MELANOPSIN SYMPOSIUM [2 CME]  
(MODERATORS: RANDEY KARDON, MD, PHD AND KENNETH SHINDLER, MD, PHD)

A distinct subpopulation of retinal ganglion cells that express the photopigment melanopsin have recently been described. Melanopsin is a unique opsin-like photopigment that has different properties than the opsin–based photopigments found in rod and cone photoreceptors. Melanopsin retinal ganglion cells can be induced to depolarize through intrinsic phototransduction mediated by melanopsin, or secondary to phototransduction in rods and cones. Melanopsin retinal ganglion cell axons project to non-image forming brain regions and have been implicated in non-visual light-mediated responses including the pupillary light reflex and control of circadian rhythms.

In this symposium, current understanding of the morphology and CNS projections of melanopsin retinal ganglion cells and the basic properties of this photopigment will be discussed. The role of these cells in mediating non-visual light responses and the implications of these functions in clinical evaluation of neuro–ophthalmologic problems will be stressed.

At the conclusion of the symposium, attendees should be able to: 1) Learn the basic properties of melanopsin retinal ganglion cells, including their intrinsic phototransduction, cellular morphology and projections to the CNS; 2) Understand the role of melanopsin retinal ganglion cells in mediating specific non–visual, light–mediated, physiologic pathways including the pupillary light reflex and circadian rhythms; and 3) Consider the clinical implications of melanopsin retinal ganglion cell pathophysiology in neuro–ophthalmologic disorders.

CONTROVERSIES IN NEURO–OPHTHALMOLOGY [2 CME]  
(MODERATORS: STEVEN GALETTA, MD AND LUIS MEJICO, MD)

The role of pharmacological testing of pupillary disorders in neuro–ophthalmic practice remains controversial. The first two speakers will provide the pathophysiological basis and the evidence for and against the need for pharmacological diagnostic testing. While much advancement has been made in the understanding of neumyelitis optica in the last decade, questions remain about its existence as a distinct entity from multiple sclerosis. Such distinction becomes of utmost importance as more effective treatment options become available. The next two speakers will provide an update on the pathogenesis and treatment options for neumyelitis optica.
As understanding of genetics, pathophysiology, clinical awareness and specificity of diagnostic tests have increased in hereditary optic neuropathy, effective treatment options have lagged behind. The speakers will present the current status of treatment strategies for these disorders.

At the conclusion of this symposium, attendees will be able to describe the pros and cons, latest understanding, and controversies surrounding the following topics: 1) Discuss the pros and cons of pharmacological testing for pupil disorders; 2) Explain the controversy surrounding the disorder of neuromyelitis optica and its implications for treatment; and 3) Understand the potential treatment options for hereditary optic neuropathy.

10:00 a.m. – 10:35 a.m. Pupil Disorders, is Pharmacological Testing Necessary? 93
Evidence presentation Randy Kardon, MD
Expert opinion & commentary Jonathan Trobe, MD
Questions & Answers

10:35 a.m. – 11:0 a.m. Neuromyelitis Optica, is it a Distinct Entity From MS and What are the Treatment Implications? 101
Evidence presentation Jeffrey Bennett, MD, PhD
Expert opinion & commentary Steven Galetta, MD
Questions & Answers

11:0 a.m. – 11:45 a.m. Hereditary Optic Neuropathy, is there a Treatment? 107
Evidence presentation Nancy Newman, MD
Expert opinion & commentary Alfredo Sadun, MD
Questions & Answers

11:45 a.m. – 12:00 p.m. NANOS Platform Presentation: Sustained Neuroprotection after a Single Intravitreal Injection of PGJ2 in a Rodent Model of NAION – Valerie Touitou, MD

1:00 p.m. – 4:00 p.m. NOVEL UPDATE Arizona Salons 1–6
Nancy T. Lombardo, University of Utah, Spencer S. Eccles Health Sciences Library
Jeanne Le Ber, University of Utah, Spencer S. Eccles Health Sciences Library

This session provides an update to NOVEL: the Neuro–Ophthalmology Virtual Education Library. Many enhancements have been made to the digital library, and new collections have been added. Specific collections, such as those of Shirley H. Wray, Robert B. Daroff, and Helmut Wilhem will be highlighted. Following the NOVEL update, free web-based online tools which can make member’s lives easier will be presented. The tools covered will include the Doodle Poll and Scheduler, a free online tool that makes organizing meetings and getting feedback a snap. Other tools from the Google suite will be demonstrated, including Google Scholar, Desktop, Docs, Calendar and using YouTube to share video clips. A brief overview of methods for sharing EndNote libraries will be covered. NOVEL help and EndNote consultations will be available at the NOVEL table outside the main lecture hall throughout the meeting.
LEARNING OBJECTIVES
1. To learn the histology and morphology of melanopsin Retinal Ganglion Cells (mRGCs) and their projections in the human CNS.
2. To understand the relationship of mRGCs structure to function.
3. To understand the relative susceptibility of mRGCs in comparison to regular RGCs to the effects of aging and mitochondrial disease.

CME QUESTIONS
1. What are the non-formed visual functions subserved by the melanopsin retinal ganglion cells (mRGCs) and through which brain nuclei?
2. In what layer of the retina do these mRGCs cells reside?
3. Are there optic neuropathies that primarily affect or spare mRGCs?

KEY WORDS
1. Melanopsin
2. mRGCs
3. Intrinsically Photosensitive Retinal Ganglion Cells
4. ipRGCs
5. LHON

1. There are about 1,000 mRGC scattered about in two layers of the human retina. Yet, this small number does not mean that the afferent input is minor. Although mRGCs only comprise approximately 1% of all retinal ganglion cells, their extensive dendritic fields provide a photosensitive bilayer within the inner retina. The large area of the mRGC dendritic fields and the dense content of the photopigment melanopsin make their contribution reflecting non-formed light input to the brain very important.

2. Anatomic features of the mRGC as determined by immunohistology and immunohistofluorescence.
   a. Density distribution in the non–human primate retina (slightly denser in the macula) and receptive field size as a function of location, compared to other ganglion cells (Dacey et al).
   b. Thick and extensive dendrites, soma of 15–20 microns and moderately thick axon compared to other retinal ganglion cells.
   c. Location within the retinal ganglion cell and inner nuclear retinal layers (M1, M2, and M3 subtypes) and current knowledge about the subtype projections in the CNS (i.e. one subtype distributes more to the SCN and the other more to the olivary nucleus of the pretectum).
   d. The melanopsin pigment is distributed densely on the cytoplasmic membrane around the soma, along the knobby and varicose branching dendrites and even along the axons in the retinal nerve fiber layer.
   e. Connections with other retinal neurons and neurotransmitters (bipolar and amacrine cells) allows for rod and cone inputs as well as direct photosensitivity.

3. Connections to the CNS and neurotransmitters.
   a. Suprachiasmatic Nucleus of the hypothalamus
   b. Olivary Nucleus of the pretectum
   c. Trigeminal Nucleus?
   d. Superior Colliculus?
   e. LGN?

4. Effects of aging and disease on human rRGCs.

   See manuscript to follow:

ABSTRACT
The non–rod/cone photoreceptors melanopsin retinal ganglion cells (mRGCs or intrinsic photosensitive RGCs = ipRGCs) have been shown in several animal models to convey the detection of light irradiance to the brain through the optic nerve to such centers as the hypothalamus, photoentraining circadian rhythms and the pretectum, contributing to the pupillary light reflex. Eyes from two LHON patients, two age–matched controls and two glaucoma patients were collected at autopsy for histological and immunohistochemical investigations. Both LHON patients carried the homoplasmic 11778/ND4 mutation and were affected by a mild and severe form of
The recent identification of a new type of photoreceptor as a subclass of retinal ganglion cells (RGCs) in rodents was seminal. These RGCs are intrinsically photosensitive through the expression of the photopigment melanopsin (mRGCs). These cells project to the suprachiasmatic nucleus of the hypothalamus (SCN), which serves as a master circadian clock, to form the retino-hypothalamic tract (RHT). Thus in some mammals, the eye, besides providing image detection function, conveys to the brain the detection of light irradiance. This function subserves the phototransmission of circadian rhythms, as well as the afferent arm of the pupillary light reflex. This non-image forming system operates in parallel to the well known image-forming pathway that depends upon rods and cones.

Recent studies in humans have suggested that mRGCs constitute less than one percent of the total number of RGCs. They originate large dendritic fields, which predominantly run in the outer sublayer of the inner plexiform layer. These dendrites contain melanopsin and respond to light by depolarization. However, the mRGCs also receive input from rods and cones through the bipolar and the amacrine cells, which modulate their activity. There is substantial evidence that melanopsin (Opn4) is a photopigment exhibiting bistability, which uses the invertebrate (rhodobacter) signal transduction pathway. The mRGCs innervate the SCN through the RHT and also target other areas of the brain, such as the olivary pretectal nucleus, through which they constitute part of the pupillary light reflex circuit.

The assumption that a “circadian photoreceptor” originated from studies of mouse models, however there are examples of human ocular disease characterized by profound disruption of the rods and cones photoreceptive system, which did not eliminate circadian rhythms. Optic neuropathies are diseases characterized by the loss of RGCs and optic atrophy. Specific patterns of RGCs loss, possibly reflecting different pathophysiological mechanisms, may lead to predominant involvement of functional, anatomic or retinal position subtypes of RGCs. For example, both inherited optic neuropathies due to mitochondrial dysfunction, Leber’s hereditary optic neuropathy (LHON) and dominant optic atrophy (DOA), are known for primarily involving the RGCs that constitute the papillomacular bundle, leading to dyschromatopsia, loss of visual acuity, cecocentral scotomas and temporal optic atrophy. Would these two neurodegenerative processes involve the mRGCs?

The present study was designed to establish the morphometry and morphometrics of mRGCs in human retina and compare normals to patients with mitochondrial optic neuropathies (using glaucoma as a positive control). The hypothesis is that mRGCs may be affected in different proportion in LHON or DOA subjects such that they may be largely spared abnormalities of circadian rhythm or papillary disturbances even with substantial blindness. In the process, we describe the characteristics and distribution of mRGCs in normal human retina and in hereditary optic neuropathies. We hypothesize that in LHON, DOA and perhaps other mitochondrial optic neuropathies, there is a specific mechanism leading to RGC death. Further, since this mechanism may specifically target RGCs, it may differentially involve mRGCs. This study was performed to test this hypothesis.

Materials and Methods
We performed an immunohistochemical analysis of melanopsin-containing RGCs (mRGCs) in human retinas and morphometric assessment of the RGC fibers in the optic nerves from normals, LHON, DOA and glaucoma.

Eyes with optic nerves were obtained post-mortem from two LHON male subjects (59 and 52 years) and from one DOA male subject (84 years). Eyes were also acquired from an eye tissue bank (Lions Eye Bank of Oregon, USA) for age and sex-matched controls (58, 54 and 85 years respectively). We excluded tissues from subjects with a history of ophthalmological or neurological disorders. All tissues were initially immersion fixed in neutral buffered formalin. The eyes and optic nerves were oriented and dissected horizontally at the meridian producing two collotes containing the entire retina bisecting the papillomacular bundle. Tissue from the superior half was embedded in paraffin and serially sectioned at 5 μm. Sections were immunostained for melanopsin (antibody provided by Dr. Jens Hannibal, Copenhagen, Denmark)
using an indirect immunoperoxidase technique with DAB as the substrate/chromogen. Immunostaining was performed on seven to ten serial sections to define the extent of each single mRGCs and establish the counting criteria for subsequent quantitative analysis. To this end mRGCs were then identified and manually counted by two independent observers on five to six sequential slides originating from every fifth sections.

