Our results are consistent with the hypothesis that an OPN4 immunotoxin, consisting of a saporin
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NIH F31 EY026787
8:30 AM–10:15 AM
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Program Number: 4123 Poster Board Number: B0242
Presentation Time: 8:30 AM–10:15 AM
Tbr2 is necessary and sufficient for the specification and maintenance of non-image forming RGCs
Andreaa Nistorica, David Feldheim. University of California Santa Cruz, Santa Cruz, CA.
Purpose: The molecular factors that specify and maintain retinal ganglion cell (RGC) circuitry in the adult mouse remain a mystery. By using a mouse knockout of Tbr2 (Tbr2CreER; Tbr2<sup>fl/fl</sup>) and ectopic Tbr2 expression, this study investigates the role of the transcription factor Tbr2 in the specification and maintenance of non-image forming RGCs.
Methods: Tbr2Cre<sup>fl/fl</sup> and Tbr2<sup>fl/fl</sup> that also harbor a tdTomato reporter are used to remove Tbr2 from adult retina while labeling the dendrites, soma, and axons of mutant cells. Retinas and brains were harvested 1 week after control and Tbr2 KO adult mice were fed tamoxifen by oral gavage. Melanopsin expression was examined by immunofluorescence on whole mount retinas. Brains were sectioned to visualize the axonal projections to retinoreceptor nuclei. Ectopic Tbr2 expression in the retina was performed through intravitreal injection of AAV-Tbr2 in the adult eye. A virus that expresses only a fluorescent reporter was used as a control. We quantified the population of cells infected with Tbr2 and control virus that coexpressed melanopsin and other RGC markers in whole mount retinas 2 weeks post infection, and also visualized the axonal connections of the infected RGCs in brain sections.
Results: Removing Tbr2 from the retina in adult mice leads to a dramatic loss of melanopsin-positive RGCs when compared to the control (n=5 retinas for mutant and controls). There is a drastic loss of axonal inputs to the vLGN, OPN, and NOT in the mutants while SCN inputs remain (n=5 for mutant and controls). Ectopic Tbr2 expression in a random population of RGCs leads to melanopsin expression in image forming RGCs (n=5 retinas). Additionally, axonal inputs from image forming RGCs ectopically expressing Tbr2 were observed in the vLGN, OPN, and NOT (n=3).
Conclusions: Our results are consistent with the hypothesis that Tbr2 is involved in maintaining RGC circuitry. Knocking out Tbr2 perturbs the pretectal connections known to be responsible for the pupillary light reflex, but not the connections to the SCN, responsible for circadian rhythms. Our results also show that Tbr2 is necessary and sufficient for melanopsin expression, and that Tbr2 expression in image forming RGCs is sufficient for rewiring axonal connections to specific non-image forming nuclei. Together, our evidence indicates that Tbr2 is necessary and sufficient for maintaining a non-image forming RGC fate.
Commercial Relationships: Andreaa Nistorica, None; David Feldheim, None
Support: NIH F31 EY026787

Program Number: 4124 Poster Board Number: B0243
Presentation Time: 8:30 AM–10:15 AM
Gap-junction networks of intrinsically photosensitive retinal ganglion cells (iRGCs)
Xiwu Zhao, Kwoon Y. Wong. Ophthalmology and Visual Sciences, University of Michigan, Ann Arbor, MI.
Purpose: We previously found that in the ganglion cell layer (GCL), all amacrine cells with spiking, tonic ON photoresponses receive gap-junction input from iRGCs (Reifler et al. 2015 Curr Biol). Here we test whether iRGCs are coupled to additional kinds of GCL cells.
Methods: Dark-adapted mouse retinas containing GFP-labeled iRGCs (Ecker et al. 2010 Neuron) were flattened and superfused by Ames medium, and GFP+ somas visualized with a two-photon laser. The gap-junction-permeable dye PoPro1 (Hoshi et al. 2006 J Histochem Cytochem) was injected using sharp electrodes into GFP+ somas to label iRGC-coupled cells, which were whole-cell-recorded and Alexa-filled to study their photoresponses and morphologies. While recording from an iRGC-coupled cell, individual nearby GFP+ somas were illuminated with a localized two-photon stimulus to excite one iRGC at a time, in order to reveal how many iRGCs signaled to that cell. PoPro1 was injected into some iRGC-coupled cells to identify cells that were coupled to them.
Results: Each iRGC was coupled to 2 – 20 GCL cells, ~70% of which were morphologically and physiologically similar to those reported in Reifler et al. 2015: S5-monostratifying and S1/ S5-bistratifying amacrine cells with medium and wide fields, all displaying spiking, tonic ON responses. The remaining iRGC-coupled cells included S1-monostratifying medium-field amacrine cells, starburst amacrine, and OFF RGCs including OFF alpha cells. Though some of these new types of iRGC-coupled cells had spiking, tonic ON photoresponses, many showed novel light responses, e.g. OFF responses to dim light but ON responses to bright light; ON responses to dim light but OFF responses to bright light; and non-spiking responses. These cells could generate melanopsin-driven light responses during pharmacological block of rod/cone signaling, confirming they received iRGC input. Each iRGC-coupled amacrine cell was coupled to other amacrinan, and could be excited by two-photon activation of up to four nearby iRGC somas.
Conclusions: iRGCs are coupled with and signal to a far greater variety of GCL cells than previously appreciated, including conventional RGCs – this is the first report of coupling between different types of mouse RGCs. Each iRGC is coupled to many amacrines and each amacrine cell may get input from multiple iRGCs. Coupling with OFF RGCs enables iRGCs (which are ON cells) to influence the OFF channel.
Commercial Relationships: Xiwu Zhao, None; Kwoon Y. Wong, None
Support: NIH NEI Grants EY023660 and EY007003

Program Number: 4125 Poster Board Number: B0244
Presentation Time: 8:30 AM–10:15 AM
Immunotoxin-Induced Ablation of the Intrinsically Photosensitive Retinal Ganglion Cells in Rhesus Monkeys
Paul D. Gamlin<sup>1</sup>, Christianne E. Strang<sup>2</sup>, Kevin Chang<sup>1</sup>, Li-Fang Hung<sup>2</sup>, Baskar Arumugam<sup>1</sup>, Laura J. Frishman<sup>1</sup>, Earl L. Smith<sup>1</sup>, Lisa A. Ostrin<sup>2</sup>. 1Vision Sci Rsch Ctr, Univ of Alabama at Birmingham, Birmingham, AL; 2University of Houston College of Optometry, Houston, TX.
Purpose: The intrinsically photosensitive retinal ganglion cells (iRGCs) are photosensitive cells in the inner retina that control pupil size and entrain circadian rhythm. Evidence suggests they are involved in a range of other non-image forming functions, such as ocular development and refractive error. The goal of this study was to develop and validate a targeted iRGC immunotoxin to ultimately examine the role of these cells in Rhesus monkeys.
Methods: An OPN4 immunotoxin, consisting of a saporin-conjugated antibody directed at the N-terminus of macaque OPN4, was prepared in solutions of 50, 10, 3.16, 1 and 0.316 µg in sterile BSS, and delivered intravitreally to the right eye of six Rhesus monkeys, respectively. Left eyes were injected with BSS only. The iRGC-driven pupil responses were recorded and OCT imaging
The photocurrents of M2- and M4-ipRGCs were recorded from 8:30 AM–10:15 AM. Photo-uncaged cyclic nucleotide in the presence of synaptic blockers. Photo-uncaging experiments were performed as described in Xue et al., 2017.

**Results:** Before injection, animals showed robust ipRGC-driven pupil responses to 1s 456nm light, with an average 6s PIPR of 0.7±0.1 to the highest intensity. Pupil constriction was sustained during 5s light pulses. After injection, delayed redilation was eliminated in all but the lowest dose, which had a 6s PIPR to 456nm of 0.88. During 5s stimulation, pupil constriction was no longer sustained. Baseline pupil size increased 12±17% and maximum transient pupil constriction to 456 nm light decreased 16±5%. For the highest concentrations of immunotoxin, some inflammation and structural changes were observed; however, eyes injected with lower concentrations appeared normal. IHC showed significantly decreased numbers of ipRGCs in experimental eyes.

