recorded in both affected and fellow eyes with a commercially available system (ER80; Kowa, Japan). The luminance of the stimulus and background were 115.7 and 8.0 cd/m^2, respectively. The stimulus duration was 100 ms (Fujinami K et al., Arch Ophthalmol 2011; 129:597–602.). The spot size was 15 degrees in diameter and the macula was stimulated under careful monitoring with the infrared fundus camera. At least 300 responses were averaged for the analysis. The amplitude and implicit time of the a-wave, b-wave, and OPs were measured. The average amplitude and peak latency of the first three peaks were used for the analysis of OPs (Terasaki H et al. Invest Ophthalmol Vis Sci 2001; 42: 229–34.). To evaluate the macular dysfunction, comparison between the affected and the fellow eyes was performed in each parameter by using Wilcoxon signed-rank test. The statistical significance was defined as $p < 0.05$.
Results: The median age of the seven patients was 54 years (range, 51-63). Two were female and five were male. None of these patients had any ocular disorder in the fellow eye. The median a-wave amplitude and implicit time of the affected/fellow eyes were 0.84 μV (0.35-1.20)/0.88 μV (0.64-1.76) and 20.1 ms (19.0-20.5)/20.0 ms (18.6-20.9), respectively; the median b-wave amplitude and implicit time were 2.04 μV (1.02-4.85)/3.81 μV (3.02-5.89) and 40 ms (35.7-44.2)/39.4 ms (37.0-40.9), respectively. The median amplitude and implicit time of the averaged OPs of the affected/fellow eyes were 0.47 μV (0.25-1.09)/0.70 μV (0.53-1.12) and 32.5 ms (30.4-35.0)/31.4 ms (30.5-31.6), respectively. Comparison analysis revealed statistically significant differences in the b-wave amplitude ($p = 0.028$) and the implicit time of OPs ($p = 0.028$). There was a suggestion of difference in the amplitude of OPs (though the calculated value did not reach the significance; $p = 0.128$).
Conclusions: The b-wave amplitude of FMERG was significantly reduced compared to the fellow eyes in patients with macula-on retinal detachment, whereas the a-wave amplitude of the affected eyes was relatively maintained. These findings indicate that the function of the inner retinal layers was affected even in the area without retinal detachment, whilst there was no evidence of the photoreceptor dysfunction in the attached macula.

12:45 Retinal dystrophy with supernormal and delayed rod ERG b-waves: a possible explanation

John Robson

University of Houston College of Optometry, Houston, Texas, USA

Purpose: To examine the hypothesis that the phenotypic features of the dark-adapted ERG seen in cases of 'retinal dystrophy with supernormal and delayed rod ERG b-waves' are the result of a reduction in size (diameter) of those parts of the rod (axon and cell body) that lie in the outer nuclear layer (ONL) of the retina.
Methods: To consider, and where possible to simulate, the effects upon the dark-adapted ERG of reducing the diameter of the proximal parts of rods in the context of the phenotypic features of this retinal dystrophy. These features are a) an a-wave in response to strong stimuli whose recovery towards the baseline from the initial peak is delayed, b) a b-wave that only appears with stronger than normal stimuli, c) a dependence of b-wave amplitude upon stimulus energy that is much steeper than normal, d) a b-wave latency (and implicit time) with weak stimuli that is longer than normal and e) a maximum b-wave amplitude that is greater than normal.
Results: (a) The initial transient portion of the a-wave in response to strong stimuli is generated by capacitive axonal membrane current flowing in the extracellular space of the ONL. This voltage would be very greatly reduced by the decrease in the electrical resistance of the extracellular path in this retinal layer that would result from a reduction in diameter of the cell bodies and axons of the rods. (b) Reducing the diameter of the rod axon would increase its electrical resistance. This would attenuate the signal reaching the synaptic terminal. The attenuation could be great enough that single photon signals from rods would no longer be effectively transmitted to rod bipolar cells. (c) The need to have n photoisomerisations in a rod to generate a signal large enough to be effectively transmitted would result in bipolar-cell response being proportional to the nth power of the stimulus energy. If n is greater than 1 then the amplitude increases more rapidly with stimulus energy than normal. (d) Attenuation of the rod signal implies that the threshold for transmission would only be reached at a later time than normal. In the limit the threshold would only be reached when the rod signal had reached its peak. (e) There does not appear to be any way in which changes restricted to photoreceptors could result in an increase in b-wave amplitude.
Conclusions: Most of the features of "retinal dystrophy with supernormal and delayed rod ERG b-waves" would be explained by a reduction in size of the proximal parts of the photoreceptors without change in basic function. The hypothesis could be directly evaluated by observation of the structure of the ONL in affected retina.

