Intrinsically Photosensitive Retinal Ganglion Cells

For 150 years of modern vision science, mammalian vision was believed to be duplex: all light signals are detected in rod and cone cells in the photoreceptor layer of the outer retina. However, studies over the last thirty years have proved the presence and importance of a third kind of photoreceptor known as the intrinsically photosensitive retinal ganglion cell (ipRGC)[1]. ipRGC morphology differs from that of traditional rods and cones in many ways, most significantly that their somas are located in the retinal ganglion cell layer of the inner retina, their dendrites arborize in the inner plexiform layer (IPL), and their axons project directly to the brain rather than other cells in the retina. ipRGCs mostly serve non-image forming functions such as photoentrainment of the circadian rhythm[2] and the pupillary light reflex (PLR)[3], but they also serve limited image-forming functions such as pattern perception as well.

ipRGCs are characterized by their expression of melanopsin, a G-protein-coupled receptor transmembrane protein. Melanopsin, along with a vitamin A based chromophore called retinaldehyde, is the component that makes ipRGCs photosensitive[4]. Melanopsin was first discovered in the melanophores of

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[1] The first evidence of the third photoreceptor was in 1927, when Harvard University graduate student Clyde Keeler discovered that virtually blind mice bred without an outer retina –which normally contains mostly rod and some cone photoreceptors– could respond to light stimuli by constricting their pupils (Keeler 1927, 1928). Keeler's work suggested that rods and cones are not the only cells that detect and cause a response to light in mammals. However, consensus in the field of vision science was deeply vested in the duplex theory of vision and the idea of a third photoreceptor was not further explored until the 1990s.

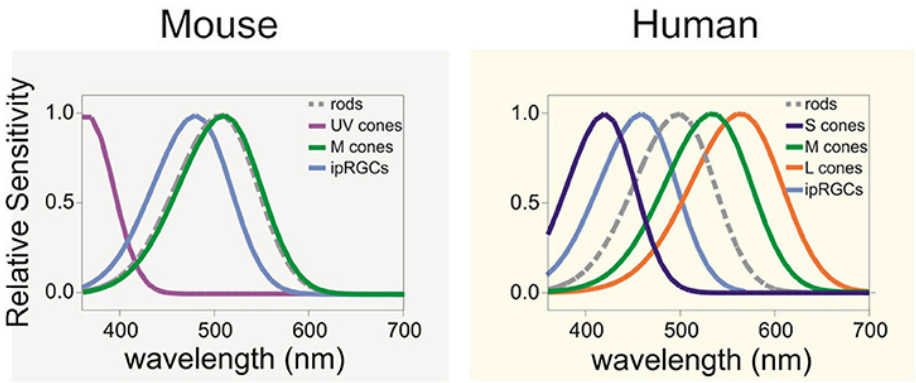
[2] Photoentrainment of the circadian rhythm means to synchronize the body's internal clock with the outside environmental time of day by encoding light signals.

[3] The PLR involves the contraction of the iris muscle in response to bright light, which limits the amount of light entering the eye and acts to balance visual sensitivity, which is supported by a large pupil, and spatial resolution, which benefits from a small pupil. This balance appears to maximize information transmission over environmental irradiances spanning many orders of magnitude (Do 2020).

[4] In the early years of ipRGC research, the blue-light-sensitive flavoproteins called cryptochromes were hypothesized to be the light-sensitive component of ipRGCs. However, Fu et al (2005) summarizes that cryptochromes are not the photopigment that identify ipRGCs and their role in circadian photoentrainment because cryptochromes use flavin adenonucleotide and/or a pterin as a chromophore, whereas opsin-based photopigments use retinaldehyde; a flavin-based photopigment is unlikely to account for the opsin-like action spectrum of ipRGCs. Additionally, mice lacking cryptochromes but have normal rods, cones, and ipRGCs show no decrease in the sensitivity of the pupil reflex or light-induced expression of SCN clock genes. Finally, cryptochromes also are expressed far too broadly in the retina to be localized within a subset of special photoreceptor cells like melanopsin is. Therefore, evidence suggests that it is not cryptochromes but rather melanopsin that drives the photosensitivity of ipRGCs.