**Conclusions:** Findings show that the newly developed OPN4 macaque immunotoxin induced a graded reduction in the ipRGC-driven pupil response with concentration and was specific for ipRGCs. Further refinement and validation of the immunotoxin will allow for better elucidation of the functions of ipRGCs in primates.

**Commercial Relationships:** Paul D. Gamlin, None; Christianne E. Strang, None; Kevin Chang, None; Li-Fang Hung, None; Baskar Arumugam, None; Laura J. Frishman, None; Earl L. Smith, None; Lisa A. Ostrin, None

**Support:** 5R01EY025555-02, R01EY03611

**Program Number:** 4126 Poster Board Number: B0245

**Program Presentation Time:** 8:30 AM–10:15 AM

Three blind mice, see how they run: Light-dependent behavior in the absence of an optic nerve

**Anna Matynia, Sachin Parikh, Steven Nusinowitz, Michael B. Gorin.** Jules Stein Eye Institute, UCLA, Los Angeles, CA.

**Purpose:** The retinohypothalamic tract is critical for light-dependent responses that drive behavior although light aversion and light-induced blinks have been observed in the absence of a functional optic nerve. This study investigates the role of the optic and trigeminal nerves in light aversion.

**Methods:** Light aversion was assessed in adult mice using an aversion index (AI) (0≤no aversion; 1=complete aversion). Wild type mice (WT) or mice lacking melanopsin expressing cells (OPN4<sup>Δhr</sup>) were subjected to either bilateral sham or one of three optic nerve manipulations that result in “blindness”: severe Optic Nerve Crush (ONC), Optic Nerve Transsection (ONT) or ciliary Trigeminal and Optic nerve, ophthalmic Artery and Sheath Transsection (TOAST). OPN4<sup>Δhr</sup>-/rd1 mice lacking all known photoreceptors were also assessed. Administration of morphine (5mg/kg) or nitroglycerin (NTG, 10mg/kg) was used to unmask light aversion.

**Results:** Without drug treatment, all WT mice with optic nerve injury (ONC, ONT, TOAST) exhibited no light aversion at 1000 Lux. Light aversion increased in WT sham mice after ONC (AI=0.79±0.09, n=8) or morphine (AI=0.98±0.01, n=8). NTG also increased light aversion in severe bilateral ONC (AI=0.46±0.16, n=8), ONT (AI=0.37±0.24, n=4) and TOAST mice (AI=0.43±0.17, n=3). Neither OPN4<sup>Δhr</sup>-/- nor OPN4<sup>Δhr</sup>-rd1 double mutants (AI=0.12±0.02, n=4) showed NTG-induced light aversion. Morphine similarly increased light aversion in WT mice with ONC (AI=0.39±0.05, n=8), ONT (AI=0.58±0.15, n=4) or TOAST (AI=0.58±0.21, n=3). By contrast to NTG, morphine induced light aversion in OPN4<sup>Δhr</sup>-/- with ONC (AI=0.41±0.08, n=8) and OPN4<sup>Δhr</sup>-rd1 double mutants (AI=0.46±0.09, n=4). Both retinal (ipRGCs) and trigeminal ganglion melanopsin-expressing neurons in OPN4<sup>Δhr</sup>-/- mice are ablated.

**Conclusions:** Light perception mediating behavioral responses can occur in the absence of the optic nerve and ensheathed trigeminal nerves. Morphine and NTG provide useful tools for identifying the neural circuit(s) underlying this latent light perception. Melanopsin expression has been observed in a subset of trigeminal ganglion neurons that respond to light ex vivo, providing a potential alternative circuit to influence light aversion. Other mechanisms for light dependent behavior in the absence of trigeminal or optic nerve innervation must also be considered.

**Commercial Relationships:** Anna Matynia; Sachin Parikh, None; Steven Nusinowitz, None; Michael B. Gorin, None

**Support:** Knights Templar Eye Foundation, Gerald Oppenheimer Family Foundation

**Program Number:** 4127 Poster Board Number: B0246

**Program Presentation Time:** 8:30 AM–10:15 AM

Novel Phototransduction Pathway in Intrinsically Photosensitive Retinal Ganglion Cells (ipRGCs)

**Zheng Jiang, Wendy W. Yue, Xianghui Sheng, Li-Hui Cao, King-Wai Yau.** 1Department of Neuroscience, Johns Hopkins University School of Medicine, Baltimore, MD; 2Department of Physiology, University of California San Francisco, San Francisco, CA; 3College of Life Sciences, Peking University, Beijing, China.

**Purpose:** M1-ipRGCs utilize a rhodameric-photoreceptor-like phototransduction pathway mediated by melanopsin, G<sub>q</sub>-subfamily members, PLC<sub>β4</sub>, and TRPC6,7 channels. Phototransduction in the other subtypes of ipRGCs has not been explored. Here we report a novel phototransduction pathway in M4-ipRGCs, as well as a substantial portion of it in M2-ipRGCs, involves HCN channels instead of TRPC channels or PLC<sub>β4</sub>.

**Methods:** The photocurrents of M2- and M4-ipRGCs were recorded by whole-cell, patch-clamp recording from flat-mount mouse retina in the presence of synaptic blockers. Photo-uncaging experiments used intracellular caged cyclic nucleotide delivered from whole-cell pipette. To disrupt HCN channel function, mouse retina was infected by intravitreal injection of AAV2 virus carrying a mutant HCN channel subunit. Animal circadian photentrainment was monitored from wheel-running activity. PLR (pupillary light reflex) was recorded as described in Xue et al., Nature 479: 67-73, 2011.

**Results:** We found that M2- and M4-ipRGCs had a persistent intrinsic photocurrent in PLC<sub>β4</sub>, TRPC<sub>6,7</sub>-genotype, even in TRPC<sub>1,3,4,5,6,7</sub>-genotype. This photocurrent was insensitive to Ruthenium Red, a wide-spectrum TRPC-channel blocker, but completely blocked by ZD7288, a HCN-channel blocker. The voltage dependence of the TRPC<sub>6,7</sub>-photocurrent was consistent with HCN-channel properties, and its amplitude was positively correlated with the hyperpolarization-induced I<sub>ur</sub> current. Immunostaining and/or genetic labeling also revealed HCN4-channel, but not CNG channel, expression in retinal melanopsin-positive cells. A virally-expressed mutant HCN channel subunit significantly reduced the light responses of M2- and M4-ipRGCs. We found that phototransduction in M1-ipRGCs, but not M2- or M4-ipRGCs, depends on G<sub>αq</sub>, G<sub>α12</sub>, and G<sub>α13</sub>. Photo-uncaged cyclic nucleotide in OPN4<sub>Δhr</sub>-/- M2- or M4-ipRGCs induced an inward current similar to the melanopsin-mediated response. Finally, TRPC<sub>6,7</sub>-/- rd1 rd1 mice (which have lost rod/cone signals and TRPC<sub>6,7</sub>-dependent signals in ipRGCs) still exhibited PLR and circadian photoentrainment, although both functions were partially impaired.

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Conclusions: In M2- and M4- ipRGCs, there is a novel phototransduction pathway mediated by HCN channels and cyclic nucleotide. This pathway makes a significant contribution to in vivo PLR and circadian photoentrainment.

Commercial Relationships: Zheng Jiang, None; Wendy W. Yue, None; Yanghui Sheng, None; Li-Hui Cao, None; King-Wai Yau, None

Support: EY014596

Program Number: 4128 Poster Board Number: B0247
Presentation Time: 8:30 AM–10:15 AM
Probing the intraretinal influences of ipRGCs using chemogenetic manipulation
Nina Milosavljevic, Annette E. Allen, Jasmina Cehajic Kapetanovic, Robert J. Lucas. Faculty of Biology, Medicine and Health, The University of Manchester, Manchester, United Kingdom.

Purpose: Melanopsin expressing intrinsically photosensitive retinal ganglion cells (ipRGCs) are thought to provide the retina’s ability to measure background light intensity (irradiance). In order to explore the intraretinal influence of ipRGCs we adopted a chemogenetic approach to selectively and acutely activate ipRGCs. Using this approach we set out to determine the effect of activating ipRGCs on the retinal light response by electroretinography (ERG) and relate this to retinal cell activation by c-Fos mapping.

