

# **Advanced Retina**

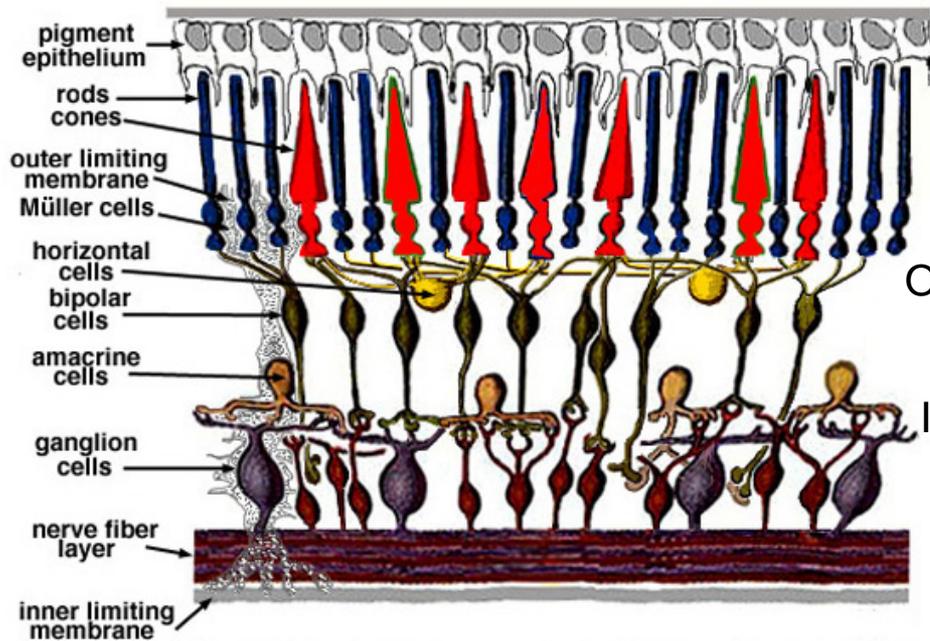
**Dr. Anthony Vugler**

**UCL-Institute of Ophthalmology**

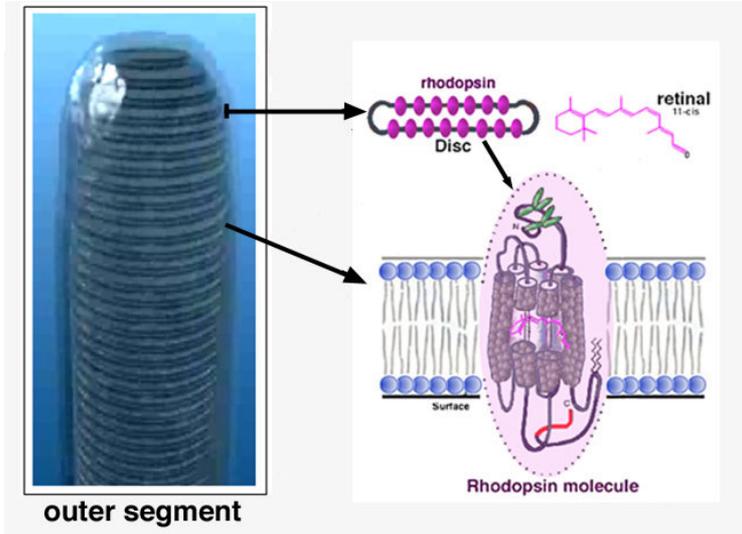
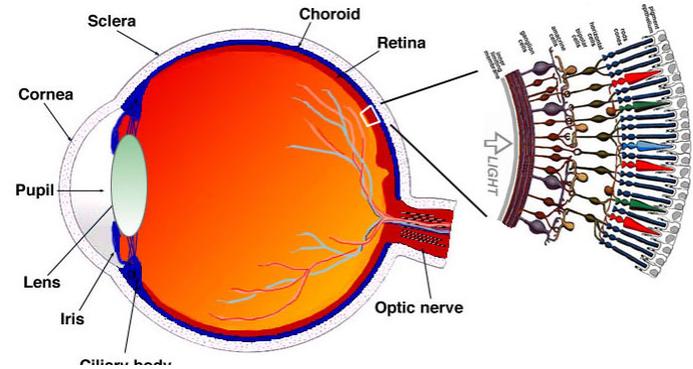
## Overview:

- The discovery of intrinsically photosensitive Retinal Ganglion Cells (ipRGCs)
- Structure and function of ipRGCs
  - Anatomy and physiology
  - How do ipRGCs contribute to visual function?

# Rods and cones account for all photoreceptive input to the mammalian CNS...

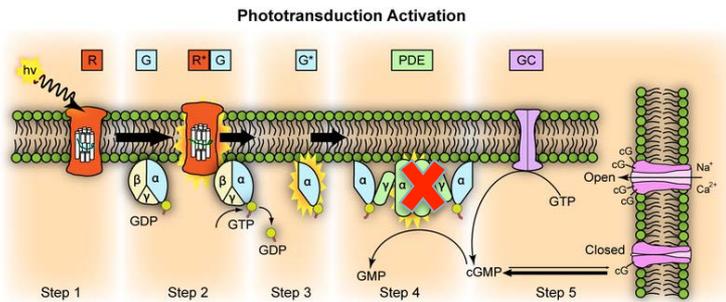


ONL  
 OPL  
 INL  
 IPL  
 GCL

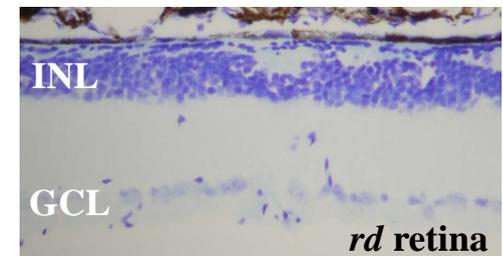
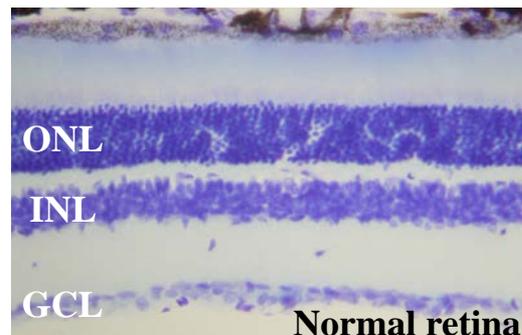
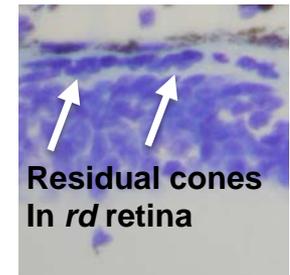
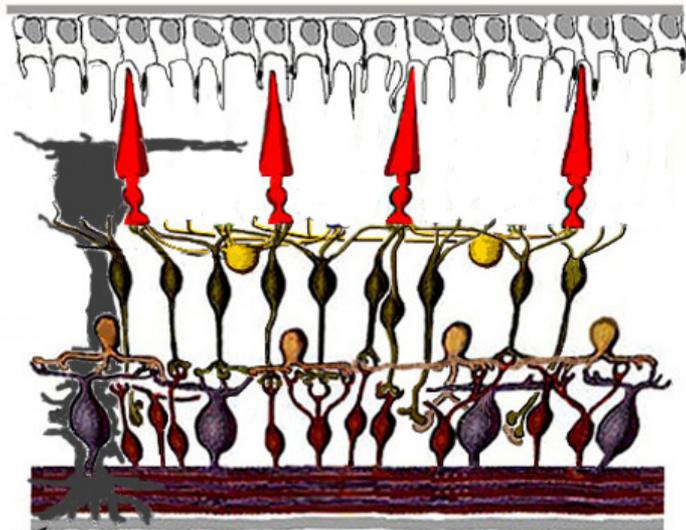


**Abbreviations:** outer nuclear layer (ONL), outer plexiform layer (IPL), inner nuclear layer (INL), ganglion cell layer (GCL).

# Could there be something else apart from rods and cones?



Evidence came from studies of retinal degenerate (*rd*) mice, which have a mutation in the  $\beta$  subunit of rod-specific phosphodiesterase (PDE). This leads to a rapid degeneration of rods followed by a slower loss of cones.



# rd mice retain a pupillary light reflex (PLR)

TABLE 1

INDIVIDUAL	EYE	CONDITION OF RETINA	AVERAGE CONTRACTION	TIME OF LATENT PERIOD					TIME OF CONTRACTION					DIAMETER OF PUPIL		
				1	2	3	4	5	1	2	3	4	5	Atropin	Sulfide of eserine	
Gray ♀ 28	Left	Normal	1.46-0.616	0.3	0.3	0.3	0.3	0.3	3.0	3.0	3.0	3.0	3.0	2.31	0.231	
Black ♂ 23	Left	Normal	1.54-0.539	0.6	0.6	0.6	0.6	0.6	3.0	3.0	3.3	3.3	3.3	2.31	0.099	
Black ♂ 23	Right	Normal	1.54-0.539	0.6	0.6	0.6	0.6	0.6	3.0	3.3	3.3	3.3	3.3	2.31	0.099	
Gray ♀ 12	Left	Normal	1.54-0.539	0.6	0.6	0.6	0.6	0.6	3.0	3.0	3.6	4.2	4.2	2.31	0.924	
Gray ♀ 12	Right	Normal	1.54-0.616	0.6	0.6	0.6	0.6	0.6	4.2	4.2	4.2	5.6	5.6	2.31	0.924	
Gray ♀ 13	Left	Normal	1.54-0.616	0.6	0.6	0.6	0.6	0.6	3.6	3.6	3.6	3.6	3.6	2.31	0.385	
Gray ♀ 13	Right	Normal	1.54-0.616	0.6	0.6	0.6	0.6	0.6	3.6	3.0	3.0	3.0	3.0	2.31	0.385	
Gray ♀ 10	Left	Normal	1.54-0.693	0.6	0.6	0.6	0.6	0.6	3.0	3.0	3.0	3.0	3.0	2.31	0.154	
Gray ♀ 10	Right	Normal	1.54-0.616	0.6	0.6	0.6	0.6	0.6	3.0	3.0	3.0	3.0	3.0	2.31	0.154	
Chinchilla ♀ 11	Left	Normal	1.54-0.616	0.6+	0.6	0.6	0.6	0.6	5.4	5.4	5.4	5.4	6.8	2.16	0.616	
Chinchilla ♀ 11	Right	Normal	1.54-0.616	0.6+	0.6+	0.6+	0.6+	0.6+	4.8	5.4	5.4	5.4	5.4	2.16	0.616	
Averages.....			1.53-0.602					0.57						3.73	2.28	0.417
Gray ♀ 31	Left	Rodless	1.54-0.62	2.4	2.4	2.7	2.7	2.4	2.4	2.4	2.1	2.1	1.8	2.31	0.154	
Gray ♀ 31	Right	Rodless	1.54-0.62	3.0	3.3	2.7	3.0	3.0	2.4	1.5	1.5	2.1	1.8	2.31	0.154	
Black ♂	Left	Rodless	2.31-1.16	3.3	3.6				6.0	6.6	Animal choked to death					
Chinchilla ♀	Left	Rodless	1.39-0.61	1.8	1.8	1.8	2.4	1.8	3.0	3.0	3.0	3.0	3.0	2.70	0.385	
Chinchilla ♂	Left	Rodless	1.39-1.16	1.2	1.5	1.8	1.8	1.8	3.0	2.4	2.4	2.4	2.4	2.70	0.308	
Brown ♂ 7	Left	Rodless	1.93-1.16	1.8	3.0	3.0	3.0	1.8	3.0	2.0	3.0	3.0	2.0	2.39	0.154	
Brown ♂ 7	Right	Rodless	1.93-1.16	0.6	0.6	2.4	1.8	2.4	2.4	3.0	1.8	3.0	2.4	2.39	0.154	
Brown ♀ 34	Left	Rodless	1.54-0.77	1.8	3.0	3.0	3.0	3.0	2.4	1.8	2.4	3.0	1.8	2.39	0.365	
Brown ♀ 34	Right	Rodless	1.54-0.77	1.8	3.0	3.0	3.0	4.8	3.0	1.8	1.8	2.4	1.8	2.31	0.365	
Brown ♀ 6	Left	Rodless	1.54-1.16	1.8	1.2	1.8	0.6	0.6	3.0	1.8	2.4	2.4	3.6	2.39	0.231	
Brown ♀ 6	Right	Rodless	1.54-1.16	1.8	1.5	1.2	0.6	0.6	2.4	2.4	2.4	3.6	3.6	2.39	0.231	
Averages.....			1.65-9.40					2.18					2.56	2.43	0.250	

All diameters are given in millimeters. All times are given in seconds.



