Melanopsin, Ganglion-Cell Photoreceptors, and Mammalian Photoentrainment

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Abstract An understanding of the retinal mechanisms in mammalian photoentrainment will greatly facilitate optimization of the wavelength, intensity, and duration of phototherapeutic treatments designed to phase shift endogenous biological rhythms. A small population of widely dispersed retinal ganglion cells projecting to the suprachiasmatic nucleus in the hypothalamus is the source of the critical photic input. Recent evidence has shown that many of these ganglion cells are directly photosensitive and serve as photoreceptors. Melanopsin, a presumptive photopigment, is an essential component in the phototransduction cascade within these intrinsically photosensitive ganglion cells and plays an important role in the retinal photoentrainment pathway. This review summarizes recent findings related to melanopsin and melanopsin ganglion cells and lists other retinal proteins that might serve as photopigments in the mammalian photoentrainment input pathway.

Key words: photoentrainment, melanopsin, ganglion cell, retina, photopigment, circadian rhythm

Light depends on ocular photoreceptors to entrain circadian activity rhythms in mammals (Yamazaki et al., 2002). The ocular mechanisms activated by light to entrain circadian rhythms, however, are poorly understood. It is known that the ocular output arises from a small population of ganglion cells that project via the retinohypothalamic tract to the suprachiasmatic nucleus (SCN) (Moore et al., 1995; Abrahamson and Moore, 2001; Pickard et al., 2002). These ganglion cells are unique in that they contain pituitary adenylate cyclase-activating polypeptide (Hannibal et al., 1997; Hannibal, 2002). Most of the SCN projecting ganglion cells also contain melanopsin (Gooley et al., 2001; Hannibal et al., 2002a; Hattar et al., 2002) and are intrinsically photoreceptive, responding to light independent of the rod and cone photoreceptors used for vision (Berson et al., 2002). Current evidence demonstrates that melanopsin plays a critical role in the photoactivation of these intrinsically photosensitive ganglion cells (Lucas et al., 2003; Panda et al., 2002; Ruby et al., 2002).

MELANOPSIN

Melanopsin's name reflects its discovery in dermal melanophores of *Xenopus laevis*. Melanosomes, the melanin-containing organelles of amphibian

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melanophores, are dispersed throughout the cytoplasm when the cells are exposed to light (Daniolos et al., 1990). In darkness, the melanosomes are aggregated around the nucleus. This photodispersion response is dependent on exogenous retinaldehyde (Rollag, 1996). Melanopsin was discovered when an amphibian melanophore cDNA library was screened for opsins, a class of proteins whose presence was suggested by the retinaldehyde requirement for melanosome photodispersion (Provencio et al., 1998).

Melanopsin is expressed in all vertebrate classes examined to date, ranging from fish (Bellingham et al., 2002) to mammals (Provencio et al., 2000; Hattar et al., 2002; Hannibal et al., 2002b). The pattern of melanopsin expression differs among the vertebrate classes. Nevertheless, its presence in scattered retinal ganglion cells is constant. Indeed, these are the only cells known to express melanopsin in mammals (Provencio et al., 2000).

Melanopsin, like other opsins, has the lysine residue in the 7th transmembrane domain needed for formation of a Schiff's base with 11-*cis*-retinaldehyde (Ebrey and Koutalos, 2001; Yeagle et al., 2001). If melanopsin functions as an opsin photopigment, light will photoisomerize 11-*cis*-retinaldehyde to an all-*trans* conformation (Burns and Baylor, 2001), triggering activation of a G-protein signal cascade. The sensitivity of opsin photopigments to light is determined in part by the phosphorylation state of the opsin cytoplasmic tail (Mendez et al., 2000). It is not yet known if melanopsin is posttranslationally modified, but its long cytoplasmic tail with numerous consensus phosphorylation sites provides a rich substrate for dynamic regulation (Provencio et al., 1998).