We counted each heavily stained mRGC with a complete soma and the nucleus visible. The length of each retina section was calculated on serial photographs overlapping on the borders, covering the entire retina.

Optic nerves were cut into cross-section 2 mm thick about 3 mm posterior to the globe. Orientation was established and the specimens processed for paraffin and plastic blocks. The paraffin tissue blocks were horseradish peroxidase and DAB as substrate/chromogen for neurofilaments. Semithin sections were cut at 1μm from plastic-embedded tissues and stained with p-phenylenediamine (PPD) for light microscopic examination and acquired with a Spot II digital camera (Diagnostic Instruments, Inc.).

Axonal counts were manually performed in the optic nerves from the two LHON subjects. The optic nerve cross section profiles were divided into five regions, each with a different axonal density, to account for the non homogeneous distribution of axonal loss. The counts for the five regions were summed to obtain the total axon count for each optic nerve.

Counting of mRGCs allowed calculation of their area density across the posterior retina in the nasal/temporal axis. The area density of mRGCs was calculated by multiplying the length of the retinal cross sections by 15 μm.

The total area density of mRGCs in each eye was calculated assuming an average total surface in human retina of 1040 mm². Total RGC numbers were calculated. Optic nerve cross sections were analyzed by axonal morphometry for normal subjects at different ages with PPD based image analysis. The ratio of iPRGCs on the total number of RGCs for the control eyes was based on the average of 1.200.000 RGCs per retina, as previously reported by our laboratory.

RESULTS
Melanopsin–Containing RGCs (mRGCs) in Human Retinas
Figures 1A, 1B and 2 illustrate the histopathology of mRGCs from retinal specimens collected at autopsy from LHON, DOA, glaucoma and control subjects. The two LHON subjects were brothers, included in a prospective clinical study and hence they were systematically evaluated prior to death. They both were part of a large Brazilian pedigree of Italian maternal ancestry carrying the 11778/ND4 mutation in mitochondrial DNA (mtDNA).

The severity of optic neuropathy differed in these two subjects. The 59-year old man (at necropsy) had a late-onset and mild form of LHON (Case 1), whereas his 52-year old (at death) brother had a classical LHON with young–adult onset and severe optic atrophy (Case 2).

Case 1 with LHON suffered visual loss at 51 years of age probably precipitated by lifelong abuse of tobacco and alcohol and his disease was stable for the eight years to his death. Fundus examination revealed mild temporal optic atrophy. Post-mortem histological evaluation of the optic nerves revealed a sectorial loss of axons that matched the fundus pattern. Four eyes from two sex and age matched controls were selected as control normals.

FIGURE 1A. Normal (control) retina. mRGC is seen in the RGC layer identified by the arrow. Note the dendritic tree that also stains for melanopsin.

FIGURE 1B. Higher magnification of the same mRGC as seen in Figure 1A. mRGC is stained dark brown. Note (arrow) the axon emanating from the soma and entering the retinal nerve fiber layer.
The Case 2 LHON patient was more typical. He suffered visual loss at 27 years of age and had a disease duration of 25 years. Fundus examination of the Case 2 LHON showed severe bilateral optic atrophy. Post-mortem evaluation of the optic nerves revealed that they were both small had had massive axonal loss throughout except for some sparing nasally.

The DOA patient was an 84 year-old man, belonging to a family of 152 members reported by Kjer in the seminal description of DOA\. This patient had a history of severe visual loss and dyschromatopsia. His visual acuities were 20/200 OD and counting fingers vision OS. Fundus examination revealed bilateral diffuse optic atrophy. Histopathology of this patient was previously reported and documented a severe loss of RGCs in the retina and extensive loss of axons primarily in the temporal optic nerve.

Manual counts of residual axons in the optic nerve cross sections revealed about 300,000 axons for the Case 1mLHON (74% loss) and about 22,500 for the Case 2 LHON (98% loss). Immunohistochemical investigations using human melanopsin antibody in the cases of LHON, DOA, glaucoma and control retinas revealed the presence of melanopsin marked by high contrast brown staining or fluorescence and distinguished by heavily stained extensive dendritic fields (Figures 1 and 2). The mRGCs were located either in the retinal ganglion cells layer (RGL) or in the inner nuclear layer (INL). This was consistent with animal studies and previous reports. The mRGCs were characterized by having a large cell body (15–20μm) with a centrally located nucleus. The pigment was largely distributed peripherally under the plasma membrane and along the dendrites. The dendrites were very long and studded with pigment containing knobs and protrusions. Fragments of dendrites were recognizable in our retina sections mostly running between the inner nuclear and the inner plexiform layers (IPL). Occasionally, delicate and thin but densely stained axons were also identifiable by the melanopsin antibody and they were seen exiting the soma and coursing into the RNFL.

Quantitative evaluation by manual counting of mRGCs resulted in a mean density of 12.0 cells/mm² for control retinas, 0.0 cells/mm² for glaucoma retina, 9.0 cells/mm² for LHON subjects and 7.5 cells/mm² for the DOA subject. The 1,200,000 fibers per optic nerve has previously reported by our group and represented the normal standard axonal counts. For LHON subjects total RGCs number was compared to this assuming each RGC contributed only one axon to the optic nerve. Thus, the percentage of mRGCs, compared to the total number of RGCs, was 1.23% for controls. By comparison, and reflecting the greater loss or regular RGCs, this number was 3.3% for the Case 1 (mild) LHON case and 39.3% for Case 2 (severe) LHON. These higher percentages reflected the relative sparing of mRGCs in LHON that became more obvious with more severe disease.

<table>
<thead>
<tr>
<th>AGE</th>
<th>Normalized mRGCs/1,000,000</th>
<th>Average Axon Counts</th>
<th>mRGCs/RGCs</th>
</tr>
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<tbody>
<tr>
<td>CTRL 1</td>
<td>58</td>
<td>1.37%</td>
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</tr>
<tr>
<td>CTRL 2</td>
<td>54</td>
<td>1.09%</td>
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<tr>
<td>LHON 1</td>
<td>59</td>
<td>0.81%</td>
<td>301,255</td>
</tr>
<tr>
<td>LHON 2</td>
<td>52</td>
<td>0.74%</td>
<td>22,632</td>
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The percentage of mRGCs located in the RGC layer was about half but varied slightly in different conditions. For example, slightly more than half (about 60%) of mRGCs were in the RGC layer (vs. the IPL) in the four eyes from controls. However in the two LHON cases the percentage of mRGCs varied from about 40% (Case 1—mild) to 63% (Case 2 severe) LHON. Likewise, this subpopulation of mRGC in the RGC layer was also lower in the older (85–year old) control down to 42%.

**Take home points**

Melanopsin RGCs (mRGCs) represent slightly more than one percent of the RGCs in the normal human retina. They are distributed across the retina without significant concentration in the macula or elsewhere. About half of these cells consist of a subpopulation in the RGC layer. The other half is in the inner nuclear layer. Both sets of...
mRGCs have extensive dendritic arborizations that stain heavily for melanopsin. These dendrites fan out with knobs and protrusions primarily at the interface between the inner plexiform layer and the inner nuclear layer. Additionally, the mRGCs emit axons that rise and run along the retinal nerve fiber layer to the optic nerve. Even these axons contain melanopsin and may therefore have the capacity for intrinsic photosensitivity.

In the hereditary neurodegenerations, such as LHON and DOA, there is relative sparing of the mRGCs. Hence, their percentage in the total population of RGCs can rise as high as 40%. Both subpopulations of mRGCs (in the RGC and INL respectively) are substantially spared. In absolute glaucoma, used as a “positive control”, all RGCs, including all mRGCs, are eliminated.

This relative sparing of mRGCs in LHON and DOA may provide an underlying anatomical substrate explanation for why many LHON and DOA patients, despite devastating loss of vision and optic atrophy, maintain good papillary reflexes and light entrainment of the circadian rhythm.

It remains to be elucidated as to what imparts robustness to mRGCs in the face of the compromise of RGCs seen in neurodegenerations such as LHON and DOA. Cybrid studies in our laboratories are exploring some possible mechanisms by which mRGCs may have fundamental resistance to various oxidative stresses. We are also conducting further post-mortem immunohistochemical studies, in aging and various stages of glaucoma, to better elucidate this resistance to mRGC loss.

CME ANSWERS
1. Light entrainment of the circadian rhythm is mediated by the suprachiasmatic nucleus of the hypothalamus and pupillary function through the olivary nucleus of the pretectum.
2. About half of the mRGCs are in the retinal ganglion layer, the other half in the inner nuclear layer.
3. Melanopsin RGCs seem particularly robust to mitochondrial optic neuropathies such as LHON, however, they are more sensitive to aging than regular RGCs.

REFERENCES


LEARNING OBJECTIVES

1. The attendee will be able to understand the basic structure of melanopsin and how it relates to other photopigments.
2. The attendee will be able recognize the properties of melanopsin light absorption and molecular pathways it uses to transduce light into electrical signals.
3. The attendee will learn the distinct properties of melanopsin photopigment regeneration.

CME QUESTIONS

1. What is melanopsin and how does it differ from photopigments in rods and cones?
2. How does melanopsin transduce light into an electrical signal?
3. What mechanism is used to regenerate melanopsin photopigment and how does this relate to photopigments in invertebrates?

KEYWORDS

1. Melanopsin
2. Photopigment
3. Phototransduction

THE MELANOPSIN PHOTOPIGMENT AND ITS RELATION TO OTHER OPSINS

Opsins are an evolutionarily conserved group of photosensitive proteins that contain seven transmembrane domains and crosslink to a vitamin A–based retinaldehyde chromophore (reviewed in Fernald, 2006). Each opsin is sensitive to photons of light within a range of wavelengths and, once stimulated, trigger photoisomerization of the chromophore and initiate an intracellular signaling cascade through a coupled G–protein. Opsins controlling vision in vertebrates, rhodopsin and cone opsins, have been studied for many years and are known to transduce light signals in retinal photoreceptors. The novel photopigment melanopsin was first identified using antiserum raised against bovine rhodopsin and screening proteins from frog eyes and from photosensitive cells called melanophores (Provencio et al, 1998). Melanopsin was subsequently found to be encoded by the Opn4 gene and expressed in a subset of retinal ganglion cells in the mammalian eye, including human inner retina (Provencio et al, 2000).

The sequence and structure of melanopsin shares features that are conserved among all classes of opsins. Interestingly, the amino acid sequence of melanopsin is more homologous with invertebrate opsins (39%) such as octopus rhodopsin, than to vertebrate opsins with which it shares about 27–30% sequence homology (Provencio et al, 1998). In critical functional regions within opsins, including the retinal/ligand binding pocket, melanopsins from various species are highly conserved with 89–100% sequence homology, whereas the melanopsins have only 40–46% sequence homology with rod and cone opsins (Kratochwil et al, 2005; Hankins et al, 2007). Some structural features, including increased length of the third intracytoplasmic loop and the length of the intracellular C terminal end of melanopsin are also similar to invertebrate opsins (Provencio et al, 1998). At the genome level, human melanopsin is encoded in an 11.8 kb region of chromosome 11q22, and additional features, including the number of exons and introns in the melanopsin gene, also suggest a stronger evolutionary relationship to invertebrate rather than vertebrate opsins (Bellingham and Foster, 2002; Provencio et al, 2000). The structural relationship of melanopsin to invertebrate opsins provides clues to its evolutionary origin, and suggests that its function as a light sensitive pigment is likely different than classic visual photopigments in rod and cone photoreceptors.