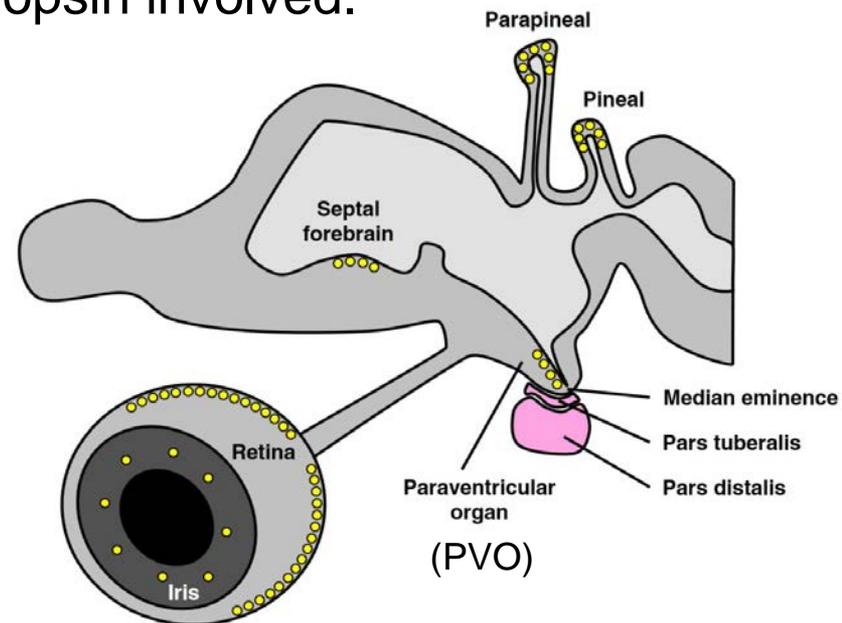
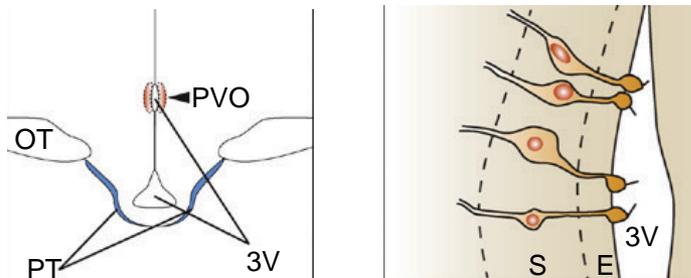
Clyde Keeler noted that rodless animals had a slower and weaker PLR than normals. He concluded that the iris may function independently of vision in rodless animals (based on work in eels from the 1840s) and that the deficits in rodless animals pointed to a regulatory system for iris constriction in normal eyes.

(Keeler, (1927) American J. Physiology 81: 107-112).

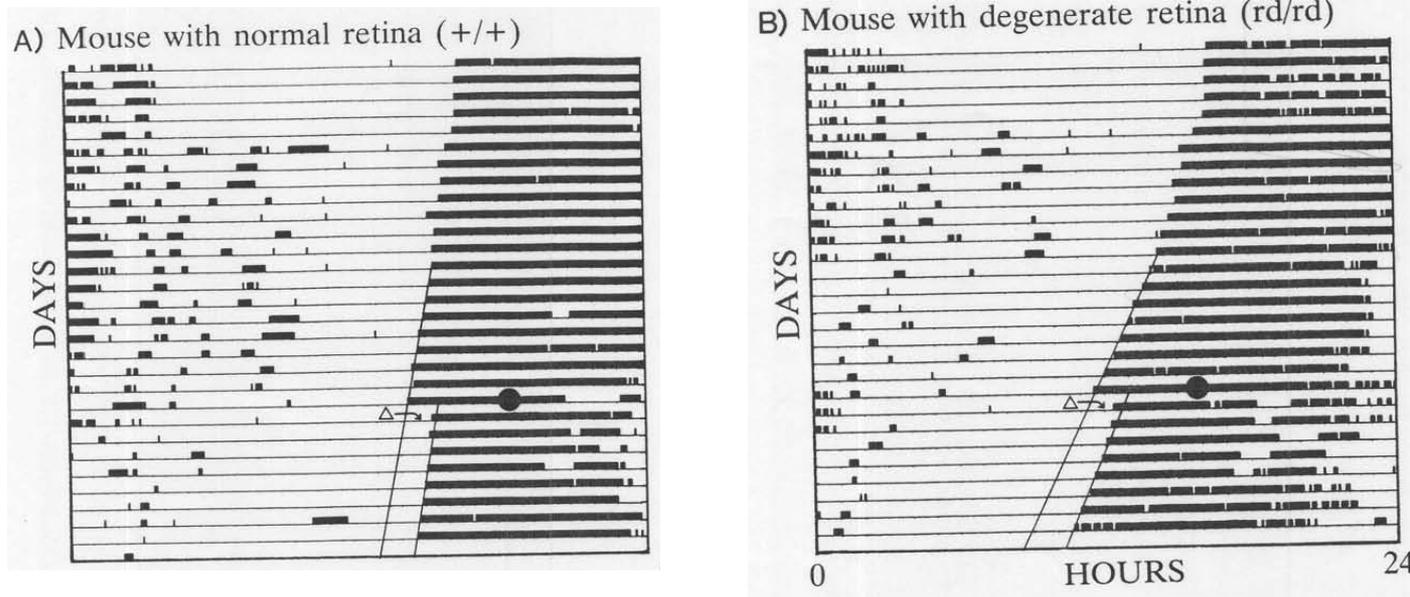
# Russell Foster

- Studied the photoperiodic response in quail
  - PhD in the Dept. Zoology University of Bristol
- Seasonal gonad maturation is mediated by deep brain photoreceptors in the hypothalamus.
  - Foster et al., (1985) Nature 313(3): 50-52.
  - Defined the action spectrum of the opsin involved.

Birds have deep brain photoreceptors in the hypothalamus

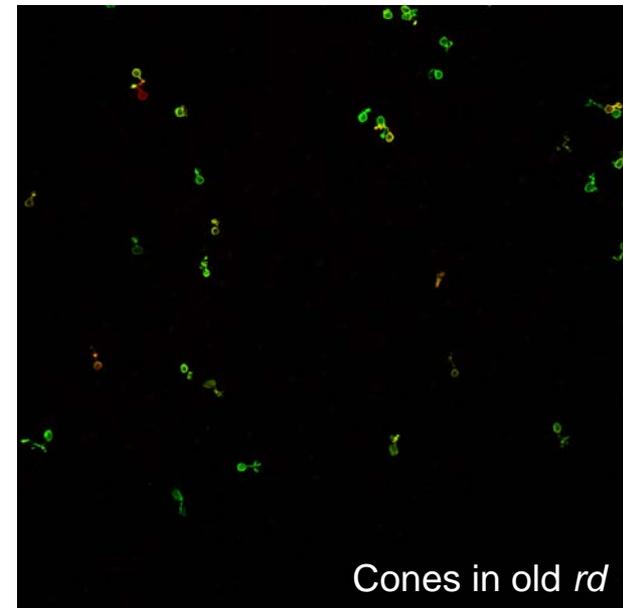
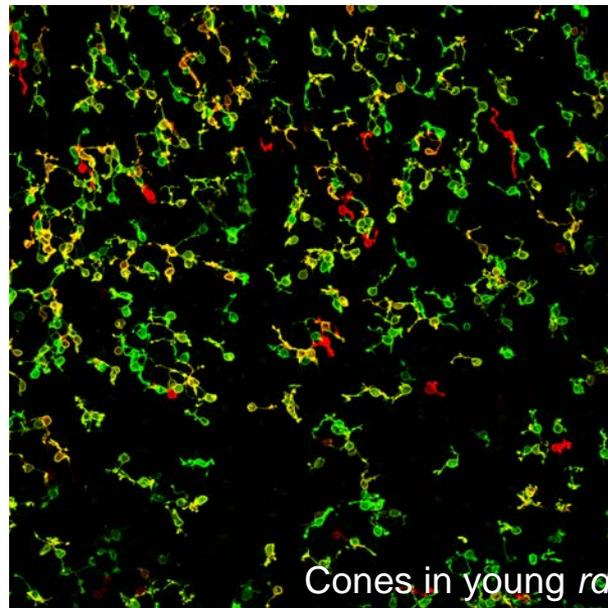
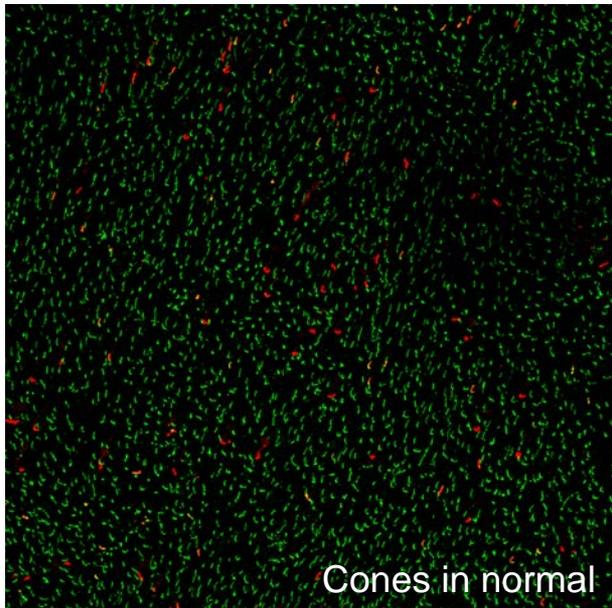


# *rd* mice retain circadian photoreception



- Mice on 12h light:dark cycle for the first 5 days, then into constant darkness for 16 days. Black bars show wheel running activity during subjective night. A 15min pulse of light at CT16 (●) causes a 90min delay in the phase shift (Δ) on subsequent days in both normal and *rd* mice. Testing at different irradiances revealed that the response in *rd* mice was indistinguishable from that in congenic wildtype (+/+) mice. This was in contrast to an earlier study by Ebihara and Tsuji in 1980, which compared *rd* mice with wildtype mice from a different strain.

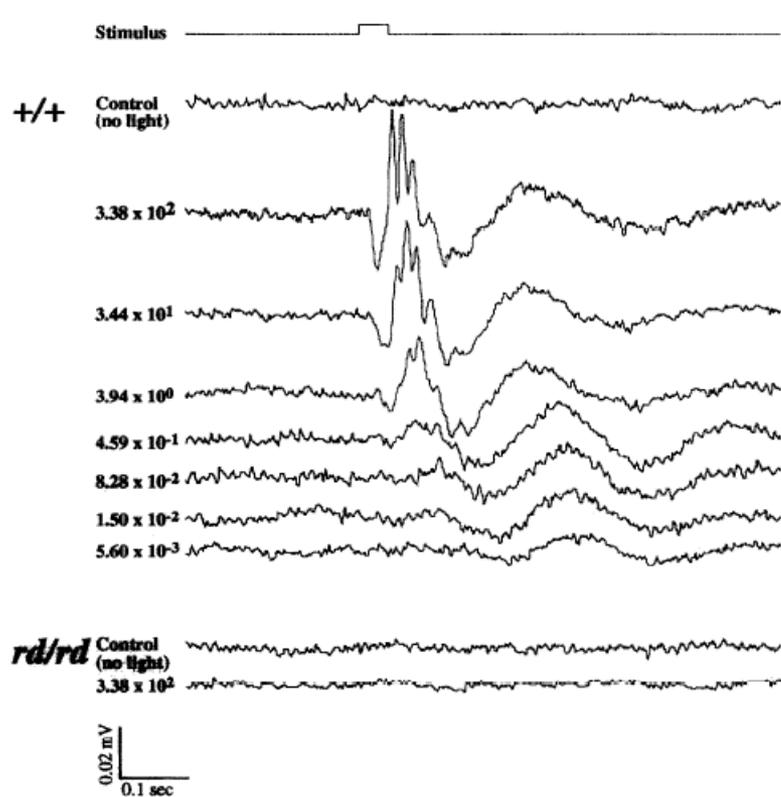
# Could cones mediate this circadian response in *rd* mice?



Images above show immunohistochemistry (antibody staining) for Short-wavelength sensitive cone opsin (red) and Long-wavelength sensitive cone opsin (green). The *rd* mouse lacks rods but retains cones, which decline in number with with age.