Methods: Melanopsin-Cre mice were intravitreally injected with a Cre-recombinase-dependent viral vector to express engineered excitatory chemogenetic hM3Dq receptors in ipRGCs. We previously showed that using this approach and applying a drug that specifically activates hM3Dq receptors (elozapine N-oxide), we can recreate the effects of bright light to this cell class only (ipRGCs) in dark-housed mice (Milosavljevic N et al, Curr Biol, 2016). Flash ERG was performed across the 9-fold intensity range on hM3Dq mice before and after chemogenetic activation of ipRGCs. To identify cells that are excited following ipRGC activation we used c-Fos immunoreactivity as an early marker of neuronal activation.

Results: Chemogenetic activation of ipRGCs suppressed a- and b-wave amplitudes of the scotopic ERG across the flash intensity range of hM3Dq compared to control mice. Examination of the normalized irradiance-response functions revealed a shift in b-wave but not a-wave sensitivity. No changes in a- and b-wave implicit times were detected. C-Fos mapping in the hM3Dq retinas revealed a significant number of cells that receive ipRGCs signalling. These c-Fos+ cells were localized mostly in the ganglion cell layer but some were found in the inner nuclear layer.

Conclusions: Acute and selective activation of ipRGCs modulates the amplitude of both a- and b-waves of the scotopic ERG, indicating that the influence of this ganglion cell class on the retinal physiology extends to the photoreceptors as well as their downstream pathways. This event is associated with a significant number of excited retinal cells (c-Fos+) following ipRGCs chemogenetic activation. In sum, although rare (~5% of RGCs) ipRGCs have a wide ranging influence on retinal physiology.

Commercial Relationships: Nina Milosavljevic, None; Annette E. Allen, None; Jasmina Cehajic Kapetanovic, None; Robert J. Lucas, None
Support: Medical Research Council (MRC)

C-terminal phosphorylation of mouse melanopsin regulates the kinetics of a subset of melanopsin mediated behaviors in mice
Preethi Somasundaram1, Glenn Wyrick2, Diego B. Fernandez3, Alireza Ghahari4, Cindy Pinhal5, Melissa Simmonds-Richardson6, Alan Rupp7, LiHong Cui7, Zhijian Wu7, Lane Brown7, Tudor C. Badea2, Samer Hattar8, Phyllis Robinson9. 1University of Maryland Baltimore County, Halethorpe, MD; 2Washington State University, Pullman, WA; 3Johns Hopkins University, Baltimore, MD; 4National Institute of Health, Bethesda, Maryland; 5National Institute of Health, Bethesda, MD.

Purpose: Melanopsin is a visual pigment, expressed in intrinsically photosensitive retinal ganglion cells (ipRGCs) of the mammalian retina, that plays a major role in non-image forming visual behaviors like the pupillary light reflex, circadian photoentrainment, and sleep. It is hypothesized that melanopsin mediated phototransduction is terminated by the phosphorylation of melanopsin’s C-termiunus by a G-protein coupled receptor kinase, followed by b-arrestin binding. Little is known about the contribution of melanopsin phosphorylation to ipRGC physiology and its influence on non-image forming behaviors. We investigated the role of melanopsin C-terminal phosphorylation on non-image forming behaviors by generating a phosphorylation deficient melanopsin mutant that lacks all putative C-terminal phosphorylation sites (C-phosphonull).

Methods: C-phosphonull mice were generated by expressing the C-terminal phosphorylation deficient melanopsin mutant in Opn4fwe cones by intravitreal delivery of an AAV2 construct for targeted expression of melanopsin in ipRGCs.

Results: We found that C-terminal phosphorylation of melanopsin has a direct influence on the dilation kinetics of the pupillary light reflex, due to the observation that the C-phosphonull mice exhibit prolonged pupil constriction to a high intensity blue light stimulus, and have a half time of dilation of 9.43 min as compared to only 6 s in wildtype animals. This phenotype is reflected in the physiology of ipRGCs as determined by both single cell and multielectrode recordings, where ipRGCs sustain action potentials for at least 15 min to a 5 s blue light stimulus. We also found that exogenous expression of melanopsin in melanopsin knockout animals enhances their ability to shift their activities to a delayed light dark cycle (a jet lag paradigm) and rescues a deficit in negative masking (reduction in activity exhibited by nocturnal animals in response to aberrant light during subjective night).

Conclusions: Our results suggest that C-terminal phosphorylation of melanopsin influences the kinetics of some non-image forming behaviors and physiology, but does not completely explain deactivation of melanopsin and that there may be alternate mechanisms that play a role in its absence.

Commercial Relationships: Preethi Somasundaram, None; Glenn Wyrick, None; Diego B. Fernandez, None; Alireza Ghahari, None; Cindy Pinhal, None; Melissa Simmonds-Richardson, None; Alan Rupp, None; LiHong Cui, None; Zhijian Wu, None; Lane Brown, None; Tudor C. Badea, None; Samer Hattar, None; Phyllis Robinson, None

Program Number: 4130 Poster Board Number: B0249
Presentation Time: 8:30 AM–10:15 AM
Circadian control of cone kinetics
Sahar Farajnia1, Maria D. Arietti2, Maarten Kamermans1. 1retinal signal processing, Netherlands institute for neuroscience, Amsterdam, Netherlands; 2Neurogenetics, Academic medical Center, Amsterdam, Netherlands.

Purpose: It is well known that cone responses are faster in the light-adapted than in the dark-adapted retina. A major part of this difference in kinetics comes from light/dark adaptational processes in the phototransduction cascade. In this study we investigated whether there is also a circadian control of cone kinetics.

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Methods: Isolated goldfish retinas were prepared under dim red light for electrophysiological recordings during the day and night (12h-12h; LD) or subjective day and night (constant darkness; DD). Naturalistic stimuli were used to determine cone frequency response relations. Two-tailed Student’s t-test was applied for statistical analysis.

Results: Voltage light responses of cones were faster during the day than during the night. This difference remained in DD conditions (n=17, p<0.001). To determine the underlying mechanism, we measured the cone frequency response relations under current and voltage clamp conditions. Responses under current clamp are shaped both by phototransduction and voltage gated currents whereas responses under voltage clamp are fully determined by the phototransduction cascade. Both current and voltage clamp recordings showed faster kinetics in the day than in the night (n=40; p<0.01). However, in DD conditions the change in kinetics was only present under current clamp conditions. This indicates that apart from light adaptation dependent change in the phototransduction, voltage gated channels control cone kinetics in a circadian fashion.

We found that Ih, the current flowing through HCN channels, is the major current that is circadian-modulated in cone photoreceptor. Ih was larger in the day than in the night both in LD and DD (n=93; p<0.01). Previously we have shown that Ih changes the cone kinetics in a dynamic way, and induces temporal contrast adaptation (Howlett et al., submitted). Since Ih is circadian regulated, we tested whether contrast adaptation was circadian regulated as well. We found reduced contrast adaptation at night compared to the day both in LD and DD conditions (p<0.01).

Conclusions: These data show that Ih is under circadian control. During the day Ih is large making cones fast and allowing contrast adaptation. During the night Ih is attenuated, cones slow down and contrast adaptation is reduced. Circadian modulation of cone membrane properties seems to prepare the retina to transmit the appropriate visual signal depending on the time of the day.

Commercial Relationships: Sahar Farahnia, None; Maria D. Arietti; Maarten Kamermans, None

Program Number: 4131 Poster Board Number: B0250
Presentation Time: 8:30 AM–10:15 AM

Mechanisms of light entrainment of the mammalian ciliary body circadian clock
Shunsuke Tsuchiya1, Ethan Buhr2, Tomomi Higashide1, Kazuhisa Sugiyama2, Russell N. Van Gelder2. 1Ophthalmology, Kanazawa University, Kanazawa, Japan; 2Ophthalmology, University of Washington, Seattle, WA.

Purpose: Intraocular pressure has a strong circadian rhythm, due in part to rhythmicity of ciliary body function. This rhythm is normally entrained to the 24-hour light-dark cycle. Recent work has demonstrated that some ocular tissues, including cornea and retina, are directly light entrained through a neuropin (OPN5)-dependent mechanism. The purpose of this study was to assess whether the mammalian ciliary body uses a similar mechanism for light entrainment.

