530 Photoreceptors
Thursday, May 11, 2017 11:30 AM–1:15 PM
Room 308 Paper Session
Program #/Board # Range: 5600–5606
Organizing Section: Visual Neuroscience

Program Number: 5600
Presentation Time: 11:30 AM–11:45 AM
Light acts directly on S-cone terminals to regulate transmitter release
Xianshi Zhang[1, 2], Yongjie Li[1], Steven H. DeVries[2]. [1]School of Life Science and Technology, University of Electronic Science and Technology of China, Chengdu, China; [2]Department of Ophthalmology, Northwestern University Feinberg School of Medicine, Chicago, IL.
Purpose: Photoreceptors are functionally and morphologically polarized, with a light transducing mechanism in the outer segment (OS) that communicates by a change in membrane voltage with synaptic release mechanisms at the terminal. In S- or short wavelength light sensitive cones, the opsin is localized to both the OS and synaptic terminal. Based on the synaptic localization of S-opsin, we tested whether light could directly modulate transmitter release at S-cone terminals.
Methods: Experiments were performed on slices from the dark-adapted retinas of 13-lined ground squirrels (*Ictidomys tridecemlineatus*). Whole cell or perforated patch (b-escin) voltage clamp recordings (holding potential = -70 mV unless specified) were obtained from S- and M- (middle wavelength sensitive) cones. Recorded cones were identified as S- or M- prior to an experiment by the ratio of their responses to LED-generated stimuli at 468 and 574 nm, and after an experiment by an antibody to SW1-opsin. Synaptic release was evoked by briefly (1 ms) stepping a cone to -30 mV at 1 s intervals. Release was measured as the amplitude of the feedback current at the cone transporter anion conductance. The terminal Ca current was obtained as a difference current using a voltage ramp from -70 to 20 mV (1 mV/ms) in control and $CoCl_2$ (2 mM) containing solution. For some experiments, the non-hydrolyzable GTP analog GTPγS was included in the pipette solution, and transporter, proton block, and horizontal cell feedback currents were suppressed by TBOA, 15 mM HEPES, and CNQX, respectively.
Results: During whole cell and perforated patch recordings, 468 nm light significantly increased synaptic release in S- but not in M-cones (19.5 ± 2.2%, mean±SEM, n=15 S- and 39 M-cones, $p<0.0001$). Responses in the two recording conditions did not differ. Including 1 mM GTPγS in the recording pipette increased glutamate release in S- relative to M-cones (80.9 ± 9.3%,n=9 S- and 23 M-cones, $p<0.0001$). The increase was the same when 10 μM CNQX was added to block horizontal cell feedback and when the OS was ablated. In the presence of GTPγS, the 468 nm stimulus increased the S-cone Ca current without affecting the M-cone current (49.5 ± 2.9%, n=14 S- and 16 M-cones, $p<0.0001$).
Conclusions: Our results suggest that S-opsin at the S-cone terminal can activate a G protein which increases the terminal Ca current. The increase in Ca current produces an increase in synaptic release.
Commercial Relationships: Xianshi Zhang, None; **Yongjie Li**, None; **Steven H. DeVries**, None
Support: NIH Grant R01 EY012141