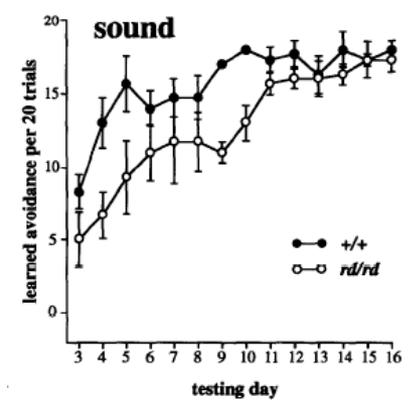
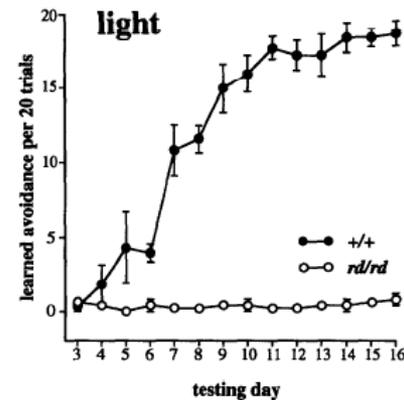
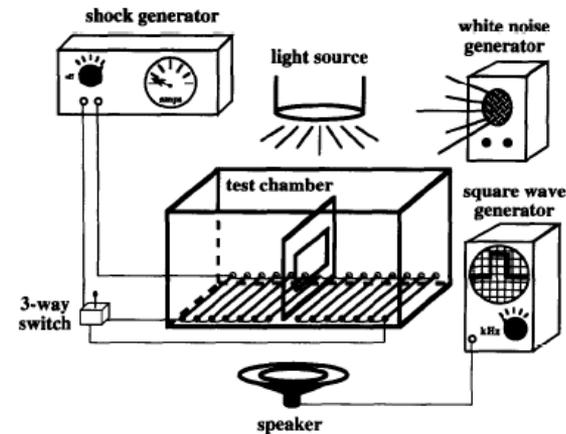
Ignacio Provencio and Russell Foster went on to show that even in old (>2 years) *rd* mice, the ability to phase shift in response to light remained indistinguishable from age matched normal mice (Provencio et al., (1994) *Vision Res.* 34(14) 1799-1806).

# Old *rd* mice appear to be otherwise blind



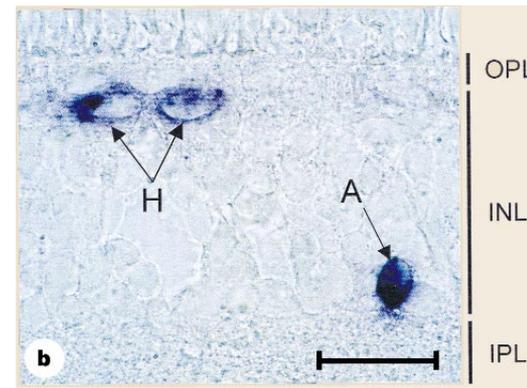
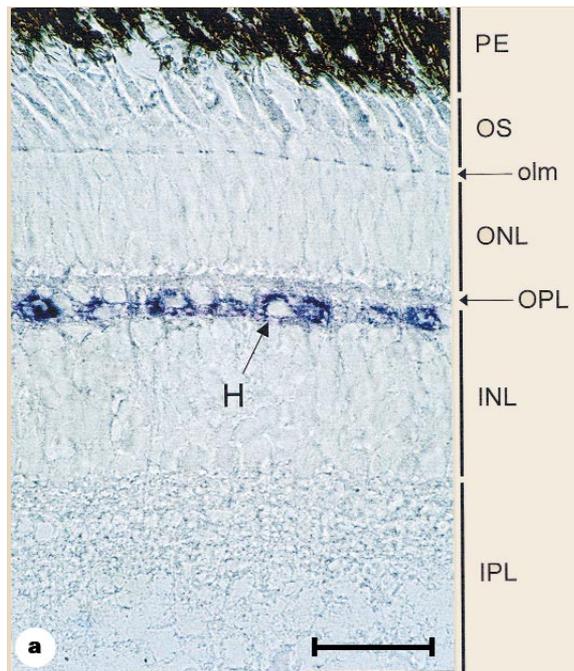
*rd* mice have no detectable ERG from 26 days old.

(Provencio et al., (1994) Vision Res. 34(14) 1799-1806)



The *rd* mice failed to associate light with impending shock.

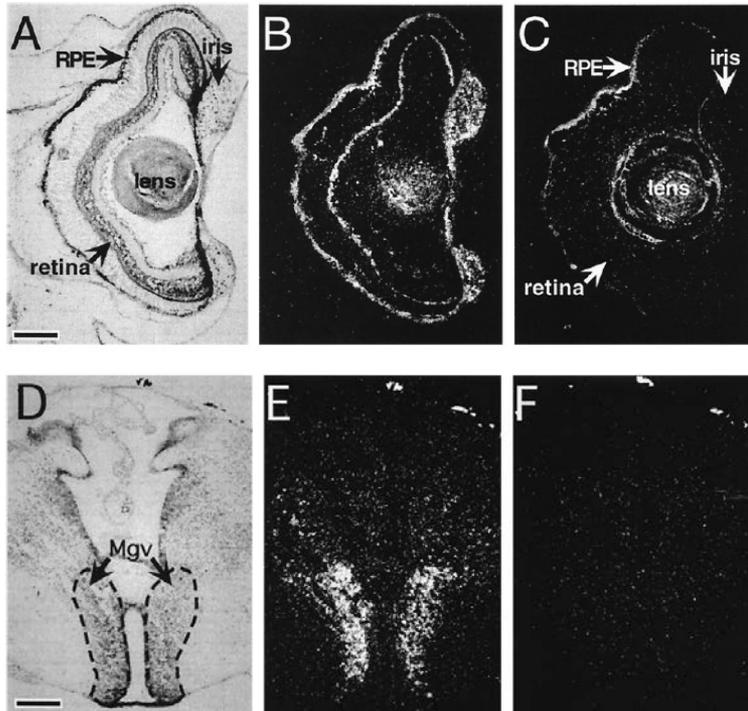
# Russell Foster was convinced there was another opsin at work in the vertebrate retina apart from rhodopsin and cone opsins...



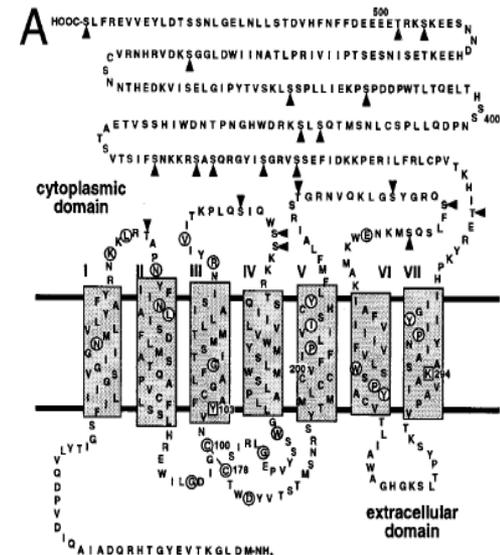
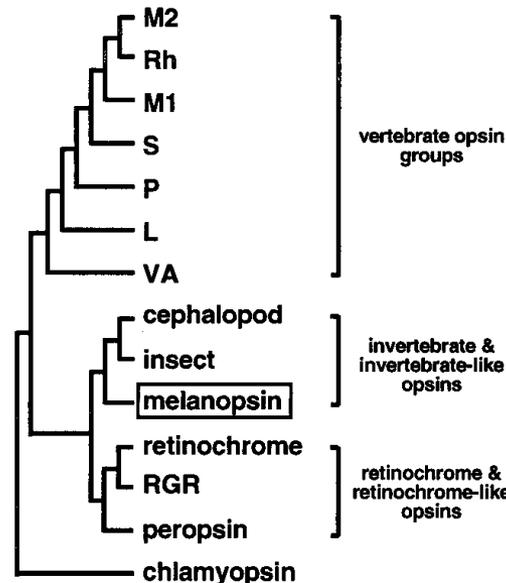
(Soni, Philip & Foster (1998) Nature 394: 27-28 )

*In situ* hybridisation histochemistry (ISHH) revealed that vertebrate ancient (VA) opsin is expressed in horizontal (H) and amacrine (A) cells of the fish retina

# Ignacio Provencio discovers melanopsin in photosensitive dermal melanophores, brain and eye of *Xenopus laevis*



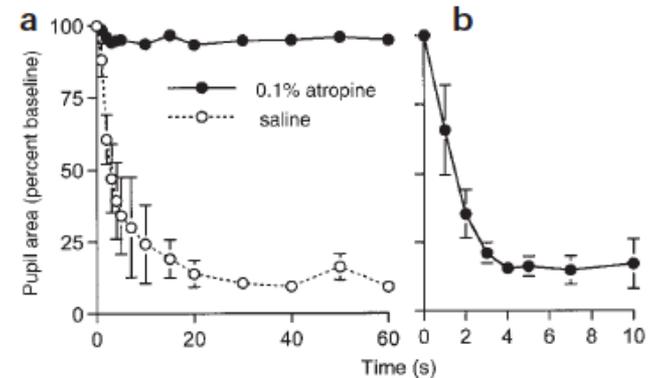
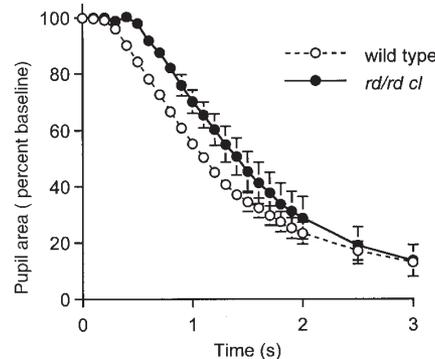
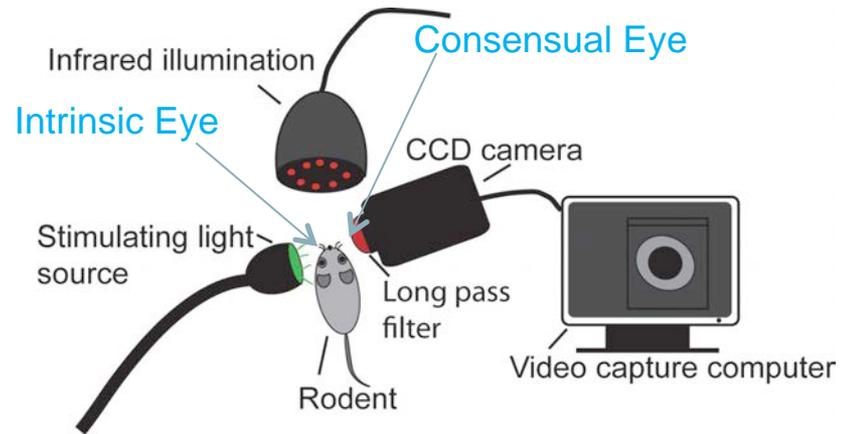
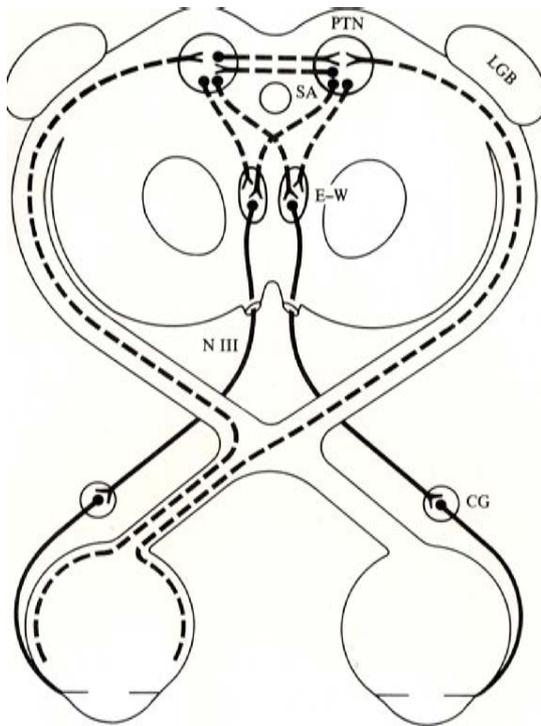
ISHH for melanopsin mRNA shows antisense signal (white) in the retina (INL) / iris (B) and hypothalamus (E). The adjacent sections (C&F) are sense probe controls.



## Foster lab at Imperial College London generated mice lacking rods and cones (*rd/rd cl* mice)

- Following a 15 minute exposure to green light the *rd/rd cl* mice still had:
  - Circadian phase shifting (Freedman et al., **Science** (1999) 284 502-504)
  - Suppression of pineal melatonin (Lucas et al., **Science** (1999) 284 505-507)
- The *rd/rd cl* mice also retain a pupillary light reflex (PLR)
  - Lucas, Douglas and Foster (2001) **Nature Neuroscience** 4(6) 621-626

# A non-rod, non-cone photoreceptor regulates the Pupillary Light Reflex (PLR)

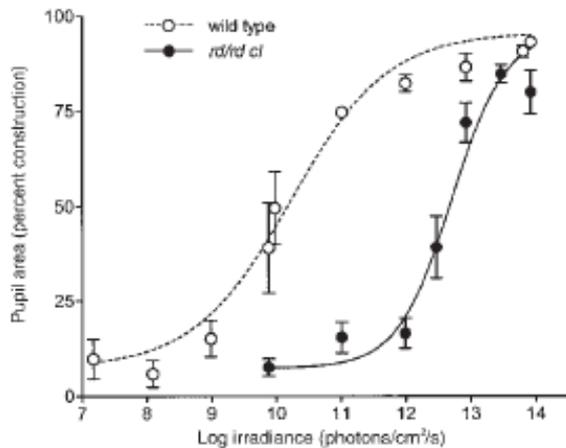


## Neural circuitry of the PLR

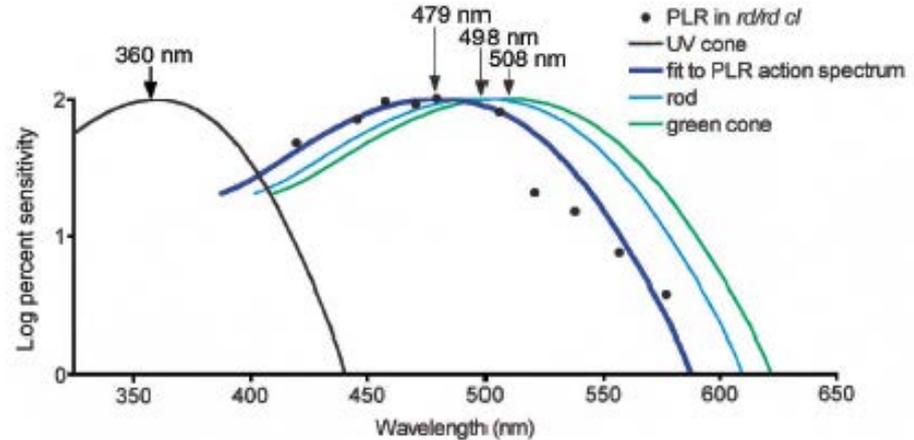
(Lucas et al., (2001) Nature Neuro. 4(6) 621-626 )

The PLR in *rd/rd cl* mice is abolished by topical atropine application (a) and can be elicited in the consensual eye (b). Therefore, the response is mediated by connections to the brain (3 mW/cm<sup>2</sup> white light).

