532 Retinal Function: ERG studies
Thursday, May 11, 2017 8:30 AM–10:15 AM
Exhibit/Poster Hall Poster Session
Program #/Board # Range: 5339–5351/B0436–B0448
Organizing Section: Visual Neuroscience

Program Number: 5339 Poster Board Number: B0436
Presentation Time: 8:30 AM–10:15 AM
Is an electroretinogram in Sudden Acquired Retinal Disorder (SARDS) really flat?
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Purpose: Sudden Acquired Retinal Disorder (SARDS) is an idiopathic cause of acute vision loss in dogs and is diagnosed based on the lack of retinal function (as measured with an electroretinogram or ERG), pupil unresponsiveness to red light, normal response to blue light and relatively normal fundus. We examined ERGs obtained from dogs diagnosed with SARDS for information pertinent to the cause and potential treatment of SARDS.

Methods: Dogs diagnosed with SARDS were examined in Texas Veterinary Ophthalmology, Fort Worth, TX. The ERGs were performed on dilated and un-sedated animals using stainless steel subdermal needle electrodes (reference and ground) and an ERG-Jet corneal electrode (active). Both scotopic and photopic function were assessed with the RETevet device (LKC Technologies). Six dogs, 7-12 years old, were examined.

Results: Photopic flash and flicker ERG as well as scotopic rod response were not measurable in any of 6 dogs. However, both scotopic mixed stimuli (3.0 cd/s/m² and 10.0 cd/s/m²) had measurable, but atypical, responses consisting of an a-wave/fast PIII component, followed by the plateau and occasionally a blink. There were no b-waves. A-wave/fast PIII amplitudes varied from 35 to 210 µV and latencies from 55 to 130 ms. Three dogs had similar amplitudes OD and OS; two dogs had amplitudes in one eye around 50% smaller than in the other; and one dog had one eye with a flat ERG while it was measurable in the other.

Conclusions: Some dogs diagnosed with SARDS produce ERGs indicative of ON bipolar cells being blocked, as similar ERG waveforms have been observed in pharmacological studies where 50 µM of a glutamate analog (APB) blocked the b-wave component of the ERG by binding to sites on the postsynaptic membrane. These findings support the glutamate excitotoxicity hypothesis proposed previously as one of the causes of SARDS. Recording ERGs from dogs with SARDS characteristics (7-10 years old, overweight, spayed female of small or mixed breed) not yet diagnosed with this incurable blindness could provide more information about the early stages of the retinal function decline and hopefully provide insight into treatment and prevention.

Commercial Relationships: Olga Kraszewska, LKC Technologies (E); Brian Cichocki, Texas Veterinary Ophthalmology (I); Quentin Davis, LKC Technologies (E)

Program Number: 5340 Poster Board Number: B0437
Presentation Time: 8:30 AM–10:15 AM
Scotopic electroretinogram a- and b-wave alterations in adult rats after an acute exposure to ozone
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Purpose: Millions of people who suffer from retinal disease live in air polluted environments. The impact of the gaseous air pollutant ozone (O₃), a strong oxidant, on the retina is unknown. The purpose of this study is to compare the electroretinographic responses between control and O₃-exposed rats and to better understand the effect of O₃ on retinal function.

Methods: Age- and sex-matched Long Evans rats were randomly separated into two groups (n=12 rats); six control (clean air) and six acute O₃-exposed (0.4 ppm for 4 hours). Full-field electroretinography (ERG) was recorded simultaneously from both eyes in each rat. The scotopic ERG, a-wave, b-wave, and photopic negative response (PhNR) was measured and compared between the two groups. Recordings were performed under general anesthesia (ketamine 70 mg/kg, xylazine 2.5 mg/kg, IP). Active corneal electrodes were designed for use in rats. A ground needle electrode was placed subcutaneously in the real flank of the animal. Pupils were dilated with 2.5% phenylephrine and 1% tropicamide eye drops. Lubrication and proper electrical conductance of the active electrode were maintained with Refresh lubricant eye drops. Rats were dark adapted for 30 minutes before scotopic responses were measured.

