Yiming Lu

Mammals, the gap junction expressed in cones has been identified as connexin36 (Cx36). However, the identity of the rod connexin is unknown. To assess the role of Cx36, we compared wild type mice with a pan-Cx36 knockout and both rod and cone specific Cx36 knockouts.

Methods: The junctional conductance between pairs of adjacent rods was estimated using a perforated-patch clamp technique in the dark. The distribution of Cx36 in the outer plexiform layer (OPL) was assessed by confocal microscopy.

Results: The junctional conductance between pairs of adjacent rods was ~150 pS in wild type mice (appropriate littermates) and close to 0 pS in pan-Cx36−/− or rod-Cx36−/− mice. In addition, the rod junctional conductance was ~75 pS in cone-Cx36−/− retinas. In the wild-type retina, most of the Cx36 in the OPL was found around the periphery of cone pedicles. A large fraction of this labeling was observed at points of contact between telodendritic processes and rod spherules. Cx36 labeling in the OPL was reduced by more than 90% in both the rod-Cx36−/− and the cone-Cx36−/− lines, except in small clusters underneath the cone pedicles where Cx36 is known to be associated with bipolar cell dendrites. In the rod-Cx36−/− line, a few remaining Cx36 plaques were found at contacts between cone telodendria.

Conclusions: Direct measurements of the rod junctional conductance demonstrate that Cx36 is required for rod electrical coupling. Intervening cones apparently account for ~50% of rod coupling. The distribution of Cx36 expression in the conditional lines indicates that rod-cone gap junctions require Cx36 on both sides (rod and cone) as the absence of Cx36 on either side prevents the formation of a plaque. This implies that 1) most of the Cx36 plaques observed in the OPL are rod-cone gap junctions. Thus, the rod/cone coupling provides an important pathway. 2) Cx36 is expressed in rods and required to form rod-cone gap junctions. 3) Altogether, the data strongly indicate that Cx36 is the rod connexin.

Commercial Relationships: Nange Jin, None; Friso Postma, None; Sean Youn, None; Eduardo Silveyra, None; David Paul; Stephen C. Massey, None; Christophe P. Ribelayga, None

Support: National Institutes of Health (grants EY018640, EY06515, EY010608, OD010768), the University of Texas System (seed grant #362469), the University of Texas Health Science Center at Houston (BRAIN Initiative and CTSA grant UL1 TR000371), the Hermann Eye Fund and an Unrestricted Challenge Grant from Research to Prevent Blindness.

Program Number: 584 Poster Board Number: B0057

Neuronatin is a novel stress responsive protein of rod photoreceptors

Priyamvada M. Pitale, Vishal M. Shinde, Wayne House, Oleg Gorbatyuk, Marina S. Gorbatyuk. School of Optometry, University of Alabama at Birmingham, Birmingham, AL.

Purpose: Neuronatin (NNAT) is a small transmembrane protein with α and β isoforms highly expressed in developing brain neurons and differentiated non-neuronal tissue. It regulates insulin signaling and glucose trafficking, and acts as a Ca2+ modulator. Although the role of NNAT in other tissues has been highlighted, no study regarding NNAT expression in the adult retina has been conducted so far. Therefore, the purpose of the study was to investigate whether NNAT was expressed in healthy and diseased adult retinas.

Methods: We used adult mammalian retinas of a wide spectrum. Cryostat retinal sections were obtained from various rod-dominant mammals including wild type (WT) rodents, transgenic rodents expressing mutant S334ter, P23H, or T17M rhodopsins, primates, and humans as well as cone-dominant tree shrews. In addition to immunohistochemical analysis, qRT-PCR and western blotting were

Program Number: 583 Poster Board Number: B0056

The Rod Connexin is Connexin36

Nange Jin, Friso Postma, Sean Youn, Eduardo Silveyra, David Paul, Stephen C. Massey, Christophe P. Ribelayga.

‘Ophthalmology & Visual Science, University of Texas McGovern Medical School, Houston, TX, ‘Neurobiology, Harvard University Medical School, Boston, MA; ‘Undergraduate Program, Rice University, Houston, TX.

Purpose: Photoreceptors are electrically coupled via gap junctions. Rod-rod and cone/cone coupling are both present while rod/cone coupling provides an alternative pathway for rod signaling. In mammals, the gap junction expressed in cones has been identified
used to detect NNAT. Isolation of primary rod photoreceptor cells was performed from the WT rat retinas.