Methods: Iris-ciliary body complexes were obtained from PERIOD2::LUCIFERASE (PER2::LUC) mice and cultured to measure bioluminescence rhythmicity using a Lumicycle. Pairs of the iris-ciliary body complex were exposed to antiphasic 9:15 light-dark cycle in vitro in the presence of 9-cis-retinaldehyde. After 4 days of exposure to light/dark cycles, bioluminescence was recorded to establish circadian phase. In addition, pairs of the iris-ciliary body complex cultured with the retinas of mice without PER2::LUC (i.e. C57Bl/6J) were exposed to light/dark cycle in vitro and their bioluminescence was obtained. Opn5 expression of the iris-ciliary body was analyzed using RT-PCR.

Results: Cultured PER2::LUC iris-ciliary body complexes showed sustained circadian rhythmicity of bioluminescence in vitro. Neither the complex nor the co-cultured complex was directly entrained by light-dark cycle in vitro. Opn5 mRNA expression could not be detected in the iris-ciliary body complex.

Conclusions: The iris-ciliary body complex does not express OPN5 and is not entrained by light-dark cycle in vitro. Local retinal signals do not appear sufficient for entrainment. This suggests that central circadian phase cues from the suprachiasmatic nucleus likely control circadian entrainment of the ciliary body in vivo.

Commercial Relationships: Shunsuke Tsuchiya; Ethan Buhr, None; Tomomi Higashide, None; Kazuhisa Sugiyama, None; Russell N. Van Gelder, None

Program Number: 4132 Poster Board Number: B0251
Presentation Time: 8:30 AM–10:15 AM

Light affects mood through a novel retina-brain circuit
Diego C. Fernandez1, Michelle Fogerson2, David M. Berson2, Samer Hattar1. 1Biology, Johns Hopkins University, Baltimore, MD; 2Department of Neuroscience, Brown University, Providence, RI.

Purpose: Light exerts profound effects on behavior, including circadian timing and mood. Alterations in regular lighting conditions lead to mood deficits; we recently showed that this direct effect of light on mood is mediated by intrinsically photosensitive retinal ganglion cells (ipRGCs). The goal of this study was to characterize the retina-brain circuits mediating the direct effects of light over affecting behaviors.

Methods: We used several viral and conventional axon-tracing techniques and immunofluorescence to trace pathways linking ipRGCs, through the thalamus, to the cortex. We used genetically modified mouse lines, behavioral tests, and assays of circadian genes to assess the role of these pathways in the mood effects of light.

Results: We identified a novel retino-brain circuit that places ipRGCs just one synapse away from mood-regulating networks in the medial prefrontal cortex (mPFC) through a thalamic region, the perihabenular nucleus (PHb). Aberrant light conditions disrupt the molecular circadian clock within the PHb. Using a genetically modified mouse line that lacks ipRGC innervation to the PHb and manipulating the function of this region, we demonstrate that the PHb is necessary and sufficient for driving the adverse effects of light on mood.

Conclusions: These findings demonstrate that the mood-related disturbances induced by irregular light cycles are attributable to a distinct output pathway of ipRGCs that influences mood centers.

Commercial Relationships: Diego C. Fernandez, None; Michelle Fogerson, None; David M. Berson, None; Samer Hattar, None

Support: National Institutes of Health grants GM076430 and 5R01EY017137

Program Number: 4133 Poster Board Number: B0252
Presentation Time: 8:30 AM–10:15 AM

Physiological Effects of Ten Days of Total Darkness in Humans
Ashutosh Jnawali1, Benjamin T. Buckas1, Elizabeth M. Quinlan1, Cristina Llerena-Law1, Suresh Viswanathan1, Nabin Joshi1, Jason Grygiel1, Lisa A. Ostrin1. 1University of Houston College of Optometry, Houston, TX; 2College of Optometry, SUNY, New York, NY; 3College of Optometry, Nova Southeastern University, Fort Lauderdale, FL; 4Biology, University of Maryland, College Park, MD.
Purpose: Light exposure impacts many physiological and metabolic processes including circadian rhythm, mood, metabolism, and eye growth. Light contributes to circadian rhythm by synchronizing diurnal activity of intrinsically photosensitive retinal ganglion cells (ipRGCs), which modulate the release of the hormone melatonin. Here, we investigated the effects of total darkness on rhythm-dependent markers and the ipRGC-driven pupil response in humans.

Methods: Two subjects, ages 21 and 27, were sequestered for 10 days in complete, continual darkness. Subjects wore an actigraph device for 30 days, beginning 10 days before the initiation of visual deprivation, for objective measures of light exposure, activity and sleep. Morning and nighttime saliva samples were collected for subsequent melatonin analysis, and body temperature, heart rate and blood pressure were recorded. During sequestration, zeitgebers were provided, and subjects had access to auditory-output timepieces. Pupil responses to short and long wavelength light were measured immediately before and after sequestration as an assessment of ipRGC activity.

Results: Actigraphs revealed a decrease in daily activity for the two subjects from 384 ± 154 activity counts per minute (mean cpm ± sd) during baseline, to 278 ± 109 cpm during dark exposure. Sleep duration increased from 389 ± 68 m to 528 ± 62 m for subject 1 (no baseline sleep data collection for subject 2). During the 10-day dark period, morning melatonin increased according to the fitted regression equation y = 1.2x + 2.4 where x was day in the dark, and nighttime melatonin decreased as y = -0.8x + 22.3. Therefore, by the end of the dark period, the morning and nighttime melatonin levels were roughly equal. The ipRGC-driven tonic pupil constriction was enhanced immediately after the dark period, compared to baseline.

Conclusions: Constant darkness disrupted systemic melatonin levels, increasing morning and decreasing nighttime melatonin, so that levels converged within 10 days. Physical activity decreased and sleep duration increased in the dark. However, phase shifts in the sleep/wake cycle were not observed, likely due to numerous cues, such as auditory clocks, meals, and scheduled events. We conclude that light deprivation is sufficient to impact circadian control of melatonin in humans, which may be mediated by the ipRGC pathway.

Commercial Relationships: Ashutosh Jnawali, None; Benjamin T. Backus, None; Elizabeth M. Quinlan, None; Cristina Llerena-Law, None; Suresh Viswanathan, None; Nabin Joshi, None; Jason Grygier, None; Lisa A. Ostrin, None

Support: R21 EY025398, K23 EY022669

Program Number: 4134 Poster Board Number: B0253
Presentation Time: 8:30 AM–10:15 AM

Human Melanopic Pupillary Responses Isolated from Outer Retinal Photoreceptor Input in LCA Patients with Severe Vision Loss

Jason Charng1, Samuel G. Jacobson1, Elise Heon2, Alejandro J. Roman3, David B. McGuigan1, Rebecca Sheplock1, Mychajlo S. Kosyk2, Artur V. Cideciyan1, Scheie Eye Institute, Dept. of Ophthalmology, University of Pennsylvania Perelman School of Medicine, Philadelphia, PA; 2Department of Ophthalmology and Vision Sciences, The Hospital for Sick Children, University of Toronto, Toronto, ON, Canada.

Purpose: Pupillary light reflex (PLR) refers to pupil size changes in response to retinal illumination. PLR is thought to be driven by rods and cones of the outer retina, and by melanopsin-expressing intrinsically-photosensitive retinal ganglion cells of the inner retina. In order to isolate the melanopic component of the human PLR, we considered patients with severe vision loss due to Leber congenital amaurosis (LCA) caused by gene mutations acting on the outer retina.

Methods: Direct PLR was recorded in the dark with achromatic full-field stimuli (2.5 log scot-cd.m-2; -13 log quanta.cm-2.s-1) in dark-adapted eyes. Videos were analyzed frame-by-frame. Patients with autosomal recessive LCA with known mutations and severe loss of visual acuity (light perception, LP, or no light perception, NLP) were evaluated first with 100 ms stimuli. A subset of patients (n=19; mean age=18.4 y) showing no PLR to the 100 ms stimulus was further examined with 5 s stimuli. Full-field stimulus testing (FST) was used to quantify light perception.