MELANOPSIN PHOTOTRANSDUCTION PATHWAY

Light absorption

Each opsin/vitamin A–based photopigment absorbs light at different wavelengths. The peak absorption spectra for melanopsin was first measured using mice deficient for both rod and cone photoreceptors (Lucas et al, 2001). Pupillary responses were measured in these mice and suggested that the non–rod, non–cone photopigment (later identified as melanopsin) could respond to monochromatic light stimuli over a range of 420 – 625 nm, with peak response at a wavelength of 479 nm. Subsequent studies have found similar maximal wavelength responses of around 480 nm for melanopsin in other mammals, including rats, monkeys and humans (Berson et al, 2002; Dacey et al, 2005; Brainard et al, 2001; Thapan et al, 2001; Hankins et al, 2002).

G–Protein Coupling, Intracellular Signaling, Ion Channels and Cell Polarization

The molecular signaling events that allow melanopsin containing cells to convert light into an electrical signal are still being studied. Based on its deduced structure and similarity to invertebrate opsins, it has been suggested...
that melanopsin may use a similar signaling cascade as in invertebrate photosensitive cells and distinct from rod and cone opsins (Hankins et al, 2007). Rod and cone opsins are coupled to the G-protein transducin. Light signaling activates the transducin which leads to activation of phosphodiesterase, hydrolysis of cGMP, and ultimately closure of cGMP-gated ion channels resulting in hyperpolarization of the cell membrane (Arshavsky et al, 2007; Koyanagi and Terakita, 2008). In one study, patch-clamp recordings of individual photosensitive retinal ganglion cells isolated and cultured from rat retina indicated that illumination drove melanopsin activation of a G\textsubscript{q}/G\textsubscript{11}-type G-protein and phospholipase C similar to signaling in invertebrate photosensitive cells (Graham et al, 2008). This intracellular signaling leads to cell depolarization by gating transient receptor potential channels, a family of Ca\textsuperscript{2+} permeable cationic channels (Sekaran et al, 2003), similar to invertebrate photosensitive cells. Indeed, coexpression of these channels with melanopsin leads to light induced current in otherwise non-photosensitive cells (Qiu et al, 2005; Panda et al, 2005). Furthermore, inhibitors of transient receptor potential channels block current generation in rat photosensitive retinal ganglion cells (Warren et al, 2006). While further studies are needed to confirm similar signaling cascades and ion channels are used physiologically by human melanopsin retinal ganglion cells, these studies suggest that melanopsin uses a signaling pathway that is conserved across a number of species and is similar to those used in invertebrate photosensitive cells.

**REGENERATION OF MELANOPSIN PHOTOPIGMENT**

Opsin photopigments use only cis-isoforms of retinaldehyde as chromophores for light responses. Following stimulation, retinaldehyde is isomerized to the trans-isoform, and therefore cannot be used for additional light responses until it gets regenerated back to the cis-isoform. In rod and cone photoreceptors, once 11-cis-retinal isomerizes to all-trans-retinal, the retinaldehyde cannot be regenerated within the photoreceptor cells themselves, instead requiring enzymatic conversion in retinal pigment epithelial cells. This regeneration, the retinoid cycle, involves several enzymatic steps beginning with reduction of all-trans-retinal to all-trans-retinol in the photoreceptor, extracellular release and entrance into the retinal pigment epithelium, esterification into all-trans-retinyl ester, hydrolysis of the retinyl ester and isomerization to 11-cis-retinol, and finally oxidation back to 11-cis-retinal that can be released from the pigment epithelium back to the photoreceptors (summarized in Arshavsky, 2002b). Thus, rods and cones are dependent on other cell types to maintain their photosensitive pigments.

Invertebrate photopigments have a unique property of bistability, in which the opsin itself can respond to different wavelengths of light to drive regeneration of its own chromophore back to the cis-isoform after the light sensing response has isomerized the chromophore to the trans-isoform. Growing evidence suggests that melanopsin may share this unique ability to regenerate its own pigment, in addition to the structural and signaling similarities it shares with invertebrate opsins. Several groups demonstrated that transfection of non-photosensitive cell types with melanopsin renders the cells light sensitive and show that at least in these in vitro systems, melanopsin is capable of regenerating its own chromophore (Panda et al, 2005; Melyan et al, 2005; Koyanagi et al, 2005). Melyan and colleagues (2005), for example, demonstrated that melanopsin expression in a neuronal cell line led to detectable current responses within cells following application of 9-cis- or 11-cis-retinal and stimulation with 420-nm or 480-nm light. Interestingly, melanopsin expressing cells also generated some current in response to light after treatment with all-trans-retinal, demonstrating that transfection with melanopsin provided the cells with the capability of converting the all-trans-retinal into a light-responsive cis-isoform. Panda and colleagues (2005) found similar results expressing melanopsin in frog oocytes.

While there is good evidence that melanopsin is capable of bistability, it has been debated whether this mechanism is used physiologically in photoresponsive retinal ganglion cells. Mure and colleagues (2007) found that by pre-stimulating with appropriate wavelengths of light, circadian light responses were restored or increased as compared to a previous, identical light stimulus. Their results suggest that the relative proportions of 11-cis and all-trans-retinal crosslinked forms of melanopsin adjust in response to light similar to invertebrate bistable pigments. Another group, however, found that pre-exposure to long-wavelength light did not increase melanopsin retinal ganglion cell mediated responses calling into question the physiologic relevance (Mawad and Van Gelder, 2008). Thus, the physiologic role of this property and the precise mechanisms used to regenerate melanopsin in vivo continue to be evaluated.

**SUMMARY**

Melanopsin is a photosensitive protein sharing sequence and structural homology to other opsins. Melanopsin appears to be more closely related to invertebrate opsins than classic visual opsins in rod and cone photoreceptors. Mounting evidence suggests that the phototransduction
pathway and pigment regeneration cycle used by melanopsin may also resemble those used by invertebrate opsins. While the pathways and functions of melanopsin continue to be identified, recognizing its distinctions from rod and cone opsins can help put into perspective some of the distinguishing properties of melanopsin retinal ganglion cells and how they mediate various light responses, distinct from the vision forming functions of rod and cone photoreceptors, that will be discussed in other sections of this symposium.

CME ANSWERS
1. Melanopsin is a photosensitive protein with seven transmembrane domains and coupling to G-proteins typical of other opsins. The amino acid sequence and gene structure of melanopsin have only limited homology to rod and cone opsins, and melanopsin responds to light at wavelengths distinct from rods and cones using different intracellular signaling cascades.

2. Light at or near 480 nm wavelength induces a conformational change in melanopsin and its associated retinaldehyde. Evidence suggests this can induce G-protein mediated activation of phospholipase C and opening of transmembrane ion channels to depolarize the melanopsin retinal ganglion cell.

3. The mechanism used to regenerate the melanopsin photopigment is currently debated in the literature. While rod and cone photopigments require separate pathways in neighboring cells for isomerization, melanopsin likely is a bistable pigment similar to invertebrates whereby direct light absorption by the melanopsin itself drives both the phototransducing isomerization from 11-cis- to all-trans-retinal as well as the reverse processing back to 11-cis-retinal.

REFERENCES
LEARNING OBJECTIVES
1. To know the important central projections of the MGCs.
2. To understand the basic properties of the intrinsic light response.
3. To understand the basic properties of the extrinsic light response.

CME QUESTIONS
1. Name the nucleus considered to be the main circadian pacemaker.
2. Name the central integrator of afferent input of the pupil light reflex.
3. In rodents at birth, the MGC generates an action potential from both an intrinsic response to light and from synaptically-mediated, extrinsic signals. True or False.
4. Which of the following is not a feature of the intrinsic light response?
   a. active in the absence of functional outer photoreceptors
   b. long latency time to first spike
   c. peak spectral sensitivity at 550 nm
   d. steady firing rate during constant illumination

KEYWORDS
1. Melanopsin
2. Intrinsically Photosensitive Retinal Ganglion Cell
3. Photoreceptor

MORPHOLOGY AND CLASSIFICATION OF MELANOPSIN-EXPRESSING RETINAL GANGLION CELLS (MGCs)
Retinal ganglion cells that express the photopigment melanopsin (MGCs) are sparsely present in the retina. In rodents, they comprise 1–3% of the total number of retinal ganglion cells. In humans, this percentage is even smaller, estimated at 0.2% or about 3000 MGCs per eye. Their distribution across the retina follows a small gradient in which the density of MGCs is greatest toward the fovea, reaching a peak density of 20–25 cells/m² in the parafoveal retina and decreases to 3–5 cells/m² in the periphery.

The cell bodies of MGCs are located primarily in the ganglion cell layer but some MGC somas can be found in the inner nuclear layer. The morphologic appearance of MGC is distinctive. The MGC is a giant-sized neuron and from its soma extend 2 to 4 long, primary dendrites which branch sparsely to form large dendritic trees (the diameter of a dendritic field ranges 200 to 800µm in a primate).

The size of the dendritic trees increase with eccentricity from the fovea, and the dendritic processes project to the inner plexiform layer (IPL) where they stratify either in the extreme outer or inner layer of the IPL.

Based on their dendritic stratification, three morphologic classes of MGCs can be identified (at least in mice). Type I cells have dendrites which stratify at the outer borders of the IPL (OFF sublamina a) and type II cells have dendrites near the ganglion cell layer in the inner sublamina of the IPL (ON sublamina b). These monostratified type I and type II cells make up the majority of MGCs (74–80%). A rare third type of MGC (type III) is bistratified with dendritic arbors that ramify in both sublamina a and b.

Baver and colleagues recently divided MGCs into 2 classes based immunocytochemical criteria, or expression of β-galactosidase reporter protein in genetically modified tau–lacZ knock–in mice. M1 cells are identified as being β-galactosidase (+) and also immunostain specifically with C-terminus melanopsin antibody. They contain greater quantities of melanopsin and have dendrites that stratify in the outer IPL. M1 cells correspond to morphologic type I cells described above and account for about half of the total number of MGCs in mouse retina. In contrast, M2 cells are β-galactosidase(−). They have larger cell bodies and dendritic fields with more complex arborisation. M2 cells correspond to type II cells.

The mouse retina contains equal numbers of M1 and M2 cells. The axons of the M1 subtype of MGCs heavily dominate the retinohypothalamic tract to the SCN whereas M2 input is favored at the PON (read section Central Projections of MGCs). In addition to their anatomic differences, M1 and M2 cells display physiologic differences in their responsiveness to light. In brief,
M1 cells appear more tuned to signalling via the intrinsic, melanopsin-mediated phototransduction. Such findings might suggest that M1 cells serve mainly to regulate the circadian photoentrainment, but the functional roles of M1 and M2 subtypes of MGCs are not yet established.