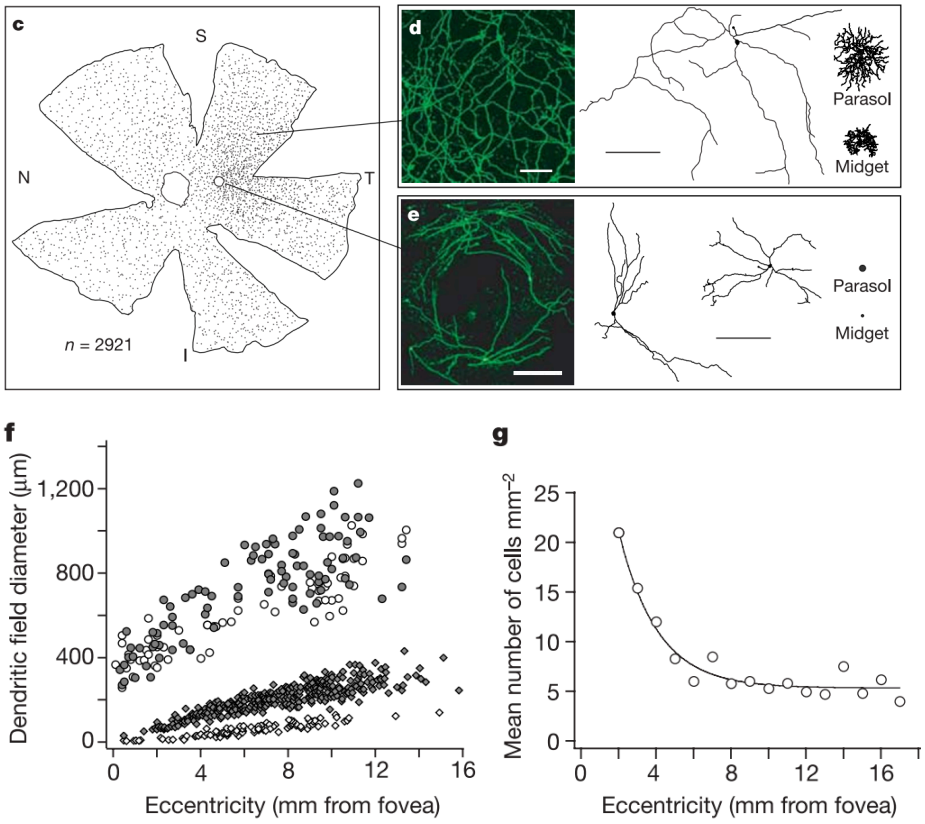
frogs, which is what its name is derived from (Provencio et al 1998), and was quickly also identified in humans (Provencio et al 2000). It is expressed by the OPN4 gene, which has been manipulated in many experiments to study the morphology, brain targets, and function of melanopsin and ipRGCs as a whole. Interestingly, melanopsin's OPN4 gene in both mice and humans is more similar to the rhabdomeric opsins (r-opsins) of invertebrate photoreceptors than the ciliary opsins (c-opsins) of vertebrate photoreceptors, which may be due to the its role in non-image forming functions of circadian rhythm entrainment that is shared between invertebrates and vertebrates alike (Provencio et al 1998).