**Results:** The expression of NNAT in the WT retina was restricted to the outer segments (OS) of photoreceptors without evidence of staining in other retinal cell types across all mammalian species. Moreover, in tree shrew retinas, we found NNAT to be co-localized with rhodopsin protein indicating its expression in rods. The rod-derived expression of NNAT was further confirmed by qRT-PCR in a primary rod photoreceptor cell line. Subsequently, we demonstrated partial mislocalization of NNAT in transgenic retinas suggesting that stress in photoreceptors leads to NNAT accumulation in the outer nuclear layer (ONL) of the retina. In addition, stressed retinas demonstrated an increase in NNAT mRNA and protein levels as compared to WT. We found 2- and 3-fold increases in NNAT mRNA and protein respectively (P<0.0001 and P<0.001) in S334ter rhodopsin retinas.

**Conclusions:** Our study is the first to provide evidence that NNAT is predominantly expressed in rod photoreceptors in adult mammalian retinas indicating its potential structural role or function involved in cell signaling. Partial mislocalization of NNAT to the ONL and its over-expression associated with retinal degeneration alludes to NNAT being a stress responsive resident protein of rod photoreceptors.

**Commercial Relationships:** Whitney M. Cleghorn, None; Michelle Giarmarco, None; James Hurley, None; Susan E. Brockerhoff, None

**Support:** None

**Program Number:** 586 Poster Board Number: B0059

**Presentation Time:** 1:30 PM–3:15 PM

**Thyroxin b2 receptor (trb2) overexpression alters cone spectra in zebrafish**

**Authors:** Annika Balraj¹, Takeshi Yoshimatsu², Ralph F. Nelson¹. ¹NINDS, National Institutes of Health, Washington, DC; ²Biological Structure, University of Washington, Seattle, WA.

**Purpose:** The trb2 nuclear receptor triggers precursors to become L-cones. When expressed in all cone progenitors (Patterson et al, 2015), the tetrachromatic (L, red; M, green; S, blue; UV) zebrafish becomes an L- cone monochromat. In gnat2:MYFP-2A-trb2 (gnat2:trb2), trb2 is expressed in differentiated cones, causing co-expression of L-opsin in M- and UV-cones at 5dpf (Suzuki et al, 2013). We hypothesized that mixed opsin cones might sensitize ERG responses at long wavelengths (wl), and desensitize UV- and M-cones on red backgrounds.

**Methods:** Both gnat2:trb2 and wild type (WT) siblings were studied at 5, 6, 7, and 12 dpf. gnat2:trb2 larvae showed MYFP fluorescence in the pupil and pineal gland. Dissected eyes were perfused with oxygenated MEM containing 20mM Na Aspartate to block postsynaptic glutamatergic receptors and isolate cone PIII responses. Using a microelectrode, ERG responses were recorded at 10 wavelengths (330-650nm). Backgrounds were either infrared (IR) or red (627nm). Spectra were fit by a ‘sum of Hill functions’ model (Nelson & Singla, 2009). The wl dependence of red to IR background sensitivity provided an index of L-opsin distribution in cone types. For 650nm stimuli, red background sensitivity was 26% of IR background sensitivity and increased towards 100% with shorter wl stimuli. A red background half-desensitized-wavelength (wl½) was measured at 63% of IR background sensitivity.

**Results:** 5-12dpf WT: The primary peak was 370nm, and the secondary peak was 550nm. The median wl½ was 490nm. 5dpf: gnat2:trb2 and WT spectra were similar on both IR and red backgrounds. The gnat2:trb2 wl½ was 490nm. 6dpf: on IR background, gnat2:trb2 sensitivity was greater than wild type for wavelengths longer than 400nm. wl½ was 400nm, suggesting L-, M- and S-cones were desensitized by red backgrounds. 7dpf: gnat2:trb2 IR background sensitivity in the UV was less than WT, but similar at longer wl. wl½ was 390nm, suggesting L-, M-, S- and UV-cones were desensitized by the red background. 12dpf: gnat2:trb2 IR spectra showed normal (WT) UV sensitivity, but an enhanced 550nm peak. wl½ was 490nm.

**Conclusions:** The results support the hypothesis of mixed opsin cones in gnat2:trb2, but only for 6 and 7dpf. At 12dpf, a red spectral enhancement persists, but red desensitization of non-L cones is lost. Mixed L-opsin expression in non-L-cones appears ultimately rejected by 12dpf.