Surprisingly, melanopsin segregates with the invertebrate opsins upon cladistic analysis of nucleotide sequences (Provencio et al., 1998; Bellingham and Foster, 2002). This invertebrate-like molecular structure is best demonstrated by the aromatic tyrosine residue at the site of the retinaldehyde Schiff's base counterion. In contrast, most vertebrate opsins employ an acidic residue as the counterion, usually glutamate (Ebrey and Koutalos, 2001). An important attribute of invertebrate opsins is that they do not rely on exogenous tissues to reisomerize the spent retinaldehyde (Gartner and Towner, 1995). Instead, all-*trans*-retinaldehyde is photoisomerized within the opsin molecule to the active *cis* isomer (Vought et al., 2000). It is not known if such retinaldehyde recycling occurs within the binding pocket of the melanopsin molecule; this possibility needs to be tested.

MELANOPSIN GANGLION CELLS

In mammals, the melanopsin-containing ganglion cells project to the SCN (Gooley et al., 2001; Hannibal et al., 2002a; Hattar et al., 2002) as well as other diencephalic and midbrain regions, including the olivary pretectal nucleus (Hattar et al., 2002). Melanopsin-containing retinal ganglion cells respond to light even when isolated pharmacologically or physically from other retinal neurons (Fig. 1) (Berson et al., 2002; Dacey et al., 2003; Hattar et al., 2002; Lucas et al., 2003). The response of these cells differs from that in rod and cone photoreceptors in several important respects. First, the light response is depolarizing in melanopsin ganglion cells, whereas it is hyperpolarizing in rods and cones. Second, while the action spectrum for photostimulation of isolated melanopsin ganglion cells has the form predicted for an opsin-mediated response, the wavelength of peak sensitivity (approximately 484 nm; Berson et al., 2002; Smith et al., 2003) clearly distinguishes it from that of rodent rods and cones. Moreover, the ganglion cell response is remarkably sustained during continuous light exposure; rods and cones, on the other hand, exhibit marked adaptation under these conditions. There are important quantitative differences when the response of intrinsically photosensitive ganglion cells is compared to that of rod and cone photoreceptors. In particular, the melanopsin ganglion cells have higher photoactivation thresholds than rods and cones, and they are surprisingly sluggish, with response latencies of up to 1 min before light-induced depolarization becomes evident (Fig. 1). One must be careful, however, not to overinterpret the quantitative attributes of the ex vivo response of the intrinsically photosensitive ganglion cells. Ganglion cell sensitivity and response latency is likely influenced by both the temperature of the experimental preparation (room temperature was used in the Berson et al., 2002, study) and the duration of the stimulus (≤ 1 min for threshold tests in the Berson et al., 2002, study).

Cells expressing melanopsin are widely distributed throughout the ganglion cell layer of the mammalian retina, with a few melanopsin positive cells located in the inner nuclear layer (Fig. 2) (Hannibal et al., 2002a,