Melanopsin has a peak absorbance of around 480 nm wavelength, corresponding to the blue light most abundant outside during the daytime. This peak absorbance does not align with the peak absorbance of rods at 500 nm or S, M, and L cones at 420 nm, 530 nm, and 560 nm respectively, suggesting that melanopsin has unique functions not covered by the traditional photoreceptors. Although evidence shows that melanopsin is at least as photosensitive as rods and cones (Do et al 2010), ipRGC responses to stimulation by light in rodents, macaque monkeys, and humans are described as slow and sluggish. Unlike rods and cones, which have a response time of less than 100 milliseconds after stimulus presentation, ipRGC signals start 3 seconds after of the presentation of the stimulus, stays throughout the duration of the stimulus, and remains present up to 30 seconds after the stimulus disappears (Mure et al 2019, Berson 2002, Dacey 2005).



Relative spectral sensitivity of the rods, cones, and ipRGCs. From: From: Mure (2021)

Within the conventional retinal ganglion cell population, ipRGCs consist of a very low percentage: about 1%-3% in mice (Berson 2002; Hattar 2002)[5] and only about 0.4%-1.5% in humans (Esquiva et al 2017, Hannibal et al 2017, Liao et al 2016, Nasir-Ahmad et al 2017). While there are no ipRGCs in the fovea, ipRGC density is highest in the peri-foveal region and it declines until 10 mm away from the fovea; beyond that, the density remains fairly consistent (Hannibal et al 2017, Liao et al 2016, Nasir-Ahmad et al 2017). In this way, ipRGC cell density parallels the decrease of density of conventional RGCs from the center to periphery of the retina (Mure 2021). Additionally, ipRGCs are considered "giant" cells and are much larger than conventional RGCs. Their somas can be up to 50 um in diameter and their dendritic fields range from about 300 um to over 1200 um in diameter, increasing in size from the center to the periphery of the retina (Dacey 2005)[6].



(c) Macaque retina tracing; dots represent melanopsin cells. T, temporal retina; N, nasal retina; S, superior retina; I, inferior retina. (d) Melanopsin cells in peripheral retina (left; scale

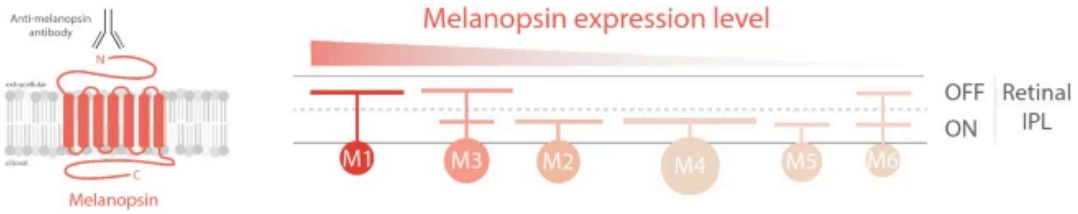
[5] David Berson at Brown University and Samer Hattar at Johns Hopkins University are often simultaneously credited with the discovery of ipRGCs in 2002.

[6] Dennis Dacey and colleagues in 2005 were the first to identify ipRGCs in primates.