Paper session 7: 14:30–16:00

Animal electrophysiology

14:30 Introductory lecture: Oxidative retinopathies in neonatal rats: subtle onset with long-term devastating consequences

Pierre Lachapelle, Anna Polosa, Allison L. Dorfman, Samaneh Chaychi, Xiaojuan Yang

Department of Ophthalmology and Neurology-Neurosurgery, McGill University - MCH, Montreal, Canada

Purpose: Our laboratory has been focused on exploring various models of environmental oxidative stress including light-induced retinopathy (LIR) and oxygen-induced retinopathy (OIR). The former mimics Retinitis Pigmentosa and Age-related Macular Degeneration and the latter mimics Retinopathy of Prematurity. While previous work of ours has involved the characterization of the acute pathophysiological processes, our more recent studies have been aimed at further elucidating the long term anatomical, biochemical and functional sequelae.
Methods: The LIR model was generated by exposing animals to bright cyclic light (12D:12L; 10,000lux), while the OIR model was generated by exposing animals to 80 % O2 for 22.5 h. In order to examine the long term features that characterize our models of oxidative stress, analysis of retinal histology and vasculature, neurotrophic factor expression, flash and multifocal electroretinograms and visual evoked potentials were performed. The latter experiments were conducted in young rats at different pre-determined time points [from postnatal day (P) 60 up until 1 year of age]. Finally, various therapeutic avenues were explored with the goal of limiting these detrimental effects of light and oxygen, respectively.
Results: Exposure of the immature retina (<P30) to light or oxygen results in relatively less pronounced damage than that which is known to characterize the adult form of these retinopathies. For example, immediately following exposure, the juvenile form of LIR is characterized by a confined region of significant outer retinal damage [outer nuclear layer thinning] with concomitant functional loss as evidenced with the significantly reduced a-wave. By sharp contrast, the pathogenesis of OIR is limited solely to the inner retina [outer plexiform layer thinning along with decreased horizontal cell count] with concomitant b-wave attenuation. With progressive maturation, both retinopathies reveal an almost completely altered retinal structure [outer and inner retina] and function [attenuated ERGs]. Exogenous antioxidant treatment (Trolox C and estrogen) can partially limit the devastating consequences of exposure, while the observed endogenous upregulation of various neurotrophic factors (FGF-2 and CNTF) shortly following exposure might help to explain the relative protection of young exposed rat pups.
Conclusions: Despite our earlier studies that suggested a particular site of susceptibility in LIR and OIR respectively, follow-up long term studies reveal not only a widespread extension of this damage towards the periphery, but also extension throughout the different retinal layers. Our findings therefore suggest that the pathogenesis of LIR and OIR is characterized by a two-phase process beginning with an early destructive phase and ending with a degenerative phase that extends into adulthood. Further elucidation of these processes will be instrumental in order to better understand the pathophysiology of the human forms of these retinopathies. Supported by CIHR, NSERC, and by CIHR and FRSQ, under the frame of E-Rare-2, the ERA-Net for Research on Rare Diseases.

15:00 Bright light exposure in photoreceptor-only rodent model: unexpected findings

Anna Polosa[1*], Suna Jung[1,2*], Fares Antaki[1*], Hyba Bessaklia[1], Pia Wintermark[2], Pierre Lachapelle[1]

[1]Department of Ophthalmology and Neurology-Neurosurgery, McGill University/MCH, Montreal, Canada; [2]Newborn Medicine, Montreal Children's Hospital, Montreal, Canada; *Theses authors contributed equally to this work

Purpose: In rodents, exposure to a bright light environment creates a retinopathy known as Light-Induced Retinopathy (LIR). In this model, the main damage occurs to the photoreceptor layer, while the inner retina remains well preserved even long after the initial insult. Our previous studies demonstrated that compared to adult rats, juvenile rats are more resistant to light-induced damage, a protection that was attributed to a higher up-regulation of neurotrophic factors secreted by the inner retina. In contrast, the Vannucci model, a model of neonatal hypoxic-ischemic (HI) brain injury, causes a retinopathy that spares the photoreceptors while destroying the inner retina. In the present study, we combined the two models to determine the importance of inner retinal contribution to photoreceptor survival.
Methods: At postnatal day (P) 10, Long Evans rats were subjected to the Vannucci procedure [unilateral ligation of the left common carotid artery combined with 2 h of hypoxia (8 % oxygen)] to induce destruction of the inner retina [HI group; $n = 9$]. Due to known variations in the severity of inner retinal layer cell loss in this model, HI rats were categorized into two groups based on the ERG b- to a-wave amplitude ratios [mild (b/a ratio > 1; $n = 3$) and severe (b/a ratio < 1; $n = 6$)] prior to the light exposure (at P17). HI pups were then exposed to 10,000 lux of bright white light (12 h light/12 h dark; both pupils dilated) for a total of 10 days (P18–28) [HI-light group; $n = 9$]. The degree of retinal damage was then assessed in both eyes with flash ERGs at P30 and P180, and with retinal histology at P180. HI-only, Light-only and untreated rats served as control.
Results: At P30, no significant difference in the amplitude of the a-wave was observed between groups [HI-light (mild): 296 ± 80 μV; HI-light (severe): 250 ± 44 μV; HI-only: 343 ± 31 μV; Light-only: 321 ± 50 μV and control: 372 ± 66 μV, $p > 0.05$], whereas the b-wave amplitude was only significantly reduced in the HI-only [38 % of control] and HI-light [mild: 18 %; severe: 63 % of control] groups. From P30 to P180, the a-wave amplitude was reduced to the same extent for the light-only, the HI-only and the HI-light severe groups [by 57, 52 and 62 %, respectively]. No significant difference ($p > 0.05$) was observed in the HI-light mild and control groups. Retinal histology revealed that while a complete loss of photoreceptors occurred in the superior central retina of the light-only group by P180, this portion of the outer retina was relatively well preserved in all HI groups.