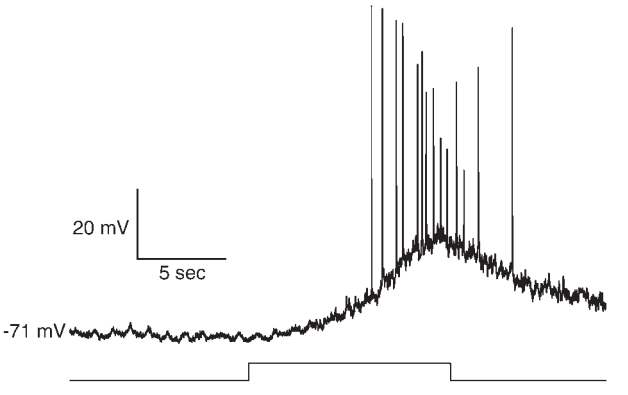

Figure 1. Light-evoked depolarization of a synaptically isolated, intrinsically photosensitive ganglion cell of the rat retina, whole-cell current-clamp recording. Note the long latency for the response to light stimulus (indicated by step below voltage trace) and the fast action potentials riding on the depolarization. This ganglion cell was labeled by retrograde transport of a fluorescent tracer from the suprachiasmatic nucleus of the hypothalamus. The retina was isolated from the retinal pigment epithelium (severely compromising rod and cone function) and bathed in a solution of agents blocking synaptic transmission to the ganglion cells from remaining retinal neurons: 2 mM $CoCl_2$ to block Ca^{2+}-mediated transmitter release; 100 μM L(+)-2-amino-4-phosphonobutyric acid (APB) to block glutamate transmission between rods and cones and ON bipolar cells; 20 mM 6,7-dinitroquinoxaline-2,3-dione (DNQX) and 50 μM DL-2-amino-5-phosphonovaleric acid (APV) to block ionotropic glutamate transmission between photoreceptors and OFF bipolar cells and between all bipolar cells and both amacrine and ganglion cells; and 50 μM picrotoxin (PTX) and 0.3 mM strychnine to block ionotropic gamma-aminobutyric acid (GABA) and glycine receptors mediating most inhibition by amacrine cells (M. Takao, F. A. Dunn, D. M. Berson, unpublished data).

2002b; Hattar et al., 2002; Provencio et al., 1998, 2002). The melanopsin ganglion cells have a relatively sparse dendritic tree that is characterized by numerous varicosities (Fig. 2) and that arborizes near the outer margin of the inner plexiform layer in all mammalian species studied to date (Berson et al., 2002; Hannibal et al., 2002a; Hattar et al., 2002; Peterson et al., 2003; Provencio et al., 2002). In mice and monkeys, there is an additional dendritic plexus near the inner margin of the inner plexiform layer that may or may not arise from a second, physiologically distinct population of melanopsin ganglion cells (Provencio et al., 2002; Peterson et al., 2003). The dendrites of melanopsin ganglion cells are photosensitive (Berson et al., 2002) and overlap to form a photoreceptive net within the inner retina (Fig. 2) (Provencio et al., 2002). Electrophysiological and ultrastructural evidence in primates and rodents indicates that melanopsin ganglion cells receive both excitatory bipolar and inhibitory amacrine cell synaptic inputs (Belenky et al., 2003; Dacey et al., 2003; Dunn and Berson, 2002).

The transduction mechanism linking activation of the photopigment in melanopsin-containing ganglion cells to their depolarization is not known. As a vertebrate opsin with invertebrate features, one could expect it to be coupled to either a vertebrate-like or invertebrate-like transduction cascade. These differ substantially from one another. Vertebrate photoreceptors (including an unusual depolarizing variety) use modulation of phosphodiesterase activity to increase or decrease intracellular cGMP and open or close cyclic nucleotide-gated ion channels in the plasma membrane (e.g., Xiong et al., 1998). Invertebrate photoreceptors, on the other hand, operate through a phosphoinositide cascade regulating intracellular calcium and plasma membrane ion channels of the TRP family (e.g., Hardie, 2001).

PHOTOENTRAINMENT DEFICITS IN MELANOPSIN-KNOCKOUT MICE

There is strong evidence that melanopsin is an essential component in the phototransduction pathway found in intrinsically photosensitive ganglion cells, but whether it is a photopigment or photoisomerase or fulfills some other function remains to be determined. In particular, melanopsin is selectively expressed in intrinsically photosensitive ganglion cells (Hattar et al., 2002). The intrinsic photosensitivity of these cells is abolished in melanopsin-knockout mice (Lucas et al., 2003). And, at least in amphibian melanophores, overexpression of melanopsin augments the photic response (Rollag et al., 2000).