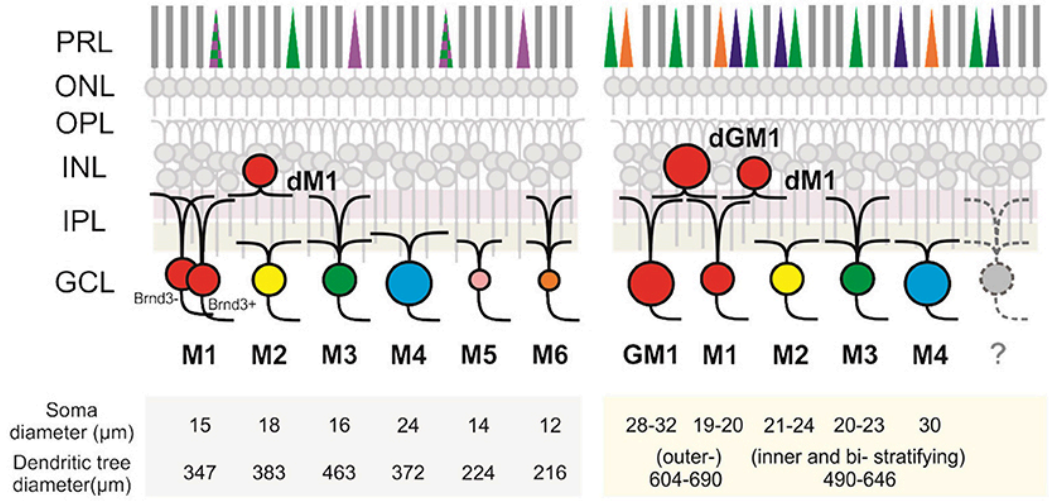
bar,100 μm). Tracing of a peripheral HRP-stained giant cell (right; scale bar, 200 μm). Parasol and midget cells (far right) are shown for comparison. (e) Melanopsin cells encircling the fovea (left; scale bar, 200 μm). Tracings of two HRP-stained giant cells ~1–1.5 mm from the fovea (right; scale bar, 200 μm). Circles (far right) indicate size of foveal parasol and midget cells. (f) Dendritic field size of melanopsin cells versus eccentricity (inner cells, filled circles, n ¼ 93; outer cells, open circles, n ¼ 63). Parasol (filled diamonds, n ¼ 333) and midget cells (open diamonds, n ¼ 93) are shown for comparison. (g) Mean cell density of melanopsin cells versus eccentricity (total 614 cells in 78 1 mm2 samples). From Dacey et al (2005).

Early studies of ipRGCs focused on mapping melanopsin-expressing cells to the suprachiasmatic nucleus (SCN) along the retinohypothalamic tract, which is known to be the brain's regulatory center for circadian rhythm entrainment. Researchers observed that blind mice and some blind humans could maintain accurate circadian rhythms, so a lot of the subsequent research focused on ipRGC connections to the SCN (Foster et al 1991, Lucas et al 1999, Freedman et al 1999, Klerman et al 2002, Zaidi et al 2007). The results from these experiments show that "most RGCs that project to the SCN express melanopsin, and a majority of melanopsin-containing RGCs project to the SCN" (Gooley et al 2001, Beier et al 2020).

Throughout the last 25 years, ipRGCs have frequently been investigated through immunostaining melanopsin; however, the class of ipRGCs can be divided into subtypes, M1-M6 in mice and M1-M4 in humans, and these subtypes do not express melanopsin equally (Mure 2021). The M1 subtype expresses the most amounts of melanopsin and is the most intrinsically sensitive to light, followed by M3 cells, then M2 cells, and finally, M4-M6 subtype cells express the least amounts of melanopsin; therefore, even the most sensitive antibody for immunohistochemistry fails to detect all ipRGCs and is not as useful for labelling ipRGC types that express low levels of melanopsin (Aranda and Schmidt 2020, Mure 2021). Often, to isolate non-M1 ipRGC subtypes, researchers use a combination of genetic markers such as the Cre labelling system instead of immunofluorescence.



Differential melanopsin expression in M1–M6 ipRGCs. From: Aranda and Schmidt (2021)



ipRGCs in the mouse (left) and human (right) retinas. (Upper panels) Diagram of murine and human retinas displaying the differences regarding the morphological subtypes of ipRGCs, their IPL dendritic stratification, and outer retina photoreceptors. (Lower panels) Morphological comparison between subtypes and species. Soma and dendritic tree measurements are rounded to the closest integer. GM1, gigantic M1; dM1, displaced M1; dGM1, displaced gigantic M1; PRL, photoreceptors layer; ONL, outer nuclear layer; OPL, outer plexiform layer; INL, inner plexiform layer; IPL, inner plexiform layer; GCL, ganglion cells layer. From: Mure (2021)