Results: Experimental data indicates, in the scotopic ERG, an acute exposure to O₃ significantly (p < 0.05) decreased the a-wave amplitude at intensities ranging from -0.001 to 25 cd.s/m². The trough of the a-wave decreased by a mean of 11 mV. The amplitudes of the b-waves were significantly higher (p < 0.01) by 7.72 mV in the O₃-exposed rats across all tested stimulation intensities. No mean significant change was observed in the photopic negative-response (PhNR) between the control and exposed groups.

Conclusions: This work demonstrates O₃ exposure leads to alterations in the rod system as indicated by the scotopic ERG changes. Results suggest air pollution may contribute to retinal deficits in sensitive populations living in air-polluted environments. It also provides useful data in establishing air quality standards to better protect the populations living in air polluted areas to prevent the possible O₃-induced oxidative stress that may contribute to retinal neuron dysfunction.

Commercial Relationships: Carlos A. Garcia, None; Jordan M. Wetz, None; Apeamokhai P. Aitsebaomo, None

Program Number: 5341 Poster Board Number: B0438
Presentation Time: 8:30 AM–10:15 AM
Rod versus cone driven ERGs at different stimulus sizes
Avinash Aher1, Declan J. McKeefry2, Neil R. Parry2, John Maguire1, Ian J. Murray1, Tina I. Tsai2, Cord R. Huchzermeyer3, Jan J. Kremers1. 1University Hospital Erlangen, Erlangen, Germany; 2University of Bradford, School of Optometry and Vision Science, Bradford, United Kingdom; 3Manchester Royal Eye Hospital, Vision Science Center, Manchester, United Kingdom; 4University of Manchester, Faculty of Life Sciences, Manchester, United Kingdom.

Purpose: To study how rod and cone driven responses depend on stimulus size in normal subjects and patients with retinitis pigmentosa (RP).

Methods: The triple silent substitution technique was used to isolate L- and M-cone and rod driven ERGs with 19%, 18%, and 33% photoreceptor contrast resp. Mean luminance was 284 cd/m²; CIE coord: (0.60, 0.39). Experiments were conducted on five normal subjects (age: 27-55 years) and three RP patients (age: 41-53 years). The ERGs in control subjects were recorded at nine different temporal frequencies (between 2 and 60 Hz) and with five different stimulus sizes: full-field (FF) and 70°, 60°, 50° and 40° diameter circular stimulus. In the experiments with the RP patients, only rod and L-cone driven ERGs were measured with FF and 40° diameter stimuli at 8 and 48 Hz. Amplitudes of the responses were defined as those of the 1st harmonic component after Fourier analysis of the ERGs.

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**Results:** In normal subjects, the rod driven responses displayed a fundamentally different behavior than the cone-driven responses. At low temporal frequencies (2-4 Hz), FF rod and cone driven responses were barely above noise. Up to 12 Hz, rod-driven signals increased by about a factor of 4 when measured with smaller stimuli. In contrast, L and M cone-driven responses in this frequency region did not change substantially with stimulus size. On the other hand, at high temporal frequencies (24 Hz and higher), rod and cone driven response amplitudes decreased with decreasing stimulus size. In contrast with responses measured in the normal subjects, 8 Hz rod driven and the 48 Hz L-cone driven ERGs to the 40° stimuli were not larger than those to FF stimuli in RP the patients.

**Conclusions:** The increased responses with smaller stimuli in normal subjects with the rod isolating conditions indicates that a fundamentally different mechanism drives the ERGs in comparison with the cone driven responses and validates the rod isolation. We propose that the increased responses are caused by stray light stimulating the peripheral retina, indicating that it is possible to isolate and study rod driven ERGs using triple silent substitution technique at photopic luminances. In RP patients both cones and rods are affected in the peripheral retina.