# The spectral properties of this new photoreceptor were defined using the PLR in *rd/rd cl* mice

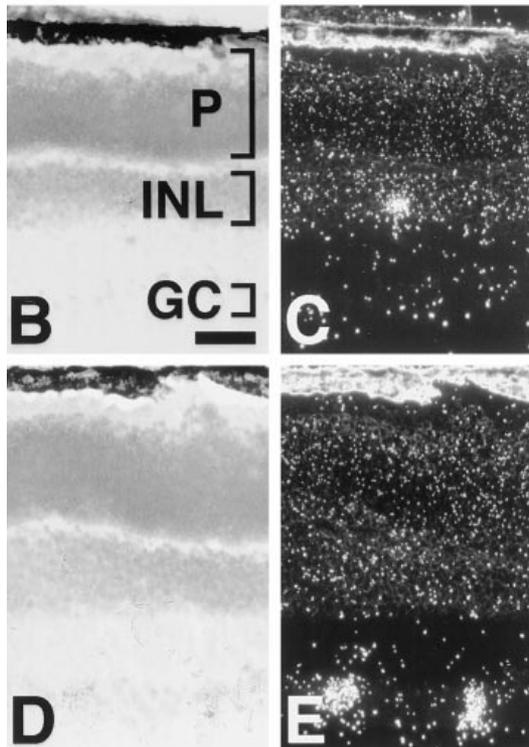


Irradiance response to 506 nm monochromatic light

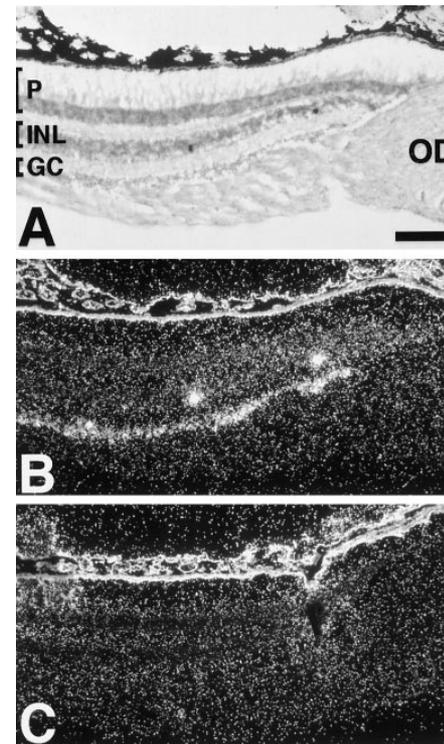


The action spectrum for the unidentified Photopigment peaks at 479nm (OP<sup>479</sup>)

# ISHH reveals melanopsin in the inner retina of mammals

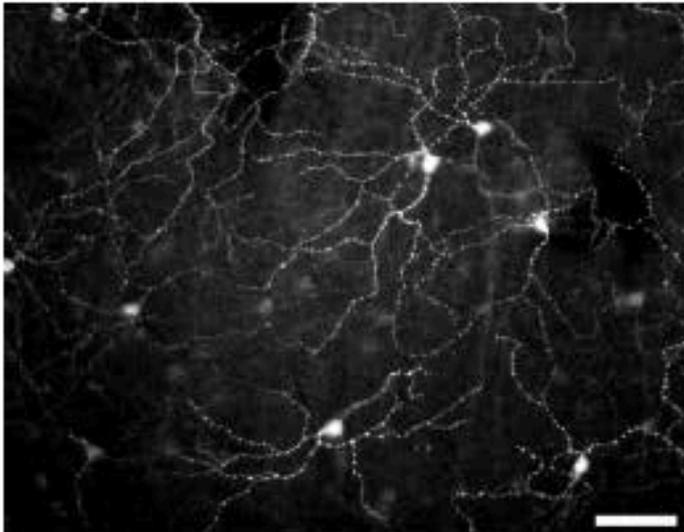


Mouse



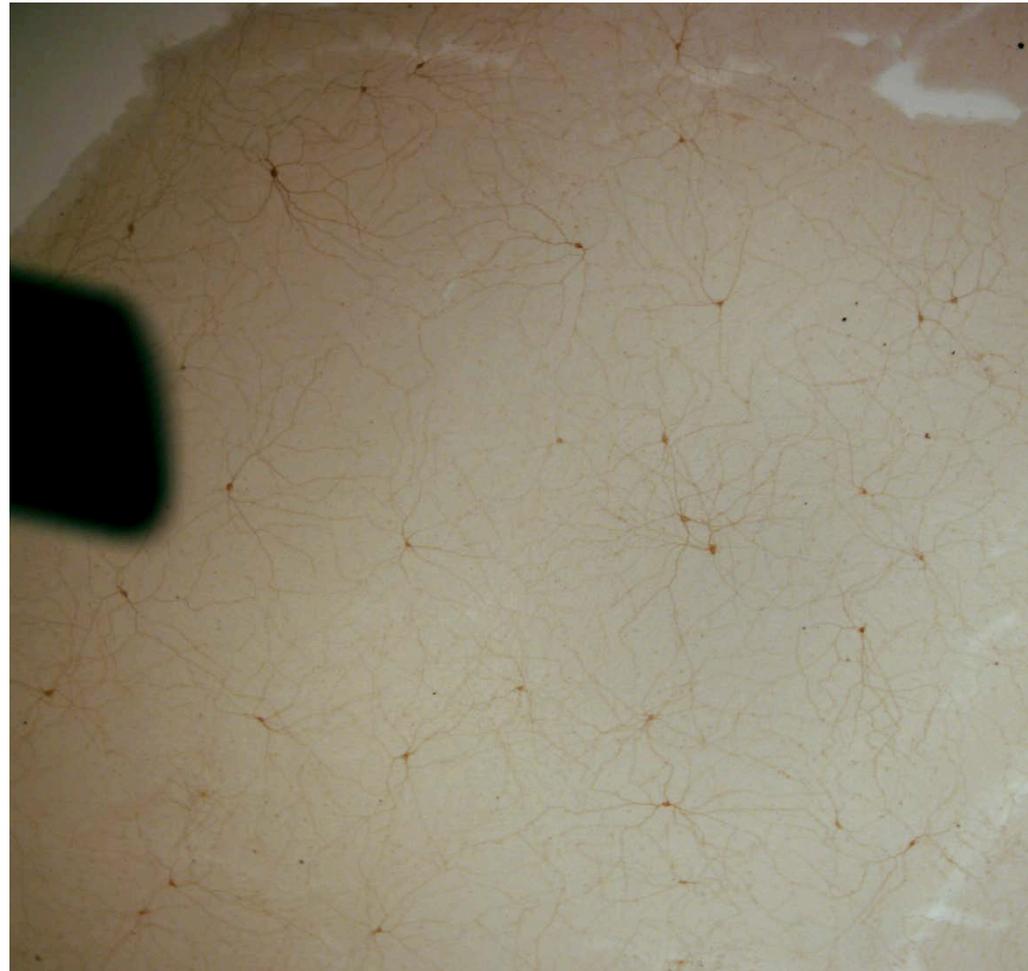
Monkey

# Antibodies to melanopsin reveal a network of ganglion cells in the inner retina

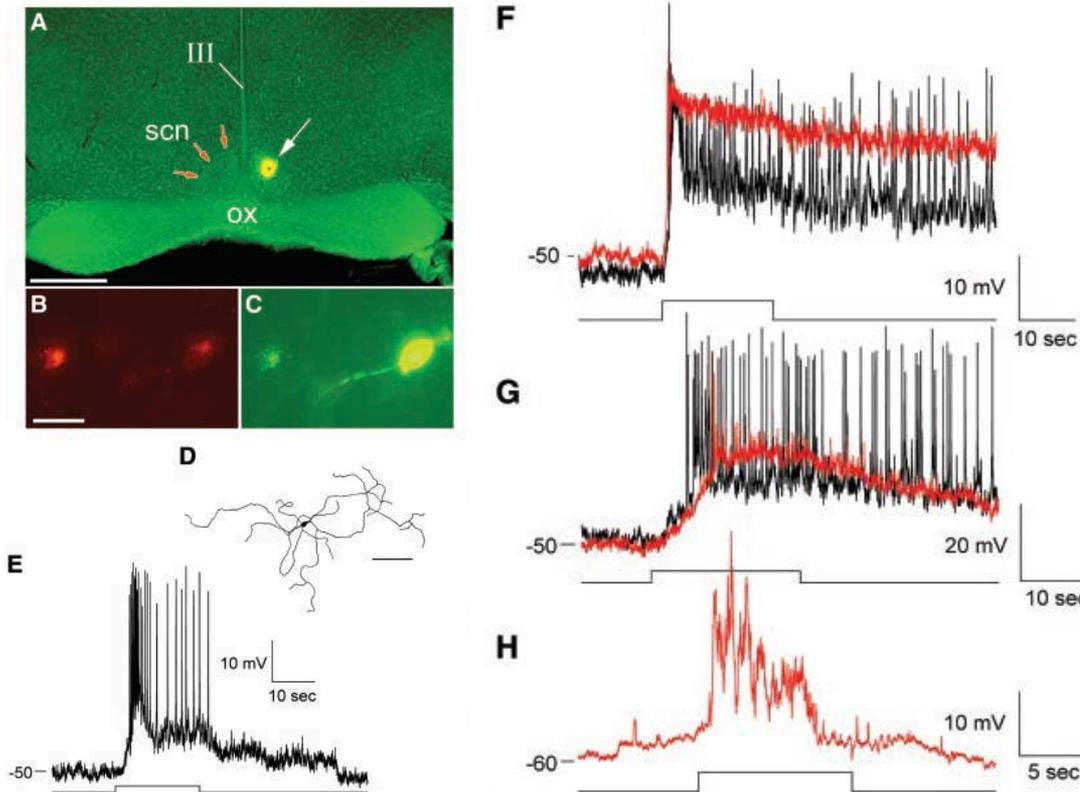


Above: Anti-melanopsin antibody revealed a network of cells in the inner retina of mice (Provencio et al., *Nature* (2002) 415 493).

Right: Melanopsin cells in the human retina



# Ganglion cells of the retinohypothalamic tract shown to be intrinsically photosensitive and referred to as intrinsically photosensitive RGCs



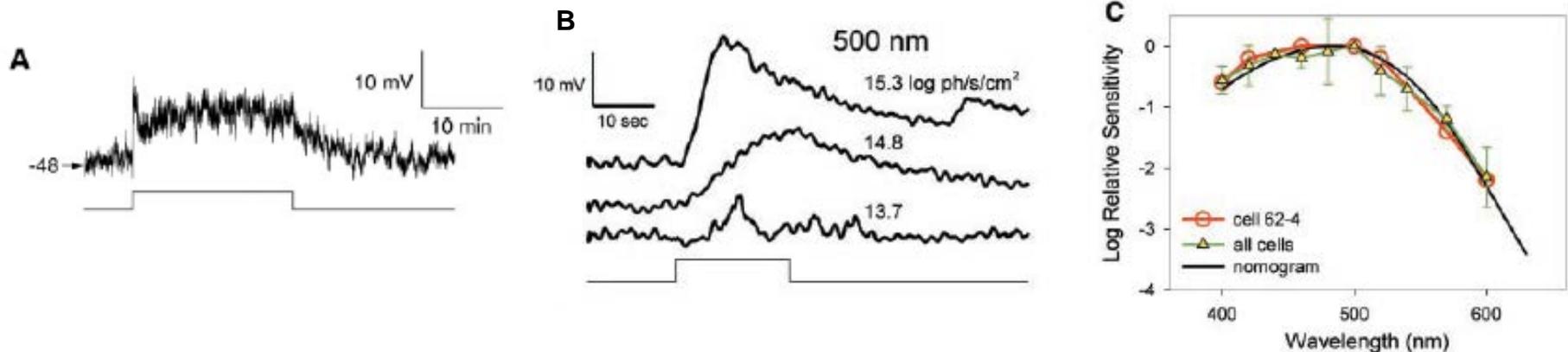
It had been suspected that retinal ganglion cells which project to a specialised region of the hypothalamus called the suprachiasmatic nucleus (SCN) may be the melanopsin positive cells.