Conclusions: Despite significant loss of the inner retina prior to the light exposure, photoreceptor structure and function were better preserved in photoreceptor-only retinas. Our findings thus suggest that the contribution of the inner retina to photoreceptor survival is less critical than originally thought and further suggests that the photoreceptor cells might depend, at least in part, on other sources for their survival, such as the RPE and/or the choroid.

15:15 Silent substitution isolation of rod and cone function in mice transgenic for human L-opsin

Tina Tsai[1,2], Jenny Atorf[1,2], Maureen Neitz[3], Jay Neitz[3], Jan Kremers[1]

[1]Department of Ophthalmology, University Hospital Erlangen, Erlangen, Germany; [2]Department of Biology, Animal Physiology, FAU Erlangen-Nuremberg, Erlangen, Germany; [3]Vision Sciences, University of Washington, Seattle, Washington, USA

Purpose: Robust isolation of rod- or M-cone only photoreceptor function using silent substitution in the normal mouse has been limited by their close absorption spectra. We applied this technique on a newly engineered mouse transgenic for the human L-opsin (Opn1lwLIAIS or LIAIS) to assess whether the increased spectral separation in this variant grants a higher degree of photoreceptor subtype-specific isolation.
Methods: Flicker ERGs tailored to rod- or cone-isolation, using double silent substitution using a six primary stimulator (Q450 SC, Roland Consult), were measured in anaesthetized (Ket:Xyl, 60:5 mg/kg) wildtype (WT; $n = 4$), heterozygous (LIAIS±; $n = 8$) and LIAIS mice ($n = 15$). Sinusoidal flicker was presented at temporal frequencies of 4–30 Hz, 39 cd/m^2 mean luminance and with the highest achievable rod and cone contrasts for each condition (cone 5 and 55 %, rod 5 and 75 % for WT and mutants, respectively). ERGs underwent Fourier analysis to extract amplitude and phase of the fundamental component, and plotted as a function of temporal frequency. Frequency-phase relationships were described with a linear regression to obtain a slope parameter of phase delay.
Results: The largest cone and rod driven amplitudes were evoked in LIAIS mice, followed by LIAIS± (65 ± 15 and 54 ± 8 % of LIAIS rod and cone ERGs, respectively), and lastly, WT mice (approx. 10× smaller than LIAIS). Despite differences in overall amplitude between mouse variants and photoreceptor types (cone 2.5× larger than rod), their amplitude and phase behaviors as a function of stimulus frequency were largely similar (one-way ANOVA between phase slopes $p < 0.01$). Amplitudes showed low-pass characteristics that gradually decreased with increasing temporal frequency. Phases were negatively and linearly correlated with frequency increase (between 11°–16°/Hz or 31–44 ms delay), with rod- and cone-driven phases shifted consistently by about 180°. Comparable linear trends were also seen in rod and cone amplitudes as a function of stimulus contrast, with cones showing a stronger increase with contrast increase.
Conclusions: Silent chromatic substitution in LIAIS did not alter post-receptoral processing as measured by the ERG, complementing previous anatomical studies. The LIAIS mouse thus provides a novel model to study the normal physiology of individual photoreceptor driven signals in the mouse retina.

15:30 Sildenafil as a potential therapy for visual impairments associated with birth asphyxia: a rat study

Suna Jung[1,2,3], Zehra Khoja[1], Aaron Johnstone[1], Pierre Lachapelle[2,3], Pia Wintermark[1,3]

[1]Department of Pediatrics, Division of Newborn Medicine, McGill University Health Centre, Montreal, Canada; [2]Department of Ophthalmology, McGill University Health Centre, Montreal, Canada; [3]Department of Neurology and Neurosurgery, McGill University Health Centre, Montreal, Canada

