Journal Club

Editor's Note: These short, critical reviews of recent papers in the *Journal*, written exclusively by graduate students or postdoctoral fellows, are intended to summarize the important findings of the paper and provide additional insight and commentary. For more information on the format and purpose of the Journal Club, please see http://www.jneurosci.org/misc/ifa_features.shtml.

New Clues Suggest Distinct Functional Roles for M1 and M2 Intrinsically Photosensitive Retinal Ganglion Cells

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Review of Pires et al.

Organisms adapt to the external world by anticipating environmental changes associated with a 24 h day. Light is the main synchronizer of mammalian circadian clocks. However, the photoreceptor types involved in the process remained the subject of debate until recently. Although rods and cones had been considered the only photoreceptors of the mammalian retina, evidence accumulated for the existence of a third type of photoreceptor involved in nonvisual photic responses. Surprisingly, human studies revealed that blind patients could retain light responses, such as the suppression of melatonin expression by bright light (Czeisler et al., 1995). In addition, mice lacking rods and cones showed normal light-induced phase shifting of circadian locomotor activity. Such light-mediated responses were abolished by eye removal, indicating the presence of a novel ocular photoreceptor responsible for circadian photoreception. Moreover, the spectral sensitivity for phase shifting of circadian locomotor activity in a retinally degenerate mouse model was predicted to have a peak at 480 nm, which does not correspond to the spectral sensitivity of the known murine rod or cone photopigments (for review, see Provencio, 2008).

Pioneering work by Berson and colleagues revealed that a small subset of retinal ganglion cells projecting to the suprachiasmatic nucleus (SCN), the master circadian clock in mammals, was intrinsically photosensitive. Action spectra of these intrinsically photosensitive retinal ganglion cells (ipRGCs) indicated a peak spectral sensitivity at 484 nm, matching the expected spectral sensitivity of the nonrod, noncone circadian photoresponses mentioned above (for review, see Provencio, 2008). At least two morphologically distinct subtypes of ipRGCs, M1 and M2, have been described. Dendrites of M1 ipRGCs ramify in the OFF layer of the inner plexiform layer (IPL), whereas the M2 arbors occupy the ON layer of the IPL. M1 cells appear more responsive to light (Schmidt and Kofuji, 2009). Questions remain regarding the origin and functional importance of these differences.

Meanwhile, ipRGCs have been shown to express melanopsin, a novel opsin that was discovered in the dermal melanophores of *Xenopus laevis*. Introduction of melanopsin into non-photosensitive cells *in vitro* transformed these cells into photoreceptors, with a peak spectral sensitivity at 479 nm, further fulfilling the criteria for a circadian photopigment, and corresponding to the spectral sensitivity of ipRGCs (for review, see Provencio, 2008). Although melanopsin knock-out mice could synchronize activity rhythms with the prevailing light/dark cycle, their capacity to phase-shift activity in response to discrete light pulses was attenuated, consistent with a role for melanopsin in circadian photoentrainment. Subsequent work showed that mice lacking functional rods, cones, and melanopsin were completely nonresponsive to light, indistinguishable from bilaterally enucleated mice (for review, see Provencio, 2008). Furthermore, three additional studies have confirmed that selective ablation of melanopsin expressing cells abolishes all known nonvisual responses to light, even in the presence of rod and cones, indicating that melanopsin expressing cells not only function as photoreceptors but also serve as conduits of essentially all rod/cone information to nonvisual centers of the brain (Goz et al., 2008; Güler et al., 2008; Hatori et al., 2008).

Surprisingly, the mouse melanopsin (*mOpn4*) gene sequence has been reported to be significantly different than that of human melanopsin, whereas the rat sequence is more similar to human than to mouse. This led Pires et al. (2009) to investigate the sequence of *mOpn4* in more detail. In a recent issue of *The Journal of Neuroscience*, they reported the existence of two distinct isoforms from the mouse *Opn4* locus: a long isoform (*Opn4L*) and a novel short isoform (*Opn4S*), which are generated by alternate splicing of a single melanopsin gene in the mouse genome.

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Comparison of the published amino acid sequences of human, mouse, and rat melanopsins reveals that the mouse *Opn4* sequence has a longer C terminus than human or rat. Whereas both human and rat *Opn4* genes consist of 10 exons spanning 11.9 and 9.2 kb of genomic sequence, respectively, the mouse *Opn4* gene has 9 exons spanning only 7.8 kb. This discrepancy was explained by the fact that exon 9 in mouse is 321 bp, compared with 144 bp in human and 141 bp in rat. Using adult mouse retina cDNA as template, Pires et al. (2009) investigated the possibility that a region downstream of mouse exon 9 was expressed in the mouse and produced a short melanopsin isoform. Primers designed to amplify the complete coding sequences revealed 1566 and 1401 bp products, of which the first 1362 nt (exons 1–8) were identical. The predicted proteins were 521 and 466 aa long, respectively. Finally, the presence of both isoforms was verified in the adult mouse retina, generating 857 bp fragments from *Opn4L* and 930 bp fragments from *Opn4S*. *Opn4L* had more putative posttranslational modification sites in its longer C-terminal tail than *Opn4S*. Although quantitative PCR indicated that both isoforms had comparable amplification efficiency, levels of *Opn4S* transcript were 40 times higher than levels of *Opn4L* transcript.