**CENTRAL PROJECTIONS OF MGCs**

In mice and primates, the MGC axons project to several deep brain centers, and the majority of fibers innervate the suprachiasmatic nucleus (SCN) in the hypothalamus which is considered to be the master circadian pacemaker. Dense projections also synapse at the ventrolateral preoptic area and intergeniculate leaflet, other important circadian control centers, and at the pretectal olivary nuclei (PON) in the dorsal midbrain, the integrating center for the pupillary light reflex. The retinal input to the SCN derives predominantly, if not exclusively, from MGCs and this percentage varies with species (70% MGC input in rat, 80% in hamster and 100% in mouse). Among the MGCs, the 2 classes of MGCs show a differential innervation of the central targets. The SCN receives retinal input predominantly from M1 cells (80% M1 vs. 20% M2) whereas the PON receives slightly more M2 than M1 input (55% vs. 45% respectively). The superior colliculus, however, has only M1 connections. Thus, M1 and M2 cells may integrate light information differently in order to convey specific signals to different central targets.

**INTRINSIC (MELANOPSIN-MEDIATED) LIGHT RESPONSES**

MGCs are intrinsically photosensitive which means they can depolarize to light stimulation in the absence of all synaptic input. Intrinsic light responses are characterized by the properties of the intrinsic light response using whole cell recordings in isolated rat retinas bathed in a pharmacologic cocktail that prevented all synaptic input to the ganglion cells. Light exposure evoked a large depolarizing voltage response with superimposed fast action potentials in the MGCs whereas non-melanopsin ganglion cells were electrically silent. The latency time to response onset was typically on the order of several seconds and was related to stimulus strength. For saturating light stimuli, the time of first spike was only a few hundred milliseconds but for stimuli near threshold, the latency time could be nearly one minute. Constant illumination depolarized the cells to a peak firing rate within 10-20 seconds and then the cells settled to a steady-state firing rate that varied little in its spike frequency. Repolarization following light termination was also slow and persistent spiking could be seen for 5 to 60 seconds after the light was turned off. Dacey et al found similar properties of the intrinsic light response in primates. Another characteristic of the intrinsic response is its very large size, excluding inefficient signal transduction as an explanation for their low light sensitivity. The low sensitivity of MGCs is believed to be due to poor efficiency of photon capture.

At birth, all activity from the MGCs is driven exclusively by intrinsic phototransduction as outer retinal photoreception does not yet contribute to ganglion cell light responses. The earliest light response is small, sluggish and transient but this early intrinsic pattern of cell firing rapidly diversifies in the first postnatal week. That the intrinsic phototransduction system is functional in very early development underlines the importance of photoentrainment of the circadian clock even at this age. Diversity of intrinsic light responses in the early postnatal period, different from adult light responsiveness, suggests that the melanopsin irradiance detection might also play a role in the maturation of the neonatal retina, including neuronal differentiation, expansion and stratification of MGC dendrites and formation of functional connections with the rod and cone systems.

The adult retina of rodless/coneless mice shows 2 patterns of intrinsic light activity correlating to the 2 classes of MGCs. M1 cells have very prolonged activity after light termination. In addition, they are 10 times more sensitive to 480 nm light compared to M2 cells and fire to a narrower range of depolarizing current, suggesting that in M1 cells, the intrinsic pathway is more influential than synaptic connections in modulating the light signal to the brain. M2 cells have a long latency to onset, especially at subsaturating light intensities as well as slow termination of activity when the light is turned off. M2 cells are capable of higher peak and sustained firing rates and spike over a greater range of depolarizing current. The ability of MGCs to maintain steady activity during constant illumination is the feature that distinguishes these cells from conventional retinal ganglion cells. Yet the classical rod and cone system is functionally linked to the intrinsic, melanopsin system. This linkage expands the ability of MGCs to encode a broader range of light stimulation with a more complex signalling pattern. Synaptically-mediated light responses evoked by rod and cone pathways emerge during the second postnatal week.
**EXTRINSIC (SYNAPTICALLY-MEDIATED) LIGHT RESPONSES**

The developmental changes in the light response of MGCs in the postnatal period have been recorded in whole cells using a genetic mouse model. To a 5 second white light, only the intrinsic pathway is functional at birth and the MGC activity is characterized by a long latency time, sustained depolarization and a small peak depolarization. During the first week, the rate of firing and peak depolarization increases and periodic bursts of action potentials independent of light stimulation appear. Between postnatal days 11 to 14 which is around the time of eye opening, some MGCs show a marked decrease in response latency time to peak depolarization and repolarization, even though spiking is still present after light offset. After the second postnatal week (postnatal days 17 to 24), the latency to light onset and offset decreases further and the peak depolarization reaches a maximum amplitude.

These differential kinetics of the MGC light response that first appear during the second postnatal week and reach a mature firing pattern in the third and fourth postnatal weeks are consistent with the emergence of rod and cone influence superimposed on the intrinsic activity. By fourth postnatal week and in adult mice, the majority of the MGCs, if not all of them, display both extrinsic and intrinsic light responses.

These extrinsic inputs allow the MGCs to respond to light with shorter response time, larger depolarization and greater sensitivity to light than is possible from intrinsic phototransduction alone. Extrinsic, or synaptically-driven, responses are evoked by light on the order of 6 log units dimmer than intrinsic light response. In addition, there is a shift in the peak spectral sensitivity of MGCs from 480nm to 510nm. Taken together, the implication of these extrinsic influences is that cones, particularly M cones, have an important role in regulating circadian entrainment.

**THE FUNCTIONAL RECEPTIVE FIELD OF MGCs**

Melanopsin is found in the soma, the dendrites and the proximal portion of the axon of MGCs. The extensive dendritic arbor thus provides multiple sites of intrinsic phototransduction. The receptive fields of MGCs are oval in shape. The size of the extrinsic receptive field approximates the size of the intrinsic receptive field (957 ± 62 µm), suggesting that excitatory (ON) bipolar cells make synaptic contacts along the entire dendritic tree of the MGC. The estimated dendritic field diameter is, however, considerably smaller (500 µm). This discrepancy may be accounted for by several factors including light scatter or measurement error. It may also be that some bipolar cells have exceptionally large dendritic fields or that atypical ON bipolar cells make at least a few synaptic contact in the OFF sublayer.

**CONCLUSION**

There is still much to be learned about the MGCs. The extent of extrinsic synaptic influences on the intrinsic photosensitivity of MGCs remains a growing area of research and may broaden understanding of the biologic functions of the 2 classes of MGCs.

**CME ANSWERS**

1. suprachiasmatic nucleus in hypothalamus
2. pretectal olivary nucleus in dorsal midbrain
3. false
4. C

**REFERENCES**


LEARNING OBJECTIVES
1. To understand how to relate transient and sustained firing properties of the melanopsin retinal ganglion cells (MGCs.) to the resulting pupil movements
2. To relate the responses of rods and cones to their corresponding pupil responses by choosing stimulus conditions which seek to isolate their responses.
3. To recognize how the unique intrinsic photosensitive properties of the melanopsin retinal ganglion cells relate to pupil movements in photopic stimulus conditions and the clinical application.

CME QUESTIONS
1. Which of the following light stimulus conditions and pupil responses would most likely be mediated by rods?
   a. Transient pupil contraction under mesopic adaptation using low intensity blue light
   b. Transient pupil contraction under mesopic adaptation using high intensity red light
   c. Sustained pupil contraction under mesopic adaptation using high intensity blue light
   d. None of the above

2. Which are pupil response characteristics that are most likely to reflex intrinsic activation of melanopsin retinal ganglion cells?
   a. Sustained contraction to constant blue light intensity
   b. Sustained contraction after blue light is turned off
   c. Pupil sustained contraction greater with blue light compared to red light of photopically matched intensity
   d. All of the above

3. A patient reports subacute unilateral vision loss and accompanying visual field loss and it has not changed. The retina and optic nerve exam appear normal. The patient’s pupil responds poorly to red light, but with blue light matched in intensity, the pupil slowly contracts down to a very small size. What is the most likely diagnosis?
   a. Optic neuritis
   b. Retinitis pigmentosa
   c. AZOOR
   d. Central retinal artery occlusion

KEYWORDS
1. pupil light reflex
2. melanopsin
3. intrinsically photosensitive retinal ganglion cell
4. photoreceptor disease

Interest in the pupil light reflex and its value as an objective test of neuroretinal function has received renewed attention due to recent advances in understanding its neural substrate. Melanopsin–expressing retinal ganglion cells are a newly–described, specialized subset of ocular neurons capable of being activated by direct, intrinsic phototransduction using a unique photopigment, melanopsin or by indirect activation via via rod and cone input1-4. In mouse, dog, and primate studies, the major source of retinal input to the pupil light reflex originates from the melanopsin–expressing retinal ganglion cells5-7. Cell depolarization occurs either following phototransduction in the rods and cones or by intrinsic melanopsin–mediated phototransduction (or both). In other words, the pupillomotor information conveyed to the midbrain may derive indirectly from the outer retina (rod and cone activation) or directly from within the inner retina (melanopsin activation)7.

Several studies indicate a similar function of the melanopsin–expressing retinal ganglion cells in primates, including humans. The spectral sensitivity of intrinsic melanopsin–mediated phototransduction is broad with a peak at around 482 nm (short wavelength light)1,6,9. Recording from a single melanopsin–expressing retinal ganglion cell isolated within a full–thickness primate retina in vitro, Dacey et al demonstrated that different patterns of cell activity could be produced by retinal
exposure to light of different wavelengths. Continuous exposure to short wavelength (blue) light at high intensity evoked a high-frequency, non-fatiguable firing rate that was consistent with intrinsic melanopsin activation of the cell. In contrast, an equivalent long wavelength (red) light evoked a burst–attenuation pattern of cell firing, indicating adaptation to light and consistent with excitatory input from L and M cones.

In an in vivo primate study, Gamlin et al demonstrated that the spectral sensitivity of the sustained pupil response to a continuous bright light corresponded to that of melanopsin–mediated light transduction. In humans, Kimura and Young showed that blue light of high intensity produced a more sustained pupillary contraction compared to a photopically equivalent red light. These studies suggest that the pupil light reflex is a clinical correlate of the melanopsin ganglion cell activity and, under specific light conditions the pupil response can reflect whether cell activity is derived primarily from a photoreceptor–generated signal or from intrinsic activation of these neurons. We have previously demonstrated this differential pupil response to chromatic light in a unique patient with unilateral severe retinitis pigmentosa causing no light perception. The pupil was unresponsive to red light at all intensities, and did not respond to blue light at low and medium intensities. However, when the blue light intensity was increased to 100cd/m2, there was a robust pupil contraction, even though the patient did not perceive the light.

We hypothesize that optimal selection of the light intensity, wavelength and duration can bias the contribution of rods, cones, and melanopsin to the overall activity of the melanopsin–expressing retinal ganglion cells which drive the pupil light reflex. Light wavelength and intensity are important differentiating parameters. Direct, intrinsic activation of melanopsin mainly occurs at brighter light intensities so we anticipated that recording pupil contractions at suprathreshold low light intensities would primarily elicit outer photoreceptor (rod and cone) mediated pupil responses. More specifically, we expected that the use of a low intensity blue light would produce pupil responses that are contributed to a great extent by rods, considering their peak spectral sensitivity in the blue light range and the magnitude of their number (approximately 92 million rods compared to 5 million cones in the primate retina).