Fluorescent beads were injected into the rat SCN. This retrogradely labeled ganglion cells in the retina which were found to depolarise in response to prolonged light exposure (measured using whole-cell patch clamp recordings).

The intrinsic light response was demonstrated by bathing the cells in 2mM  $\text{CoCl}_2$  (red traces), either alone (F), or together with additional drugs to block glutamatergic signals from rods/cones (G).

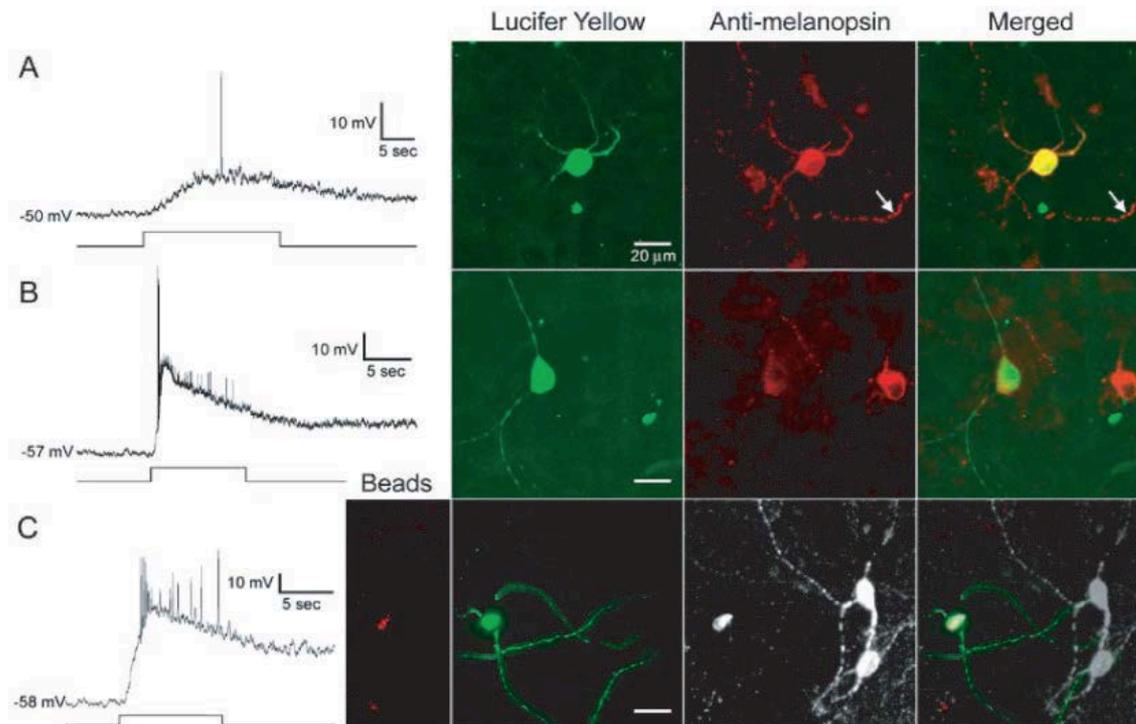
Physically isolated cells (H) also retained their intrinsic light response.

# Properties of the intrinsic light response are similar to the irradiance response properties of the PLR



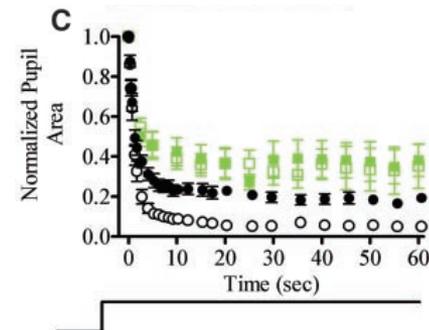
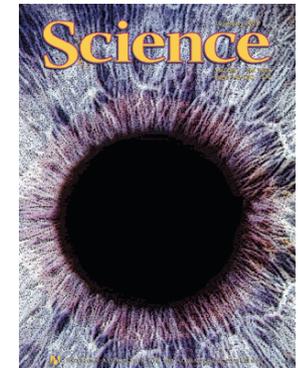
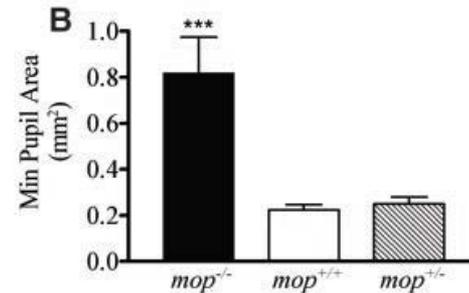
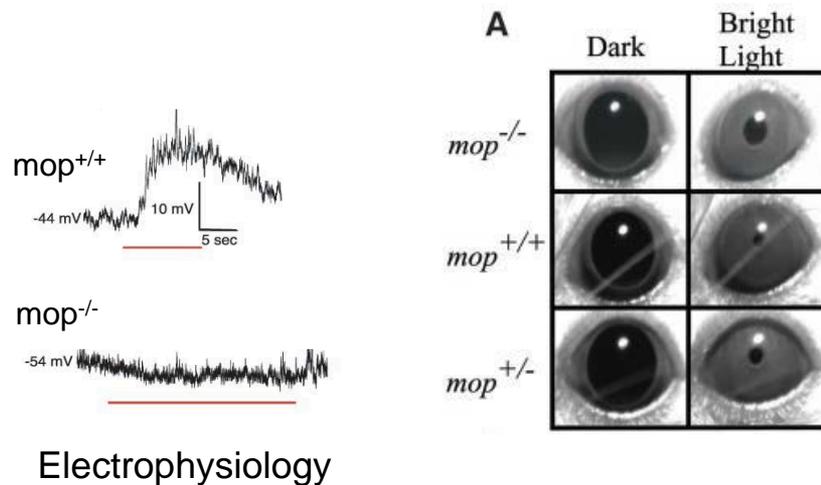
The electrophysiological response of intrinsically photosensitive retinal ganglion cells can be sustained for long periods of time (**A**) and is dependent on irradiance (**B**), with a peak sensitivity around **480nm** (**C**).

# These cells also express melanopsin



Retinal ganglion cells were retrogradely labeled (from the SCN) with fluorescent beads to enable whole-cell recordings. At the end of recording, cells were filled with the fluorescent dye Lucifer yellow (green). Labeling of these cells with an antibody against rat melanopsin confirmed their identity.

# Melanopsin-knockout eliminates the intrinsic light response of ipRGCs and reduces the PLR at high Irradiance

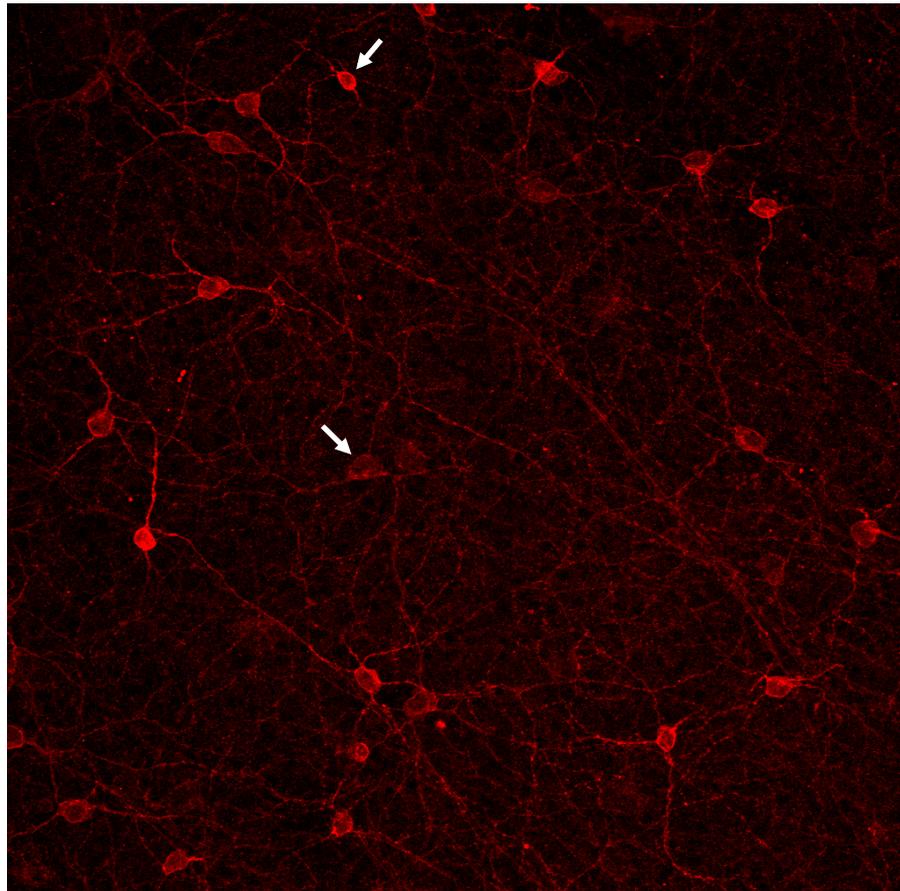


Melanopsin-knockout (*mop*<sup>-/-</sup>) mice were generated, where the ipRGCs remain but lack melanopsin and do not respond intrinsically to light (see intrinsic light responses on the left). As shown in **A** and **B**, unlike wildtype (*mop*<sup>+/+</sup>) and heterozygote (*mop*<sup>+/-</sup>) mice, *mop*<sup>-/-</sup> mice could not quite achieve a full pupil constriction under bright light (monochromatic 480nm, 145μW cm<sup>2</sup>). The *mop*<sup>-/-</sup> mice can sustain pupillary constriction for 60 seconds like wildtypes (**C**) and can sustain the same level of constriction under low irradiance (0.12μW cm<sup>2</sup>, green squares) but not high irradiance (110 μW cm<sup>2</sup>, black circles).

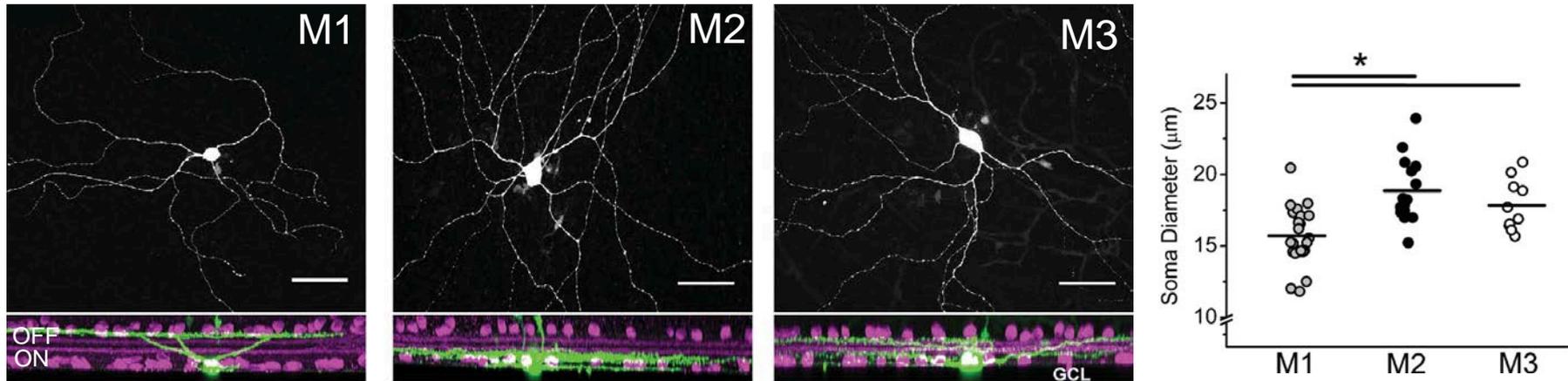
## Overview:

- The discovery of intrinsically photosensitive Retinal Ganglion Cells (ipRGCs)
- Structure and function of ipRGCs
  - Anatomy and physiology
  - How do ipRGCs contribute to visual function?