Direct evidence that melanopsin plays a role in photoentrainment comes from 2 independent studies using melanopsin-knockout mice (Panda et al., 2002; Ruby et al., 2002). Bright light phase shifts the activity rhythm in melanopsin-knockout mice but not as much as in mice expressing melanopsin (Fig. 3A). At low light intensities, the impairment in phase shifting in melanopsin-knockout mice is even more pronounced. Furthermore, the lengthening of the circadian period typically observed when rodents are housed in constant light is attenuated in melanopsin-knockout mice relative to wild-type sibling controls (Fig. 3B). It should be noted that melanopsin is not involved in cir-

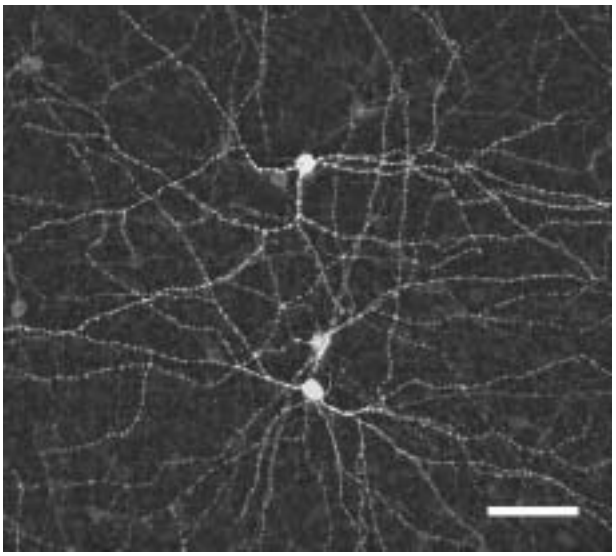

Figure 2. Melanopsin ganglion cells form a photoreceptive net in the human retina. The melanopsin-stained ganglion cells in this human retina flatmount display an extended dendritic tree characterized by numerous varicosities. The retina was immunohistochemically stained with antibody UF028 raised against the 15 N-terminal amino acids of the human melanopsin protein. Eye bank tissue had been formalin fixed prior to shipment. Scale: 100 um (A. M. Castrucci, I. Provencio, M. D. Rollag, unpublished data).

cadian clock function per se since the knockout mice do not exhibit any overt defect in circadian activity rhythms under constant darkness.

ADDITIONAL CANDIDATES FOR OCULAR CIRCADIAN PHOTOPIGMENTS

Some photopigment other than melanopsin must be invoked to explain why melanopsin-knockout mice retain a residual capacity to phase shift in response to light (Panda et al., 2002; Ruby et al., 2002), as well as to photic induction of *c-fos* expression in the SCN (Ruby et al., 2002). Electrophysiological recordings indicate that the photic responses of SCN neurons are driven in part by rod and cone input (Aggelopoulos and Meissl, 2000). Indeed, the SCN neurons respond to rod and cone input when the light intensity is insufficient to cause phase shifts. This input could be relayed through melanopsin ganglion cells, which, as noted above, receive synaptic inputs from rod and cone networks. Alternatively, the rod and cone input could be relayed through ganglion cells that lack melanopsin (Gooley et al., 2001) and that excite SCN cells either directly or through polysynaptic circuits in the brain. The strongest argument against a central role of rods and cones in photoentrainment is derived from studies showing that rodless/coneless mice have normal photic thresholds for circadian phase shifts (e.g., Freedman et al., 1999). It is possible, however, that the chronic loss of rods and cones in these experimental animals is compensated by enhancement of melanopsin ganglion cell response or the response of some other inner retinal photoreceptor. If such developmental compensation occurs in rodless/coneless mice, then the importance of rod and cone output for photoentrainment of mammalian circadian rhythms would need to be reexamined.