Another differentiating feature is based on the discharge pattern of action potentials (Figure 1). Based on electrophysiologic single cell recordings in primate retina, Dacey et al demonstrated that direct depolarization of the melanopsin–expressing ganglion cell has a prolonged latency to first action potential and thereafter the firing rate builds slowly to reach a maximal rate that is linearly proportional to light intensity and maintained without fatigue. In contrast, when rods and cones hyperpolarize in response to light activation, the latency time to ganglion cell depolarization is short, with an initial discharge that is at maximal rate and then rapidly attenuates, indicating early adaptation. Because the pupil behavior reflects ganglion cell activity, we predict that the pupil contraction would show much greater adaptation, or escape, during continuous light stimulation when it is mediated by rods and cones. However, once the light intensity becomes bright enough and within the peak blue spectral sensitivity of direct cell activation, a more sustained pupil contraction during light stimulation, i.e., without escape, is the expected response.

In our previous studies we sought to characterize the transient and sustained pupil contractions to continuous stepwise increases in red and blue light intensity over a 2 log unit range under mesopic conditions in normal human eyes (Figure 2).
The dynamics of the pupil movements to this stimulus paradigm are shown in Figure 3. Not surprisingly, the pupil tracings show both a transient and a sustained contraction that correspond to the action potential firing pattern shown in Figure 1, recorded from individual melanopsin retinal ganglion cells from a monkey retina when activated by either rods, cones, or by intrinsic activation of the cells.

FIGURE 3: Normal eye transient and sustained pupil responses to stepwise increases in photopically matched red and blue light stimuli. Note that at the onset of each step of light there is a transient pupil contraction followed by a mild pupil escape before leveling off to a sustained contraction. However, with bright blue light, there is hardly any pupil escape due to the intrinsic sustained firing of the melanopsin retinal ganglion cell.

We have since prospectively studied a large number of patients with inherited photoreceptor diseases and a small number with acquired photoreceptor disease. In addition, our group has reported the results of pupil testing with red and blue lights in dogs with acquired autoimmune retinopathy and almost complete blindness associated with non-recordable electroretinograms (ERG)\textsuperscript{14}. Pupil testing in patient examples of photoreceptor degeneration in humans (Figure 4) and veterinary patients serve to validate the important work already performed in measuring the contributions of the photoreceptors and intrinsic activation of melanopsin containing retinal ganglion cells in laboratory animals\textsuperscript{15-17}.

FIGURE 4: Pupil “signature” of a patient with retinitis pigmentosa having mixed cone and rod dysfunction and narrowed retinal vessels. In the upper left graph the normal pupil reactions are shown to the stepwise increased in intensity to red and blue light. The vertical bars denot the amplitudes of transient and sustained pupil contraction. Upper right graph is the pupil tracings from the patient with retinitis pigmentosa; not the diminished contractions to the red and blue light, but not the bright blue light (intrinsic melanopsin activation mediating the pupil response does not need photoreceptor input. Bottom left: kinetic perimetry of the patient showing typical ring scotoma. Bottom right; box plot of normal transient and sustained pupil contractions with patient data superimposed as circled dots, showing decrease in contraction compared to normal eyes.

In conclusion, the development of a thoughtful light stimulus protocol using red and blue light varying in intensities and at different levels of light adaptation will allow us to associate transient and sustained pupil contractions with their respective rod, cone and intrinsic melanopsin mediated retinal responses. Our results support the hypothesis that the different sources of retinal input to the pupil light reflex can be individually tested and contribute to our understanding of the functional role of the melanopsin–expressing retinal ganglion cells in humans. A chromatic light pupil test is likely to become further refined, which could be used to non-invasively differentiate outer retinal dysfunction (rod and cone damage) from inner retinal ganglion cell loss (optic neuropathy).
CME ANSWERS

1. a) since the rods have a similar spectral sensitivity as melanopsin, but are more sensitive, they can be preferentially activated with low intensity blue light, below the threshold for melanopsin activation, which occurs under more photopic conditions.

2. d) the intrinsic activation of the melanopsin retinal ganglion cells results in a sustained volley of action potentials with bright blue light that is reflected in a sustained pupil contraction. Even after termination of the bright blue light, the pupil is slow to redilate. The intrinsically mediated melanopsin pupil contraction is most activated by blue light and very little activation occurs at wavelengths greater than 620nm.

3. c) the lack of pupil response to red light, but preserved sustained contraction to blue light indicates photoreceptor diseases. Since it was unilateral and acquired, the most likely cause would be AZOOR.

REFERENCES


LEARNING OBJECTIVES
1. The attendee will be able to describe the effect of exposure to morning and evening light on circadian rhythms.
2. The attendee will be able to name two conditions in which there is misalignment between the circadian and sleep-wake systems.
3. The attendee will be able to describe the effects of aging on the circadian system.

CME QUESTIONS
1. Please describe the effects on the circadian system of exposure to light in the morning and in the evening.
2. Please name two conditions in which the circadian system is not aligned with the sleep-wake system.
3. Please describe the effects of aging on the circadian system.

KEY WORDS
1. Entrainment
2. Circadian rhythms
3. Suprachiasmatic nucleus

CIRCADIAN RHYTHMS
Every creature on earth adapts to the cycle of day and night. We are familiar with these behaviors, such as the sleep–wake cycle in humans, wheel running in lab rats, or feeding in rabbits. Physiologic function varies over a period of about 24 hours, too. It is called a circadian period (circa diem, about a day). In humans, the circadian period is 24.2 hours. In mammals, body temperature and secretion of melatonin, cortisol, thyrotropin, growth hormone, and prolactin all follow a circadian rhythm, with the nadir of body temperature and maximum melatonin secretion used as time makers in the circadian cycle. These occur around the middle of the circadian night. These rhythms persist in people and animals in constant environments, without any cues to the time of day.

SUPRACHIASMATIC NUCLEUS (SCN)
The main pacemaker, or oscillator, of the brain and body is the suprachiasmatic nucleus (SCN), a paired nucleus on either side of the third ventricle, just above the optic chiasm. Individual cells of the SCN, and the nucleus as a whole, have a firing rate that varies over a period (tau) of about 24 hours. The daily fluctuations of body temperature and hormone levels depend on changes in its electrical output. Each cell of most organs of the body contains independent oscillators. The SCN, the “master pacemaker”, drives and synchronizes them.

The SCN electrical output consists of inhibitory impulses to the pineal via the nearby paraventricular nucleus (PVN) and the sympathetic nervous system. The sympathetic nervous system sends a tonic signal to the pineal gland, stimulating it to make melatonin. The inhibitory signals from the SCN turn off that stimulus.

FIGURE 1: Cell in the supachiasmatic nucleus (SCN)

The circadian day begins when CLOCK and BMAL1 proteins form a dimer in the nuclei of SCN cells. This dimer turns on transcription of per and cry genes by binding to their gene promoters in the nucleus. Per and cry mRNA migrate to the cytoplasm, where they are translated into PER and CRY proteins. PER and CRY proteins form dimers. As these proteins accumulate in the cytoplasm they increase the firing rate of the SCN.
As PER–CRY dimers accumulate in the cytoplasm, some migrate back into the nucleus where they interact with the BMAL1–CLOCK dimer and turn off transcription of per and cry genes. Less PER and CRY proteins accumulate; the SCN firing rate drops; the PVN, disinhibited, stimulates the pineal to secrete melatonin. This is dim light melatonin onset (DLMO), the start of the circadian night.

ENTRAINMENT

The human circadian period is 24.2 hours long, slightly longer than the earth’s day-night cycle. Most animals have circadian periods that do not quite match the earth’s rotation. If left to themselves, as in temporal isolation studies, the oscillators and the animals they control would free run. Creatures with circadian periods longer than 24 hours would go to sleep and wake up a little later each day. Their activity would gradually shift to an inappropriate part of the day-night cycle—bats and owls active during the day; cows, sunflowers, and chickens active at night. By being active during a consistent part of the 24 hour day an animal can avoid predators, find food and otherwise optimize the benefits and minimize the hazards of its environment.

Light can synchronize an animal’s circadian rhythm to the day-night cycle of the earth. This synchronization is called entrainment.

One of the strongest markers of the circadian cycle is the temperature minimum (Tₘᵦᵣₖ), which usually occurs 2–3 hours before waking. The circadian cycle is most susceptible to phase shifting by light just before and after the temperature minimum. Light stimulus before Tₘᵦᵣₖ causes a phase delay. Light stimulus after Tₘᵦᵣₖ causes a phase advance. If a person is exposed to light in the evening hours, before the temperature nadir he will stay awake later and sleep later the next day. This is a phase delay. On the other hand, if a person is exposed to light early in the morning, after the temperature nadir, he will wake up earlier and go to bed earlier the next day. This is a phase advance.

The peak action spectrum (inversely proportional to the number of photons needed to get a biological response) for entrainment is 450–500 nm, which matches the action spectrum of melanopsin. This wavelength is in the lower part of the light spectrum. The human lens becomes yellow with age and does not absorb as much short wavelength light as that of younger individuals. This has implications for entrainment.

For an animal to make the best use of its environment, and to avoid predators, its activity and physiologic functions need to be synchronized to the earth’s day-night cycle. Light transmitted to the brain resets the clock each day. The pathways are not those of the visual system that lets you see shape and movement, but a system that detects luminance. It is a non-image forming visual system.

About ten years ago scientists identified a group of retinal ganglion cells sensitive to melanopsin, a photopigment like those found in amphibians. Although they receive input from outer retinal photoreceptors, these cells function as photoreceptors as well as retinal ganglion cells. Their peak sensitivity is to light with a wavelength around 480 nm, which is also the peak frequency of absorption for melanopsin. Their axons form the retinohypothalamic tract (RHT), which projects to the SCN. It allows the SCN to adjust the timing of behavior, temperature, and hormone secretion to match the 24-hour light–dark cycle.

The data proving this comes from blind mice and men. Mice without rods and cones have a circadian rhythm of rest and activity. Exposure to 480 nm light shifts the activity, and the cycle, to a different time. Mice with rods and cones respond best to 500 nm light, suggesting that a photopigment of more than 500 nm affects entrainment, too.

In 1995, before melanopsin was discovered, Czeisler, Rizzo et al found that 3 of 11 NLP blind subjects did not free run. Their sleep-wake and temperature cycles were synchronized with the 24–hour clock. All 11 subjects were exposed to bright light (10,000 lux) an hour before the expected temperature minimum. The light suppressed melatonin secretion in the three subjects who were entrained, but not in the others. This showed that, in these people, light reached the SCN even though they did not perceive it. This was some of the first evidence of a non-image forming visual system.

SCN INPUT

The retinohypothalamic tract (RHT) also projects to the supraventricular zone (SPZ) which helps regulate the circadian timing of sleep and motor activity, and to the ventral lateral preoptic nucleus (VPLO), a key site for the regulator of sleep an arousal. Some melanopsin retinal ganglion cells (MRGCs) project to the intergeniculate leaflet (IGL) via the optic tract. The IGL is a secondary pathway for photic entrainment.
Melanopsin retinal ganglion cells form the retinohypothalamic tract (RHT) which transmits light to the circadian pacemaker in the SCN. The tract is bilateral, with a little more than half projecting to the contralateral SCN. The RHT’s neurotransmitters are PACAP (pituitary adenylate cyclase activating peptide) and glutamate. The SCN also receives input from the median raphe nucleus, whose neurotransmitter is serotonin (5–HT) and from the intergeniculate leaflet (IGL) which uses neuropeptide Y (NPY) and gamma-aminobutyric acid (GABA) as transmitters. The median raphe is important for arousal and sleepiness. Input to the SCN from the IGL and raphe antagonizes that from the RHT.