**Commercial Relationships:** Annika Balraj; Takeshi Yoshimatsu, None; Ralph F. Nelson, None
**Support:** Supported by Basic Neurosciences Program, NINDS NIH

**Program Number:** 587  **Poster Board Number:** B0060

**Presentation Time:** 1:30 PM–3:15 PM

**The Role of Thyroid Hormone Receptor B2 (trβ2) in Photoreceptor Opsin Development**

Sara Patterson1,2, Takeshi Yoshimatsu1, Tara Suresh1, Ralph F. Nelson2. 1Neuroscience, University of Washington, Seattle, WA; 2Neural Circuits Unit, NINDS-NIH, Rockville, MD.

**Purpose:** In the transgenic zebrafish line cxx:MYFP-2A-trβ2, the highly conserved cone rod homeobox (crx) gene promoter drives thyroid hormone β2 (trβ2) expression (Suzuki et al., 2013). Crx is expressed in photoreceptor progenitors and trβ2 is a necessary for subsequent red cone development. This transgenic provides the opportunity to study thyroid hormone in photoreceptor development.

**Methods:** Both cxx:trβ2 and wildtype (WT) cousins were studied as larvae at 5-12 days post-fertilization (dpf) and adults at 6-9 months old. Eyes were removed and perfused with oxygenated MEM containing 20mM L-Aspartate or 10mM CNQX to isolate the photoreceptor and ON-bipolar responses, respectively. Microelectrode ERG responses were recorded to 9 wavelengths (330-650nm), each at 7 irradiances. A 627nm background was used for red adaptation trials. Spectral sensitivity was calculated with an ERG model summing 4 Hill functions, one for each zebrafish cone type (Nelson & Singla, 2009). To examine Vitamin A2 L-opsin expression, a 5th Hill function was added using a 600nm max.

**Results:** In cxx:trβ2, photoreceptor ERG responses showed increased long-wavelength sensitivity and diminished, but not entirely absent, short-wavelength sensitivity. By adulthood, cxx:trβ2 photoreceptor spectra resembled WT spectra with only a small increase in long-wavelength sensitivity UV- and S-opsin immunostaining found expression at 5 and 12dpf. While S-opsin was widespread, UV-opsin appeared primarily in the peripheral retina. Red adaptation altered only long-wavelength photoreceptor sensitivity but decreased ON-bipolar sensitivity at all wavelengths in both larvae and adults. A2 sensitivity was stronger in cxx:trβ2 than WTs and decreased from 5 and 12dpf in both genotypes.

**Conclusions:** Zebrafish photoreceptor differentiation is normally complete by 4dpf. However, photoreceptor ERG responses in cxx:trβ2 developed towards WT sensitivity between 5dpf and adulthood. The large decrease in both short- and long-wavelength ON-bipolar sensitivity in red-adaptation trials suggests cxx:trβ2 may also impact photoreceptor-bipolar cell connectivity. The decrease in Vitamin A2 600nm sensitivity from 5 to 12dpf suggests thyroid hormone may be involved in the early retinal development of zebrafish. Understanding how short-wavelength opsins and spectral sensitivity returns in cxx:trβ2 could have important implications for photoreceptor development and regeneration research.

**Commercial Relationships:** Sara Patterson, None; Takeshi Yoshimatsu, None; Tara Suresh, None; Ralph F. Nelson, None

**Program Number:** 588  **Poster Board Number:** B0061

**Presentation Time:** 1:30 PM–3:15 PM

**GABA-mediated horizontal cell signaling switches back and forth between cones at night and ON-cone bipolar cells in the day**

Stuart C. Mangel. Neuroscience, Ohio State Univ Coll of Med, Columbus, OH.

**Purpose:** Horizontal cells (HCs) can signal cone bipolar cells (cBCs) directly and indirectly by providing feedback inhibition to cones. Although HCs evoke cBC surround responses, the identity of the HC transmitter and whether the two HC pathways have similar functions remain unclear. My lab has shown that dopamine D Rs (on ON-cBC dendrites) and D Rs (on cones), by modulating intracellular PKA, increase the expression and activity of GABA Rs on 1) ON-cBC dendrites in the day following maintained (>30 min) illumination (Chaffiol et al, submitted) and 2) cone terminals at night following maintained darkness (Mangel et al, 2015, ARVO). We thus studied whether HC signaling to ON-cBCs and to cones depend on GABA Rs, D Rs, D Rs, and the time of day or night.

**Methods:** The effects of artificially polarizing HCs on nearby ON-cBCs in rabbit retinal slices and on nearby cones in intact goldfish retinas were studied in the day and night in the presence and absence of gabazine (GBZ, GABA R antagonist), SCH23390 (SCH, D R antagonist), spiperone (SP, D R antagonist) and MFA (gap junction blocker) (HCs: sharp pipettes; ON-cBCs, cones: patch pipettes).