Results: 12 of 19 patients with no PLR to the 100 ms stimulus showed a detectable contraction with the 5 s stimulus; 7 were non-responders. There was no difference in acuity between the two groups.
Melanopsin-expressing ganglion cells in human retina

Ulrike Grunert1,2, Subha Nasir Ahmad1,2, Sammy C. Lee2, Paul R. Martin1,2, Save Sight Institute, University of Sydney, Sydney, NSW, Australia; 1Clinical Ophthalmology, University of Sydney, Sydney, NSW, Australia.

Purpose: Melanopsin-expressing cells are spared from ganglion cell loss in some retinal diseases, yet are more vulnerable in other diseases. The present study aimed to extend the knowledge about density and spatial distribution of melanopsin containing ganglion cells across normal human retina.

Methods: Five post mortem human donor eyes, from one female and four males aged 44, 48, 53, 59, and 64 years, were obtained from the Lions NSW Eye Bank at Sydney Hospital and Sydney Eye Hospital with ethical approval from the University of Sydney Human Research Ethics Committee. The posterior eye cup was fixed in 2% paraformaldehyde and the retina dissected and processed with a rabbit antiserum against human melanopsin protein (kindly provided by Dr. King-Wai Yau). In addition vibratome sections of retinal paraformaldehyde and the retina dissected and processed with a rabbit antiserum against human melanopsin protein (kindly provided by Dr. King-Wai Yau). In addition vibratome sections of retinal

Results: Two types of melanopsin-expressing cells were distinguished based on their dendritic stratification: inner stratifying and outer stratifying types. Outer stratifying cells make up on average 60% of all melanopsin-expressing cells. About 80% of the outer melanopsin cells have their soma displaced to the inner nuclear layer. Inner stratifying cells have their soma exclusively in the ganglion cell layer and include a small proportion of bistratified cells. The dendritic field diameter of melanopsin cells ranges from 250 µm (near the fovea) to 1000 µm in peripheral retina. Melanopsin cells have an average peak density of about 20 to 40 cells per/mm² at about 2 mm eccentricity. The density drops to below ~10 cells/mm² at about 8 mm eccentricity. Both the outer and inner-stratifying dendrites express postsynaptic density (PSD95) immunoreactive puncta suggesting that they receive synaptic input from bipolar cells.

Conclusions: Our results support previous findings showing that melanopsin-expressing cells in human retina form two morphological populations. These populations have higher average densities than their counterparts in macaque retina (Liao et al., 2016). In addition, our findings indicate that outer and inner cells are differentially distributed across the retina, namely that there might be higher proportions of inner stratifying cells in central than in peripheral retina.

Commercial Relationships: Ulrike Grunert, None; Subha Nasir Ahmad, None; Sammy C. Lee, None; Paul R. Martin, None

Support: National Health and Medical Research Council project grant 1042609; Australian Research Council Centre of Excellence for Integrative Brain Function (CE140100007)

Program Number: 4136 Poster Board Number: B0255
Presentation Time: 8:30 AM–10:15 AM
Novel roles for ipRGC neurotransmitters, glutamate and PACAP, in pupil constriction and circadian phototransient

William T. Keenan, Annette Wang, Samer Hattar. Biology, Johns Hopkins University, Baltimore, MD.

Purpose: Intrinsically photosensitive retinal ganglion cells (ipRGCs) utilize at least two neurotransmitters, glutamate and PACAP. Despite a variety of studies on the topic, the contribution of glutamate or PACAP to ipRGC-dependent visual behaviors remains unclear. In this study, we endeavor to identify the critical role of each neurotransmitter in two ipRGC-dependent visual behaviors: phototransient and pupil constriction.

Methods: In order to study the individual contributions of glutamate or PACAP, we utilized mutant mouse lines capable of removing PACAP or glutamatergic signaling specifically from ipRGCs (Opn4fl/fl; Vglut2fl/fl or Adcyap1fl/fl). First, we investigated the pupillary light reflex using scroffed unanesthetized mice. Previous work has focused on constriction when light increases with little attention payed to dilation in response to decrements of light. We have included analysis of dilation in our study by simply recording post-stimulation for several minutes to hours. Second, we investigated phototransient using wheel running activity in light controlled boxes. We subjected mutant and control mice to a normal 12hr light/12hr dark (LD) cycle, an ultradian light cycle (T7), constant light (LL), and constant darkness (DD).

Results: This study identifies a critical role for glutamate in pupil dilation and for PACAP in circadian phototransient/masking. By focusing on dilation in response to decrements of light, we find that mice lacking glutamatergic signaling lacked any coherent dilation response after bright light exposure and required several hours to return to their baseline pupil sizes. In contrast, control mice and mice lacking PACAP signaling were fully dilated a few minutes post-stimulation. For phototransient we find mice lacking PACAP display a drastically enhanced masking response under the T7 and LL light cycles resulting in highly disrupted and fragmented rhythms. Control and mice lacking glutamatergic signaling retain readily discernible wheel running rhythms under all light cycles.

Conclusions: Our results highlight novel roles for glutamate and PACAP in pupil dilation, masking, and phototransient. Glutamatergic ipRGC signaling provides a potent OFF signal actively modulating masking/photoentrainment in order to maintain coherent rhythmic activity under disruptive or dynamic light cycles.

Commercial Relationships: William T. Keenan, None; Annette Wang, None; Samer Hattar, None

Support: NIH Grant EY024452
We have modified a commercially available projection.

Shi-Jun Weng, Wen-Long Sheng, Xue Gong, Xiong-Li Yang, Yong-Mei Zhong.
Fudan University, Shanghai, China.

Purpose: The melanopsin-containing intrinsically photosensitive retinal ganglion cells (ipRGCs) are under circadian modulation, but the underlying mechanism is not clear. We sought to explore whether and how melanin, a neurohormone involved in various retinal circadian events, exerts modulatory effects on rat ipRGCs.

Methods: Melanopsin-driven light responses (M-responses) of ipRGCs in whole-mount retina were isolated with a glutamateergic blocker cocktail, and recorded using a multi-electrode array (MEA). Melatonin was administered in vitro in bath solution (for testing acute effects) or in vivo by intravitreal injection (for testing long-term effects while avoiding run-down). MEA data were fitted with the Michaelis-Menten equation to plot Irradiance-Response (I-R) curves for determining response thresholds. Whole-retina melanopsin levels were assessed using quantitative Western blotting.

Results: Short-term (< 0.5 hour) bath application of melatonin failed to alter M-responses significantly, indicating the absence of an acute modulation. However, when the time of melatonin exposure was elongated to 2+ hours, achieved by intravitreal melatonin injection, M-responses were significantly suppressed in a dose-dependent manner, suggesting a long-term modulation of melanin on ipRGC activities. The effect of melanin was completely abolished by luzindole, a melatonin receptor antagonist, suggesting that the observed melanin-induced suppression is a specific effect derived from melatonin receptor activation. Such a suppression was, at least in part, due to a decline in photopigment levels. The evidence was two folds. First, whole-retina melanopsin levels were significantly down-regulated in melanin-treated eyes, as revealed by quantitative Western blotting. Second, M-responses in melanin-treated eyes exhibited a rightward-shifted I-R curve, with a significantly elevated mean threshold, as compared with those in vehicle-treated eyes.

Conclusions: Retinal melanin modulates ipRGC photoresponsitivity by down-regulating melanin expression, which may contribute to the previously reported circadian fluctuation in ipRGC activities.

Commercial Relationships: Shi-Jun Weng, None; Wen-Long Sheng, None; Xue Gong, None; Xiong-Li Yang, None; Yong-Mei Zhong, None

Support: Ministry of Science and Technology of China (2011CB504602, 2015AA020512); the National Natural Science Foundation of China (31100796, 31171005, 31421091, 31571072, 31571075, 81430007)

A Circadian Clock Controls the Transmission of Scotopic Signals from the Rod to Rod Bipolar Cell in the Mouse Retina

Christophe P. Ribelayga, Nange Jin, Zhijing Zhang, Eduardo L. Silveyra.
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Purpose: Under dim, scotopic conditions, vision relies on the detection of single photons by rods and the synaptically transfer of the rod single-photon responses to rod bipolar cells (RBCs). It has been proposed that a threshold non-linearity in RBCs rejects continuous noise from rods while preserving signals that exceed a criterion amplitude. We recently reported that electrical coupling between rods is controlled by a circadian clock so that coupling is weak during the day and much stronger at night. An increase in rod electrical coupling at night is expected to reduce the amplitude of the rod single-photon responses and therefore reduce signaling in the rod pathway, unless other mechanisms are at play. Here, we sought to determine whether performance of the RBC changes between day and night.