**Commercial Relationships:** Avinash Aher, None; Declan J. McKeefry, None; Neil R. Parry, None; John Maguire, None; Ian J. Murray, None; Tina I. Tsai, None; Cord R. Huchzermeyer, None; Jan J. Kremers, None

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**Program Number:** 5342 **Poster Board Number:** B0439
**Presentation Time:** 8:30 AM–10:15 AM

**Characterization of Focal Electroretinogram and Visual Evoked Potential in Normal Pigmented Rats**

Yossi Mandel, Adi Gross, Nairouz Farah, Optometry and Visual Sciences, Faculty of Life Sciences and Bar-Ilan Institute for nanotechnology and Advanced Materials (BINA), Bar-Ilan University, Ramat Gan, Israel.

**Purpose:** Measurements of focal retinal function are highly important in assessing intervention efficiency in various research models where a localized area of the retina is treated (e.g. retinal prostheses, genetic therapy). This work characterized the focal Electroretinogram (fERG) and Visual Evoked Potential (fVEP) in response to a photopic localized visual stimulus projected on the rat retina.

**Methods:** fERGs and fVEPs signals were recorded in Long-Evans anesthetized rats in response to LED flashes relayed through circular apertures which are incorporated into a fundus camera (Micron IV, Phoenix Research Lab) optical path. Stimuli with varying irradiances, repetition rates, and spot diameters were investigated at various background illumination. VEP signals were recorded using intracranially implanted screws over the primary visual cortex (V1) and ERG signals were recorded using a corneal contact electrode. Raw data were analyzed by a customized software.

**Results:** The fERG bwave amplitude increased with light intensity reaching a plateau at 4*10^12 cd/s/m^2, and decreased with increasing stimuli repetition rate and increasing background illumination. The fVEP amplitude (N1P2) demonstrated a similar trend, however, a plateau was observed at a lower stimuli luminance (1*10^10 cd/s/m^2) suggesting a smaller cortical dynamic range as compared to the retina. fERG and fVEP b-wave amplitude increased with stimuli spot size reaching a plateau at smaller spot size for high intensity illumination levels, suggesting a contribution of the scatter effect. The b-wave latency decreased with increasing stimuli luminance, reaching a minimum at luminance levels above 10cds/m^2. The photopic stimuli elicited a robust photopic negative response (PhNR) with increasing amplitude and latency for increasing stimuli irradiance and spot size.

**Conclusions:** We present a thorough characterization of the effect of various stimuli parameters on fERG and fVEP signals in normal pigmented rats. The obtained signals reveal a robust PhNR component and suggest a scatter effect at high irradiance stimuli. Furthermore, our results demonstrate the larger retinal dynamic range as compared to the cortical dynamic range under photopic conditions. This study can serve as a basis for evaluating localized retinal function as an important research tool for investigating retinal diseases in rodents.

**Commercial Relationships:** Yossi Mandel, None; Adi Gross, None; Nairouz Farah

**Program Number:** 5343 **Poster Board Number:** B0440
**Presentation Time:** 8:30 AM–10:15 AM

**Scotopic dim blue and white flash responses closely correlated in full-field electroretinography**

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**Purpose:** The full-field electroretinogram (ERG) is a widely utilized tool to assess mass retinal function. While the International Society for Clinical Electrophysiology of Vision (ISCEV) specifies standard flashes as broad-spectrum and visibly white, colored filters or colored light-emitting diodes (LEDs) have been used to enhance the separation of rod and cone responses and may offer certain advantages. ¹ This retrospective study compares responses to dim blue and white flashes under scotopic conditions.

**Methods:** Data was collected from full-field ERG responses measured in right eyes of 268 subjects in an ERG database at Mount Sinai Hospital in New York, NY, obtained between 2011-2015. Scotopic responses were recorded after dark-adaptation with single flashes of blue light (0.01 cd-s/m^2) and white light (0.05 cd-s/m^2). B-wave amplitude (mV) and implicit time (milliseconds) were measured and recorded for blue and white flashes, from averaged waveforms. A Pearson correlation coefficient was used to quantify the similarities between the responses.