**Results:** Preliminary rabbit HC/ON-cBC paired recordings revealed that in the day 1) artificially hyperpolarizing HCs reduced ON-cBC surround responses (as did GBZ or SCH), and 2) in the absence of light stimuli and with APB in the Ringer, depolarizing and hyperpolarizing HCs depolarized and hyperpolarized ON-cBCs, respectively (i.e. sign-conserved signaling), effects that were blocked by GBZ or SCH. Preliminary fish HC/cone recordings revealed that in the absence of light stimuli and with MFA in the Ringer HC polarizations altered cone voltage in a sign-inverted manner at night in the dark and in the day following SP (effects that were blocked by GBZ), but not in the day without SP.

**Conclusions:** The results suggest that 1) in the day when the D Rs of ON-cBCs are activated HCs provide a GABA R-mediated sign-conserving (excitatory) signal to ON-cBCs that contributes to surround responses, and 2) at night when the D Rs of cones are NOT activated HCs provide a GABA R-mediated sign-inverting (inhibitory) signal to cones that decreases cone light responses. Because cones and ON-cBCs do not exhibit surrounds at night, the findings also suggest that in addition to operating at different times, HC signaling to cones and to ON-cBCs have different functions.

**Commercial Relationships:** Stuart C. Mangel, None

**Support:** Plum Foundation

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**Conclusions:** Our current study uncovers versatile functional roles of HCs in the retina circuit.

**Commercial Relationships:** Taro Chaya, None; Akihiro Matsumoto, None; Yuko Sugita, None; Satoshi Watanabe, None; Rysuuke Kuwahara, None; Masao Tachibana, None; Takahisa Furukawa, None

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**Program Number:** 590 Poster Board Number: B0063
**Presentation Time:** 1:30 PM–3:15 PM

**Membrane topology analysis of the ON bipolar cell transduction channel subunit TRPM1**

Melina A. Agosto, Theodore G. Wensel. Biochemistry and Molecular Biology, Baylor College of Medicine, Houston, TX.

**Purpose:** Neurotransmission between photoreceptors and downstream ON bipolar cells is mediated by a GPCR signaling cascade, for which the TRPM1 channel subunit is required for function. A high-resolution structure of TRPM1 has not yet been determined. Limited homology between the transmembrane (TM) domain of TRPM1 and those of other TRP family channels suggests that TRPM1 has six TM helices. However, no experimental data regarding the topology of the protein has been reported, and different TM prediction algorithms yield varying results. The purpose of this study is to experimentally determine the topology and orientation of TRPM1 in the membrane.

**Methods:** A fluorescence protease protection assay, in conjunction with live cell imaging, was employed to assess the membrane topology of TRPM1 and nyctalopin. Human embryonic kidney (HEK293) cells expressing GFP fusion proteins were treated with digitonin to selectively permeabilize the plasma membrane, followed by protease K to digest regions accessible to the cytoplasm.

**Results:** Mouse nyctalopin (NYX), which is known to have an extracellular or ER-luminal N-terminus and a cytoplasmic C-terminus, was used as a control. As expected, the fluorescence of cells expressing NYX-GFP decreased rapidly upon addition of protease. The fluorescence after 4 min of treatment was greatly reduced compared to control cells treated with digitonin but not protease (p<0.001, Mann-Whitney test). In contrast, GFP-NYX was resistant; cells with and without protease treatment were not significantly different (p=0.176). Both GFP-TRPM1 and TRPM1-GFP were localized to intracellular membranes in HEK293 cells. Unlike nyctalopin, the fluorescence of cells expressing TRPM1 with GFP at either the N- or C-terminus was sensitive to protease treatment (p<0.001 after 4 min).

**Conclusions:** The fluorescence protease protection assay revealed that both the N- and C-termini of TRPM1 are cytoplasmic, consistent with the presence of six TM helices. Future studies will further dissect the topology of TRPM1 and the location of the TM helices by testing the accessibility of epitope tags and protease cleavage sites engineered into predicted luminal and cytoplasmic loops.

