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.

A Novel Role for the Visual Retinoid Cycle in Melanopsin Chromophore Regeneration

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Review of Zhao et al.

The mammalian retina contains three classes of photoreceptors that convert light information into electrical signals: rods, cones, and intrinsically photosensitive retinal ganglion cells (ipRGCs). These photoreceptors are capable of responding to a wide range of light intensities, which can subdivided into scotopic (<9.5 log photons cm^{-2} s^{-1}), mesopic (between 9.5 and 12 log photons cm^{-2} s^{-1}), and photopic (>12 log photons cm^{-2} s^{-1}) light intensities. Rods primarily mediate vision in dim, scotopic lighting conditions, and cones primarily mediate vision in photopic lighting conditions, whereas both rods and cones mediate mesopic vision. ipRGC-mediated behaviors, such as circadian photoentrainment and the pupillary light reflex, function primarily in photopic lighting conditions.

Photoreceptors contain photopigments composed of a light-sensing chromophore (11-*cis*-retinal), and an opsin protein, which is a 7 transmembrane G-protein-coupled receptor. 11-*cis*-retinal is isomerized to all-trans retinal in response to light, and this activates the opsin, triggering a cellular response. Subsequent regeneration of 11-*cis*-retinal through a process called the visual retinoid cycle is required for photoreceptors to continue responding to light. Chromophore regeneration in rod photoreceptors relies solely on a tissue adjacent to the rod and cone layer of the retina called the retinal pigment epithelium (RPE). Cone photoreceptors rely both on the RPE and Müller glia, which traverse the entire depth of the retina. However, chromophore regeneration in ipRGCs is still not well understood.

ipRGCs express an opsin called melanopsin, which closely resembles invertebrate opsins despite its vertebrate origins. Invertebrate opsins are bistable, which means they can convert all-trans retinal back to 11-*cis*-retinal autonomously when exposed to long-wavelength light. For this reason, researchers predicted that melanopsin was also a bistable photopigment. Indeed, melanopsin has been shown to be bistable when expressed in heterologous systems (Melyan et al., 2005; Panda et al., 2005; Qiu et al., 2005). Furthermore, circadian photoentrainment and the pupillary light reflex, both of which are melanopsin-driven visual behaviors, have been demonstrated to be independent of the RPE, consistent with the hypothesis that melanopsin can regenerate chromophore autonomously (Doyle et al., 2006; Tu et al., 2006). However, ipRGCs have also been shown to regenerate 11-*cis*-retinal and potentiate their light responses in the dark, which cannot be explained by a model in which chromophore regeneration is accomplished solely by exposure to long-wavelength light (Wong et al., 2005; Walker et al., 2008). These observations led to the hypothesis that ipRGCs also partially rely on the visual retinoid cycle for chromophore regeneration. To date, there have been no clear demonstrations of this, but in a recent study in *The Journal of Neuroscience*, Zhao et al. (2016) explore a novel mechanism by which ipRGCs use the visual retinoid cycle for chromophore regeneration.

Zhao et al. (2016) first examined whether the RPE plays a role in melanopsin chromophore regeneration. To test this, they used an *in vitro* rat eyecup preparation, which allowed them to record the spike output of ipRGCs using multielectrode arrays (MEAs) either in the presence (i.e., with the RPE attached to the retina) or absence (i.e., with RPE function pharmacologically inhibited or the RPE physically removed) of functional RPE. First, the authors showed that detaching the RPE from the retina abolished the sustained, melanopsin-based firing of ipRGCs in response to a 1 h background light stimulus. The authors then applied drugs that perturb the visual retinoid cycle to RPE-attached retinas. Specifically, they used sodium iodate, which poisons RPE cells (Sorsby, 1941) and 13-*cis*-retinoic acid and α-*phenyl*-*N*-*tert*-butylnitrone (Sieving et al., 2001; Mandal et al., 2011), both of which are visual retinoid cycle inhibitors. They found that melanopsin-based sustained firing of ipRGCs was abolished in the presence of these drugs at low photopic (12.6 log photons cm^{-2} s^{-1}) and high photopic (14.6 log photons cm^{-2} s^{-1}) light intensities. These results suggest that the deficit in sustained firing in ipRGCs resulted from the absence of RPE and not from me-

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chanical damage that occurred when removing the RPE, and thus indicate that the RPE may play a role in chromophore regeneration in ipRGCs.