Methods: We patch-clamped RBCs in CBA/Ca mouse retinal slices and recorded their light-evoked voltage responses at different times in the circadian cycle.

Results: During the subjective day, we found strong non-linearity in the light responses of RBCs. As expected, this high non-linearity translated into a high signal-to-noise ratio (SNR) of the single-photon responses in RBCs (4.16). In contrast, during subjective night, the average voltage response-intensity function appeared more linear compared to the daytime, as evidenced by a decrease in the Hill coefficient (from 1.45 to 1.00). We also found that the amplitude of the single-photon responses were larger (from 1.6 mV to 2.6 mV) and the SNR was lower (from 4.16 to 2.26) in RBCs at nighttime. Whereas the amplitude of the rod single-photon voltage-response decreases between subjective day and subjective night (from 2.5 mV to 1 mV), the amplitude of the single-photon response in RBCs increases; the data indicate that the voltage gain of the rod-to-RBC synapse increases at nighttime by a factor of 4.

Conclusions: Our data suggest that rod-to-RBC signaling is controlled by a circadian clock on a daily basis. Specifically, the rod-to-RBC synapse operates at low gain during the subjective day and high gain at night. The nighttime increase in gain may represent a compromise of the decreasing false negative rate in order to cope with the small amplitude of the rod single-photon responses and increasing false positive rate.

Commercial Relationships: Christophe P. Ribelayga, None; Nange Jin, None; Zhijing Zhang, None; Eduardo L. Silveyra, None

Support: This work was supported by the National Institutes of Health (grants EY018640), the Hermann Eye Fund and an Unrestricted Challenge Grant from Research to Prevent Blindness.

A melanopsin contribution to the representation of spatial patterns in the mouse visual system

Annette E. Allen1, Riccardo Storch1, Robert Bedford2, Franc P. Martial1, Robert J. Lucas1.
1Faculty of Biology, Medicine and Health, University of Manchester, Manchester, United Kingdom; 2National Institute for Health Research, NHS, Manchester, United Kingdom.

Purpose: The discovery of melanopsin, a new photoreceptor in the retina, has revolutionized our understanding of how changes in background light intensity are measured to drive sub-conscious responses such as circadian photoentrainment. As yet it has not altered our view of how more conventional visual tasks such as detecting spatial patterns are undertaken. Responsibility for that aspect of vision remains solely attributed to rods and cones. Here, we set out to define the spatiotemporal resolution of melanopsin vision in visually intact mice.

Methods: We have modified a commercially available projection system so that each of the R, G and B channels is instead a combination of up to five, independently controlled wavelengths (λ = 405, 455, 525, 561, 630nm). With this stimulus, we have adapted a psychophysical method (receptor silent substitution) to produce patterned stimuli that are only visible to only rods and cones.
cones, only melanopsin, or all photoreceptors. We have used multi-channel recording electrodes to record light-evoked activity in the dorsal lateral geniculate nucleus (dLGN) of urethane anaesthetised OptiLmΔ mice; these mice display a long-wavelength shifted spectral sensitivity of green cones, which allows us to achieve maximal contrast for each photopigment.

**Results:** We find that melanopsin can support the representation of spatial patterns in ~20% of dLGN neurons. Receptive fields of individual dLGN neurons arising from either melanopsin alone, or rods and cones, are spatially contiguous. However, melanopsin is important in maintaining the representation of spatial patterns over longer time frames. By using natural images and simulated head/eye movements, we show that the dLGN relies upon melanopsin to avoid ‘fade out’ of coarse spatial patterns under periods of relative visual fixation. We have modelled the temporal contribution of melanopsin using an autoregressive exogenous model, with which we explored the spatiotemporal contribution of melanopsin in the dLGN.

**Conclusions:** Visual responses arising exclusively with melanopsin are detectable at the level of the dLGN at relatively modest contrasts, and on a physiologically relevant spatial and temporal scale. Our data imply that melanopsin makes a distinct contribution to encoding spatial patterns, and allows a reappraisal of the circuits and processes responsible for spatial vision.

**Commercial Relationships:** Annette E. Allen, None; Riccardo Storchi, None; Robert Bedford, None; Franck P. Martial, None; Robert J. Lucas, None

**Support:** ERC (268970)

**Program Number:** 4141 Poster Board Number: B0260
**Presentation Time:** 8:30 AM–10:15 AM
**Retinal circadian clock modulates cone photoreceptor viability in mice**

Kenkichi Baba, Ilaria Piano, Gauadro Gargini, P. Michael Iuvone, Gianluca Tosini, 1 Morehouse School of Medicine, Atlanta, GA; 2 Università di Pisa, Pisa, Italy; Emory University, Atlanta, GA.

**Purpose:** The widespread control of signaling, metabolism, and gene expression exerted by the ocular circadian clocks suggests that the molecular clockworks, or their output signals, may contribute to ocular disease and pathology as well as to normal visual function. For example, signaling by the retinal clock and its outputs seem to play a role in the regulation of eye growth and refractive errors. Furthermore, the mammalian retinal clock influences cell survival and growth processes in the eye, including the susceptibility of photoreceptors to degeneration from light damage. Bmal1 gene is a key component of the mammalian circadian clock. Bmal1 knock-out (KO) mice at young age do not show any circadian rhythmicity and develop several pathologies. Bmal1 KO mice show premature aging and the lifespan is significantly reduced (about nine months). The purpose of our study is to investigate the role of Bmal1 removal on cone photoreceptor function and viability during aging using neural retina specific Bmal1 KO mice.

**Methods:** Photoreceptor viability was assessed in the retina of neural retina specific Bmal1 KO (Chx10Δm/Bmal1ΔmΔmΔm) mice at young (3 month old) and old (26 month old) ages. The thickness of photoreceptors layers (PRL) was first assessed non-invasively using spectral domain optical coherence tomography (SD-OCT). The eyes of Chx10Δm/Bmal1ΔmΔmΔm mice were subsequently explanted, and sectioned for immunohistochemical analysis. Chx10ΔmΔmΔm mice were used as controls.

**Results:** SD-OCT analysis indicated that the PRL thickness of Chx10Δm/Bmal1ΔmΔmΔm mice at 26 months of age is significantly reduced with respect to the PRL thickness of 3 month old Chx10ΔmBmal1ΔmΔmΔm or 26 month old controls. Our immunohistochemical analysis also revealed a significant reduction in the number of cone photoreceptors in 26 old months Chx10Δm→Bmal1ΔmΔmΔmΔm mice. Finally significant alterations in the circuitry of outer plexiform layer were observed not only in 26 months old Chx10Δm→Bmal1ΔmΔmΔmΔm, but also in 3 and 12 months old mice, indicating a possible defect in retinal development.

**Conclusions:** Our results indicate that removal of a functional circadian clock from the cones may affect the viability of these cells during aging and maybe affect the development of entire retinal structure.

**Commercial Relationships:** Kenkichi Baba, None; Ilaria Piano, None; Gauadro Gargini, None; P. Michael Iuvone, None; Gianluca Tosini, None

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**Program Number:** 4142 Poster Board Number: B0261
**Presentation Time:** 8:30 AM–10:15 AM
**Retinal remodeling throughout hibernation in the 13-lined ground squirrel**

Benjamin S. Sajdak, Brent A. Bell, Alexander E. Salomon, Wei Li, Dana K. Merriman, Joseph Carroll.

1 Cell Biology, Neurobiology, & Anatomy, Medical College of Wisconsin, Milwaukee, WI; 2 Ophthalmic Research, Cleveland Clinic, Cleveland, OH; 3 Unit of Neurophysiology, National Eye Institute, Bethesda, MD; 4 Biology, University of Wisconsin Oshkosh, Oshkosh, WI; Ophthalmology & Visual Sciences, Medical College of Wisconsin, Milwaukee, WI.