Input (light) from the MRGCs and RHT causes phase shifts at night and prevents phase shifts during the day; whereas input from the IGL and raphe nuclei cause phase shifts during the day and inhibit phase shifts at night. Think about going to bed early the night after you have been up on call, or about getting up earlier when bright light floods into your bedroom at 6:00 on a summer morning. These mutually inhibitory systems maintain sleep at night and wakefulness during the day.

SCN OUTPUT
Like many hypothalamic nuclei the SCN exerts its effects directly, by neurotransmission, and indirectly, by influencing the timing of hormone secretion. The SCN affects the daily rhythms of melatonin secretion, cortisol secretion, sleeping and waking, and temperature.

Kalsbeek et al in 2006 showed that the SCN controls daily fluctuations in the autonomic nervous system’s output two ways—by direct neuronal input and indirectly through the sympathetic and parasympathetic nervous system. Melatonin secretion is a good example of an autonomic pathway. The paraventricular nucleus of the hypothalamus (PVN) gives tonic stimulus to the intermediolateral cells in the thoracic spinal cord, which connect to the superior cervical ganglion (SCG), which stimulates the pineal to secrete melatonin. (Figure 3) During the day, GABAergic neurons from the SCN mediate the effect of light and turn this pathway off, stopping melatonin secretion. At night, a small group of SCN neurons, expressing glutamate, stimulate melatonin production.

The SCN uses both direct and indirect pathways to set the daily rhythm of cortisol secretion. Vasopressin-containing SCN neurons project to the PVN/dorsomedian area of the hypothalamus (DMH). The DMH also expresses vasopressin, which inhibits the hypothalamic–pituitary–adrenal (HPA) axis early in the day. In the evening, it expresses a different, unknown neurotransmitter which stimulates the HPA axis. The SCN also affects the timing of cortisol secretion by activating the sympathetic nervous system through the PVN.

SLEEP-WAKE STATE
Information from the SCN helps maintain sleep or wakefulness, adjusting the brain’s state to the appropriate part of the circadian day. The SCN has two main types of outputs. It regulates the diurnal variations of hormone production and of the autonomic nervous system. It also synchronizes these metabolic processes with the sleep–wake system.

FIGURE 3: Pathway from the retina to the pineal
The two systems, working together, maintain wakefulness during the circadian day and sleep during the circadian night. Light is the key zeitgeber, or entrainer, that keeps the circadian and sleep–wake systems synchronized. There are three sleep states: REM sleep, nonREM sleep (including stage 2 sleep and slow wave sleep), and wakefulness. REM sleep relies completely on the circadian system. Slow wave sleep depends completely on the sleep–wake homeostat, and nonREM sleep and wakefulness depend on both. 16

Some may want to know the pathways by which the SCN uses light to entrain the sleep–wake mechanism to match the earth’s rotation. The output from the SCN projects to the dorsomedial nucleus of the hypothalamus (DMH). The DMH projects to the ventrolateral posterior (VPLO), tuberomammillary (TMN), and lateral nuclei of the hypothalamus as well as to the locus ceruleus and raphe nuclei in the brainstem. 5 These nuclei control sleep and wakefulness. If they work they prevent an animal from being half-awake and half in REM or nonREM sleep (Think narcolepsy when they don’t.). 17 There is some evidence that the sleep–wake system may affect the firing rate of SCN neurons. 5

What happens if an animal or person is not entrained to the day–night cycle? Think of working the night shift as an intern. Cognitive function, measured by calculation, recall, vigilance, and reaction time is worst around or just after the temperature minimum. 16 (Figure 5) This circadian effect is magnified by sleep deprivation. Sleep efficiency also suffers. The most efficient sleep happens when sleep starts at the end of the “wake–maintenance zone” before melatonin secretion. If it starts after the temperature minimum the person tends to wake up a few hours into the sleep episode. Other consequences will be discussed below.

Seasonal Affective disorder (SAD) is the most common mood disorder among women of childbearing age in the temperate and northern parts of North America during the fall and winter. Lewy 18, 19 argues that it is at least partly caused by misalignment between the circadian and sleep–wake cycles, measured by dim light melatonin onset (DLMO) and mid–sleep. DLMO is the time of day when serum melatonin rises above 10 picograms /ml. It is fixed by the circadian clock, and is linked to the temperature minimum. Mid–sleep, the midpoint of the nocturnal sleep episode, is set by the sleep–wake cycle, which is subject to behavioral and environmental factors. It is not the same as Tmin. Mid–sleep is a behavioral measurement of the sleep–wake cycle. Tmin, the temperature nadir, is a biologically determined marker of the circadian system. Ideally, the time between DLMO and midsleep, which Lewy calls the phase angle, is six hours. Patients with SAD have phase angles longer or shorter than six hours. Lewy et al showed that the severity of depression in these patients was proportional to the difference between their DLMO—mid–sleep time and 6 hours. In other words, the farther the phase angle was from 6 hours, the more depressed the patient. The effectiveness of treatment correlated with how closely it brought the phase angle to 6 hours—how well it aligned the circadian and sleep–wake cycles. (Figure 6)

The treatments used to realign the circadian clock with the sleep–wake cycle are bright light and melatonin 19. Light exposure in the morning shifts the circadian clock earlier, or causes a phase advance. Melatonin in the afternoon or early evening has the same effect. If a person whose DLMO is at 9 PM goes to bed at midnight and sleeps 8 hours, his mid–sleep is at 4 AM. His phase angle is 7 hours. Treating him with light at least 8 hours before DLMO, or melatonin less than 8 hours before DLMO, causes a phase advance.
Melatonin treatment can be difficult to administer. Using too high a dose or using it too late in the day can make some of it spill over into the hours after the temperature minimum, causing a phase delay instead of a phase advance, as predicted by the phase-response curve discussed earlier.

Most patients with SAD are phase delayed—they stay up late. In Lewy’s study a minority had a DLMO too close to mid-sleep. For instance, a person whose DLMO is at 8 PM and who goes to sleep at 9 PM has a phase angle of 5 hours—too short. He is phase advanced. The correct treatment for him would be bright light in the afternoon or evening, less than 8 hours before DLMO, or melatonin after waking in the morning, more than 8 hours before DLMO. This would enable him to stay awake later at night and sleep later in the morning.

Poor sleep quality is very common in patients with psychiatric disease. Part of this may be due to the circadian-sleep wake cycle mismatch. People who try to sleep at the wrong part of their sleep-wake cycle have frequent awakenings and non-restorative sleep. There are individual case reports or small series that suggest involvement of circadian-sleep cycle mismatch in bipolar affective disorder, attention deficit hyperactivity disorder, post-partum depression, and bulimia. Darkness stops rapid cycling in mania. Light therapy can exacerbate mania in patients with bipolar affective disorder. Lithium and valproate can increase the length of the circadian period. Haloperidol can disrupt circadian rhythms.

There have been a few studies which have shown that short wavelength light (around 460 nm) is more effective at phase shifting and melatonin suppression than full wavelength light. The studies are small but intriguing. One, with 11 subjects, showed that 460 nm light produced twice as much phase delay as 555 nm light at the same intensity. 555 nm is the sensitivity peak of the photopic system.

**JET LAG**

Misalignment between the circadian and sleep-wake systems can also cause jet lag. When you fly across several time zones your circadian clock is misaligned with the light-dark cycle when you first arrive at your destination. Jet lag is the name given to the symptoms of daytime sleepiness, nocturnal insomnia, irritability, and impaired thinking that prevail until the two clocks are resynchronized. To align the clocks a person flying east needs to phase advance and a person flying west needs to phase delay. The human circadian period is a little longer than 24 hours, so it is easier to adapt to the new time zone on westward than on eastward flights. There is a limit to how much the circadian clock can shift, though. In one study, the maximum phase shift was a phase advance of 1 hour/day, and a phase delay of 1 ½ hours/day.

Techniques for phase shifting use the phase-response curves for light and melatonin. Figure 7) Melatonin given in the afternoon several hours before DLMO causes a phase advance. Melatonin has little effect during the circadian night, while the body is producing it. Melatonin given in the morning, as intrinsic production declines and afterward, causes a phase delay. Exposure to bright light after DLMO and before the temperature minimum (Tmin) causes a phase delay. A smaller dose of melatonin (0.5 mg) works as well as a larger dose (3 mg), without causing as much sedation. It can be given closer to bedtime, and is less likely to have melatonin ‘spill over’ into the time past Tmin when exogenous melatonin causes an effect opposite to the one intended.

**FIGURE 6: Phase Angle Difference (PAD) between dim light melatonin onset (DLMO) and mid-sleep.**


**FIGURE 7: From Eastman & Burgess, Sleep Med Clin, 2009, with permission.*

Human Phase Response Curves To Bright Light and Melatonin
Exposure to bright light (approx 3500 lux) after Tmin, early in the morning, causes a phase advance. Exposure to light during the day has little effect on circadian phase. Light exposure after DLMO and before Tmin causes a phase delay.

Take the example of a traveler from San Francisco to Hong Kong. These cities have a 9-hour time difference. Going westward, his circadian clock can phase delay about 1½ hours each day. He will be most jet “lagged” the first few days, because his Tmin will occur during his waking period. He can be re-entrained to Hong Kong time in 5 days, but his symptoms will start to improve in 3 days, as soon as he adjusts enough that Tmin occurs during sleep. (Figure 8)

Returning eastward, entrainment is more difficult. The traveler can phase advance only an hour a day, so it takes him longer to adjust to his new time zone. His Tmin won’t enter the sleep period for 6 days, at best. Some people traveling many time zones eastward do not phase advance at all. They are exposed to enough light late in their circadian day that they phase delay instead. In this example, our traveler would have to phase delay thirteen hours instead of advancing nine hours. His Tmin would not reach this sleep period for a week. Until then, he would sleep and wake at inappropriate hours in his new time zone. Only after his Tmin occurs during sleep will he begin to feel better.

Jet lag can be minimized by adjusting one’s sleep-wake schedule to one’s destination time zone starting a few days before departure, by rescheduling sleep and meals, and exposing oneself to light and taking melatonin at appropriate times. These actions move Tmin into the sleep period. Jet lag symptoms start to improve as soon as this happens. Moving Tmin into the sleep period as soon as possible after arrival, and keeping it there throughout the trip, is the circadian goal in treating the circadian/light-dark mismatch that occurs with transmeridian travel.

**SHIFT WORK**

Sleep work causes a chronic misalignment of the circadian and sleep-wake systems. The night worker needs to be awake when his circadian system says that he should be asleep. It is estimated that up to 20% of night or rotating shift workers have shift work sleep disorder, one of the well-recognized circadian sleep disorders. This includes sleepiness during the night work shift, with attendant inefficient work and increased likelihood of errors and accidents, and insomnia during the daytime sleep period. Night and rotating shift workers also have an increased risk of high blood pressure, metabolic syndrome, gastric and duodenal ulcers, coronary artery disease, and an increased risk of frequency of miscarriages, premature births, and low birth weight.

The risk of this disorder among night workers increases with a preference for morning rather than evening activity, excessive weekend working, increasing age, and long commute times.