Program Number: 5601
Presentation Time: 11:45 AM–12:00 PM
Circadian clock gene *Bmal1* is necessary to establish the S-opsin gradient in murine cone photoreceptors
Onkar Sawant[1], Amanda Horton[1], Ivy S. Samuels[2], Sujata Rao[1]. [1]Ophthalmic Research, Cleveland Clinic, Cleveland, OH; [2]Ohio Department of Ophthalmic Research, Louis Stokes Cleveland Veterans Affairs Medical Center, Cleveland, OH.
Purpose: We recently reported that cyclic light-dark (LD) exposure during early postnatal period is crucial for photoreceptor development and these light dependent developmental processes in the eye could be mediated by thyroid hormones (TH). In the dark reared (DD) animals the retinal circadian clock genes as well as TH components were altered. Based on these observations we hypothesized that the clock genes control TH signaling mediated photoreceptor development.
Methods: To test this hypothesis, photoreceptor specific Crx Cre and Hgrp Cre lines were used to achieve conditional deletion of clock genes *Bmal1* and *Per2* from developing photoreceptors. ERGs were performed to assay for visual function. Chromatin immunoprecipitation (ChIP) method was used to determine the downstream targets of BMAL1. Immunofluorescence data was confirmed by qRT-PCR and immunoblots.
Results: Deletion of *Bmal1* from the cone photoreceptors results in ectopic expression of S-opsin cones in the dorsal retina and a complete loss of the dorsal-ventral S-opsin gradient. Conversely, deletion of *Per2*, a negative regulator of the circadian clock assembly exhibited the opposite phenotype with an overall decrease in S-opsin expression. *Bmal1* mutant animals demonstrated increase in light-adapted b-wave amplitude to 365 nm of light (short wavelength) but decrease in amplitude to 452 nm, 523 nm and 596 nm of lights (medium-long wavelengths). In DD animals we observed a significant decrease in the retinal expression of type II deiodinase (*Dio2*). *Dio2* is required for converting prohormone thyroxin (T4) into biological active triiodothyronine (T3). In the *Dio2* null mice we detected ectopic expression of the S-opsin in the dorsal retina which is similar to the *Bmal1* mutant phenotype. This suggests an interaction between Bmal1 and Dio2. To validate this interaction we performed ChIP experiments on retinal tissues. Preliminary data indicates that BMAL1 binds to the Dio2 promoter via E-boxes. Accordingly in *Bmal1* mutant animals we detect lower levels of *Dio2* mRNA and the double heterozygotes for *Bmal1* and *Dio2* have defects in S-opsin expression while the single heterozygotes are normal.
Conclusions: Based on these findings, we propose a new molecular cascade involving circadian clock gene *Bmal1* that establish cone opsin gradient via thyroid activating enzyme *Dio2*. Our data suggests that *Bmal1* is required for cone development and function.
Commercial Relationships: Onkar Sawant, None; **Amanda Horton**, None; **Ivy S. Samuels**, None; **Sujata Rao**, None
Support: This study was supported in part by the NIH-NEI P30 Core Grant (IP30EY025585-01A1) and Unrestricted Grant from The Research to Prevent Blindness, Inc., awarded to the Cole Eye Institute. This research is also funded by R01 EY027077-01 (SR), RPB1503 (SR), E.Matilda Ziegler Foundation - MZF1408 (SR), Knights Templar Eye Foundation award (SR), VA Merit Award i01-BX002754 (IS) and Cleveland Clinic Foundation startup funds (SR).