# There is more than one type of ipRGC in the mouse retina



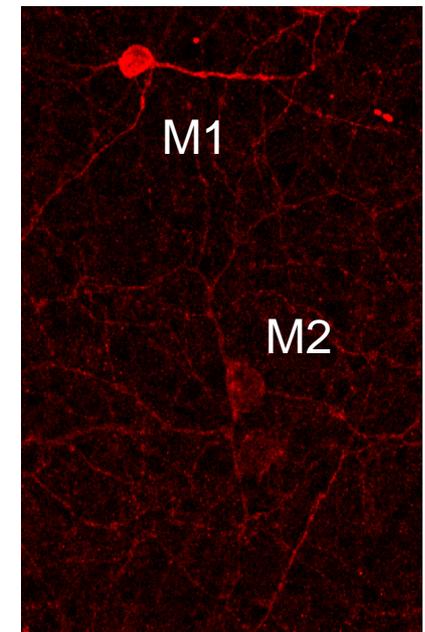
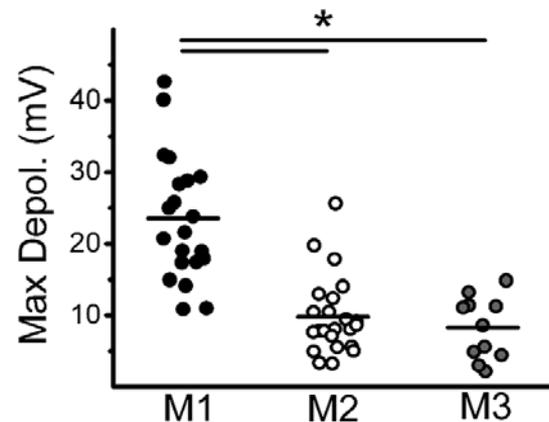
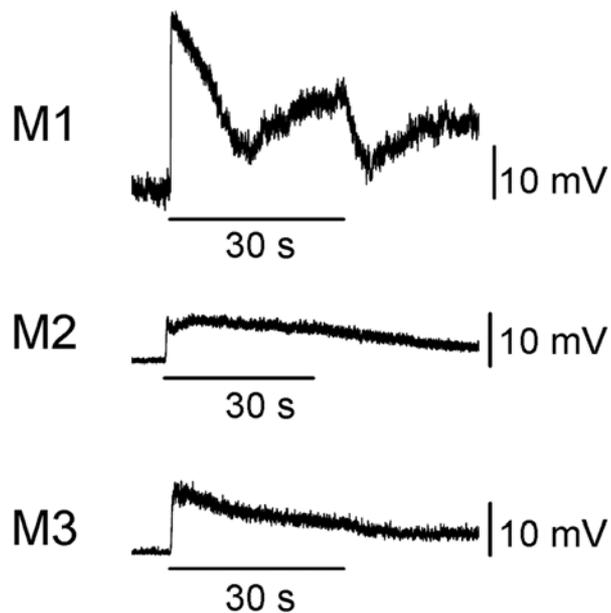
# Three main types of ipRGC were originally distinguished on the basis of dendritic stratification



The three types of ipRGC (M1, M2 and M3) are shown in green (filled with neurobiotin), with a marker for cholinergic amacrine cells in magenta (to delineate ON and OFF sub-regions of the inner plexiform layer). The M1 cells (smallest soma diameter) extend dendrites into the OFF subdivision, while M2 cells extend dendrites into the ON subdivision only. M3 cells extend dendrites into both ON and OFF regions (Schmidt and Kofuji *J. Comp Neurol.* (2011) 5(19) 1492-1504).

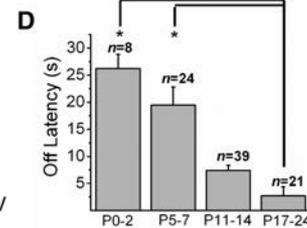
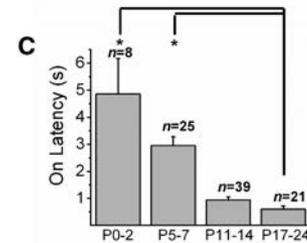
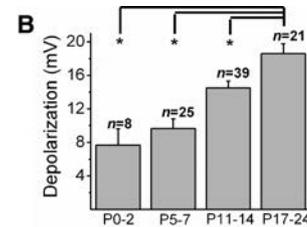
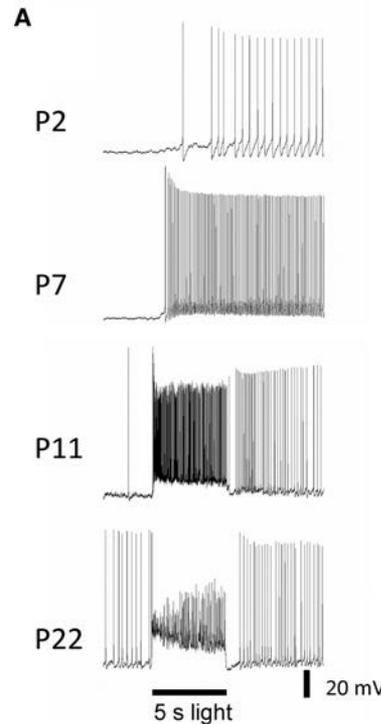
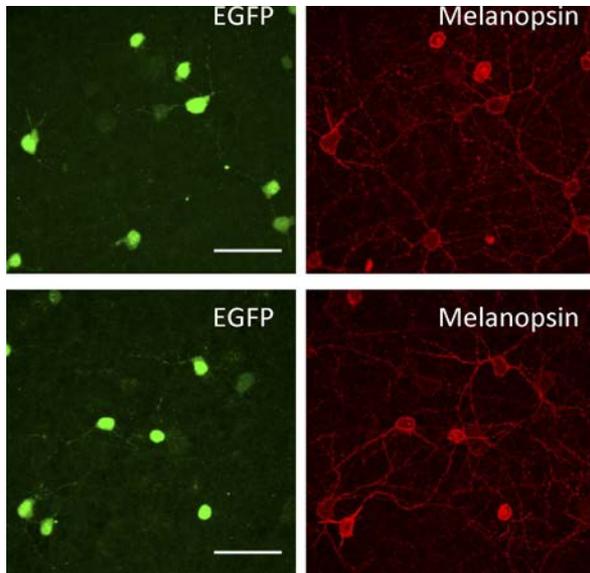
In general, M1-type ipRGCs have smaller cell bodies and express higher levels of melanopsin.

# The different types of ipRGC have distinct electrophysiological responses to light

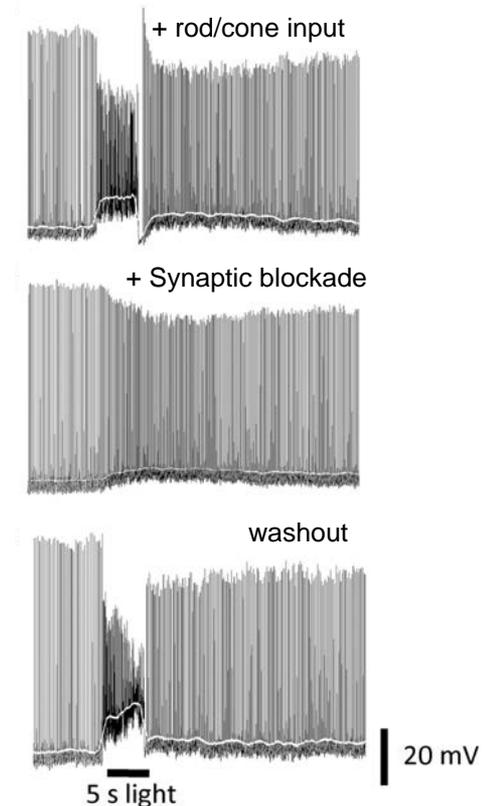


Patch-clamp recordings from ipRGCs in *Opn4-EGFP* mice (in the presence of synaptic blockade), reveal a stronger depolarisation to bright white light in M1-type cells. This is because M1 ipRGCs contain the highest levels of melanopsin (*Opn4*, stained red).

# Outer retinal input modulates the ipRGC light response



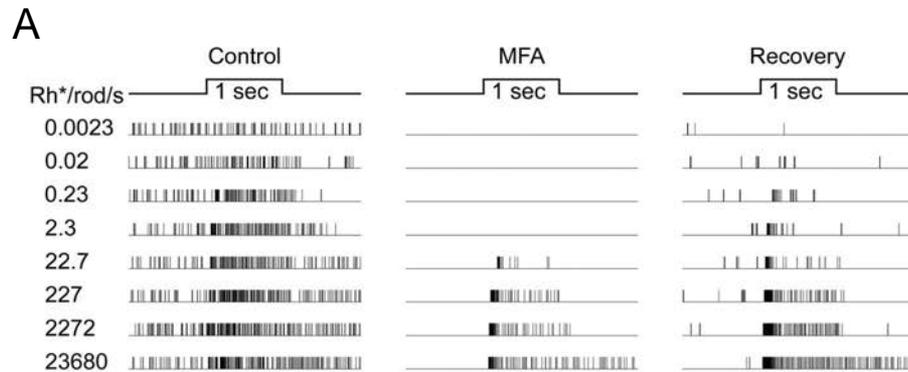
## P21 ipRGC response



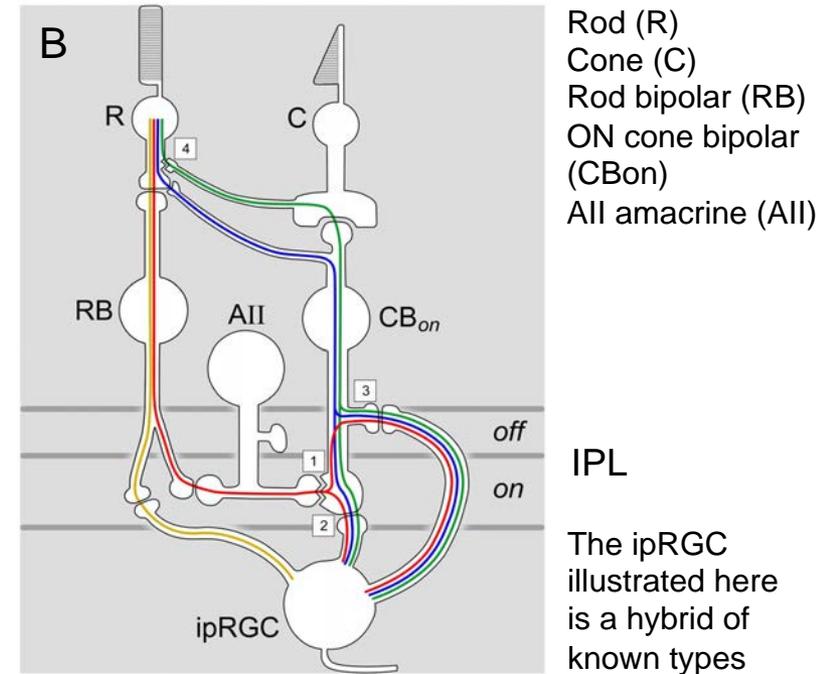
Schmidt et al., *J. Neurophysiol.* (2008) 100: 371 - 384

Whole cell patch-clamp recordings were made from ipRGCs in mice where enhanced green fluorescent protein (EGFP) is expressed under control of the melanopsin promoter. Strong evidence of rod/cone input from P11, with increased amplitude of ipRGC depolarisation and reduced On and Off latencies. A similar response is seen in postnatal day 21 (P21) mice plus or minus synaptic blockade induced by a cocktail of drugs that block glutamate receptors (right panel).

# ipRGCs receive sustained ON-type input from rods and cones

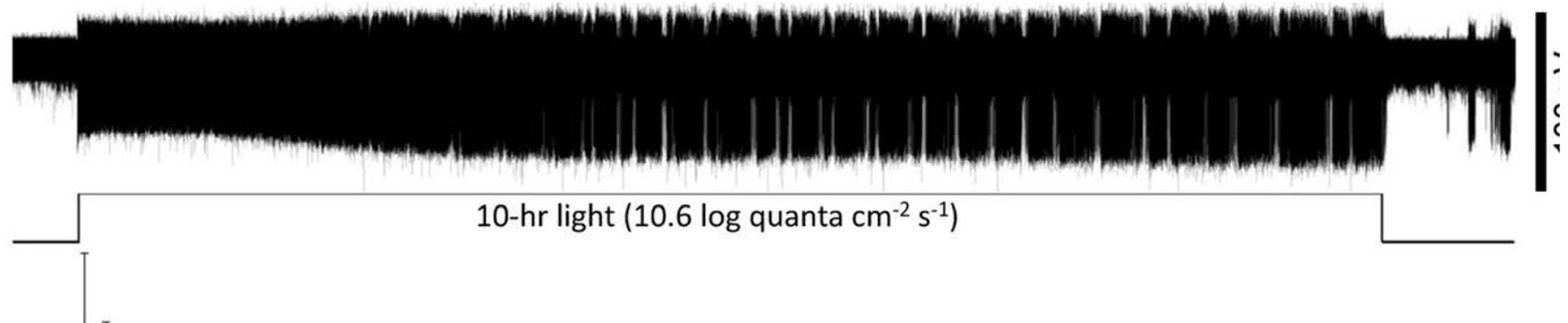


Weng et al., PLoS One. (2013) 8(6), e66480



**A.** Pharmacological blockade of gap junctions with 100µM meclofenamic acid (MFA) reduces sensitivity of synaptic input to ipRGCs. Light intensity is in Rh\*/rod/S (average rate of photoisomerisations/rod/second) and each line represents responses from a single ipRGC exposed to light stimuli (500nm) of increasing intensity (~1log unit increase per line). **B.** Schematic summary of routes by which rod signals could reach ipRGCs. Jagged lines indicate gap junctions at sites 1 and 4. The primary rod pathway is in red, secondary rod pathway in green and other novel pathways in blue and gold. It has been shown that M1 type ipRGCs predominantly receive “ON” type input despite ramifying in the “OFF” sublamina of IPL. This is due to “*en passant*” synapses made by ON bipolar cells as they pass through the OFF sublamina of IPL (Dumitrescu et al., J. Comp. Neurol. (2009) 517, 226-244; Hoshi et al., J. Neurosci. (2009) 29, 8875-83).