Cryptochromes are also popular candidate circadian photopigments (Sancar, 2000; Selby et al., 2000; Van Gelder, 2002). Cryptochromes are employed as photopigments by *Drosophila* to mediate the direct effect of light on the clockwork in the circadian pacemaker (Ceriani et al., 1999; Hall, 2000). Importantly, cryptochromes are expressed in the mammalian inner retina (Miyamoto and Sancar, 1998), though in far too many cells to be restricted to the photosensitive ganglion cells. The possible involvement of cryptochromes as photopigments in the mammalian photoentrainment pathway is supported by the finding that the SCN remains responsive to light in retinaldehyde-depleted mice (Thompson et al., 2001). Cryptochromes, as flavoproteins, do not depend on retinaldehyde as a chromophore and represent the best candidate photopigment when opsins are excluded. Efforts to prove cryptochrome involvement in ocular photoentrainment pathways have been confounded by the critical role that cryptochromes play within SCN neurons to generate the circadian oscillation (Griffin et al., 1999; Okamura et al., 1999; Vitaterna et al., 1999). Because cryptochromes are required for circadian clock function, mice with the 2 known mammalian cryptochrome genes knocked-out lack a self-sustaining circadian rhythm in constant dark. Hence, genetic deletion experiments are unable to reveal at a behavioral level whether cryptochromes play a role in photoentrainment in addition to their involvement in clock oscillatory mechanisms. Such cryptochrome knockout mice do, however, have reduced photic responsiveness in the SCN as judged from *c-fos* expression (Selby et al., 2000), although it is uncertain if the basis for this defect lies in the retina or in the hypothalamus. This mouse model has also been used to evaluate the involvement of these flavoproteins in the control of the pupillary constriction response remaining in *rd/rd* mice (Lucas et al., 2001), a response likely regulated by intrinsically photosensitive gan-