**Results:** B-wave amplitudes showed strong correlation between blue and white flashes with a conversion factor of 1.3 and a correlation of R^2 = 0.88. B-wave implicit time showed correlation between blue and white flashes with a conversion factor of 0.98 and R^2 = 0.41. Results are illustrated in Figures 1 and 2, below.

**Conclusions:** These results show excellent correlation between B-wave amplitude and strong correlation between B-wave implicit time for blue and white flashes, suggesting that either may be used to record the scotopic rod-isolated ERG component.


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Purpose: Cynomolgus Macaque (CM) plays an important role as an experimental subject in studies of visual abnormalities and development of potential retinal therapeutics. At the same time normal parameters of its full field electroretinograms (ERG) recorded with bipolar Burian-Allen electrodes have not been reported. We determined the parameters of full field ERG recorded in CM with bipolar Burian-Allen electrodes following the current standards of the International Society for Clinical Electrophysiology of Vision.

Methods: Twenty male and female adult CM were used in this study. Each animal/eye had a full ophthalmological exam and retinal imaging with optical coherence tomography to ensure that there were no anomalies that might impact retinal function. The ERG sessions were carried out under the dim red light conditions. Full-field stimulation was produced with a custom made Ganzfeld stimulator. A Diagnosys LLC (Lowell, MA) Espion workstation controlled the stimulator and acquired the signals. ERGs were recorded with bipolar Burian-Allen electrodes (Hansen Labs, Coralville, IA). The intensities of flash stimuli used (cd s m⁻²) were: 0.01, 3 and 10 dark-adapted; 3 and 10 light-adapted.

Results: We obtained the following normal values for the ERG components (adaptation state, flash intensity, component, amplitude in mV, (standard deviation)): dark, 0.01, rod b-wave, 30 (13.1); dark, 3, a-wave, 48.9 (19.8); dark, 3, b-wave, 126 (29.5); dark, 10, a-wave, 89.4 (31.4); dark, 10, b-wave, 147.7 (34.8); light, 3, a-wave, 16.4 (4.9); light, 3, b-wave, 57.3 (16.6); light, 10, a-wave, 30 (9.8); light, 10, b-wave, 61 (17.6).

Conclusions: We determined normal values of ERG components in the CM thus establishing the baseline for other researchers interested in using ERG as a means of assessing retinal function in Macaque fascicularis.

Figure 1

Figure 2

Commercial Relationships: Gaurav M. Chandra, None; Scott E. Brodie, None

Program Number: 5344 Poster Board Number: B0441
Presentation Time: 8:30 AM–10:15 AM
Normal parameters of the full field ERG recorded with bipolar electrodes in Cynomolgus Macaque (Macaque fascicularis) Arkady Lyubarsky1, 2, Erik Wielechowski1, Tomas S. Aleman4, Albert M. Maguire1, 4, Gui-Shuang Ying1, Erin Bote1, Leah Makaron1, James Wilson1, Jean Bennett1, 4, Anna P. Tretiakova1. 1Center for Advanced Retinal and Ophthalmic Therapeutics, SOM Univ. of Pennsylvania, Philadelphia, PA; 2Vision Research Center, University of Pennsylvania, Philadelphia, PA; 3Gene Therapy Program, University of Pennsylvania SOM, Philadelphia, PA; 4Scheie Eye Institute, University of Pennsylvania SOM Ophthalmology, Philadelphia, PA.
Here we demonstrate a technique for creating spatial corneal maps of $I_{1/2}$, that can be interpreted for local changes in $I_{1/2}$ at the retina.