Program Number: 5602
Presentation Time: 12:00 PM–12:15 PM
Apo-opsin forms a photoactivated rhodopsin-like state
Shinya Sato[1], Beata Jastrzebska[2], Andreas Engel[2, 3], Krzysztof Palczewski[2], Vladimir J. Kefalov[1]. [1]Department of Ophthalmology and Visual Sciences, Washington University School of Medicine in St. Louis, Saint Louis, MO; [2]Department of Pharmacology, Case Western Reserve University School of Medicine, Cleveland, OH; [3]Department of Bionanoscience, Technical University Delft, Delft, Netherlands.
Purpose: Bleaching adaptation in rods is mediated by apo-opsin, which activates phototransduction with an estimated activity 10^6-fold lower than that of photoactivated rhodopsin (Meta-II). It is unclear whether every opsin molecule has low constitutive activity or if opsin exists in equilibrium between a predominantly inactive state and an intermittent active state. To address this question, we studied opsin signaling by electrophysiological recordings from mouse rods.
Methods: We studied opsin signaling in two models, guanylate cyclase activating proteins knockout mice ($GCAPs^{-/-}$) and retinal pigment epithelium specific 65 kDa protein knockout mice ($RPE65^{-/-}$). First, we examined $GCAPs^{-/-}$ mouse rods, which have ~5 times higher sensitivity than wildtype rods, in an effort to resolve the signal from individual opsins. Prior to the recordings, dark-adapted mouse retinas were dissected and a small fraction of opsin was produced by bleaching <1% of rhodopsin by light. Then, activation of the phototransduction cascade by opsin was measured from rod outer segments by single-cell suction recordings in the dark. Next, we stueied $RPE65^{-/-}$ chromophore-deficient rods. Here, prior to recordings, almost all of the opsin was converted into unbleachable rhodopsin by regeneration with exogenous locked 11-*cis*-7-ring retinal. Resistance of this 11-*cis*-7-ring rhodopsin to photoactivation and bleaching was confirmed biochemically. The signaling of the residual small fraction of apo-opsin in these rods was then measured by the same methods as above.
Results: Surprisingly, we observed frequent photoresponse-like events in the dark from bleached $GCAPs^{-/-}$ rods. The rate of these photoresponse-like events was similar from 2 hours to 12 hours after the bleach, arguing against a contribution from Meta-II. Consistent with this interpretation, dark activity returned to pre-bleached levels by regenerating bleached opsin into rhodopsin with exogenous 11-*cis* retinal treatment. These data suggest that opsin can form an active Meta-II like state. We observed similar events in $RPE65^{-/-}$ rods regenerated with the unbleachable rhodopsin analogue, further ruling out the involvement of Meta-II and its decay intermediates in these photoresponse-like events.
Conclusions: Our data suggest that, contrary to current beliefs, bleaching adaptation in rods is mediated by opsin that exists in equilibrium between predominantly inactive and intermittently Meta-II like states.
Commercial Relationships: Shinya Sato, None; **Beata Jastrzebska**, None; **Andreas Engel**, None; **Krzysztof Palczewski**, None; **Vladimir J. Kefalov**, None
Support: NIH Grant EY025696, the Arnold and Mabel Beckman Foundation

Program Number: 5603
Presentation Time: 12:15 PM–12:30 PM
Unravelling the mystery of the Stiles Crawford Effect
Ulrich Schraermeyer[1], Sigrid Schultheiss[1], Theo Oltrup[2], Thomas Bende[2], Sebastian Schmelzle[3], Alexander Tschulakow[1]. [1]Centre for Ophthalmology, Experimental Vitreoretinal Surgery, University Hospital Tuebingen, Tuebingen, Germany; [2]Centre for Ophthalmology, Experimental Ophthalmic Surgery, University Hospital Tuebingen, Tuebingen, Germany; [3]Department of Biology, Technical University Darmstadt, Darmstadt, Germany.
Purpose: The Stiles Crawford Effect of the first kind (SCE I) was regarded as one of the most important discoveries in visual science of the last century. The SCE describes the phenomenon that light entering the eye near the edge of the pupil produces a lower photoreceptor response compared to light of equal intensity entering near the center of the pupil. The reduction in the photoreceptor response is significantly greater than would have been expected from a mere reduction in the photoreceptor acceptance angle of light entering near the edge of the pupil, but its cause is still not understood.
Methods: Serial ultrathin and semithin sections were made through human foveolae and used to construct 3D models of central Müller cells. In addition, we used human foveae from flat mounted isolated retinae and measured the transmission of collimated light under the light microscope at different angles.
Results: We found extremely large Müller cells in the central foveola (200 μm in diameter) in which cell organelles are rarely present. We discovered that when the light enters the fovea directly, there is a very bright spot exactly in this area which is composed only of cones and Müller cells. However, when the angle of the transmitted light was changed to 10 degrees, the bright foveolar center became dark and the SCE-like phenomenon became directly visible (Fig. 1). Measurements of the intensities of light transmission in the central foveola for incident angles 0 and 10 degrees resemble the relative luminance efficiency for narrow light bundles as a function of the location where the beam enters the pupil as reported by Stiles and Crawford. The effect was observed in all human foveae and persisted in one fovea after carefully brushing away the outer segments.
Conclusions: Here we show that unique Müller cells in the center of the fovea guide the light based on the incident angle by their specific anatomy and thereby reduce light transmission through the retina, probably causing the SCE of the first kind.