Alertness and cognitive performance vary a lot when the sleep pressure is high (when a person has been awake a long time). Night workers are often sleep-deprived. They sleep 10 hours less each week than day workers.

Cognitive performance also shows a circadian decline near the temperature minimum. Circadian and sleep-wake factors align to make performance best for day workers. Misaligned, they worsen the performance of those who must work at night. It is not surprising that night workers are inefficient, sleepy, and accident prone on the job.

The solution to his problem is re-entrainment, or adjusting the circadian clock of night workers to match the times they need to stay awake. This is harder than re-entrainment for jet lag because night workers need to be awake during the daytime on their days off. One solution is partial re-entrainment. Its goal is to move the Tmin (the time of maximum circadian sleepiness, 7 hours after dim light melatonin onset (DLMO) into the daytime sleep period. In a series of studies in Chicago subjects were exposed to four 15-minute pulses of bright light (4100 lux) between 12:45 and 4:00 AM. They wore blue-light-blocking glasses on the drive home at the end of the “shift” in the laboratory at 7:00 AM. They went to bed by 8:30 AM and slept in a darkened room until 3:30 PM. They exposed themselves to light during the afternoon as a “light brake” to prevent excessive phase delay. By the end of eight night shifts (with 2 days “off” in the middle when subjects slept 2:00 AM to noon) 8 of 9 subjects shifted their sleepiest circadian phase into their daytime sleep period. Only 4 of 10 control subjects did.

The phase shift of the experimental subjects was significantly different from that of the controls, measured by DLMOff (p<0.001). Subjects who shifted their circadian phase most effectively were more alert during the night shifts than those who did not shift.
Another study in Quebec City showed that night workers in a postal distribution center who wore blue-blocking sunglasses for 2 hours after T_min improved their daytime sleep quality and had some improvement in alertness. Twenty-eight subjects were evaluated. This is the only study I have found that use phase-shifting techniques in a workplace.

Light has been used to try to phase shift night “workers” in laboratories for at least 15 years. The later studies use slightly dimmer light (4100 lux vs. 5000 lux) and short pulses of light (four 15 minute pulses over 3 hours) instead of a six-hour exposure. The shorter pulses of light may be more practical for workplaces to use. They also use dark sunglasses instead of welders’ goggles to reduce the exposure to sunlight on the drive home. If the drive home occurs after T_min, light exposure causes phase advancement. It will prevent the desired phase delay to move the sleepest part of the circadian cycle into a daytime sleep period.

There are practical limits to these techniques. First, the sunglasses cannot be so dark that they make it dangerous to drive. Second, many night workers have daytime responsibilities such as child care. They cannot sleep in a dark room as soon as they get home.

AGING

Circadian photoreception changes with age. The pupil gradually gets smaller, reducing the amount of light that enters the eye. The lens pigmentation changes. Yellow chromophores build up. They increase absorption of short wavelength light, so less of it is transmitted to the retina. Transmission of light to the retina drops by about 10% per decade of life after age 15. This is most pronounced for short-wavelength light. (Figure 9) Because of this loss, it can take more light to entrain an older person than a young one. Although exposure to bright light (3500 lux) caused similar phase shifts in old and young patients in one study, another study showed that exposure to dim blue (456nm) light (12 lux) caused melatonin suppression in young women, but not in older women. One way to improve transmittal of blue light is with implanted lenses. Whether lenses that block blue light confer more benefit by improving circadian rhythms or cause more risk because of potential retinal damage is still controversial.

Sunlight is the most effective light for photic entrainment. It has abundant luminance, much of which is in the blue spectrum. But older people, who need more of it to entrain, get less of it. Young adults in industrialized societies often get between 202 and 120 minutes of exposure to 1000 lux or more during the day. (An overcast day is brighter than that.) Elderly adults get only 1/3 to 2/3 as much, and institutionalized elderly are exposed to sunlight for less than that. (Figure 10) This may contribute to the disrupted sleep, impaired cognitive function, and depression common among institutionalized elderly. One study of elderly residents in assisted care facilities in the Netherlands found that having 1000 lux in the day room from 9:00 AM to 6:00 PM caused as much improvement in cognition as acetylcholine reuptake inhibitors, improved depression, and reduced the functional limitations of the residents.

Older people usually have an earlier circadian phase than younger people. Their DLMO and T_min are earlier. The earlier phase may contribute to poor sleep maintenance and early morning insomnia. Circadian sleepiness, near T_min, keeps people asleep near the end of the night. If T_min is early, it is harder to stay asleep then. This is another example of misalignment between the circadian and sleep-wake systems. The same study that showed that older subjects phase delayed as well as younger ones in response to bright light found that the longer phase delays were associated with later wake times, or a longer interval between the midpoint of sleep and wake time.
ADVERSE EFFECTS OF TREATMENT WITH LIGHT

Light therapy, especially if used for weeks or months at a time, can cause harm. One study showed that 23 of 34 patients who received 10,000 lux 30 minutes a day for a week to treat seasonal affective disorder had at least one side effect, most commonly headaches, "eye or vision problems", nausea or vomiting, hypomania or agitation, dizziness, and anxiety. Many of these subside after a few days of treatment.


Although light can injure the eye by photooxidation. Molecules and compounds that absorb light are called chromophores. As light interacts with these molecules it increases their energy level, producing free radicals that damage nearby ocular tissues. Photoreceptors and the retinal pigment epithelium are particularly sensitive because of the high oxygen content of the outer retina.

Photosensitizing medicines including amiodarone, chloroquine, levofloxacin, ketoprofen, phenothiazenes, tricyclics, sulfasalazine, and tetracyclines can accelerate this damage.

SAFETY OF PHOTOTHERAPY

Ocular damage from light therapy is cumulative over a person’s lifetime. It depends on the intensity and duration of light exposure, the spectrum of light used, the distance from the light source, and filtering properties of the ocular media. The higher the energy of the light source, the more potential for damage. Shorter wavelength light carries more energy than light of longer wavelength. Ultraviolet light, at 215–400 nm, is the most powerful. Current light boxes filter most or all of this. Visible light is in the range of 400–700 nm. In this spectrum, 435–445 nm is felt to be the most hazardous wavelength. Infrared light (over 750nm) carries less energy, but can still cause ocular damage.

The cornea absorbs most UV light at wavelengths less than 300 nm. In children and young adults the lens absorbs the rest. After age 40 these protecting molecules are destroyed. The human lens turns yellow with age. This yellowing filters 400–500 nm light. The retina is protected from damage, but at the cost of less input to the melanopsin retinal ganglion cells and less circadian entrainment.

Light–induced oxidative damage may contribute to the development of age–related macular degeneration. Exposure to blue light has been shown to damage the retinas of rats and monkeys and has been postulated as a cause and accelerator of macular degeneration in humans. Its contribution is still controversial (D. Albert, personal communication).

What level of light therapy is safe? In the June 2009 issue of Sleep Medicine Clinics Lewy et al recommend 1 to 2 hours of exposure to 2500 to 10,000 lux. The Canadian Consensus Guidelines for the Understanding and Management of Seasonal Depression recommend a starting dose of 10,000 lux for 30 minutes a day with white, fluorescent light with UV light filtered out. In the United States the FDA has not approved (or disapproved) light therapy, so it has published no guidelines.
In summary, melanopsin retinal ganglion cells are the gatekeepers for the circadian biologic system in humans. This non–image forming visual system affects our ability to sleep, work, and travel. It sets the rhythm of many of our metabolic functions and entrains us to the cycle of day and night.

CME ANSWERS
1. Exposure to light in the morning causes a phase advance. Exposure to light in the evening causes a phase delay.
2. The circadian system is dissociated from the sleep–wake system in seasonal affective disorder and shift work sleep disorder. In jet lag, the traveler’s circadian system is misaligned with the light–dark cycle at his or her destination.
3. Aging is associated with yellowing of the lens and with a reduction of pupil size. These things reduce the amount of short wavelength light that reaches the retina, which interferes with entrainment. Elderly people are often exposed to less sunlight than younger ones, further limiting entrainment.

REFERENCES
PHOTOPHOBIA AND THE MELANOPSIN RETINAL GANGLION CELL: A CONNECTION?

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LEARNING OBJECTIVES
1. List common causes of photophobia.
2. Discuss how the melanopsin pathway may relate to photophobia.
3. Describe the anatomic connections of melanopsin pathway related to photophobia.

CME QUESTIONS
1. Name 3 common conditions that have associated photophobia.
2. List 3 properties of melanopsin cells.
3. Name 5 structures that the ipRGC project to.

KEY WORDS
1. Melanopsin
2. Photophobia
3. Migraine
4. Blepharospasm

BRIEF OVERVIEW OF PHOTOPHOBIA
Photophobia is a common symptom in many individuals. Some people have it chronically (blepharospasm, migraine) or episodically (migraine). For such a common symptom, so little is known about its causes and pathophysiology.

Known causes of photophobia are listed in table 1.

TABLE 1: Causes of photophobia

<table>
<thead>
<tr>
<th>Ocular causes: Causes that can usually be diagnosed by an ophthalmologist</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Anterior Segment:</strong></td>
</tr>
<tr>
<td>- Ocular inflammation: Iritis</td>
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<tr>
<td>- Conjunctivitis</td>
</tr>
<tr>
<td>- Corneal diseases</td>
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<tr>
<td>- Uveitis</td>
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<tr>
<td>- Blepharitis</td>
</tr>
<tr>
<td>- Dry eyes: the MOST Common cause of photophobia</td>
</tr>
<tr>
<td><strong>Posterior Segment:</strong></td>
</tr>
<tr>
<td>- Vitreal disease (vitreitis) and uveitis</td>
</tr>
<tr>
<td>- Retinal causes: Problems with cones, retinal diseases like: albinism, Achromatopsia, Retinitis pigmentosa</td>
</tr>
<tr>
<td><strong>Optic Nerve:</strong></td>
</tr>
<tr>
<td>- Optic Neuritis</td>
</tr>
<tr>
<td>- Papilledema</td>
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<tr>
<td><strong>Chiasmal:</strong></td>
</tr>
<tr>
<td>- Pituitary Tumors</td>
</tr>
<tr>
<td>- Hypophysitis (inflammation of your pituitary)</td>
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<tr>
<td><strong>Neurologic Conditions:</strong></td>
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<tr>
<td>- Migraine: the MOST common neurologic cause of photophobia</td>
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<tr>
<td>- Blepharospasm:</td>
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<tr>
<td>- Cyclic vomiting syndrome (a form of migraine where there is vomiting)</td>
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<tr>
<td>- Progressive Supranuclear Palsy</td>
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<tr>
<td>- Head injury: causes headache and sometimes photophobia</td>
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<tr>
<td>- Meningeal irritation:</td>
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<tr>
<td>- Meningitis (infection of the covering of the brain)</td>
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<tr>
<td>- Sub-arachnoid hemorrhage (bleeding into the brain)</td>
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<tr>
<td><strong>Psychiatric Conditions:</strong></td>
</tr>
<tr>
<td>- Known to cause light sensitivity</td>
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<tr>
<td>- Depression (the most common psychiatric cause of light sensitivity)</td>
</tr>
<tr>
<td>- Agoraphobia (fear of being in crowds)</td>
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<tr>
<td>- Anxiety disorder; panic disorder</td>
</tr>
<tr>
<td>- Hang-over headache</td>
</tr>
<tr>
<td><strong>Medications (we do not know why these drugs cause light sensitivity):</strong></td>
</tr>
<tr>
<td>- Barbiturates, Benzodiazepam, Chloroquine, Methylphenidate, Stimulants</td>
</tr>
<tr>
<td><strong>Other:</strong></td>
</tr>
<tr>
<td>- Neurasthenia or chronic fatigue</td>
</tr>
<tr>
<td>- Fibromyalgia</td>
</tr>
<tr>
<td>- Measles, Rabies, systemic infections</td>
</tr>
<tr>
<td>- Inflammatory Bowel Disease</td>
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</tbody>
</table>
There are clinical clues about its pathophysiology. First, everyone has a light threshold. Studies show that some individuals have lower thresholds than others. These individuals tend to have migraine or blepharospasm. Second, pain and light are connected. Pain decreases an individual’s light threshold and light stimulation in subjects with migraine can decrease pain thresholds compared with normal controls. In one study individuals endured progressive light stimulation until discomfort was reported and then each individual underwent algometric procedures (progressive pressure algometer, a device to measure pain sensitivity) over predetermined three trigeminal sites, occipital nerves, and temporal muscles. Algometry readings occurred before light stimulation, directly after and delayed readings. All migraine subjects had reduced thresholds of light sensitivity (not unexpected), but unexpectedly they also had significant and sustained (beyond the second testing) lowering of pressure and pain sensitivity in both trigeminal and cervical sites. Controls did not exhibit this same phenomenon. So, some individuals exposed to light have a longer lower pain threshold than others.