**Commercial Relationships:** Melina A. Agosto, None; Theodore G. Wensel, None

**Support:** NIH grants R01 EY07981, R01 EY11900, T32 EY007102, and F32 EY200672, the Welch Foundation, and the Knights Templar Eye Foundation

**Program Number:** 591 Poster Board Number: B0064
**Presentation Time:** 1:30 PM–3:15 PM

**Vesicular ATP pools in zebrafish horizontal cells**

Salvatore L. Stella, Dillon McDevitt. Neural and Behavioral Sciences, Penn State University College of Medicine, Hershey, PA.

**Purpose:** The discovery of a vesicular nucleotide transporter (VNUT) has established that ATP can be concentrated and stored in neuronal vesicles. VNUT expression has been localized to horizontal cells in the outer retina. Therefore, we hypothesize that an ATP pool is present in horizontal cells and contributes to ATP release which can serve as a precursor to adenosine during nocturnal conditions.

**Methods:** Experiments were performed on both intact retinas and isolated horizontal cells from zebrafish retina. Confocal live cell imaging of quinacrine or MANT-ATP were used to monitor ATP levels and release. Immunohistochemical analysis using confocal microscopy of zebrafish retina was performed on vertical sections and whole mounts. Vesicle cycling was monitored using a VNUT antibody targeted to the luminal face of the protein conjugated to CF488 in order to characterize vesicle turnover in horizontal cells.

**Results:** Vesicles containing ATP labeled stores co-localized with VNUT in horizontal cells. Antibodies targeted to the luminal face of VNUT transporters labelled cycling vesicles within horizontal cell processes. Uptake was stimulation and Ca²⁺-dependent arguing in favour of a vesicular mechanism, and reduced in the absence of Ca²⁺ and Ca²⁺ channel blockers. Synaptic vesicle recycling and retrieval was demonstrated with sequential Ca²⁺ labelling of vesicles with fluorescently labelled VNUT antibody followed by a secondary antibody targeted to the VNUT antibody.

**Conclusions:** These findings support the hypothesis that horizontal cells contain ATP in VNUT expressing vesicles and release ATP from horizontal cells in the outer retina. Thus, during nocturnal conditions it is possible that outer retinal adenosine is derived form ATP.

**Commercial Relationships:** Salvatore L. Stella, None; Dillon McDevitt, None

**Support:** The Plum Foundation (Studio City, CA), Penn State College of Medicine

**Program Number:** 592 Poster Board Number: B0065
**Presentation Time:** 1:30 PM–3:15 PM

**Simulation analysis of negative feedback in the outer retina**

Hiroaki Kunisada, Yoshimi Kamiyama. Information Science and Technology, Aichi Prefectural University, Nagakute, Japan.

**Purpose:** Synaptic interactions between cones and horizontal cells play a key role in the center-surround receptive field organization of the retina. Recent physiological studies suggest that complex feedback systems exist between horizontal cells and cones. Three major feedback mechanisms (1.ephaptic, 2.pH-mediated and 3.GABAergic) have been proposed and studied, however, the functional consequences have not yet fully understood. The purpose of the present study is to evaluate how the each mechanism contributes to the feedback function quantitatively through computer simulation.

**Methods:** We constructed a mathematical model of cone-horizontal cell network based on physiological characteristics. We developed a cone model by describing phototransduction mechanism in the outer segment, the membrane ionic currents in the inner layer (I\textsubscript{Glu}, I\textsubscript{Na}, I\textsubscript{Ca}, I\textsubscript{Cl(Ca)}, I\textsubscript{L}) and the synaptic terminal (I\textsubscript{Ca}, I\textsubscript{Cl(Ca)}, I\textsubscript{L}). Horizontal cell is modeled with the ionic currents (I\textsubscript{Cl}, I\textsubscript{Na}, I\textsubscript{Ca}, I\textsubscript{Cl}, I\textsubscript{L}). IGlu is modulated by glutamate released from the cone synaptic terminal. The effects of the ephaptic and pH-mediated feedback were modeled as functions of horizontalcell membrane potential. I\textsubscript{Ca} in the cone synaptic terminal was assumed to be directly modulated by the feedback.

**Results:** In simulation, the shift of cone I\textsubscript{Ca} activation-range was reproduced with the combination of the ephaptic and pH-mediated feedback mechanisms. Simulated cone responses to a flash of light showed the characteristic responses such as initial transient hyperpolarization and delayed depolarization similar to those
observed experimentally. Those response properties could not be reproduced with a single feedback mechanism. **Conclusions:** The results suggest that both the ephaptic and pH-mediated mechanisms are required in the cone-horizontal cell synapse, i.e., the dynamic characteristics of the cone light responses can not be explained by a single feedback system. The results also suggest the functional roles of each feedback system; ephaptic feedback elicits cone voltage shift in the negative direction and the pH-mediated feedback regulates the amplitude of cone calcium current.