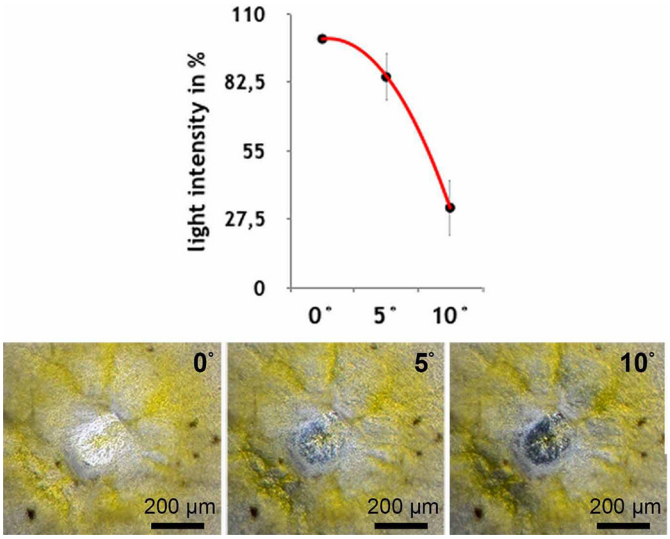
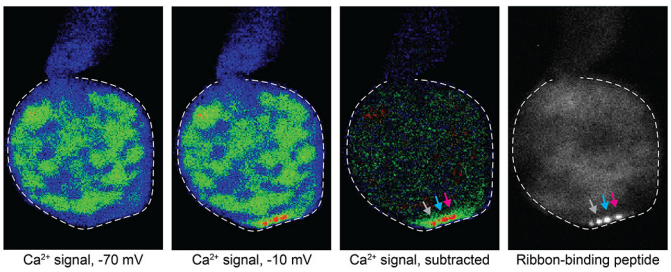


Fig. 1: A human foveolar center is shown (lower panel) in translucent light entering at 0, 5 and 10 degrees respectively with the correspondent measurements of light intensities (upper graph).
Commercial Relationships: Ulrich Schraermeyer; **Sigrid Schultheiss**, None; **Theo Oltrup**, None; **Thomas Bende**, None; **Sebastian Schmelzle**, None; **Alexander Tschulakow**, None

Program Number: 5604
Presentation Time: 12:30 PM–12:45 PM
Functional independence of synaptic ribbons in cone photoreceptors
Justin J. Grassmeyer[2], Matthew J. Van Hook[1], Wallace B. Thoreson[1]. [1]Ophthalmology and Visual Sciences, University of Nebraska Medical Center, Omaha, NE; [2]Pharmacology and Experimental Neuroscience, University of Nebraska Medical Center, Omaha, NE.
Purpose: Cone photoreceptors can have up to 50 presynaptic ribbons. We asked whether Ca^{2+} responses vary among individual ribbons in cones, as ribbon-to-ribbon differences could potentially shape contrast responses of second-order neurons.
Methods: In retinal slices of *Ambystoma tigrinum*, we combined whole-cell recordings and confocal Ca^{2+} imaging with low affinity indicator Oregon Green 488 BAPTA-5N to visualize ribbon-localized Ca^{2+} entry. Ca^{2+} responses to depolarizing stimuli were measured, normalized, and fit with Boltzmann functions adjusted for Ca^{2+} driving force to determine the voltage of half activation (V_{50}) at each ribbon site. We assessed measurement sensitivity by shifting superfusate pH by 0.2 units, which caused readily detectable 3.3 mV shifts in both I_{Ca} and Ca^{2+} signal V_{50}. Trial-to-trial differences in positioning the focal plane or region of interest for Ca^{2+} signal measurements did not significantly increase V_{50} variability.
Results: Ca^{2+} signals varied in spatial extent, but not amplitude, with ribbon size, suggesting similar Ca^{2+} channel density among ribbons. V_{50} of whole-cell Ca^{2+} currents (I_{Ca}) varied modestly among cones (SD=2.5 mV). V_{50} of Ca^{2+} signals at cone ribbons showed greater variability (SD=3.5 mV). Some of this variability reflects cell-to-cell differences in I_{Ca}, but also reflects uncertainties in determining V_{50} from optical Ca^{2+} measurements (SD=1.7 mV). After subtracting variance from both sources, the remaining genuine ribbon-to-ribbon variability in Ca^{2+} signal V_{50} was small (SD=1.4 mV). For these experiments, we had blocked inhibitory synaptic feedback from horizontal cells (HCs) to cones. With feedback active, depolarizing a simultaneously voltage-clamped HC from -60 to 0 mV significantly inhibited ribbon Ca^{2+} signal amplitude and shifted V_{50} values by +2.2 ± 0.5 mV (n=15).
Conclusions: We found modest V_{50} variability among ribbons in the absence of HC to cone feedback. HC feedback at individual ribbons can produce additional V_{50} changes. Small differences can have significant effects: at a cone's membrane potential in darkness of -40 mV, a V_{50} shift of 2.8 mV can alter Ca^{2+} influx by >30%.
The balance of inputs from heterogeneously-activating ribbons could shape bipolar cell contrast sensitivity. For example, a bipolar cell receiving input from multiple ribbons with different V_{50} values will be sensitive to a wider range of intensities than one receiving inputs from identical ribbons.