Photophobia seems to be a “net sum”—that is, that viewing with two eyes creates more discomfort than viewing with one eye. The color of the light matters: blue light seems to give most uncomfortable light sensation. Blue light also enhanced EEG beta activity.

Light stimulation causes the blink reflex. The blink reflex is present in humans and the latency tends to shorten with age. The exact afferent pathway for reflexive blinking to a light source is unknown. Some researchers report that there is a blink reflex in monkeys who have had bilateral striate cortex removal, and multiple reports of continued light blink reflex when cortical blindness has occurred. One case report of a man who suffered a cardiac arrest with intact blink to light reflex despite necrosis of cerebrum, basal ganglia, hypothalamus, several brainstem nuclei and superior colliculus suggests that the afferent pathway may involve the pretectum not the superior colliculi. In fact, Itoh and Takada reported such a pathway (a pretectal–facial motor nucleus pathway) in cats. Others have shown that in monkey lesion studies, destruction of the superior colliculus does not stop a light reflex blink pathway, but a lesion in the pretectal nucleus does. Other conditions that affect the midbrain preferentially have been shown to cause photophobia. Photophobia is present in Progressive Supranuclear Palsy as compared to Cortical Basilar degeneration and Parkinson’s disease. Given the above information, it is likely that the midbrain region and pretectal region is important for photophobia and the blink reflex.

DO YOU NEED FORMED VISION TO HAVE LIGHT SENSITIVITY?

The answer is no. Lebensohn reviewed what was known about photophobia and reported that Siegwart reported in 1920 that of 46 blind patients (phthisis, atrophy, congenital amaurosis) 3 had light sensitivity. They proposed that the patients with light sensitivity had to have some vision. They also proposed that patients are only light sensitive to the visible spectrum and not infrared light. Lebensohn concluded from these early studies that for photophobia to be present there must be an intact optic nerve and trigeminal nerve. More recently, we reported findings in 20 migraine patients with legal blindness (6 with no light perception and 14 with at least light perception). Of the 6 with no light perception, none had light sensitivity during or with migraine. Of the 14 with variable amounts of formed vision, 6 had light sensitivity in between attacks and all had severe light sensitivity with attacks. Ziadi et al reported a woman without rods and cones who was no light perception, yet she could detect “brightness” when a blue light was shined in her eyes. He reported light sensitivity in patients without a functioning outer retina. Others have reported light sensitivity in blind painful eyes. Some have found that patients with damaged optic nerve still complain of light sensitivity. We reported a case of a woman with bare light perception, poor pupillary light reflex and severe light sensitivity. Clearly formed vision is not necessary to have photophobia. Whether one needs any light perception to experience photophobia is not completely clear, but there are cases such as those reported above who clearly had photophobia with even no light perception.

MELANOP SIN AND PHOTOPH OBIA

This leads us to consider how light might cause photophobia without formed vision. The recently discovered melanopsin pathway may be important. Melanopsin cells are within the retina and are intrinsically reactive to light. The wave length that these cells respond to best is a blue spectrum light. While it takes a lot of light to get these cells to fire, once they fire, they are difficult to disengage. A single photon can stimulate these cells, and once stimulated can continue to fire for a long period of time in response to very little light. Furthermore, researchers have found that melanopsin projects to the suprachiasmatic nucleus, intergeniculate leaflet of the thalamus, the sympathetic pathway, and pretectal olivary nucleus. Interestingly, the pretectal olivary nucleus also projects to the facial nucleus involved with blink. Melanopsin ganglion cells may be more resistant to damage by things like ocular hypertension—therefore this pathway may remain when the formed visual pathway is destroyed.
WHY IS THE MELANOPSIN PATHWAY A CANDIDATE TO PARTICIPATE IN PHOTOPHOBIA?

Intrinsically light sensitive neurons which contain melanopsin are an attractive contributor to our understanding of photophobia. First, ipRGC are intrinsically light sensitive, without the need for rod or cone input. Hattar in 2002 reported that melanopsin cells signaled light in the retino–hypothalamic track and that most of its projections were to the suprachiasmatic nucleus but there were also projections to IGL, OPN, VLP. Lucas found that even their dendrites were photosensitive. Berson in 2002 also showed that even if you get rid of rod and cone input, these cells still signal the suprachiasmatic nucleus. In patients with no light perception, light behavior continues and the circadian pathway persists.

In mice with rod–cone degenerations, the ipRGC are greater in number than the mice without rod–cone degenerations.

Could this fact suggest that in rod–cone degenerations, there are more ipRGC and these could contribute to the associated light sensitivity seen in these individuals? This pathway persists in rodless, coneless individuals and continues the circadian rhythm despite no perceived light through traditional rod/cone pathways. In patients with rod and cone dystrophies where the function of either rods, cones or both are abnormal, light sensitivity or photophobia is a major feature. Furthermore, this could explain photophobia even in the face of blindness. This fact helps us understand why patients who are blind may retain light sensitivity.

This ipRGC pathway may be affected by shorter seasons of less light and may explain some of the seasonal affective disorder and light may be a potent treatment.

Photophobia is reported to be increased in depressed individuals.

Furthermore, the wave length of melanopsin is 484 nm—in the blue spectrum—the same wave length that is least comfortable to humans. Vandewalle et al used functional MR testing and exposed 15 normal subjects to blue, violet and green light. The blue light had activated the thalamus, hippocampus, amygdala and bilateral brainstem. These authors propose that melanopsin pathways (responsive to the blue light) exert an important effect on light processing in the brain and that it is modulated by brainstem and thalamic structures. The effect on the amygdala and limbic system may be important in our understanding of depression and emotional processing of light. Blue light has been known to activate the brain by increasing alpha activity on EEG.

Blue light increasing alpha waves is known to increase levels of alertness as well.

While it may be contradictory to the previous discussion of melanopsin and depression, blue light stimulation seemed in one study to promote less depression in rats by the swim test. In this test a burst of blue light brought a shorter duration of immobility than white light or red light. They interpreted this finding as showing an anti–depressant affect.

Once melanopsin cells are excited—and it takes a while to be excited—they are hard to suppress. This seems to go along with the clinical phenomenon that an individual with light sensitivity has discomfort and it is hard to inhibit the sensation.

Melanopsin expression is increased after exposure to darkness and can be suppressed after prolonged exposure to light. This trait of melanopsin is similar to patients with photophobia who claim after being in the darkness that feels more comfortable, coming into the light greatly enhances their sense of discomfort with light.

The ipRGC also seems more impervious to damage than the RGC—in fact, studies have shown that these axons are more impervious to ocular hypertension. Could this explain why light sensitivity would persist in almost blind individuals from optic nerve/chiasmal disease and accompanying photophobia?

Gooley et al found that the melanopsin system had its own pattern of innervation and didn’t always project to the formed visual pathway. The RGC projected to suprachiasmatic nucleus, ventral subparaventricular zone, ventral lateral pre–optic nucleus, pretectal area and the intergeniculate leaflet of the lateral geniculate nucleus. Interestingly parts that are mainly formed visual pathway—the dorsal lateral geniculate and the superior colliculus did not receive input from the melanopsin pathway.

The melanopsin cells project to some of the same areas that have been implicated in our understanding of photophobia: suprachiasmatic nucleus, paraventricular nucleus, inter–geniculate leaflet, olivary pretectal nucleus, with links to the superior cervical ganglion, and interomedia lateral nucleus.

CAN WE EXPLAIN PAIN AND MELANOPSIN?

Does the melanopsin pathway connect to the trigeminal system and pain system? Yes. Burstein and colleagues have mapped projections of ipRGC looking for trigeminal innervated structures linking these ipRGC to the trigeminal system in rats. They found that these cells projected contraterally between the lateral posterior and anterior pretectal nuclei to the dorsal caudal region of the posterior thalamic nuclear group. Labeling of all retinal ganglion cells (rods and cones as well as melanopsin) was in the same area. This is then a novel retino–thalamic pathway. He then used extracellular single unit recording devices and was able to show that in the areas of trigeminal input in the posterior thalamus light shined into the contralateral eye caused firing—indicating that light connected in areas where trigeminal connections were. This then could explain pain associated with melanopsin firing and photophobia especially in migraine.
**IS THERE A TREATMENT FOR PHOTOPHOBIA?**

Can knowing about the melanopsin pathway help us with treatment? Treating the underlying condition is probably the best treatment of photophobia. Treatment of migraine, depression, and co-morbid anxiety may be helpful here.

Calcium influx for the ipRGC is blocked by L-type voltage gate calcium channel blockers like verapamil and diltiazam. These are also implicated in improving migraine. Could calcium channel blockers be more helpful in treatment of photophobia associated with migraine because of its affect on melanopsin?

Tinted lenses have been shown to help with photophobia as well. Red contact lenses have been shown to be a helpful treatment in cone dystrophies. FL-41 tint was originally reported by Good et al to improve migraine in children. We showed that blue-blocking lenses, FL-41 tint, may be helpful in decreasing disability and improving quality of life in patients with photophobia who have blepharospasm and reduced blinking in patients with blepharospasm. Clearly, more work needs to be done to show that these lenses are participating in blocking melanopsin or ipRGC stimulation.

While it is likely that there are other causes of photophobia—such as rod and cone input into thalamic structures, enhancement of photophobia with sympathetic stimulation, or up-regulation of Calcitonin gene-related peptide released by trigeminal nerve endings—melanopsin is an attractive candidate to explain some of the features of photophobia.

**CME ANSWERS**

1. Migraine, Blepharospasm, Dry Eyes Depression, Meningitis, Progressive Supranuclear Palsy (PSP).

2. Most sensitive to blue light spectrum; take a long time to respond, but once they respond, continue to fire even after the stimulus is gone; somewhat more resistant to compression and damage.

3. Melanopsin projects to the suprachiasmatic nucleus, intergeniculate leaflet of the thalamus, the sympathetic pathway, and pretectal olivary nucleus.

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