Work from the last 15 years has been focusing on finding the morphological and functional distinctions between ipRGC subtypes. Each subtype also targets different areas of the brain, as well as uses different phototransduction pathways to cause a cascading signal. The ipRGC subtype most likely identified by Berson 2002 and Hattar 2002 that primarily projects to the SCN is the M1 subtype. This subtype is the most studied and the most understood by the vision science community. Its dendrites arborize exclusively in the outer (OFF) sublamina of the inner plexiform layer (IPL). In addition to the SCN, the other major brain target for M1 ipRGCs is the shell of the olivary pretectal nucleus (OPN), which controls and plays a huge role in pupil constriction. Minor targets include the intergeniculate

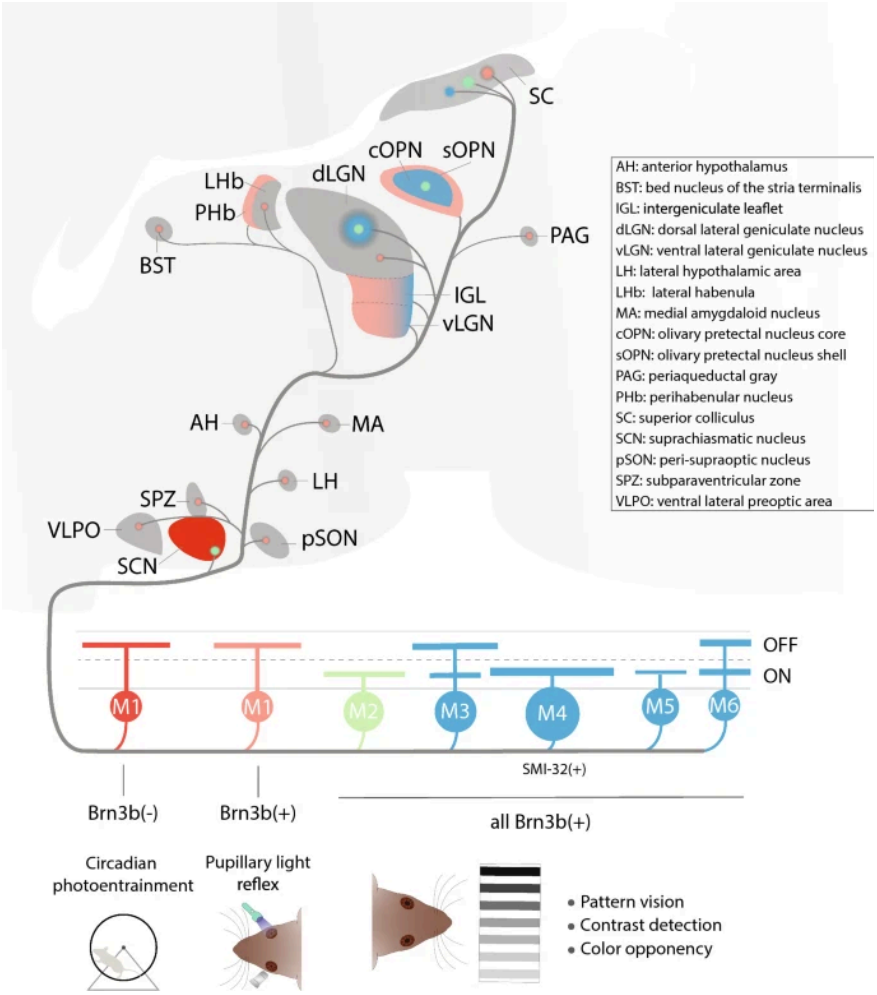
leaflet (IGL; photic and non-photic regulation of the SCN), ventrolateral preoptic area (VLPO; sleep-wake cycle), medial amygdala (MA; mood), and more. On the whole, M1 ipRGCs are mostly involved in the non-image forming centers of the brain and contribute to functions that are more dependent on the encoding of irradiance (the overall intensity of illumination) than to contrast (local, spatiotemporal differences in illumination).