**Methods:** Normally-sighted Long Evans rats (n =26) were dark adapted, anesthetized, and pupils were dilated. Multi-electrode electroretinography (meERG) was used to record a-wave potentials at 25 locations on the cornea elicited by two flash strengths, one saturating and one approximately half-saturating. At each corneal location, the a-wave amplitudes elicited by the two stimuli were fit with a function $A/A_{1/2} = 1/(1 + I/I_{1/2})$, where $A$ is the a-wave amplitude for stimulus strength $I$, $A_{1/2}$ is the saturated a-wave amplitude, and $I_{1/2}$ was the free parameter. The resulting 25 $I_{1/2}$ values were then used to create corneal maps of sensitivity for each animal, and a normative range was established. Similar $I_{1/2}$ maps were also created for 13 animals two days after receiving experimental retinal lesions (photo- or cryo-coagulation) resulting in complete disruption of between 0.9-14.9% (mean 5.1%) of the retina.

**Results:** The spatial distribution of $I_{1/2}$ across the cornea of healthy rat eyes was approximately uniform (all locations within one standard deviation of the spatial mean), unlike the distinct nasal-temporal asymmetry in the a-wave potentials from which $I_{1/2}$ is derived. The normal distribution of $I_{1/2}$ values was altered in the presence of experimental lesions. Eyes with lesions could be classified with 77% sensitivity, 65% specificity, based on local values of $I_{1/2}$.

**Conclusions:** Spatial differences in the distribution of photoreceptor sensitivity ($I_{1/2}$) seen at the cornea are affected by local areas of damage at the retina, and may be useful for detection of early, localized pathophysiology that affects this parameter prior to cell death.

**Commercial Relationships:** John R. Hetling, RetMap, Inc. (S), RetMap, Inc. (I), RetMap, Inc. (P); Brian Kunzer, None

**Support:** UIC Colleges of Engineering and Medicine Bridge Funding

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**Results:** The anesthetic used was shown to have a major impact on the amplitude of the ERG responses. KXA and KX (75/7.5 mg/kg) scotopic b-wave amplitudes (647±280 and 626±148 uV, respectively) were approximately 2-fold higher than with other cocktails. Latency times were unaffected. FMM and FDM offered the most flexibility in anesthesia duration. Anesthesia lasted up to 2 hours in most animals, but administration of the reversal agent was effective within minutes of administration, which improves recovery and reduces risks associated with ocular drying and prolonged periods of anesthesia.

**Conclusions:** In conclusion, the anesthetic agent for ERG recording has a significant impact on the magnitude of the response, which needs to be taken into consideration during the study design phase and when comparing between results in different studies. In this study, FMM and FDM were considered to be optimal for ERG recording, based on ERG waveforms obtained, levels and duration of anesthesia, and reversibility.

**Commercial Relationships:** Kelly Tenneson, Charles River (E); Mark Vezina, Charles River (E)

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**Program Number:** 5347 **Poster Board Number:** B0444
**Presentation Time:** 8:30 AM–10:15 AM
**Evaluations of Vascular endothelial growth factor (VEGF) on electroretinogram (ERG) amplitude in rats are mediated by bradykinin**

*Allen C. Clermont*, Nivetha Murugesan, Tuna Ustunkaya, Brianna Mastromarino, Lloyd P. Aiello, Edward P. Feener, Beetham Eye Institute, Joslin Diabetes Center, Boston, MA; Vascular Cell Biology, Joslin Diabetes Center, Boston, MA.

**Purpose:** The mechanisms that contribute to VEGF-induced visual dysfunction in diabetic macular edema (DME) are not fully understood. Recently, we have shown that VEGF’s effects on retinal edema in rodents are partly mediated via plasma kallikrein (PKal) and bradykinin (BK). This study investigates the effects of intravitreal VEGF and the kallikrein kinin system (KKS) on electroretinographic (ERG) responses in rats.