Purpose: Brain injury associated with birth asphyxia is the most important cause of pediatric visual impairments in developed countries. However, we have previously demonstrated in a rat model of neonatal hypoxic-ischemic encephalopathy that there is concomitant retinal injury, affecting predominantly the inner retina. The purpose of this study was to test the neurorestorative role of sildenafil in retinal injury following neonatal hypoxia–ischemia (HI).
Methods: Neonatal HI was induced in male Long-Evans rat pups at postnatal day 10 (P10) by left common carotid ligation followed by 2-h exposure to 8 % oxygen. Sham operated rat pups served as control. Both groups were administered 0 (vehicle), 2, 10 or 50 mg/kg of sildenafil twice daily by oral gavage, starting 12 h post-HI for 7 consecutive days. Flash electroretinograms (ERGs) were recorded from both eyes at P29 to assess the retinal function. Subsequently, retinal histology was performed to assess the retinal structure.
Results: The ERGs recorded from the left (ipsilateral to the carotid ligation) eyes of HI rat pups treated with vehicle showed a significant reduction in the scotopic and photopic b-wave amplitudes, and to a lesser extent a reduction in the a-wave amplitude. Consistent with these findings, structural damage occurred predominantly in the outer plexiform layer and the inner retinal layers. HI rat pups treated with a low dose of sildenafil (2 mg/kg) showed a preservation of the a-wave amplitude, but not the scotopic and photopic b-wave amplitudes, with an increase in the outer plexiform layer thickness compared to the HI-Vehicle group. In contrast, HI rat pups treated with higher doses of sildenafil (10 and 50 mg/kg) showed a preservation of both the a-wave and the scotopic and photopic b-wave amplitudes. These rats also displayed a greater structural preservation, showing no significant difference in the thickness of the retinal layers compared to control.
Conclusions: Our findings demonstrate that sildenafil may improve retinopathy following neonatal HI at the level of function and structure. More specifically, these restorative effects appear to be dose-dependent.

15:45 Modulating of ocular inflammation with macrophage migration inhibitory factor is associated with notch signaling in experimental autoimmune uveitis

Bo Lei[1,2,3], Hongxia Yang[1], Shijie Zheng[1], Yingying Mao[4], Hongwei Li[4], Zihe Chen[1]

[1]Ophthalmology, The First Affiliated Hospital of Chongqing Medical University, Chongqing, China;

[2]Chongqing Key Laboratory of Ophthalmology, Chongqing, China; [3]Chongqing Eye Institute, Chongqing, China; [4]School of Biotechnology, Southern Medical University, Guangzhou, China

Purpose: Macrophage migration inhibitory factor (MIF), a proinflammatory cytokine operating in innate and adaptive immunity, is associated with many immune-mediated diseases. We examined the function of MIF in a mouse model of experimental autoimmune uveitis (EAU) and explored the underlying mechanism.
Methods: Mutant AAV8 (Y733F)-CBA-MIF or AAV8 (Y733F)-CBA-eGFP vector was delivered subretinally into B10.RIII mice, respectively. Three weeks after vector delivery, EAU was induced with subcutaneous injection of IRBP161-180 peptide (SGIPYIISYLHPGNTILHVD). At day 14 post immunization, the effect of MIF on EAU was evaluated by using the severity of intraocular inflammation and the function of the retina, as well as by the expression of Notch receptors. heir ligands and a target gene Hes-1 were detected with Western blotting in both MIF and eGFP injected EAU mice groups. An inhibitor of Notch signal pathway DAPT was administrated to MIF injected EAU group. Its effect on intraocular inflammation was evaluated in the MIF injected EAU group and the MIF injected EAU plus the DAPT group. On the other hand, a MIF antagonist ISO-1 was intraperitoneally injected into EAU mice, and the effect of ISO-1 on intraocular inflammation and Notch signal pathway were examined in the EAU plus vehicle and EAU plus ISO-1 groups. Intraocular macrophage recruitment was examined by detecting F4/80+ cell staining. Intracellular IFN-γ and IL-17 in CD4+ T cells were measured by flow cytometry. The levels of proinflammatory cytokines TNF-α, IL-1β and IL-6 were detected by real-time PCR and ELISA. Retinal function was evaluated with electroretinography (ERG).
Results: Expressions of MIF and its two receptors CD74 and CD44 increased in the EAU mouse retina. Compared to eGFP injected and untreated EAU mice, the levels of proinflammatory cytokines TNF-α, IL-1β and IL-6 in the retina increased, ERG amplitudes decreased, and Notch1, Notch4, Dll4, NICD and Hes-1 expressions increased in MIF injected EAU mice. DAPT inhibited the expressions of proinflammatory cytokines in MIF injected EAU mice. Besides, ISO-1 attenuated intraocular inflammation, reduced intraocular macrophage recruitment and inhibited the differentiation of Th1 and Th17 in EAU. ISO-1 inhibited the activity of Notch signal pathway.
Conclusions: The expressions of MIF and its receptors are elevated in EAU mice. Over-expression of MIF exaggerates ocular inflammation. The exaggerated inflammation is associated with activation of the Notch signaling. Our data suggest that the MIF-Notch axis may play an important role in the pathogenesis of EAU. MIF and Notch signal pathways may be promising targets for developing novel interventions for uveitis.

Adachi award lecture: 19:00–20:00

Metabolic disease with choroidal atrophy: gyrate atrophy and LCHADD

Richard Weleber

Casey Eye Institute, Oregon Health and Science University, Portland, OR, USA

This presentation will review two metabolic disorders: gyrate atrophy with hyperornithinemia from deficiency of ornithine-delta-aminotransferase, and long-chain 3-hydroxyacyl-CoA dehydrogenase (LCHAD) deficiency, also referred to as LCHADD. These two diseases represent systemic deficiencies of enzymes that result in a severe atrophy of the choroid and retina as well as involvement in organ systems other than the eye. The genetic basis, enzyme deficiency, biochemistry, pathophysiology, and findings on evaluation, including electrophysiology, will be reviewed for these two diseases. During the final portion of this lecture, a new and innovative analysis of the mfERG will be presented.

