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Neuroscience: A Chromatic Retinal Circuit Encodes Sunrise and Sunset for the Brain

Alyssa M. Rivera^{1,2} and Andrew D. Huberman^{1,*}

¹Department of Neurobiology, and Ophthalmology, Stanford University School of Medicine, Stanford, CA 94305, USA

²Molecular and Cellular Biology, Oregon State University, Corvallis, OR 97331, USA

*Correspondence: adh1@stanford.edu
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A new study reveals the retinal circuit for encoding the types of light prominent at sunrise and sunset. The output of that circuit is conveyed to the brain's master circadian clock. Subconscious processing of sky color changes may therefore be the key stimulus for conveying morning and evening information to the circadian timing system in the brain.

All animals must coordinate their physiology and behavior to the external light–dark cycle. The basic elements of this process are known. The suprachiasmatic nucleus (SCN) — a dense aggregate of neurons in the hypothalamus, exhibits an intrinsic 24-hour rhythm that by chemical and electrical signaling synchronizes the cells of the brain and body to the same overall schedule. The SCN is entrained to a variety of external cues such as feeding, social interactions and temperature but the most powerful time-keeping cue for the SCN is light [1]. Namely, the light–dark cycle generated by the 24-hour rotation of the earth on its axis once each day. Unveiling the specific features of light that drive circadian entrainment and the retinal circuits that encode those

features remains an area of intense study. In this issue of *Current Biology*, Patterson *et al.* [2] describe the discovery of a neural circuit in the primate retina that ensures the unique chromatic features of light associated with sunrise and sunset are relayed to the SCN for accurate 24-hour (aka ‘circadian’) time keeping.

The SCN is the master circadian clock. It resides deep in the brain, shielded from direct light exposure by the thickness of skull. Thus, all information about ambient lighting must arrive to the SCN indirectly via signaling from other neurons. The neurons that perform that role are called retinal ganglion cells (RGCs). RGCs include ~40 different types, each of which conveys distinct signals to the brain about the presence of specific features of light

present in the immediate environment, such as dark or bright edges, objects moving, their colors, etc. [3]. One particular group of RGC types is intrinsically photosensitive due to its expression of an opsin called melanopsin. These so-called ‘ipRGCs’ thus act both as photoreceptors *and* retinal relays to brain. There are at least 6 types of ipRGCs. The M1 type connects to pacemaker neurons in the SCN to drive photic entrainment of circadian rhythms. Mice, birds, fish and primates, including humans, all have M1 ipRGCs that perform this role [1,3,4]. Given the indispensability of circadian entrainment for mental and physical health [1], understanding the biology of M1 ipRGCs, their circuits in the eye, and the way in which they signal the SCN is of crucial importance.



In addition to making melanopsin, which renders ipRGCs sensitive to bright short wavelength ‘blue’ light, M1 ipRGCs also respond to light absorbed by conventional cone photoreceptors [4,5]. In primates such as macaques and humans, the specific cone inputs to ipRGCs render them ‘spectrally opponent’ to yellow versus blue light. Their receptive fields (i.e., their ‘preferred stimuli’) are yellow-ON, blue-OFF, meaning they respond best to increments in yellow light absorbed by the long and medium wavelength cones (L/M cones) and to decrements in blue light absorbed by short wavelength cones (S cones) (Figure 1). Primate M1 ipRGC receptive fields are therefore L/M-ON, S-OFF [5].

Cones do not communicate directly with RGCs. They communicate to RGCs about the presence of increments or decrements in specific light wavelengths via ON-bipolar or OFF-bipolar interneurons. Even though the M1 ipRGCs that connect to the SCN have L/M-ON, S-OFF receptive fields, there is no evidence for S-OFF bipolar cell input to M1s. This raised the mystery as to how M1 ipRGCs acquire their characteristic ‘S-OFF’ responses.

A clue came from the finding that pharmacologic agents that block all ON bipolar cell activity also eliminate the S-OFF response in M1 ipRGCs. That suggested there could be a sign-inverting mechanism that converts S-ON bipolar signals into S-OFF responses in the M1s. Until now, the nature of that circuit and its relevance to the circadian timing system, remained unknown. To hunt down such a cell or circuit in the primate retina is the equivalent of finding a ‘needle in a haystack’. Patterson *et al.* [2] therefore undertook a ‘targeted brute force’ approach. They prepared macaque retinas for serial-sectioning electron microscopy (EM) [6], cutting the retinal tissue into nanometer-thin slices across its entire thickness spanning from the photoreceptor layer to the RGC layer. That ensured all the cells in the M1 circuit would be included. EM provides unsurpassed resolution of the morphology and synaptic connections between neurons, but in the absence of genetically encoded markers of M1 ipRGCs, which do not



M1 ipRGC spectral receptive field and spectral tuning

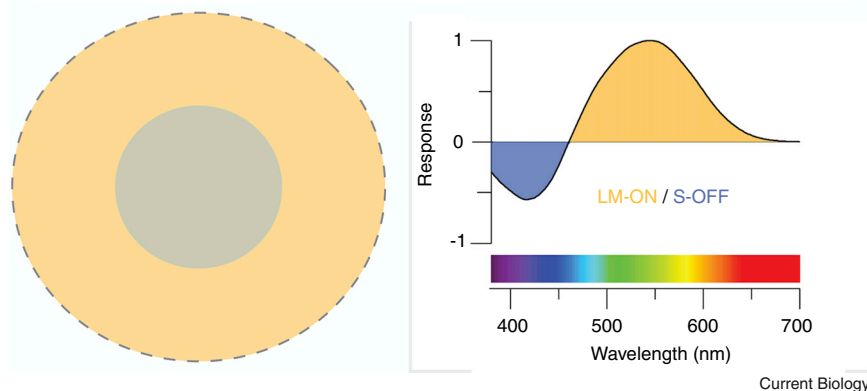


Figure 1. Light features extracted by retinal circuits for the circadian timing system in the brain.