**Methods:** Ocular PKal activity was measured by IVIS Spectrum CT using fluorogenic PKal substrate. Rat retinal neuronal function was measured using full field dark-adapted ERG at baseline, 2, 24 and 48 hours following intravitreal (IVT) injections (5μL of VEGF (10ng/eye), BK (2µM), PKal (50ng/eye), and saline control. Maximal scotopic responses were obtained using a 5ms white light flash (1.4x10^6 cd/m²).

**Results:** At 24 hrs after IVT, VEGF increased flavoponece of PKal substrate in the vitreous compared to saline injected eyes (12.7±1.5 vs 4.9±1.3 avg radiance p<0.05). IVT injection of VEGF did not affect ERG at 24 hrs but increased A- and B-wave amplitudes at 48 hrs by 75% (251±10 v 144±12µV, p<0.01) and 73% (619±26 v 357±29µV, p<0.01), respectively, compared to baseline. The B to A-wave amplitude ratio was not different between PBS and VEGF at 48 hrs (2.53 vs 2.46). Administration of selective PKal inhibitor VA999272 via subcutaneous osmotic pump (0.65mg/kg/d) reduced VEGF induced B-wave amplitude by 69% (454±46µV, p=0.002) at 48 hrs. BK IVT increased B-wave amplitude at 2, 24 and 48 hrs by 28% (437±30 v 341±20µV, p=0.015), 75% (461±46 v 262±40µV, p<0.001) and 64% (494±32±32 v 301±33µV, p<0.001). PKal IVT increased B-wave amplitude at 24 hrs by 50% (559±42 vs 372±37µV, p<0.01), which was reduced by 59% (448±32µV, p<0.05) with subcutaneous administration of B2 and B1 receptor antagonists HOE140 + desArg1-HOe140 (10µg/kg/h). Oral treatment with nitric oxide inhibitor L-NAME (0.1mg/mL) reduced VEGF induced B-wave amplitude at 48 hrs by 75% (from 455±20 to 325±25µV, p<0.05) compared to PBS (281±19µV).

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Conclusions: Intravitreal injection of VEGF causes activation of the KKS leading to increased ERG signal amplitudes. KKS activation acts upon the neuroretina through BK, which is blocked by BK receptor antagonism and nitric oxide inhibition. These data suggest that the effects of VEGF on visual dysfunction are mediated, in part, via the KKS and nitric oxide.

Commercial Relationships: Allen C. Clermont, None; Nivetha Murugesan, None; Tuna Ustunkaya, None; Brianna Mastro marin, None; Lloyd P. Aiello, None; Edward P. Feener, None

Support: NH Grant EY019029-07, Massachusetts Lions Eye Research Fund, NH DK036836

Program Number: 5348 Poster Board Number: B0445 Presentation Time: 8:30 AM–10:15 AM
Assessment of the photopic negative response in full-field electroretinogram in optic nerve-sectioned young chick eyes

Clement Afari, Daphne L. McCulloch, Chung K. Fung, Akshay Gurdita, Vivian Choh

School of Optometry and Vision Science, University of Waterloo, Waterloo, ON, Canada.

Purpose: The photopic negative response (PhNR) is known to reflect the functions of retinal ganglion cells in mammals but its origin in non-mammalian species is established. This study was undertaken to determine whether the PhNR from optic nerve-sectioned young White Leghorn (Gallus gallus domesticus) chicks reflect RGCs functions and also describe the nature of longitudinal PhNRs.

Methods: Full-field photopic electroretinograms (ERGs) were recorded bilaterally from hatching chicks (N = 4) before optic nerve section (ONS) on one eye and sham surgery on the fellow control eye. ERGs were recorded again on days 3, 5 and 7 post-ONS. Stimuli were 4 ms red (λ = 650 nm) flashes in increasing half log steps from 0.1 to 5 cd.s/m² on rod suppressing blue (λ = 462 nm) background (30 cd/m²). The a-waves, b-waves and PhNRs were measured, with the latter calculated relative to the b-wave.