Saturday, 27.06.2015

Paper session 8: 9:00–10:45

Retinal function and structure

9:00 Evidence for an asymmetrical effect of type 1 diabetes on retinal structure and function

Thomas Wright[1], Alan Poon[2], Ahmed Salem[1], Carol Westall[1,2]

[1]Neuroscience and Mental Health, Hospital for Sick Children, Toronto, Canada; [2]Institute Of Medical Sciences, University of Toronto, Toronto, Canada

Purpose: Diabetic retinopathy has been classically considered a microvascular complication of diabetes. Current research has demonstrated sub-clinical deficits in both the neuronal and vascular retina occurring early in the disease. This study describes changes observed in the outer neuronal retina of an adolescent and young adult population with type 1 diabetes (T1D) before the onset of clinically recognized diabetic retinopathy.
Methods: An adolescent and young adult population with T1D and no background diabetic retinopathy underwent standard mfERG testing and adaptive optics enhanced retinal imaging (AO). Subjects were followed longitudinally for a period of up to 6 years. An age similar control population also underwent testing. MfERG recordings were divided into retinal hemispheres (nasal/temporal) and analyzed for the amplitude and implicit time of the first peak component (P1). AO images of the cone photoreceptor mosaic were obtained for the four retinal quadrants at a distance of 7 degrees from the fovea. Cone photoreceptors were manually identified and cone densities calculated. Linear mixed-effect modeling was used for analysis; hexagons and tests were treated as repeated measures.
Results: 54 participants with T1D (mean age 15.8 $\pm$ 2.0 years) and 71 control participants (17.5 4.7 years) underwent mfERG testing. Of these, 30 participants with T1D and 46 control participants also underwent AO imaging. In both groups there was a significant difference in P1 amplitude and implicit time between the retinal hemispheres ($p \ll 0.001$). Nasal responses were reduced and delayed compared with responses from the temporal retina. There was reduction in mfERG P1 amplitude ($p = 0.03$) and delay in P1 implicit time associated with T1D ($p < 0.001$). The interaction between retinal hemisphere and diabetes status was also significant for P1 amplitude ($p = 0.05$). Post-hoc analysis revealed that as the duration of the disease increased, the amplitude difference

between the nasal and temporal hemispheres decreased. The AO imaging data also showed a reduction in cone density of the nasal vs. temporal retina ($p = 0.03$) but no effect of T1D ($p = 0.4$). Interestingly there was a suggestion of a hemisphere specific decrease in cone density, preferentially affecting the nasal retina ($p = 0.08$) in participants with T1D.
Conclusion: These findings confirm previous reports of changes opf retinal function occurring before the onset of diabetic retinopathy. These results suggest that the nasal hemisphere of the retina is more susceptible to early damage. Previous study of the interhemispheric differences in mfERG responses in typically developing participants indicated that the greatest difference occurs in the peripheral retina (Sutter E., Tran D.; Vis Res 1992;3:433-46). It remains to be seen if the peripheral retina is also more susceptible to early damage from diabetes.

9:15 Comparing the retinal structure, function and biochemistry in an animal model of spontaneous diabetes

Serge Rosolen[1], Sylvie Lavillegrand[2], Stephanie Lafarge-Beurlet[3], Pierre-Francois Isard[4], Charles Cassagnes[5], Thomas Dulaurent[4], Serge Picaud[1], Patricia Crissanti[6], Marianne Berdugo[6]

[1]Institut de la Vision, INSERM UMR_S 968, UPMC Univ Paris 06, CNRS UMR 7210, Paris, France; [2]Clinique Vétérinaire Voltaire, Asnieres, France; [3]Vébio, Arcueil, France, Arcueil, France; [4]CHV Saint Martin, Saint Martin Bellevue, France; [5]Clinique Vétérinaire Saint-Augustin, Nice, France; [6]INSERM UMRS 1138 Equipe 17 (Behar-Cohen), Centre de Recherche des Cordeliers, Paris, France