**Commercial Relationships:** Hiroaki Kunisada; Yoshimi Kamiyama, None
**Support:** JSPS KAKENHI #25330340

**Program Number:** 593 **Poster Board Number:** B0066
**Presentation Time:** 1:30 PM–3:15 PM
**Divergence of visual signals in parallel ON cone bipolar cell pathways of the mouse retina**
Sidney P. Kuo1,2, Haruhisa Okawa1, Justin Pacholec1, Rachel O. Wong1, Fred Rieke1,2. 1Physiology and Biophysics, University of Washington, Seattle, WA; 2Howard Hughes Medical Institute, Seattle, WA; Biological Structure, University of Washington, Seattle, WA.

**Purpose:** Cone photoreceptor signals are conveyed to the inner retina by ~12 distinct subtypes of cone bipolar cells. This divergence of cone signals into different feed-forward bipolar pathways forms the initial basis for parallel retinal circuits dedicated to specific visual functions. We examined how distinct bipolar cell types contribute to differential temporal processing of visual signals by comparing the light response properties of two types of ON cone bipolar cell with distinct axonal morphologies and stratification patterns (type 5 and type 6), as well as excitatory synaptic currents in specific retinal ganglion cell (RGC) targets of these bipolar cells.

**Methods:** We used patch-clamp recordings to measure visual stimulus-evoked responses from bipolar cells and RGCs in isolated mouse retinal tissue. Recordings were restricted to ventral retina and light stimuli were delivered from a short wavelength (395 nm peak) LED at a photopic background light level. ON cone bipolar cells were targeted using 2-photon microscopy in retinal slices prepared from transgenic mice with selective fluorescent protein expression in specific bipolar cell subtypes. Bipolar cell responses were recorded using gramicidin perforated-patch recordings. Whole-cell voltage-clamp recordings were obtained from RGCs in a flat mount preparation. To identify postsynaptic targets of type 5 bipolar cells, we biolistically transfected RGCs in transgenic mouse tissue with DNA coding for a fluorescently labeled postsynaptic marker.

**Results:** We found light-evoked voltage responses of type 5 and type 6 cone bipolar cells were not significantly different from one another. However, excitatory synaptic currents in RGCs measured in response to the identical stimuli as used in bipolar cell recordings exhibited substantial differences between ON alpha RGCs, a major target of type 6 bipolar cells (Schwartz et al., 2012), or a previously uncharacterized ON RGC type we identified as a postsynaptic target of type 5 bipolar cells. For example, excitatory synaptic input to this latter RGC, which we term ‘ON transient’, was less sensitive to small, rapid fluctuations in visual contrast and had distinctive kinetics compared to excitatory currents in ON alpha RGCs.

**Conclusions:** These results suggest that bipolar cell type-specific synaptic mechanisms play a critical role in divergence of visual signals across the parallel ON pathways we examined here.

**Commercial Relationships:** Sidney P. Kuo, None; Haruhisa Okawa, None; Justin Pacholec, None; Rachel O. Wong, None; Fred Rieke, None

**Support:** NIH Grant EY11850; Howard Hughes Medical Institute

**Program Number:** 594 **Poster Board Number:** B0067
**Presentation Time:** 1:30 PM–3:15 PM
**Dopamine D1 receptors are involved in light-adapted changes to bipolar cell spontaneous inhibition and receptive field surrounds**
Erika D. Eggers1,2, Reece Mazade1, Michael Flood1. 1Physiology, University of Arizona, Tucson, AZ; 2Biomedical Engineering, University of Arizona, Tucson, AZ.

**Purpose:** Retinal inhibition is vital for shaping visual signals to highlight important aspects of the visual scene and may play an important role in retinal adaptation to increased background luminance. We previously showed that light adaptation reduced spontaneous inhibitory activity and the spatial extent of inhibition to OFF bipolar cells (BC). Dopamine release is the main neuromodulator of light adaptation and can modulate GABA receptors on retinal neurons. However, the contribution of dopamine to changes in BC inhibition due to light adaption has not been studied. To investigate this, spontaneous and light-evoked spatial inhibitory inputs were measured from OFFBCs in the mouse retina under different background luminance conditions and while either stimulating or blocking dopamine D1 receptors that are located on inner retinal neurons.