Results: ERGs were clearly recordable from all eyes showing the expected luminance-response functions, with saturation at log 3 cd.s/ m². At all luminance levels, PhNR amplitudes for control eyes (63.77 ± 4.81 μV) were similar (p = 0.16) to those of ONS eyes (54.49 ± 4.09 μV). The a-wave and b-wave amplitudes were also not different between the eyes for all time points and luminance levels (p = 0.31). Differences in ERG amplitudes with maturation were not significant (p ≥ 0.14).

Conclusions: Although the PhNR of the ERG is diminished in conditions affecting ganglion cell function in mammalian models, our data do not support this conclusion in young chicks.

Commercial Relationships: Clement Afari, None; Daphne L. McCulloch, None; Chung K. Fung, None; Akshay Gurdita, None; Vivian Choh, ARVO AMPC (S)

Support: VC: Natural Sciences and Engineering Council of Canada Discovery Grant; VC: Canadian Foundation Innovation; CA: Ontario Trillium Scholarship

Program Number: 5349 Poster Board Number: B0446 Presentation Time: 8:30 AM–10:15 AM

Dynamics of adaptation process to different light levels in the mouse retina studied with ERGs

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Purpose: To date, most studies on in vivo electroretinography in mice are performed on steady state light or dark adapted animals. In the present study, we focused on the dynamics of light and dark adaptation processes in the mouse retina.

Methods: After an initial 12 h dark adaptation animals were adapted to 25 cd/m² white light and the responses to 6.3 cd.s/m² flashes were recorded every 60 sec for a 15 min period. Subsequently, the mice were dark adapted again and the ERGs to 6.3 cd.s/m² flashes were recorded every 60 sec for up to 30 min. In a second series of experiments, we recorded the changes in the responses to sinusoidal luminance modulation (12 Hz; 100% Michelson contrast) during the adaptation to a 25 cd/m² and a 1 cd/m² mean luminance, respectively.

Results: flash ERGs: Significant increases of the amplitudes of b-wave (p < 0.001), PhNR (p < 0.05) and OPs (p < 0.05) were found during light adaptation. Furthermore, the implicit times of b-wave (p < 0.001) and the 2nd (p < 0.005) and 3rd OP peak (p < 0.001) decreased significantly. The changes in most parameters were completed after 10 min of light adaptation. The subsequent reduction of the background illumination led to an instantaneous decline of the ERG signal. Afterwards the amplitudes of a- (p < 0.001) and b-waves (p < 0.001) and the OPs (p < 0.001) increased during dark adaptation. Also the ratio between a- and b-wave amplitude increased strongly (p < 0.001) indicating the presence of additional adaptation processes in the OPL. Furthermore we observed a significant decrease of the implicit times of 2nd (p < 0.05) and 3rd (p < 0.001) OP peaks.

sinusoidal flicker ERG: We found an interesting difference between light and dark adaptation. The fundamental phase decreased significantly during light adaptation (p < 0.001) and were constant during dark adaptation. The amplitude decreased during light adaptation (p < 0.005), but increased during dark adaptation.

Conclusions: Light and dark adaptation in ERG responses are highly dynamic processes that take several minutes to complete. Additionally, our data indicate that adaptation can take place at several stages in the retinal circuitry. That the response amplitude decreases to sine-wave modulation after light adaptation is at odds with the changes in the flash ERG components where the component amplitudes increase. This indicates that adaptation may be dissimilar in different signal pathways.

Commercial Relationships: Anneka Joachimsthaler, None; Tina I. Tsai, None; Jan J. Kremers, None

Program Number: 5350 Poster Board Number: B0447 Presentation Time: 8:30 AM–10:15 AM

Contributions of Second- and Third-Order Retinal Neurons to Cone Electroretinograms after Loss of Rod Function in Rhodopsin P347L Transgenic Rabbits

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Purpose: To determine the contribution of second- and third-order retinal neurons to the photopic electroretinograms (ERGs) after the degeneration of the rods in rhodopsin P347L transgenic rabbits (Tg).