Purpose: To compare retinal structure, function and biochemistry in a family of diabetic cats.
Methods: Six domestic shorthaired males (aged 10–11 years old; weight: 5.7–7.7 kg) and four domestic shorthaired females (aged 10–11 year old; 4.1–6.1 kg) from the same family were assessed. For two males and one female, glycemia and plasma fructosamin levels were >4 g/L and >400 µmol/L, respectively. They were considered to be diabetic cats (DC). For all animals, media were clear allowing for retinal SD OCT examinations of two retinal areas: one centered on the area centralis (AC) (cone-dominated retina) and the other on the retinal periphery (RP) (rod-dominated retina). Total retinal thickness (TRT), neuro-retinal thickness (NRT) and choroidal thickness (CT) were measured at AC and RP. Retinal function was assessed by full-field ERG elicited with different flash and flicker stimulations under photopic and scotopic conditions. Plasma glutathione peroxidase (GP) activity, albumin (Alb) and haptoglobin (Hapto) plasma levels were measured.
Results: In DC, the mean TRT values were significantly higher ($p < 0.01$) in the AC (517.7 ± 8.9 µm) compared to non-DC (459.4 ± 31.4 µm) while they were in RP (DC: 478.1 ± 17.9 µm; non-DC: 474.9 ± 28.4 µm). The mean CT values were significantly higher ($p < 0.01$) in the AC of Diabetic Cats (319.6 ± 18.5 µm) compared to non-DC (275.3 ± 30.0 µm) while the mean CT values were similar in the RP of Diabetic Cats (273.6 ± 13.7 µm) compared to non-DC (279.3 ± 36.7 µm). Irrespective of retinal location (AC or RP), there were no significant differences in NRT between DC and non-DC. ERGs parameters did not show significant differences between DC and non-DC. Alb mean values were significantly ($p < 0.05$) lower in DC (26.0 ± 0.1 g/L) compared to non DC (28.6 ± 1.7 g/L) while Hapto and GP were significantly ($p < 0.05$) higher in DC (0.9 ± 0.2 g/L and 24,053.7 ± 1632.8 UI/L, respectively) compared to non-DC (0.3 ± 0.1 g/L and 21,254.9 ± 1920.2 UI/L, respectively).
Conclusions: Our results show that an increase in plasma levels of oxidative stress and inflammation biomarkers is associated with a thickening of the choroid in Diabetic Cats especially in AC where there is a higher density of cones.

9:30 Assessment of macular function and structure in patients with idiopathic epiretinal membrane treated by pars plana vitrectomy

Wojciech Lubiński[1], Wojciech Gosławski[1], Leszek Kuprjanowicz[1], Karol Krzystolik[1], Maciej Mularczyk[2]

[1]Clinic of Ophthalmology, Pomeranian Medical University, Szczecin, Poland; [2]Doctoral Study, Pomeranian Medical University, Szczecin, Poland

Purpose: To estimate macular function and structure before and after epimacular membrane surgery.
Methods: Thirty two eyes of 32 patients (mean age: 70.8 ± 6.7 years) with epimacular membrane (EMM) were examined by best corrected distance visual acuity (DBCVA-logMAR), pattern electroretinogram (PERG-amplitudes (A) of P50 and N95—waves, implicit time (IT) of P50-wave; ISCEV standard) and OCT (foveal thickness) tests before and 12 months after successful 25G pars plana vitrectomy with EMM removal and internal limiting membrane (ILM) peeling. To estimate the differences between the mean values of the considered characteristics a *t* test or Wilcoxon matched pair were used (Statistica 10 software). Correlation between preoperative data (OCT, PERG) and final DBCVA were investigated using Pearson correlation analysis. A receiver operating characteristic (ROC) curve was constructed to obtain a cutoff value allowing prediction of visual prognosis. We tried to determine the P50 and N95 amplitudes cutoff value needed to predict a good visual outcome (DBCVA of 0.3 or less). The level of statistical significance was set at $p < 0.05$.
Results: Twelve months after surgery mean DBCVA significantly improved in comparison with the preoperative value (0.31 ± 0.12 versus 0.6 ± 0.15; $p < 0.000$). Twenty of thirty two eyes (72 %) achieved visual recovery of 2 and more Snellen lines. In PERG testing, a significant increase of the mean amplitudes of P50 and N95-waves (P50: 3.41 ± 1.48 µV vs 2.38 ± 1.23 µV—$p < 0.000$; N95: 5.46 ± 1.72 µV vs 3.75 ± 1.48 µV—$p < 0.000$) was achieved as was a significant decrease of the mean implicit time of P50-wave (55.00 ± 3.60 vs 56.75 ± 5.78 ms—$p < 0.000$). In OCT testing, a significant reduction of mean foveal thickness (313.34 ± 47.01 vs 509.03 ± 93.88 µm; $p < 0.000$) was obtained. Twelve months after surgery DBCVA was significantly

correlated with the preoperative foveal thickness ($r = 0.48$; $p = 0.006$), IT P50 ($r = 0.39$; $p = 0.027$), AP50 ($r = -0.68$; $p = 0.000$ and AN95 ($r = -0.73$; $p = 0.000$). The cut-off point for achieving successful VA with AP50 was 2.32 μV (sensitivity: 64.3 %; specificity: 88.9 %) and with AN95 it was 4.47 μV (sensitivity: 71.4 %; specificity: 88.9 %).

Conclusions: Removal of idiopathic epimacular membranes with internal limiting membrane peeling provided not only an increase in visual acuity and a reduction of foveal thickness but also showed an improvement in function of the innermost retinal layer in the macular region. Pattern ERG testing might be a valuable tool in predicting the postoperative visual acuity.