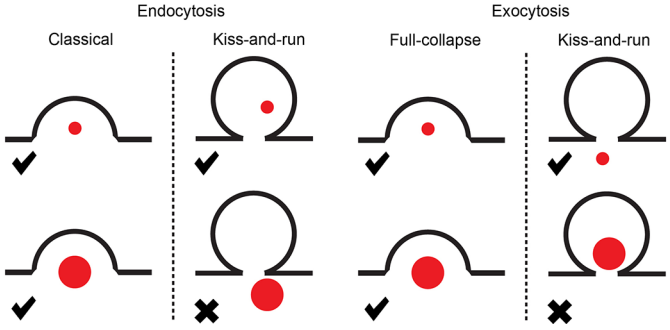


Commercial Relationships: Justin J. Grassmeyer, None; **Matthew J. Van Hook**, None; **Wallace B. Thoreson**, None

Program Number: 5605
Presentation Time: 12:45 PM–1:00 PM
Cambrian origin of the rhodopsin/porphyropsin switch
Gordon Fain[1, 5], Matthew Toomey[2], Ala Morshedian[1], Gabriel Pollock[1], Rikard Frederiksen[3], Jennifer Enright[2], Steve McCormick[4], Carter Cornwall[3], Joseph Corbo[2]. [1]Integrative Biology and Physiology, UCLA, Los Angeles, CA; [2]Pathology and Immunology, Washington University, St Louis, MO; [3]Physiology and Biophysics, Boston University Sch Medicine, Boston, CA; [4]Biology, University of Massachusetts, Amherst, MA; [5]Ophthalmology, UCLA, Los Angeles, CA.
Purpose: Lamprey are jawless vertebrates, which diverged from jawed vertebrates (fishes, amphibians, reptiles, and mammals) during the Cambrian period (~500 Mya). Lamprey are also anadromous, migrating between freshwater and marine environments during their life cycle. In the 1950s, Wald detected vitamin A_1-based rhodopsin in juvenile lamprey but red-shifted vitamin A_2-based porphyropsin in adults. Here, we sought to ask the question: do lampreys use the same mechanism of A_1-to-A_2 conversion (expression of the enzyme, CYP27C1) as fresh-water jawed vertebrates?
Methods: Single-cell measurements were made from sea lamprey (*Petromyzon marinus*) photoreceptors with suction-electrode recording. Photoreceptors were also studied by microspectrophotometry. HPLC, PCR, and in situ hybridization were used to characterize retinoid content and *CYP27C1* gene expression.
Results: Juvenile lamprey rods and cones resemble those of adults. Rods respond to single photons, have longer integration times, and are 70-80 times more sensitive than cones. Spectral sensitivity and microspectrophotometry show that both juveniles and adults have only one spectral class of rod and one cone with best-fitting λ_{max}'s for juveniles of 504 nm (rods) and 551 nm (cones); and for adults of 522 nm (rods) and 592 nm (cones). HPLC shows that vitamin A_2 is present in adult eyes but not in those of juveniles. Quantitative PCR for expression of the vitamin A_1 3,4-dehydrogenase *CYP27C1* gene indicates that levels are significantly higher in the adult RPE compared to juvenile. In situ hybridization shows that the *CYP27C1*