Next, Pires et al. (2009) assessed the localization and function of the two isoforms. When expressed in a retinal ganglion cell line, both isoforms trafficked to the plasma membrane; whole-cell patch-clamp recordings showed that both isoforms encoded a functional retinaldehyde-dependent sensory photopigment. However, in the Neuro2A system, there was no significant difference in the amplitude, kinetics, or spectral sensitivity of light-evoked responses of *Opn4L* and *Opn4S* isoforms. Immunolabeling of wild-type retinas with isoform-specific antibodies revealed expression of both isoforms in a subset of mouse retinal ganglion cells. Some melanopsin-positive cells expressed both isoforms, and the processes of these cells were present in the OFF layer of the IPL (corresponding to M1 ipRGCs). Another set of cells expressed only Opn4L and had processes confined to the ON layer of the IPL (corresponding to M2 ipRGCs). Most of the cells that expressed only Opn4L (M2 ipRGCs) had relatively low levels of staining, and their processes were more difficult to visualize than those of Opn4S-positive cells, which is consistent with previous immunostaining results (Baver et al., 2008) and provides a potential explanation for sensitivity differences between M1 and M2 ipRGCs.

Collectively, the study by Pires et al. (2009) provides strong evidence indicating that the mouse *Opn4* gene indeed has two isoforms, *Opn4S* and *Opn4L*. Furthermore, both isoforms can form functional photopigments *in vitro*. However, one notable piece of data lacking in this paper is the spectral sensitivity of the Opn4S isoform. Spectral sensitivity is the identifying characteristic of a photopigment. Based on previous behavioral, *in vivo* and *in vitro* physiological studies, the spectral sensitivity peak of, respectively, phase shifting, ipRGCs and Opn4L were all ~480 nm. It will be of interest to see what the peak spectral sensitivity of Opn4S is. This piece of data would not only confirm that Opn4S is indeed a functional photopigment *in vivo* but would also provide additional clues about differing functional roles of Opn4S and Opn4L.

The fact that differential expression patterns of Opn4S and Opn4L were related to IPL ramification is intriguing. The IPL has ~10 strata, which represent 10 distinct functional stacks (Roska and Werblin, 2001). Strata near the outer nuclear layer are termed the OFF layer, whereas strata located near the ganglion cell layer are called the ON layer. These strata are formed by a family of transmembrane Ig molecules that guide laminar targeting for specific types of bipolar, amacrine, and ganglion cells *in vivo* (Yamagata and Sanes, 2008). Opn4S and Opn4L were expressed in M1 ipRGCs, which have dendrites ramified in the OFF layer. In contrast, only Opn4L was expressed in M2 ipRGCs, which arborize in the ON layer of the IPL. IpRGCs serve as conduits of nonvisual information from rods and cones, and they receive input from rods and cones via bipolar and amacrine cells (for review, see Provencio, 2008). Thus, ramification in different layers of the IPL indicates that M1 and M2 ipRGCs receive distinct excitatory and inhibitory signals, which is expected to differentially modulate M1 and M2 intrinsic light response. The different expression patterns of Opn4S and Opn4L in M1 and M2 ipRGCs is therefore correlated with distinct synaptic input to M1 and M2 ipRGCs.

In addition to receiving different inputs, M1 and M2 ipRGCs have different outputs. M1 ipRGCs predominantly innervate the SCN, and M2 ipRGCs comprise the majority ipRGC input to the olivary pretectal nucleus, a crucial brain area responsible for pupillary light reflex (Baver et al., 2008). Developing methods, such as Opn4 isoform-specific RNA interference and specific ablation of M1 or M2 ipRGCs according to their Opn4 isoform expression differences, may allow the assessment of the functional roles of M1 and M2 ipRGCs.

Although important questions remain, the study by Pires et al. (2009) takes a critical step forward in understanding the gene structure of melanopsin, the functional roles of melanopsin cell subtypes, and their corresponding melanopsin isoforms. A more comprehensive study of melanopsin might shine light on the potential of this photopigment as a drug target for people suffering from sleep or metabolic disorders caused by shift work, time zone changes, and medications.

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