Methods: Four wild type rabbits (WT) and four Tg rabbits were studied at 18 months-of-age. The photopic ERGs elicited at stimulus onset and offset were analyzed. To block different retinal pathways, 2-amino-4-phosphonobutyric acid (APB) and 6-cyano-7-nitroquinoline-2-3 (1H,4H)-dione (CNQX), and tetrodotoxin (TTX) and N-methyl-DL-aspartic acid (NMDA) were injected intravitreally. Digital subtraction of the post-drug ERGs from the pre-drug ERGs was used to determine the contributions of the
ON-components blocked by APB, the OFF-components blocked by CNQX, and the third-order neurons blocked by TTX+NMDA.

**Results:**
Contribution of the cone photoreceptors to the photopic ERGs in Tg rabbits was about 10% of that in WT rabbits. The amplitudes of the positive waves of the ON-components at stimulus onset in Tg rabbits were about one-half as large as those in WT. On the other hand, the amplitudes of the positive waves of the OFF-components at stimulus offset in Tg rabbits were about 1.4 to 2.3 times larger than those in WT. Tg rabbits had a positive wave at stimulus offset which was reduced after the TTX+NMDA injection.

**Conclusions:**
A reduced ON-component and an augmented OFF-component with abnormal responses of the third-order neurons contributed to the cone ERGs after the loss of rod function in Tg rabbits. Our results suggest a complex synaptic remodeling of the residual retinal cells in the advanced stage of Tg rabbits.

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**Pre-drug ERGs recorded from 4 WT rabbits (left columns) and 4 Tg rabbits (right columns) elicited by long-duration stimuli (A), saw-tooth rapid-ON stimuli (B), and saw-tooth rapid-OFF stimuli (C).**

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**Purpose:** Photoreceptors in the mouse retina express much of the molecular machinery necessary for phototransduction and glutamate release prior to eye opening at postnatal day 12 (P12; Regus-Leidig et al., 2009 and Sherry et al., 2003). Light responses can be observed from photoreceptors via electroretinogram (ERG) recordings after eye opening and as early as P13 (He et al., 2013). However, it is not known if photoreceptors are electrically active or photoresponsive prior to eye opening. Previous studies suggest that light reaches the retina prior to P12. For example, light has been shown to modulate critical developmental processes such as retinal wave activity and regression of the embryonic hyaloid vasculature through inner-retina photoreceptors (Renna et al., 2011 and Rao et al., 2013). However, the impact of light on outer retinal photoreceptors prior to eye opening is not known. We hypothesize that outer retinal photoreceptors are physiologically active and electrically responsive to light prior to eye-opening.

**Methods:** We recorded responses from developing wild-type mouse retinae at P8, P10 and P12 using ex-vivo ERG. Dark-adapted retinae were isolated and mounted on a custom-made ERG chamber. Oxygenated Ames solution was perfused at 37°C and barium chloride and D-LAP4 were used to isolate the a-wave (photoreceptor responses) after exposure to a 20ms flash. A paired t-test was used to calculate statistical significance between the baseline and the peak amplitude of the a-wave.

**Results:** At P8 there was no detectable response to light (n=2; p>0.05). At P10 we found a small (on the order of 10-20 microvolts) a-wave in response to light that was statistically significant relative to baseline prior to light flash (n=4; p ≤ 0.05). In p12 retinas the responses were significantly larger in amplitude with the same stimulus (n=4; p ≤ 0.05).

**Conclusions:** Photoreceptors are electrically functional and photoresponsive prior to eye opening, earlier than previously thought. During postnatal development melanopsin ganglion cells extend dendrites to the outer-retina and are closely opposed to cone photoreceptor terminals. These outer retinal dendrites appear to express postsynaptic glutamate receptors and are in position to receive inputs from photoreceptors between P8 and P12. This appears to coincide with the time in which photoreceptors become electrically responsive to light.

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