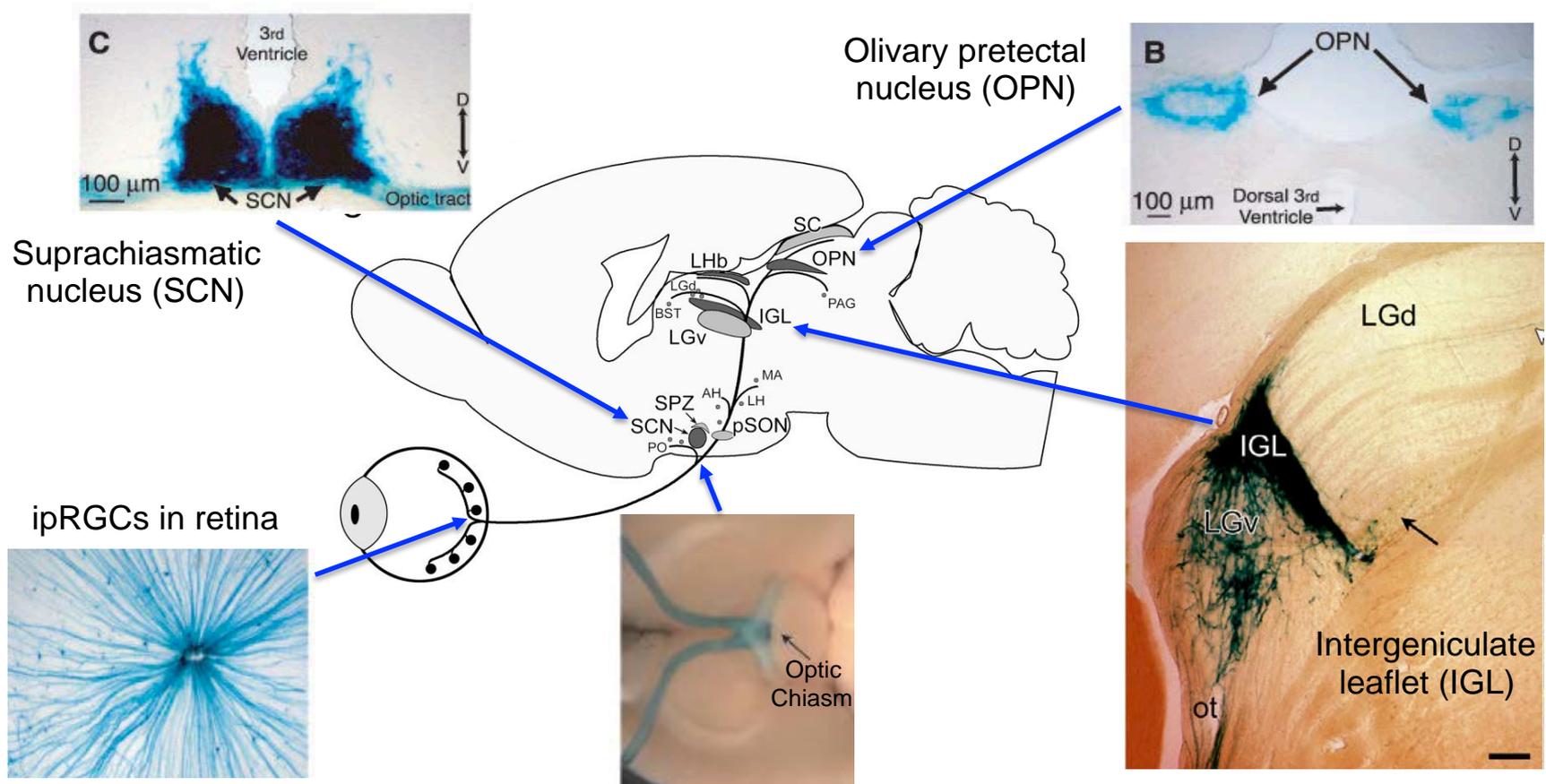
# ipRGCs signal irradiance throughout the day



Wong, *J. Neurosci.* (2012) 32(33): 11478 - 11485

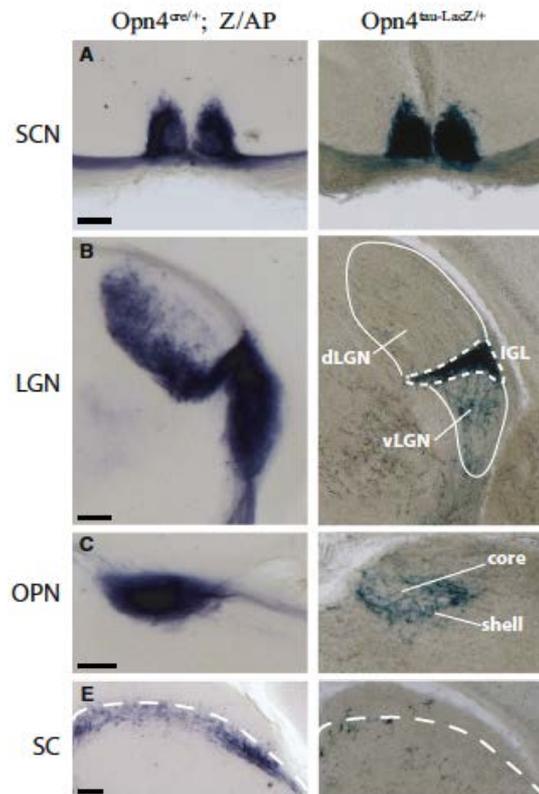
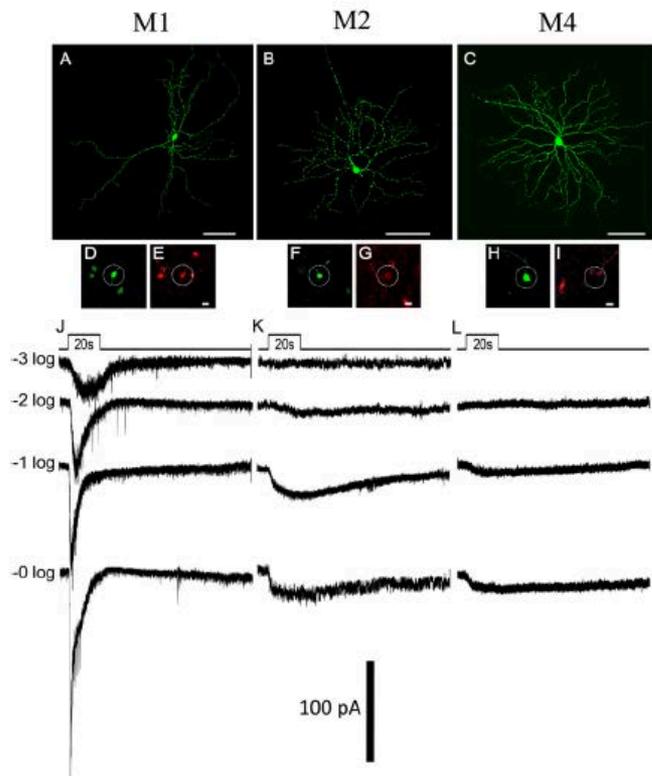
The recording above is from a single ipRGC in the absence of synaptic blockade. Similar recordings from ipRGCs in *Opn4*<sup>-/-</sup> mice also revealed tonic firing in ipRGCs driven by rods/cones.

# Where do ipRGC axons project to in the brain?



The sub-cortical targets of ipRGCs were first identified using melanopsin-knockout (*Opn4<sup>-/-</sup>*) mice, where the melanopsin gene (*Opn4*) is replaced by a gene for *tau-LacZ*. Axons can be visualised in these mice because the  $\beta$ -galactosidase enzyme is transported along axons due to the inclusion of *tau*. ipRGC axons are stained blue using the enzyme substrate (X-Gal staining). The main brain targets are shown in dark gray in the central diagram (sagittal plane).

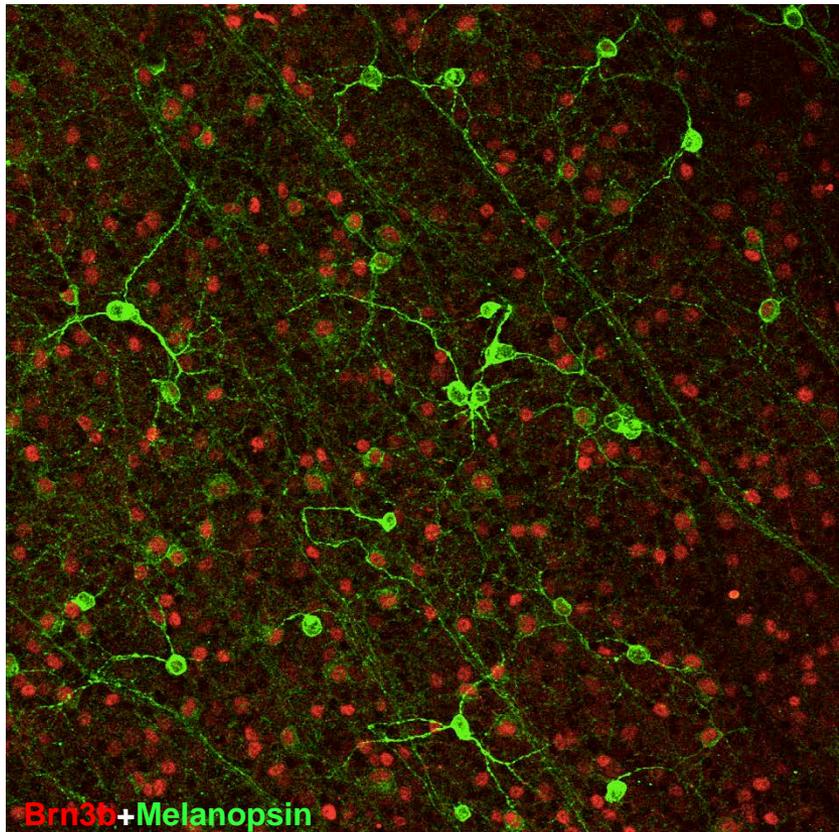
# A different reporter mouse reveals new types of ipRGC and more extensive projections to the dLGN and SC



The use of *Opn4<sup>cre</sup>* mice, where the Cre recombinase gene is knocked into the melanopsin gene sequence, has revealed new types of ipRGC (M4 and M5), together with more extensive projections to other brain regions than was previously seen with the *Opn4<sup>tau-LacZ</sup>* mouse (see panel on right for comparison). The most interesting of these are the dorsal Lateral Geniculate Nucleus (dLGN) and superior colliculus (SC).

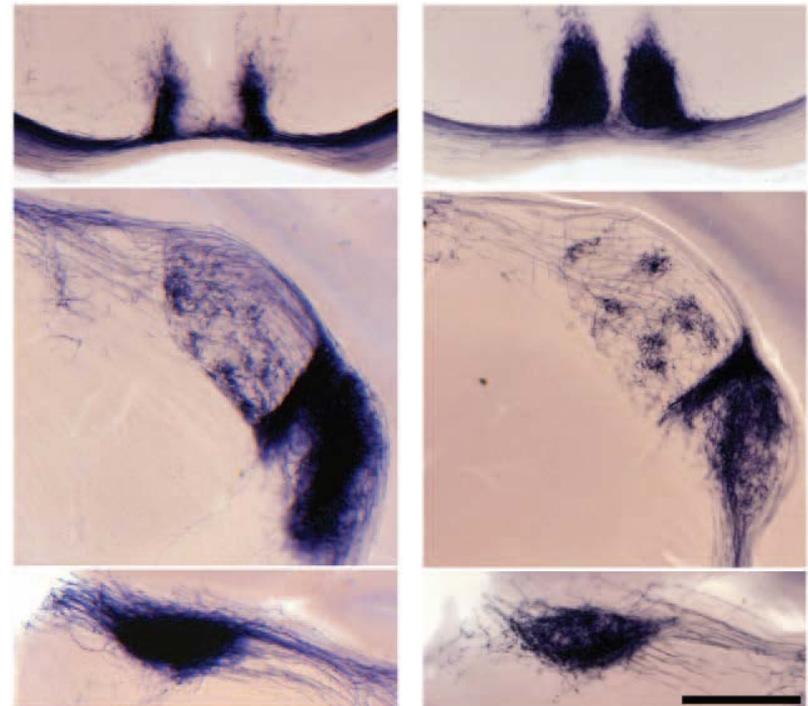
The panel to the left shows electrophysiological recordings from single M1, M2 and the new M4 type of ipRGCs. Note the large dendritic field and small intrinsic light response of M4 cells.