**Purpose:** To evaluate structural changes in the ground squirrel retina during distinct metabolic states of hibernation.

**Methods:** Retinas of 13-lined ground squirrels (13LGS; n=18) were imaged during four natural metabolic states (euthermia, pre-hibernation, torpor, or induced arousal) using a Bioptigen Envisu R2200 optical coherence tomograph (OCT). Each state was verified by examining body temperature (Tb) and behavioral phenotype. Layer thickness was measured at the visual streak (VS) from logarithmic vertical B-scans that were registered and averaged using custom software. Longitudinal reflectance profiles (LRP) from linear grayscale images were averaged from three locations (VS, 500μm inferior to VS, and 500μm superior to VS) to assess morphological changes between metabolic states.

**Results:** Consistent metabolic state-dependent changes were detected (see table and figure). The inner nuclear layer (INL) was thickened in all states relative to euthermia. The distance between the interdigitation zone and Bruch’s membrane increased during torpor. Choroidal thinning was seen during torpor which returned to euthermic levels 60 minutes into arousal. Hyper-reflectivity was seen throughout the retina (most prominently in the INL) during torpor. Hyper-reflective blood was seen in all retinal vasculature during pre-hibernation when Tb is falling to room temperature. The ellipsoid zone, interdigitation zone (also called inner segment/outter segment junction and photoreceptor outer segment tips) respectively, and Bruch’s membrane all had decreased reflectance in torpor and arousal when compared to euthermia.

**Conclusions:** The source of outer retinal band reflectivity is controversial yet critical to interpretation of OCT images in any species. Metabolic changes in 13LGS retina across the hibernation cycle provide a natural model of reversible retinal remodeling that can help explain the origin of OCT reflectance and may be informative for therapeutic neuroprotection.

1 Steureungi PMID:24755500
2 Jonnal PMID:25324288
Retinal layer thickness and reflectance data for each metabolic condition (mean±SD). Significant differences are shown relative to euthermia (𝑡-𝑡𝑒𝑠𝑡, *𝑝 ≤ 0.05, **𝑝 ≤ 0.01, ***𝑝 ≤ 0.001, ****𝑝 ≤ 0.0001).

<table>
<thead>
<tr>
<th>Metabolic State</th>
<th>Euthermia</th>
<th>Pre-Hibernation</th>
<th>Torpor</th>
<th>Arousal</th>
</tr>
</thead>
<tbody>
<tr>
<td># of 13LGS</td>
<td>7</td>
<td>5</td>
<td>7</td>
<td>2</td>
</tr>
<tr>
<td>Body Temperature (°C)</td>
<td>35-37</td>
<td>22-30</td>
<td>10-37</td>
<td>16-19</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Retinal Layer Thickness (μm)</th>
<th>Whole Retina</th>
<th>Nerve Fiber Layer</th>
<th>Ganglion Cell Layer</th>
<th>Inner Plexiform Layer</th>
<th>Inner Nuclear Layer (INL)</th>
<th>Outer Nuclear Layer</th>
<th>Inner Segments</th>
<th>Outer Segments</th>
<th>Retinal Pigment Epithelium</th>
<th>Choroid</th>
</tr>
</thead>
<tbody>
<tr>
<td>thickness</td>
<td>293±12.9</td>
<td>310±18.9</td>
<td>293±14.2</td>
<td>310±12.8</td>
<td></td>
<td></td>
<td>15±7.4</td>
<td>6±3.4</td>
<td>7±0.9</td>
<td>50±6.1</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Reflectance (a.u.)</th>
<th>Outer Plexiform Layer</th>
<th>External Limiting Membrane (ELM)</th>
<th>Inner Segments</th>
<th>Ellipsoid Zone (EZ)</th>
<th>Intercalation Zone (IZ)</th>
<th>Bruch’s Membrane</th>
<th>Sclera</th>
</tr>
</thead>
<tbody>
<tr>
<td>thickness</td>
<td>23±2.2</td>
<td>32±6.2</td>
<td>21±5.9</td>
<td>102±22.6</td>
<td>857±13.9</td>
<td>87±8.5</td>
<td>105±37.8</td>
</tr>
</tbody>
</table>

Representative 13LGS visual streak OCTs from the four metabolic states. Below are magnified linear gray scale images and example LRP s from each state (orange line denotes ELM location, green box denotes LRP sampling region).

Commercial Relationships: Benjamin S. Sajdak, None; Brent A. Bell, None; Alexander E. Salmon, None; Wei Li, None; Dana K. Merriman, None; Joseph Carroll, None

Support: T32EY014537 (BSS, AES); P30EY001931 (JC);

Unrestricted Grant from the Research to Prevent Blindness (BAB)

Program Number: 4143 Poster Board Number: B0262

Presentation Time: 8:30 AM—10:15 AM

Morphological and physiological diversity of M1 intrinsically photosensitive retinal ganglion cells

Seul Ki Lee, Tiffany M. Schmidt. Neurobiology, Northwestern University, Evanston, IL.

Purpose: Melanopsin-expressing, intrinsically photosensitive retinal ganglion cells (ipRGCs), comprise five subtypes, M1 to
M.5, according to their different morphological and physiological properties. Recent work demonstrated that the M1 subtype can be subdivided into two different molecular subpopulations based on whether they express the transcription factor Brn3b (Brn3b-positive or Brn3b-negative cells). These two populations show divergent brain targeting and functions in non-image-forming behaviors. However, whether these cells have distinct physiological and morphological properties is unknown.

**Methods:** To compare distributions of Brn3b-positive and Brn3b-negative M1 cells, whole mount retinas of Opn4<sup>cre</sup>-<sup>-</sup>lacI<sup>-</sup> mice (N=4), in which M1 ipRGCs are exclusively labeled with β-galactosidase (β-gal), were immunostained for β-gal and Brn3b. To compare physiological properties, in vitro single cell recording was performed in a whole cell configuration. M1 cells were fluorescently identified by a Cre inducible reporter gene in the Opn4<sup>cre</sup> mouse line (N=6) and morphologically identified using fluorescence-conjugated hydrazide included in the internal solution. The recorded cells were filled with neurobiotin during recording and then incubated with streptavidin to more clearly analyze morphological properties, and were immunostained for Brn3b. Statistical analysis was performed using one-way ANOVA with Fisher’s LSD post hoc test.

**Results:** We found that Brn3b-positive and Brn3b-negative M1 cells were differentially distributed across the retina, with a higher proportion of Brn3b-positive M1 cells in the ventral retina. Surprisingly, we found that Brn3b-positive and Brn3b-negative M1 cells exhibited differences in their resting membrane potential, intrinsic membrane properties, and light response characteristics. These M1 subtypes also differed in their morphological properties. Brn3b-positive M1 cells have a longer total dendritic length and larger dendritic field than Brn3b-negative M1 cells.

**Conclusions:** Brn3b-positive and Brn3b-negative M1 cells differ across multiple parameters including distribution, physiological properties, and morphological characteristics. These findings support the idea that different molecular M1 populations differ in their connectivity in the brain and influence on non-image forming behaviors.

**Commercial Relationships:** Seul Ki Lee, Tiffany M. Schmidt, None

**Support:** The Kirchgessner

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**Program Number:** 4144 Poster Board Number: B0263

**Presentation Time:** 8:30 AM–10:15 AM

**Light-dark cycle drives the daily oscillation of gut microbe through ipRGCs**

*Shih-Kuo Chen, Chih-Chan Lee.* Department of Life Science, National Taiwan University, Taipei, Taiwan.

**Purpose:** It has been shown that the gut microbes can influence the development of the brain and even trigger the degeneration of the neurons in the brain. In addition, the composition of gut microbes can be altered by providing animals with different food or housing in different environments. For example, chronic jetlag can modulate the gut microbe composition and dampen the daily oscillation of gut microbes. However, little is known about whether environmental stimuli can directly modulate the composition of gut microbes or indirectly through circadian clock system.

**Methods:** To test whether light can modulate the gut microbiota, we housed mice under dim light during the night time for 2 weeks. The food is restricted during the active phase of the mice to minimize the shift of circadian clock. After 2 weeks of dim light at night treatment, body weight, glucose tolerance and insulin tolerance were measured. Finally, fecal samples were collected every 4 hours and gut microbiota metagenomic analysis is performed with 16s rRNA NGS sequencing.