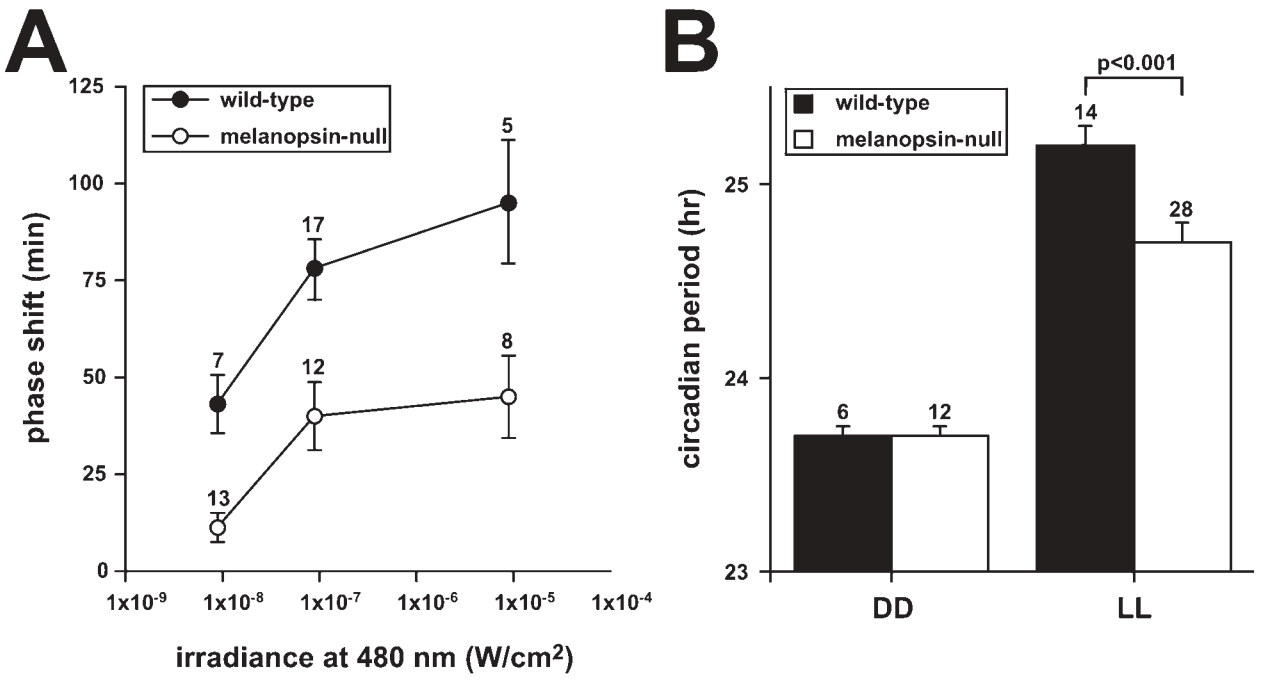

Figure 3. Attenuated circadian light input in melanopsin-null mice. (A) A single 15-min pulse of monochromatic light (480 nm; 10 nm half-peak bandwidth) was administered at circadian time 15. Melanopsin-null mice responded with smaller phase shifts in the onsets of the circadian locomotor rhythms than did wild-type littermate controls, at all irradiances tested. (B) An attenuated lengthening of circadian period was observed in melanopsin-null mice (black) relative to wild-type littermate controls when housed in constant light. Differences in circadian period were not observed in constant darkness. Mean (±SEM) of phase shift are shown; number of animals are indicated above each data point. Data were analyzed by *t*-test (two tailed, equal variance), and a statistically significant difference ($p < 0.05$) was observed between the genotypes at all irradiances tested (redrawn from Panda et al., 2002).

glion cells projecting to the pretectum (Hattar et al., 2002). Pupillary responses of *rd/rd* mice with both cryptochrome genes knocked out are markedly less sensitive to blue light than those of *rd/rd* controls (Van Gelder et al., 2003). These findings indicate that cryptochromes may play a role in mammalian non-visual irradiance detection.

There are various novel vertebrate opsins that might serve as candidate circadian photopigments including pinopsin (Okano et al., 1994; Max et al., 1998), vertebrate ancient opsin (Soni et al., 1998; Kojima et al., 2000), parapinopsin (Blackshaw and Snyder, 1997), ERrod-like opsin (Mano et al., 1999; Philp et al., 2000), and teleost multiple tissue opsin (Moutsaki et al., 2003). These opsins have not been demonstrated to be expressed in mammals, and a search of the available mammalian genomic sequences reveals no corresponding orthologues (I. Provencio I, M. D. Rollag, unpublished data). Consequently, their involvement in ocular mechanisms of mammalian photoentrainment remain speculative. Additional opsins known to be found in mammals have not yet been championed as circadian photopigments, largely because they have not been shown to be expressed by retinal neurons that could serve as photoreceptors. These include RGR (Jiang et al., 1993; Chen et al., 2001), peropsin (Sun et al., 1997), and encephalopsin (Blackshaw and Snyder, 1999).

SUMMARY

One of the emerging themes regarding mechanisms in mammalian photoentrainment is that multiple ocular photopigments may be involved. The mechanisms within the eye and SCN that integrate the different photopigment responses need to be identified before a full understanding of the dynamic regulation of the sensitivity of the input pathway to light can be obtained. We need to know how the intrinsic light response of melanopsin ganglion cells is modulated by bipolar and amacrine cell inputs, the identity of 2nd messenger pathways and ion channels that

lead to depolarization of melanopsin ganglion cells, and how the retinaldehyde chromophore is regenerated in ganglion cells after photoisomerization. It is also important to know whether circadian rhythms intrinsic to the retina affect the melanopsin ganglion cells and, conversely, whether these ganglion cells affect the retinal clock. Since some ganglion cells that project to the SCN do not contain melanopsin whereas others do, it will be of interest to learn whether their functionally distinct output signals target different populations of SCN neurons at the retinal ganglion cell level needs to be considered.

It is anticipated that a thorough understanding of the photoentrainment pathway will facilitate the development of phototherapeutic regimens to optimize performance and minimize stress in shift workers and jet travelers who routinely require phase adjustments of their circadian system. It is also expected that an understanding of the relationships between ocular mechanisms underlying vision and photoentrainment will lead to a better understanding of the morbidity experienced by people suffering from common retinopathies, including, but not limited to, retinitis pigmentosa, age-related macular degeneration, diabetes, and glaucoma. The quality of life for many blind people could be substantially improved if circadian disruption of sleep rhythms could be easily avoided. The phototherapeutic parameters that can be optimized once we obtain a thorough understanding of basic ocular mechanisms in photoentrainment include the wavelength, intensity, and duration of light therapy.

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