Subtypes M2 through M6 express less melanopsin, are less photosensitive, are less understood, and tend to impact image forming functions rather than non-image forming functions more than M1 cells.

Reports as early as Dacey 2005 have identified ipRGC targeting the dorsal lateral geniculate nucleus (dLGN) and superior colliculus (SC), brain regions essential for pattern vision, brightness perception, and contrast detection. These cells likely belong to the M2 subtype of ipRGCs, which have dendrites that stratify in the inner (ON) sublamina of the IPL, have larger somata, and have larger, more complex dendritic arbors than M1 cells. What makes the M2 subtype unique is that it projects to both image forming (SC and dLGN) and non-image-forming brain areas (SCN and OPN). While similar to M2 cells in terms of soma size and dendritic arbor size and complexity, M3 cells have dendrites that bistratify in both the outer and inner (OFF and ON) sublaminas of the IPL. While M3 ipRGCs have been reported to project to the SC, no other targets have been identified, likely because this subtype has not been as extensively studied.

The M4 subtype has the largest soma size and largest dendritic arbors of all ipRGCs, stratifies in the inner (ON) sublamina of the IPL, and has the lowest levels of melanopsin expression of all the subtypes. M4 ipRGCs are synonymous with ON sustained alpha RGCs and are also the only ipRGC subtype that is labeled with an antibody to a non-phosphorylated form of the neurofilament heavy chain protein (SMI-32). We know the least about M5 and M6 subtypes, and it is not yet known if these subtypes found in mice have corresponding cells in primates. While M5 dendrites only stratify in the inner (ON) layer of the IPL, M6 dendrites stratify in both layers. These two ipRGC types have the smallest somas and highly branched dendritic arbors (Ecker et al 2010). Interestingly, M5 ipRGCs have been shown to exhibit opponent responses to different wavelengths of light, displaying the ability for melanopsin to distinguish color opponency (Stabio et al 2018). M4-M6 cells all project their axons to the dLGN and SC, which are important for image-forming

functions of vision, however, the specific and unique contributions of each subtype to image-forming vision is not yet understood (Aranda and Schmidt 2020).



Schematic representation of the main brain targets of ipRGCs. Brn3b-negative M1 ipRGCs are sufficient to drive circadian photoentrainment and project mainly to the SCN, while Brn3b-positive M1 ipRGCs project to sOPN and are necessary for the PLR. Brn3b-positive M1 ipRGCs constitute the majority of sparse M1 ipRGC innervation of the thalamus, hypothalamus, and midbrain (VLPO, SPZ, pSON, AH, MA, vLGN, IGL, dLGN, PAG, BST, PHb, and LHb). M2 cells project to both image forming (SC and dLGN) and non-image forming (SCN and OPN) visual areas and M4–M6 cells project to brain nuclei involved in image-forming vision such as dorsal geniculate nucleus (dLGN) and superior colliculus (SC). While M3 ipRGCs have been reported to project to the SC, no other targets have yet been identified. From: Aranda and Schmidt (2021)

In summary, intrinsically photosensitive retinal ganglion cells (ipRGCs) represent a distinct class of photoreceptors that play critical roles in both

non-image-forming and, to a lesser extent, image-forming visual functions. Unlike rods and cones, ipRGCs reside in the inner retina, express the photopigment melanopsin, and respond to light with sustained, slower signals. Although they comprise only a small fraction of retinal ganglion cells, their influence is significant: regulating circadian rhythms, pupil responses, sleep, and mood. ipRGCs are divided into several subtypes (M1–M6 in mice; M1–M4 in humans), each with unique structural features, melanopsin expression levels, and brain targets. While the M1 subtype is well-studied for its role in circadian photoentrainment and pupillary light reflex, the functions of other subtypes, particularly in image-forming vision, remain an active area of research. Continued study of these cells seeks to deepen our understanding of how ipRGCs detect, process, and transmit light information to the brain.

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