**Methods:** Whole-cell voltage clamp was used to record light-evoked and spontaneous inhibitory postsynaptic currents from dark-adapted mouse OFFBCs, identified via fluorescent labeling. A white OLED screen was used to set the background light and to generate 25 μm bars of light flashed for 1 sec to map spatial inhibition. D1 receptors were activated with SKF 38393 (SKF, 20 μM) and blocked with SCH 23398 (SCH, 50 μM). The charge transfer and peak amplitude of light-evoked responses and the peak amplitude and frequency of spontaneous events were measured. The spatial distributions were averaged and compared between conditions.

**Results:** Under dark-adapted conditions, application of D1 receptor agonist SKF partially mimicked the light adapted decreases in total, glycinergic, and GABAergic evoked spatial input to the OFFBCs. However, SKF decreased OFFBC spontaneous inhibition to the same extent as light adaptation. Under light-adapted conditions, application of D1 receptor blocker SCH before adaptation partially blocked the decrease in evoked-spatial input.

**Conclusions:** These data show that dopamine signaling through D1 receptors plays a prominent role in modulation of inner retinal inhibition to OFFBCs. Modulation of spontaneous inhibition by light adaptation could be completely mimicked by activation of D1 receptors, but there are likely other important factors that contribute to the narrowing of OFF bipolar surround receptive fields seen with light-adaptation.

**Commercial Relationships:** Erika D. Eggers, None; Reece Mazade, None; Michael Flood, None
**Support:** EY018131 (EDE), W911NF-15-1-0613 (EDE), University of Arizona NIH Graduate Training in Systems and Integrative Physiology grant 5T32GM008400 (REM), and the ARCS Foundation (REM).

**Program Number:** 595 **Poster Board Number:** B0068
**Presentation Time:** 1:30 PM–3:15 PM
**Na-K-2Cl cotransporter deficiency in the retinas affects the visual contrast sensitivity**
Wen Shen. Biomedical Science, College of Medicine, Florida Atlantic University, Boca Raton, FL.

**Purpose:** To investigate the function of the Cl uptake co-transporter in retinal signal transduction and adaptation. To test a hypothesis that...
degradation of the co-transporter in the aging retina may affect the retinal electrophysiology.

**Methods:** The wild-type (WT) and NKCC1-deficient (NKCC1-/-) mice at age 1-4M and the WT mice at age 18-20M were used in the study. To assess the changes in retinal function, corneal flash electroretinogram (ERG) from the NKCC1-/- and WT were compared in dark- and light-adapted conditions. The influence of NKCC1 on cell membrane potential and light response were studied from horizontal cells (HCs) in intact retina. The ultimate effect of NKCC1 on the retinal signal outputs was studied in the ganglion cells (GCs) in whole-cell recording. The cell morphology, synaptic proteins and NKCC1 localization were detect in immunocytochemistry. The NKCC1 protein levels in the young and old age mouse retina were quantitatively analyzed in Western blotting.

**Results:** NKCC1 was found highly expressed in the distal retina, HCs and the ON-bipolar cell dendrites, in young adult animals; but the expressing levels declined in the retinas from the old age animals. Flash ERG recordings showed that the lack of functional NKCC1 led to a reduction of the b-wave amplitude in both scotopic and photopic conditions, but oscillatory potential (OP) amplitude was unchanged, detected from the NKCC1-/- mice compared to the WT littermates. A reducing of b-wave amplitude was also observed in the old age WT animals. The cell densities and ultra-structures of photoreceptor terminals and ON-bipolar cell dendrites in the NKCC1-/- retina appeared normal. However, the axon processes of HCs are reduced in the NKCC1-/- retina, although the density of HCs seems unaffected in the NKCC1-/-.. The light intensity-response curve of HCs was left-shifting in the NKCC1-/-.. A full range of contrast responses was tested from HCs and the GCs, showing that NKCC1 deficiency caused light-responses to saturate at relative low contrast compared to the control.

**Conclusions:** The lack of functional NKCC1 in distal retinal alters the cell communications in the retinas. Both the NKCC1-/- and old age mouse showing poor or less sensitive to contrast stimulation suggest that a deficient expression of the Cl transporter in the aging process could be one of the risk factors for age-related degradation of contrast sensitivity in vision.

**Commercial Relationships:** Wen Shen, None

**Support:** NSF, IOS1021646

**Program Number:** 596  **Poster Board Number:** B0069  **Presentation Time:** 1:30 PM–3:15 PM

**Rod bipolar cells in the adult mammalian retina actively search for and selectively synapse with healthy rod photoreceptors**

Corinne Beier1, Anahit Hovhannisyan2, Sydney Weiser2,  
Seung Jun Lee4, Jennifer Kung1, Dae Yeong Lee1, Philip Huie1,  
Roopa Dalal1, Daniel V. Palanker1,2, Alexander Sher1.