Next, the authors investigated whether this RPE-dependent deficit in sustained firing had behavioral consequences. To address this, the authors examined whether acutely perturbing the visual retinoid cycle affects the pupillary light reflex, which is a visual behavior primarily mediated by ipRGCs (Lucas et al., 2003; Güler et al., 2008; Hatori et al., 2008). Similar experiments were done previously by Tu et al. (2006) in which the visual retinoid cycle was acutely inhibited by administration of all-trans retinylamide. Tu et al. (2006) demonstrated that pupillary light reflex was not affected in these experiments at light intensities between 10 and 14 log photons $cm^{-2}\ s^{-1}$, which suggested that ipRGCs do not rely on the visual retinoid cycle for chromophore regeneration. Zhao et al. (2016) injected a mixture of synaptic blockers into the eyes of the animals to isolate melanopsin-mediated pupillary light reflex and also injected animals with 13-*cis*-retinoic acid to acutely inhibit the retinoid cycle. Consistent with Tu et al. (2006), the authors found that, at 13.9 log photons $cm^{-2}\ s^{-1}$, peak pupil constriction and sustained pupil constriction were not significantly altered in animals treated with 13-*cis*-retinoic acid. However, at a light intensity brighter than those tested by Tu et al. (2006) (15.6 log photons $cm^{-2}\ s^{-1}$), sustained pupil constriction was significantly reduced. These results suggest that the ipRGCs that mediate the pupillary light reflex only rely on the visual retinoid cycle in high photopic lighting conditions.

There is a discrepancy between the *in vitro* and behavioral results mentioned above. The authors demonstrated a deficit in sustained firing of ipRGCs in the presence of 13-*cis*-retinoic acid at intensities as low as 12.6 log photons $cm^{-2}\ s^{-1}$, but not in sustained pupillary light reflex at even brighter light intensities (13.9 log photons $cm^{-2}\ s^{-1}$). The most likely explanation for this is that the spikes detected in MEA recordings do not arise solely from M1 ipRGCs, the ipRGC subtype that mediates the pupillary light reflex (Güler et al., 2008; Chen et al., 2011). ipRGCs are comprised of at least 5 subtypes (termed M1-M5) with distinct physiological and morphological properties (for review, see Schmidt et al., 2011), so it is likely that the majority of cells that the authors recorded with MEAs were non-M1 ipRGCs (M2-M5). In support of this, the peak firing rates that the authors record in ipRGCs are >40 Hz, which is significantly higher than previously reported maximal firing rates of M1 ipRGCs (Schmidt and Kofuji, 2009; Hu et al., 2013; Walch et al., 2015). This suggests that perhaps non-M1 ipRGCs rely more heavily on the RPE for chromophore regeneration than M1 ipRGCs. Future work should focus on how different ipRGC subtypes rely on the visual retinoid cycle.

An important question that still remains is as follows: how are retinoids from the RPE transported to ipRGCs? The RPE is far away from the ganglion cell layer of the retina, which would make it impossible for direct transport of chromophore between the RPE and ipRGCs. The authors hypothesized that Müller glia are responsible because they span the entire depth of the retina. To test this, they treated RPE-attached retinas with DL-2-aminoadipic acid, which is a toxin specific for Müller glia (Pedersen and Karlsen, 1979), and found that melanopsin-driven sustained firing of ipRGCs was abolished but could be partially restored with application of 9-*cis*-retinal. These results suggest that Müller glia are required for RPE-dependent chromophore regeneration in ipRGCs. In cone photoreceptors, Müller glia have been shown to support the visual retinoid cycle through two mechanisms. Wang and Kefalov (2009) demonstrated that Müller glia provide 11-*cis*-retinol to cone photoreceptors, which cone photoreceptors can convert to 11-*cis*-retinal. This was demonstrated by showing that application of 11-*cis*-retinol restored photosensitivity of bleached cone photoreceptors. If ipRGCs also relied on Müller glia through a similar mechanism, then application of 11-*cis*-retinol should also restore sustained firing in ipRGCs. However, when Zhao et al. (2016) applied 9-*cis*-retinol (an analog of 11-*cis*-retinol) to RPE-detached retinas, they did not observe any restoration of sustained firing in ipRGCs. Xue et al. (2015) showed that Müller glia can also directly transport 11-*cis*-retinal from the RPE to cone photoreceptors using a protein called cellular retinaldehyde-binding protein. It is possible that Müller glia also use cellular retinaldehyde-binding protein to transport 11-*cis*-retinal to ipRGCs, but this was not tested by Zhao et al. (2016). Therefore, the mechanism by which Müller glia participate in chromophore regeneration in ipRGCs still remains a question for future studies.

Together, these results suggest that, in low photopic conditions and for short durations, melanopsin does not rely on the visual retinoid cycle. But in high photopic conditions, melanopsin relies on the RPE and Müller glia for 11-*cis*-retinal. ipRGC dependence on Müller glia for chromophore regeneration has been postulated previously, and this study by Zhao et al. (2016) clearly demonstrates such an influence. This study warrants future work investigating the molecular mechanisms of Müller glia transport of 11-*cis*-retinal and the importance of this cycle for other ipRGC-mediated behaviors.

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