transcript localizes to the adult RPE and is not detected in the juvenile eye.
Conclusions: Lamprey rods and cones respond to light in a nearly identical fashion in juvenile and adult forms, with one spectral class of rod and one cone having predominantly A_2-based pigments in fresh-water adults and A_1-based pigments in marine juveniles. Lamprey convert vitamin A_1 to vitamin A_2 with the same enzyme used by jawed vertebrates, the 3,4-dehydrogenase CYP27C1. Moreover in both lamprey and jawed vertebrates, this enzyme is localized to the RPE. We conclude that porphyropsin and the mechanism of converting A_1 to A_2 were present in the retina before jawed and jawless vertebrates diverged, not long after the origin of chordates during the Cambrian radiation.
Commercial Relationships: Gordon Fain, None; **Matthew Toomey**, None; **Ala Morshedian**, None; **Gabriel Pollock**, None; **Rikard Frederiksen**, None; **Jennifer Enright**, None; **Steve McCormick**, None; **Carter Cornwall**, None; **Joseph Corbo**, None
Support: NIH Grants EY0001844, EY024958, EY0001157

Program Number: 5606
Presentation Time: 1:00 PM–1:15 PM
Assessing Contributions of Kiss-and-run to Synaptic Exocytosis and Endocytosis from Photoreceptors with Optical Measurements of Fusion Pore Size
Xiangyi Wen, Grant Saltzgaber, Wallace B. Thoreson. University of Nebraska Medical Center, Omaha, NE.
Purpose: Photoreceptors release glutamate continuously in darkness. Neurotransmitters can be released by kiss-and-run or full-collapse fusion of synaptic vesicles. It is unclear how much kiss-and-run contributes to synaptic release from various neurons including photoreceptors. We therefore assessed contributions of kiss-and-run vs. full-collapse to exocytosis and endocytosis in salamander photoreceptors using optical methods.
Methods: We used different size dyes (SR101, 1 nm; 3-kDa dextran-conjugated Texas Red or Alexa Fluor 488, 2.3 nm; 10-kDa Texas Red, Alexa Fluor 488, or pHrodo, 4.6 nm; 70-kDa Texas Red, 12 nm) to measure fusion pore diameter and calculate proportions of kiss-and-run. To measure endocytosis, we incubated rods and cones with various dyes and then measured either single vesicle loading by total internal reflection fluorescence microscopy (TIRFM) or whole terminal fluorescence. To assess exocytosis, we imaged individual vesicles using TIRFM and then distinguished vesicle release events by their rapid disappearance.
Results: After 10 min incubation, the concentration of small dyes (SR101; 3-kDa Texas Red) loaded into rod and cone terminals was 8-12 times higher than large dyes (10-kDa Texas Red, 10-kDa pHrodo, 70-kDa Texas Red) suggesting the existence of kiss-and-run events with fusion pores between 2.3-4.6 nm. Using TIRFM, we found that when rods were co-incubated with Texas Red or Alexa Flour 488 conjugated to 3-kDa or 10-kDa dextran, significantly more vesicles loaded with small dyes than large dyes ($P<0.05$). When vesicles were loaded with SR101, 3-kDa Texas Red, or pHrodo, the population of release events identified by TIRFM constituted ~20-22% of all measured events. For 10- and 70-kDa Texas Red, this population was only 6-10% of the total. While pHrodo itself cannot permeate a small pore, its fluorescence can be rapidly quenched by proton extrusion during kiss-and-run.
Conclusions: Measurements of exocytosis and endocytosis showed the presence of kiss-and-run with a fusion pore diameter of 2.3-4.6 nm in photoreceptors. Assuming that large dyes can be released only by full-collapse whereas small dyes can be released by both modes, kiss-and-run accounts for >50% of synaptic release from photoreceptors. Thus, kiss-and-run fusion may facilitate continuous release at photoreceptor ribbon synapses.



Commercial Relationships: Xiangyi Wen, None; **Grant Saltzgaber**, None; **Wallace B. Thoreson**, None
Support: NIH Grant EY10542, Research to Prevent Blindness, China Scholarship Council