9:45 Unmasking ERG ON–OFF interactions with the discrete wavelet transform (DWT)

Mathieu Gauvin[1,2,*], Maja Sustar[3,*], Jean-Marc Lina[4,5], John M. Little[1,2], Robert K. Koenekoop[1,2], Pierre Lachapelle[1,2]

[1]Department of Ophthalmology and Neurology-Neurosurgery, McGill University, Montreal, Canada; [2] Montreal Children's Hospital Research Institute, Montreal, Canada; [3]Eye Hospital, Ljubljana University Medical Centre, Ljubljana, Slovenia; [4]Département de génie électrique, École de Technologie Supérieure, Montreal, Canada; [5]Centre de Recherches Mathématiques, Montreal, Canada; *These authors equally contributed to this study

Purpose: We have previously shown that the 20 and 40 Hz descriptors of the photopic b-wave, identified with the DWT, are specifically attenuated in diseases affecting the ON and OFF pathways, respectively and consequently reflected the contribution of the two retinal pathways to the response. Given that the DWT approach allows a more precise quantification of ERG components, we revisited the ON–OFF interactions in ERGs evoked to varying flash durations and luminances.

Methods: Time–frequency descriptors (20 Hz, 40 Hz and 40/20 Hz ratio) of the b-wave were extracted from the DWT of ERGs of normal subjects ($N = 10$). ERGs were evoked to short (5 ms) and long (10, 20, 50, 150 ms) flashes (intensities: 0.8, 1.3, 1.8, 2.3, 2.8 cd s m^2; background: 20 cd m^2). Results obtained from the different stimulus combinations were compared using ANOVAs and *t* tests. Pearson scores were used to determine if correlations existed between short (SF) and long flash (LF) ERG components.

Results: A flash of 150 ms completely separated the ON and OFF responses (ONR and OFR). The 40/20 Hz ratio of ONRs (mean ± SD: 0.49 ± 0.04) was significantly lower ($p < 0.05$) than that of OFRs (mean ± SD: 1.71 ± 0.18), due to a significantly ($p < 0.05$) higher contribution of the 20 and 40 Hz components to the ONRs and OFRs, respectively. The latter confirmed that the 20 and 40 Hz descriptors characterized the activity of the ONR and OFR, respectively. With progressively brighter stimuli, both the ONR and OFR increased steadily ($p < 0.05$) to reach maximal value with a flash of 2.8 and 2.3 cd s m^2 in intensity, respectively. For bright flashes, a progressive shortening of the flash duration from 150 to 5 ms steadily increased the OFR (↑6,4; $p < 0.05$), while for dimmer flashes, the OFR increased (↑2,5; $p < 0.05$) for flash duration from 150 to 20 ms, after which shorter flashes reduced the OFR. In contrast, the ONR value remained relatively constant (averaged maximal variation <25 %; $p < 0.05$) and was maximal with a flash duration of 10 ms irrespective of stimulus intensities. ANOVAs confirmed that the stimulus duration/luminance-dependence of the ON and OFF components were significantly different ($p < 0.05$) from each other. Of interest, correlations between the ONR of the SF and LF responses were measured ($r = 0.82$; $p < 0.05$) as were similar correlations for the OFR ($r = 0.84$; $p < 0.05$), suggesting that the LF-ONR and LF-OFR can be fairly predicted from the SF ERG.

Conclusions: In conditions where the ONR and OFR interact to form a single mixed response (flashes < 50 ms) our technique was still able to specifically quantify the ONR and OFR based on their separable time–frequency features. When shortening the flash duration, the ONR remained relatively stable, while the OFR exponentially increased, suggesting a facilitation of the OFR with shorter stimulus durations. Therefore, the interaction of the ON and OFF pathways to the building/shaping of the SF response is mostly OFF-dependant. Finally, our technique will help to better identify the origin of retinal anomalies in patients affected with various retinal conditions.

Funding: CIHR, FRQ-S, vision network and e-rare-2.

10:00 Does flicker ERG depend on stimulus color?

Olga Kraszewska[1], Quentin Davis[1], Colette Manning[2]

[1]LKC Technologies, Gaithersburg, MD, USA; [2]Wedgwood Optometry, Fort Worth, TX, USA

Purpose: According to ISCEV standards, a white flash stimulus having a color temperature near 7000 K should be used in electroretinography. However, no specific tolerance of color variability is noted. The closest CIE color coordinates to a 7000 K blackbody are (.31, .32); however, (.33, .33) equally stimulate all three cone types. This study measures the color-dependence of the ERG flicker stimulus.