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3Ophthalmology, Stanford University, Stanford, CA;  
4Hansen Experimental Physics Laboratory, Stanford University, Stanford, CA.

**Purpose:** The adult retina is well known to be destructively plastic in response to injury or disease, however, recently we have presented evidence that the retina can regain visual sensitivity following local photoreceptor ablation. Healthy photoreceptors migrate into the lesion and make synaptic contact with the restructured dendrites of deafferented rod bipolar cells (RBCs). We investigated if RBC dendrites actively search for photoreceptors, if this search is selective in choosing synaptic partners, and if cone bipolar cells exhibit similar constructive plasticity.

**Methods:** Line-shaped lesions were produced in rabbits with a 532-nm laser, using beam diameter 100 μm, scanned at 1.7m/s along the 1.5mm of inferior retina. 200μm and 300μm wide lesions were made by staggering multiple scans. Bipolar cells and their synapses with photoreceptors were visualized with immunohistochemistry (PKCa, secretagogin, CtBP2 and mGluR6) and confocal Z-stacks were used for subsequent data analysis. The dendritic reach, defined as the distance between the dendritic tip and the point at which the dendrite exits the cell body, of healthy filled RBCs was compared to the reach of restructured dendrites of deafferented RBCs. A cone pedicle was termed as being ‘approached’ by a RBC if a dendrite terminated within or next to the cone pedicle.

**Results:** Rod bipolar cell dendrites restructure to send a single thickened dendrite out of the lesion into ribbon-rich areas. The thickened dendrites terminate in multiple mGluR6 doublet-ribbon pairs. The dendritic reach of the restructured RBCs is 40% longer than in the intact retina (p<0.01). Cone pedicles within the vicinity of restructured dendrites are not approached more often by RBC dendrites than cone pedicles in the healthy retina. Deafferented cone bipolar cells retain at least some of their fine dendrites and their dendritic network appears to be disturbed. However, we did not observe thickened cone bipolar cell processes reaching out to photoreceptors.

**Conclusions:** Adult RBCs retain the capacity to make new and correct synaptic connections following local photoreceptor loss. In contrast, the restructuring of the cone bipolar cell dendrites is subtler, suggesting the structural plasticity mechanisms available to adult rod and cone bipolar cells are different.

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**Connectivity map of bipolar cells and photoreceptors in the outer mouse retina**

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**Purpose:** In the mouse retina, two types of cone photoreceptors (short (S) and medium (M) wavelength-sensitive cones) provide input to 13 types of cone bipolar cells. Type 1 bipolar cells is supposed to predominantly receive input from M-cones, whereas type 9 bipolar cells selectively contact S-cones (reviewed by Euler et al., 2014). Additionally, one type of rod photoreceptors (rods) contacts rod bipolar cells (RBCs). However, how cone and rod pathways are interconnected at the level of individual photoreceptor-bipolar cell synapse is unknown. Additionally, it is unknown to which degree the remaining cone bipolar cell types selectively sample input from S- and/or M-cones.

**Methods:** We exploit the serial block-face scanning electron microscopy dataset of the mouse retina provided by Helmstaedter et al. (2013) to systematically analyze the connectivity between cones, rods and bipolar cells in the outer plexiform layer. Using volume segmentation and contact classification, we reconstruct 163 cone and 2176 rod terminals and identify 13 S-cones based on their specific contacts with type 9 bipolar cells. Using an automated classification
approach, we analyzed the contact types between photoreceptors and bipolar cells.

**Results:** First, in addition to the input from rods, 63% of RBCs (n=141) receive input from cones indicating that two functionally distinct groups of RBC exist. Second, XBC cone bipolar cells contact very few cones (1.0 cones/XBC, 95% CI [0.14, 2.14], n=7). Third, type 1 and type 3A OFF CBCs make contacts with both S- and M-cones, but in type 3A cells M-cone input dominates cone input (with type 3A cells on average contacted by 0.31 S-cones (95% CI [0.08, 0.54]) and by 0.79 M-cones (95% CI [0.67, 0.93]), n=22) suggesting that this type may form the ‘vertical green pathway’ in the mouse retina.

**Conclusions:** We found that the large scale outer retinal connectivity map can be used for anatomical classification of bipolar cell types and provides important insight in the putative function of bipolar cells.

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