**Results:** Compared to mice housed in normal light-dark cycle, mice housed under dim light at night had distinct compositions of gut microbiota, and the daily oscillation of gut microbes was significantly dampened. Mice with photopigment melanopsin knockout showed similar gut microbiota composition under both normal light-dark cycle and dim light conditions. Furthermore, genetically elimination of ipRGC strongly dampened the daily oscillation of gut microbes.

**Conclusions:** Our results suggest that melanopsin signaling can provide the information of environmental light-dark cycle to modulate the composition of gut microbes. In addition, the light-dark cycle information transmitted by ipRGC, but not the circadian clock, is the primary driving force for daily oscillation of gut microbes.

**Commercial Relationships:** Shih-Kuo Chen, Chih-Chan Lee, None

**Support:** Taiwan MoST Grant

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**Program Number:** 4145 Poster Board Number: B0264

**Presentation Time:** 8:30 AM–10:15 AM

**ipRGCs utilize non-overlapping circuits to drive phase shifts, masking and circadian photoentrainment**

*Jennifer Langel<sup>1</sup>, Alan Rupp<sup>1</sup>, James O’Donnell<sup>2</sup>, Samer Hattar<sup>3</sup>.<sup>1</sup>* Biology, Johns Hopkins University, Baltimore, MD; <sup>2</sup>*University of Michigan, Ann Arbor, MI.

**Purpose:** Intrinsically photosensitive retinal ganglion cells (ipRGCs) are essential for many light-mediated behaviors such as circadian photoentrainment, pupillary light reflex and acute regulation of activity (masking) and sleep. The specific circuits mediating these behaviors, however, are not completely understood. In addition, most studies use wheel running as a measure of locomotor responses to light, a behavior that might be regulated by light differently than general activity or core body temperature. Here to we sought to determine whether ipRGC circuits regulating light information for circadian photoentrainment are similar to those driving phase shifting and the acute regulation of general activity and body temperature.

**Methods:** To investigate the ipRGC circuits that drive distinct light-mediated behaviors, we used mice that lack most ipRGC subtypes (*Opn4<sup>DTA</sup>*)<sup>-</sup>, but are still able to photoentrain to a light/dark (LD) cycle. *Opn4<sup>DTA</sup>* and control mice were implanted with telemetry devices to measure core body temperature and general activity in response to 3-h light pulses across a range of light intensities (0.1, 1, 10, 100 and 500 lux) during the dark period of a 12:12 LD cycle. Masking responses and phase shifts to these 3-h light pulses were calculated and compared between the different genotypes. In addition, we examined photoentrainment to a skeleton photoperiod, where animals are exposed to two 1-hour pulses of light corresponding to dawn and dusk.

**Results:** As expected, both wild-type and *Opn4<sup>DTA</sup>*<sup>-</sup> mice photoentrained to a 12:12 LD cycle, however, *Opn4<sup>DTA</sup>*<sup>-</sup> mice exhibited deficits in masking to 3-h pulses of light across all light intensities. Wild-type mice displayed a shift in the onset of general activity and core body temperature in response to the 3-h light pulses at all light intensities (surprisingly, even as low as 0.1 lux), whereas *Opn4<sup>DTA</sup>*<sup>-</sup> mice displayed a similar shift only at high light intensities (500 lux). Despite the lack of phase shifts in *Opn4<sup>DTA</sup>*<sup>-</sup> mice in the onset of their general activity/core body temperature to a 3-h light pulse at 0.1 lux, remarkably, these animals were still capable of photoentrainment to a skeleton photoperiod at this light intensity.

**Conclusions:** These results suggest that the circuits required for masking and phase shifting to light may be distinct from those regulating circadian photoentrainment.

**Commercial Relationships:** Jennifer Langel, None; Alan Rupp, None; James O’Donnell, None; Samer Hattar, None

**Support:** NIH grant GM076430 and EY024452
Melanopsin sets the contrast detection threshold of M4 ipRGCs

Takuma Sonoda, Tiffany M. Schmidt. Neurobiology, Northwestern University, Evanston, IL.

**Purpose:** Intrinsically photosensitive retinal ganglion cells (ipRGCs) were initially thought to only mediate non-image forming visual behaviors such as circadian photoentrainment. However, recent evidence suggests that melanopsin phototransduction in ipRGCs is required for behavioral contrast sensitivity. Here, we tested the hypothesis that melanopsin is required for normal contrast sensitivity at the cellular level. We specifically focused on the M4 subtype of ipRGCs, which are more commonly known as ON-alpha RGCs.

**Methods:** We made patch clamp recordings from M4 ipRGCs in whole mount retinas of adult WT and melanopsin (Opn4) KO mice. Contrast sensitivity was quantified by measuring the contrast which evoked 50% of the maximum F1 response (C_{50}) in response to drifting sine-wave gratings with mean luminances ranging from scotopic to photopic light intensities. For rescue experiments, the retinas of Opn4^{+/-} mice were virally infected with Gq-DREADDs and 10nM clozapine N-oxide (CNO) was bath applied to activate Gq-DREADDs.

**Results:** We found that M4 ipRGCs lacking melanopsin exhibited significant deficits in contrast sensitivity at not only bright, photopic light intensities, but also at mesopic and even scotopic light intensities. This effect is cell-autonomous because when we reintroduced the Gq-cascade in ipRGCs of Opn4^{+/-} mice with Gq-DREADDS, we found that the contrast sensitivity of M4 ipRGCs could be rescued with application of CNO in infected M4 ipRGCs, but not in uninfected M4 ipRGCs.

We have identified two possible mechanisms by which melanopsin could influence the contrast sensitivity of M4 ipRGCs. First, we found that WT M4 cells are significantly more depolarized compared to M4 cells lacking melanopsin in the presence of background light from scotopic to photopic light intensities. Second, we found that melanopsin phototransduction increases the excitability of M4 cells in the presence of background light, which would allow them to respond more robustly to small synaptic currents.

**Conclusions:** These results demonstrate that the behavioral deficits in contrast sensitivity we observe in Opn4 KO animals begin at the cellular level in M4 ipRGCs, allowing us to link the signaling properties of a defined RGC population to a specific behavioral output. Melanopsin phototransduction primes M4 cells, through multiple mechanisms, to respond robustly to low contrast stimuli across a surprisingly wide range of light intensities.

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Melanopsin is a unique visual pigment expressed in a small subset of retinal ganglion cells in the mammalian retina, and is involved in non-image forming tasks such as circadian photoentrainment and pupil constriction. Data from both *in vitro* electrophysiology and calcium imaging assays, suggests that the activation kinetics of the melanopsin phototransduction cascade are sluggish, and the response has a unique sustained property in response to light, unlike the transient rod and cone responses. While several key players of melanopsin’s signaling cascade have been described (such as melanopsin’s coupling to GRKs & β-arrestins), there are additional signaling molecules that remain to be elucidated. First, we aim to describe the phosphorylation profile of melanopsin’s long C-terminus tail, which we hypothesize is contributing significantly to both receptor activation and deactivation. Second, we hypothesize that melanopsin interacts with a serine/threonine phosphatase, specifically, of the protein phosphatase family, and this interaction is important for resensitization of the receptor.

**Methods:** We aim to test the first hypothesis through mutagenesis of melanopsin’s gene, expression in HEK293 cells, and *in vitro* calcium imaging to measure signaling kinetics. To test the second hypothesis, will perform RT-PCR, immunohistochemistry, and protein interaction assays using retinal samples.

**Results:** We have found that there are six serines and threonines between residues 385 and 396 on the C-terminus that are required for signaling deactivation, and five additional serines and threonines in either proximal or distal direction that are needed for complete signaling deactivation. Additionally, we have evidence suggesting phosphorylation of the proximal C-terminus plays a role in receptor activation, possibly involving an important tyrosine residue (Y382). Finally, we have found diverse expression of protein phosphatases (subclasses 1-5) within the murine retina, and are beginning to explore which one of these interact with melanopsin.

**Conclusions:** We present these findings as contributors to melanopsin’s unique signaling properties within the mammalian retina.
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