Image of sunset to illustrate high yellow–blue contrast at the level of the horizon and the corresponding retinal receptive field of M1 ipRGCs that encodes this specific spectrally opponent feature. In reality, the yellow and blue spatial receptive fields are co-extensive (overlap), rather than center-surround. Details of the precise retinal circuit including the newly discovered S-cone amacrine cells that underlie these circadian-entrainment events can be found in the new study from Patterson *et al.* [2].

yet exist for the primate retina, the authors had to home in on the M1 circuitry some other way. First, they found and reconstructed the S-cones on the basis of their unique morphologies and signature patterns of synapses

made with S-ON bipolar cells. They confirmed the identity of the S-ON bipolar cells by virtue of their signature lack of input from L or M cones. Those features provided a narrow but highly reliable wedge into the dense

jungle of otherwise indistinguishable cells and synaptic connections in the EM-stack.

Their next task was to find and reconstruct the M1 ipRGCs. They followed the processes of S-ON bipolar cells down to the inner retina where they first reconstructed the easily identifiable ‘small bi-stratified RGCs’. These cells are not ipRGCs, but they do get input from S-ON bipolar cells. They knew that reconstructing the small bi-stratified RGCs would put them in the neighborhood of the M1s. Eventually they found and reconstructed the M1 ipRGCs, first by looking for RGC somas proximal to the small bistratified cells that had dendritic processes extending to the furthest region of the so-called inner plexiform layer (IPL) (Figure 1). The IPL is a remarkably organized region of information transfer in the central nervous system whereby the axons of specific bipolar cells synapse onto the dendrites of RGCs at different depths or ‘strata’. That stratification ensures that specific combinations of cone-response-related circuitry impinge on specific RGC types to endow them with their unique response properties to light.

The communication between bipolar cells and RGCs is further shaped by another type of interneuron, the amacrine cells, which comprise ~50 different types, most all of which are inhibitory. Patterson *et al.* found an amacrine cell type that gets direct input from S-ON bipolar cells and that, in turn, synapses directly onto the dendrites of M1 ipRGCs. These cells had relatively evenly spaced cell bodies that were of consistent size and shape which, along with their stereotyped wiring patterns, indicated they comprised a single ‘cell type’. Patterson *et al.* refer to this newly identified cell type as an S-cone amacrine cell.

The functional connectome of primate M1 ipRGCs and the basis for their chromatic opponency therefore is now known. M1 neurons receive ON signals from L-cones and M-cones via convergent synaptic input onto L/M-ON bipolar cells, and they receive S-OFF signals from S-cones that connect to ON-bipolar cells but for which the S (blue) ON-signal gets ‘flipped’ into an

S-OFF signal by the newly discovered S-cone amacrine (Figure 1). These findings of Patterson *et al.* are an elegant example of using the known physiological properties of neurons and serial EM connectomics to identify highly detailed, even cryptic, aspects of circuit wiring diagrams. In this instance it also revealed a cell type that previously no one knew existed. Given the current absence of cell-type-specific transgenic labeling tools in primates, it is hard to imagine obtaining such detailed information using any other combination of approaches.

What does the presence of L/M-ON, S-OFF responses in M1 ipRGCs mean for circadian entrainment? In fact, one wonders why a retinal circuit that primarily serves to convey changes in overall light levels would bother to encode color opponency at all. Indeed, the retina-to-SCN pathway is entirely non-image-forming; M1 ipRGCs don’t entrain our circadian clocks by allowing us to consciously perceive the sunrise and sunset, or any other light for that matter. Many people who are completely pattern vision blind can entrain their circadian rhythms to ambient light [7]. Primate M1 ipRGCs respond to contrast between ‘yellow’ and ‘blue’ wavelengths of light [5]. The work of Patterson *et al.* informs us there is even a designated cell type, the S-cone amacrine cell, that creates S-OFF responses in M1s. Thus, color opponency was deliberately ‘wired’ into our eyes. The results of Patterson *et al.* suggest that color opponency in primate M1s may have arisen to detect the sunrise and sunset, which are marked by reductions in blue (hence the S-OFF amacrine). This idea was actually suggested much earlier on the basis of findings from the Neitz lab in fish [8]. Recent results from the mouse recently published in *Current Biology* [9] also show that blue/yellow color signaling provides a sky-averaging readout of luminance at dawn and dusk (even when there are clouds). At sunrise, the sky transitions from black to blue, then to yellow, whereas at sunset the sky transitions from blue to yellow, to orange, before turning dark blue and, eventually, black. Built into these transitions is yellow–blue contrast with

blues getting progressively darker and yellows brighter. This makes primate M1 ipRGCs essentially detectors of contrast between yellow and blue light, even though the M1–SCN pathway has nothing to do with conscious vision. It also raises questions as to whether color opponency may have evolved first in the circadian entrainment, non-image-forming system. Meanwhile, the work of Patterson *et al.* [2] provides a solid anatomical picture of the organizational logic that the primate retina uses to extract color opponency related to the viewing of sunrises and sunsets that dictate our internal biological sense of circadian time.

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