Methods: Flicker ERG (28.3 Hz) recordings were made with the RETeval device (LKC Technologies, Inc.) and Sensor strip skin electrodes in a randomly chosen un-dilated eye of each patient with a retinal illuminance energy of 32 Td-s with 320 Td background (equivalent to the ISCEV standard assuming a 3.7 mm pupil). Measurements were taken using 46 colored stimuli and 10 white (0.33, 0.33) stimuli presented randomly to the subject in a continuous fashion. The colors were synthesized via RGB LEDs having color coordinates of red (0.70, 0.30), green (0.15, 0.69), and blue (0.14, 0.044). The 46 colors included six hexagonally-spaced in CIE 1931 (x,y) 0.02 units away from pure white (0.33, 0.33), and six more hexagonally-spaced 0.04 units away (rotated 30°) in order to densely sample

colors near pure white. The remaining colors continued the hexagonal tessellation with 0.08 spacing, except for the last three colors, which used the individual RGB LEDs. Recording time was 6 min.
Results: Initial results (n = 5) suggest that when using the fundamental of the flicker response, neither the implicit time nor the amplitude is affected in a systematic way by color. When using waveform harmonics in the analysis, the far red causes a 2 ms average increase in implicit time and a 16 % increase in amplitude. Green, blue, and white flashes all yielded similar ERGs.
Conclusion: In terms of how close to 'white' should 'white' be for a flash, these data suggest that wide latitude is reasonable, for example, (0.33,0.33) ± (0.1,0.1). The explanation for why red light causes a different response may lie in phylogenetically late development of trichromatic vision in primates, red being the latest addition. While these results should generalize to other cone-response tests, further research is needed to determine the color requirements for dark-adapted tests. These preliminary results pertain only to subjects with normal vision without color vision abnormality.

10:15 Correlation between macular cone density and the focal macular electroretinogram in normal eyes

Hiroko Terasaki, Kennichi Kawano, Yasuki Ito, Taro Kominami, Shinji Ueno

Nagoya University School of Medicine, Department of Ophthalmology, Nagoya, Japan

Purpose: To determine whether a significant correlation exists between the amplitude of the focal macular electroretinogram (fmERG) and macular cone density measured by adaptive optics (AO) imaging in normal eyes.
Methods: fmERGs were recorded in 30 normal eyes of 15 subjects (average age: 29.0 ± 6.4 years) with a 10° stimulus spot. Macular cone mosaic images were obtained using an AO fundus camera (rtx1: Imagine eyes, France). Parafoveal cone packing density at 2 degrees superior, inferior, temporal and nasal from the fovea was measured and the average was calculated.
Results: The mean amplitude of a- and b-wave was 0.72 ± 0.36 and 1.72 ± 0.74 μV, respectively. The mean implicit time of a- and b-wave was 23.2 ± 1.3 and 43.0 ± 1.7 ms, respectively. The average parafoveal cone packing density was 26,786 ± 3491/mm^2. The amplitude of both a- and b-wave were significantly correlated with cone density (both $p < 0.001$). The implicit time of both a- and b-wave were not significantly correlated with cone density.
Conclusions: The significant correlation between amplitude of a- and b-wave and cone density may suggest that the amplitude of the fmERG reflects the number of cone cells in the macular area.

10:30 When function, structure and electrophysiology don't agree

Michael Marmor

Byers Eye Institute, Department of Ophthalmology, Stanford University School of Medicine, Palo Alto, United States

Purpose: To challenge standard assumptions about the correspondence between functional, electrophysiological and anatomical measures of the retina.
Methods: Tests described include macular ERG, ERG, EOG, D-15 color test, dark adaptometry, Goldmann fields, macular and wide field photography and autofluorescence, and high-resolution SD-OCT.
Results: Sometimes our tests seem inconsistent. Consider a 32 year-old man was first seen in 1990 for complaints of diminished vision (roughly 20/50 OU), but no specific complaints of color, night or side vision loss. The fundus was clinically normal, as were peripheral fields and endpoint dark adaptation. However, he showed an achromatic axis on color testing, and no cone limb on dark adaptation. Macular ERG showed marked reduction, and full field ERG showed cones roughly 70 % and rods 85 % of normal. EOG was somewhat reduced. A year later acuity had fallen to 20/200 and CF, and the ERG was further reduced, especially for cones. This seemed a straightforward cone-rod dystrophy. He next returned at the end of 2014 with CF acuity in both eyes and complaints of photophobia, and his ERG was now flat. However, his fundus was still pretty normal, he did not complain of night blindness, and his Goldmann field was largely unchanged with good peripheral isopters. Fundus autofluorescence was normal in the macula and showed only subtle mid-peripheral RPE loss. SD-OCT (unavailable in 1990) showed mild foveal and parafoveal thinning, but a remarkably good outer nuclear layer (ONL) everywhere with excellent definition of the ellipsoid zone (EZ) and inter-digitation zone (IZ) lines.
Conclusions: How do we explain—and understand—these types of result? We associate a flat ERG with loss of photoreceptors, poor night vision and field constriction but these were not present. He complained of poor acuity and photophobia. However, the preserved photoreceptor tip markers (EZ and IZ lines) did not match his poor visual acuity and low macular ERG. The ERG had progressed from a strong signal in 1990 to no response in 2014 with little functional change. What does a flat ERG mean when the ONL, EZ and IZ are preserved along with peripheral field? How does a dystrophy cause poor acuity with good foveal anatomy? We hear about "function before structure," but not lasting over a 25 year interval. And here we have a disconnection between electrical function (absent) and visual function (preserved). Can we reconcile such findings with our understanding of electrophysiological tests?