# An increasing diversity of ipRGCs...



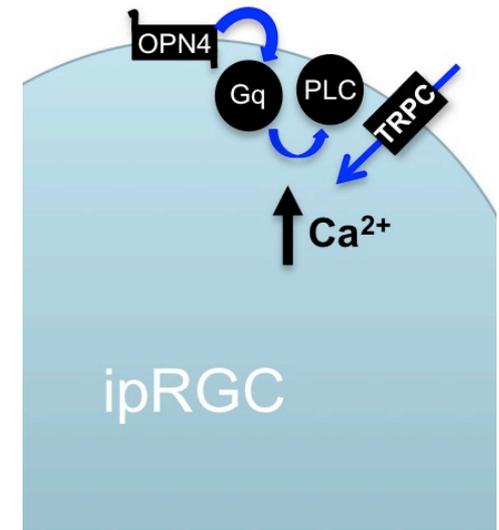
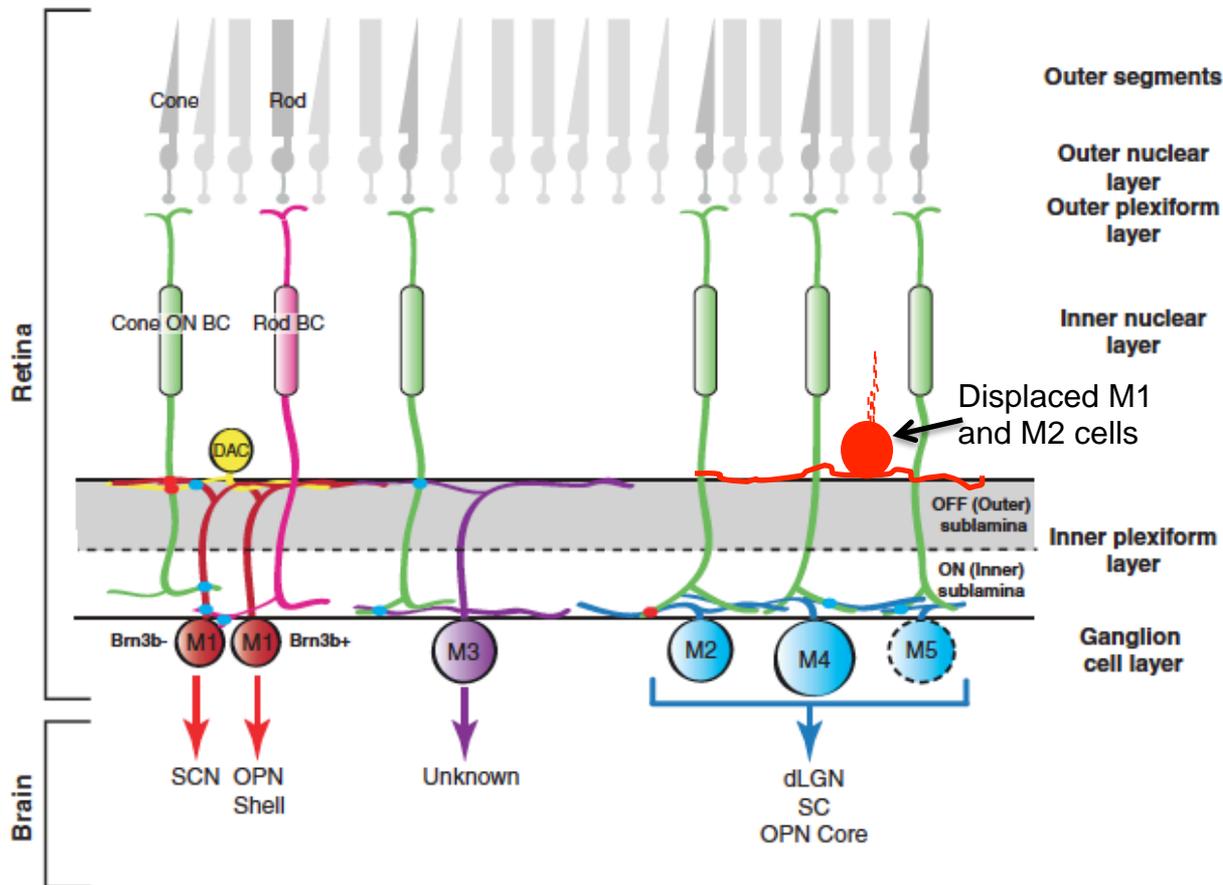
Only Brn3b expressing  
Melanopsin cells labeled

All melanopsin cells  
labeled



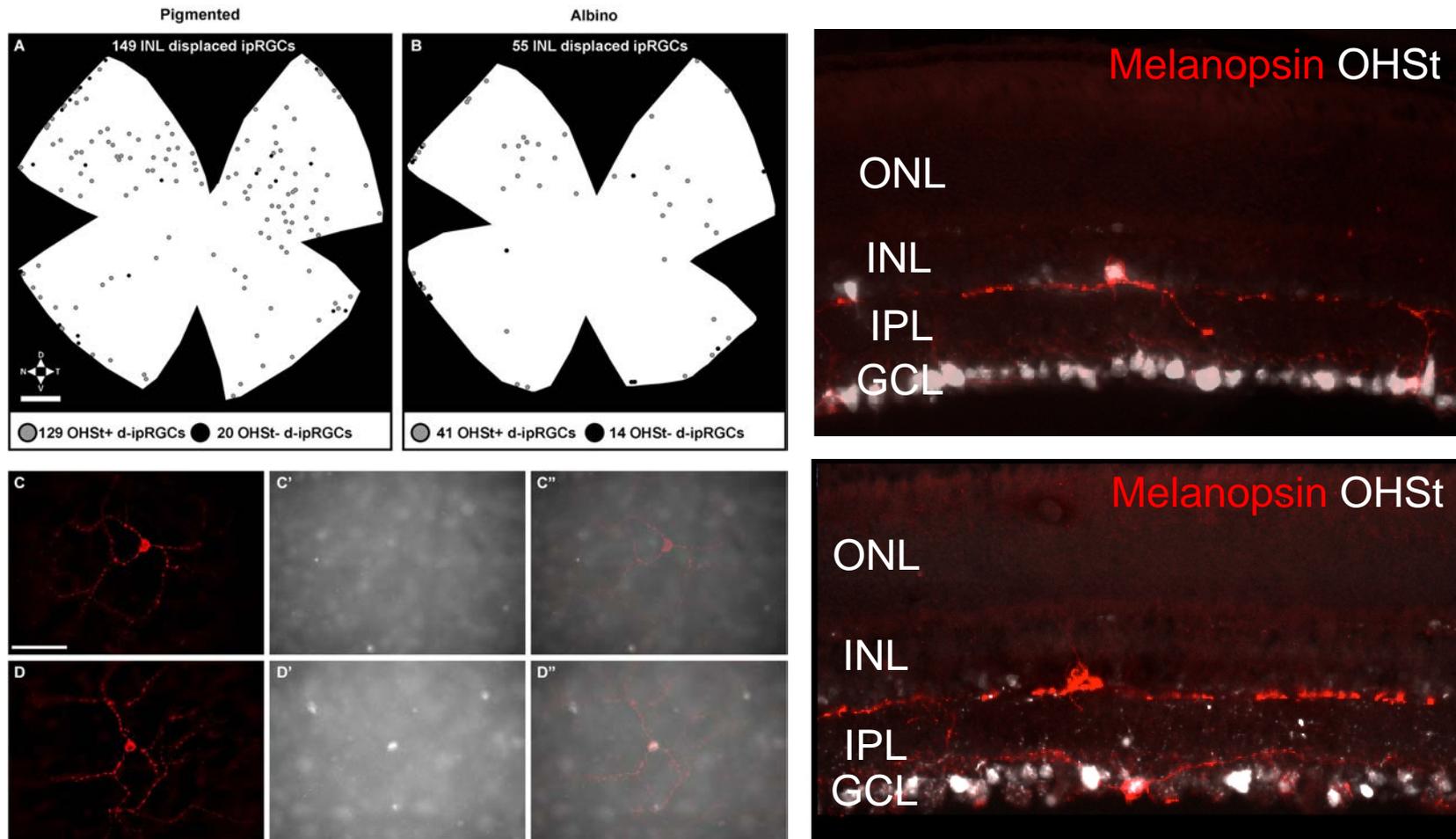
Some M1 type ipRGCs are negative for the transcription factor Brn3b. It has been found using transgenic reporter mice that these Brn3b negative M1 ipRGCs (approximately 200 cells) innervate the SCN and are sufficient to drive circadian photoentrainment.

# Summary of different ipRGC subtypes and their sub-cortical projections.



The phototransduction signaling cascade used by ipRGCs is “invertebrate-like”

# Some ipRGCs are displaced to the INL and ~14% of these also lack an axon to the brain



Retrograde tracer (OHSt) was applied to the severed optic nerve head.

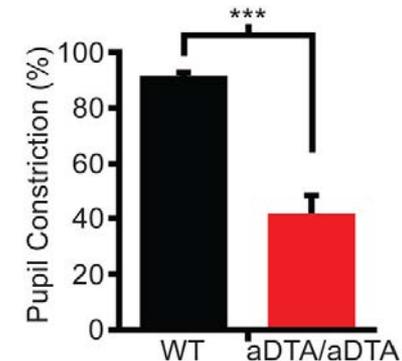
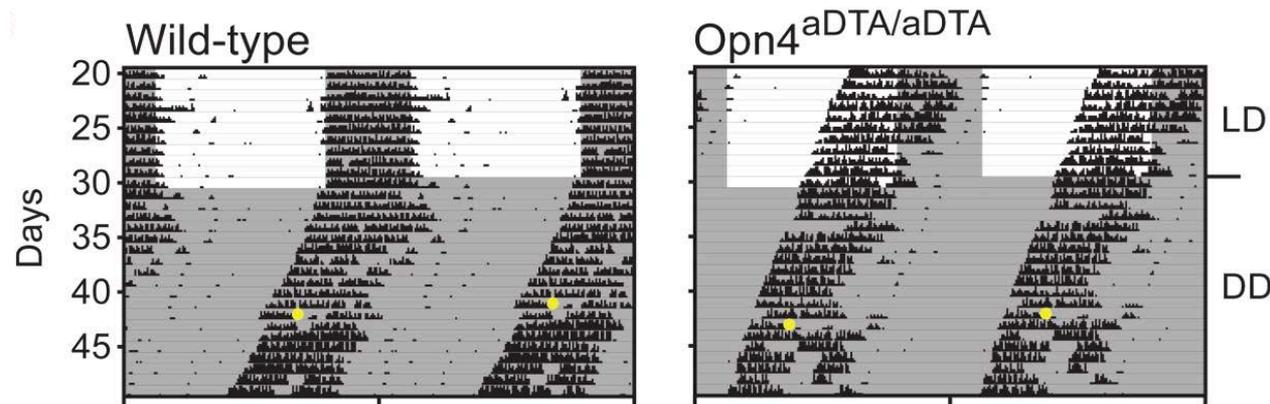
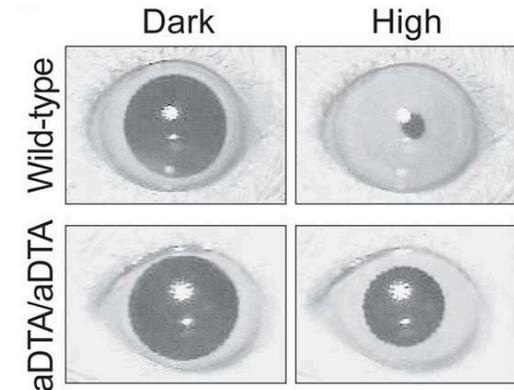
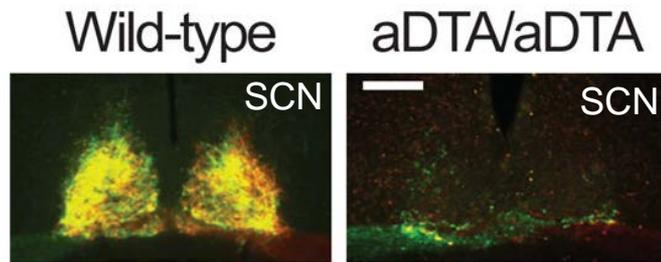
## Overview:

- The discovery of intrinsically photosensitive Retinal Ganglion Cells (ipRGCs)
- Structure and function of ipRGCs
  - Anatomy and physiology
  - How do ipRGCs contribute to visual function?

# Visual function

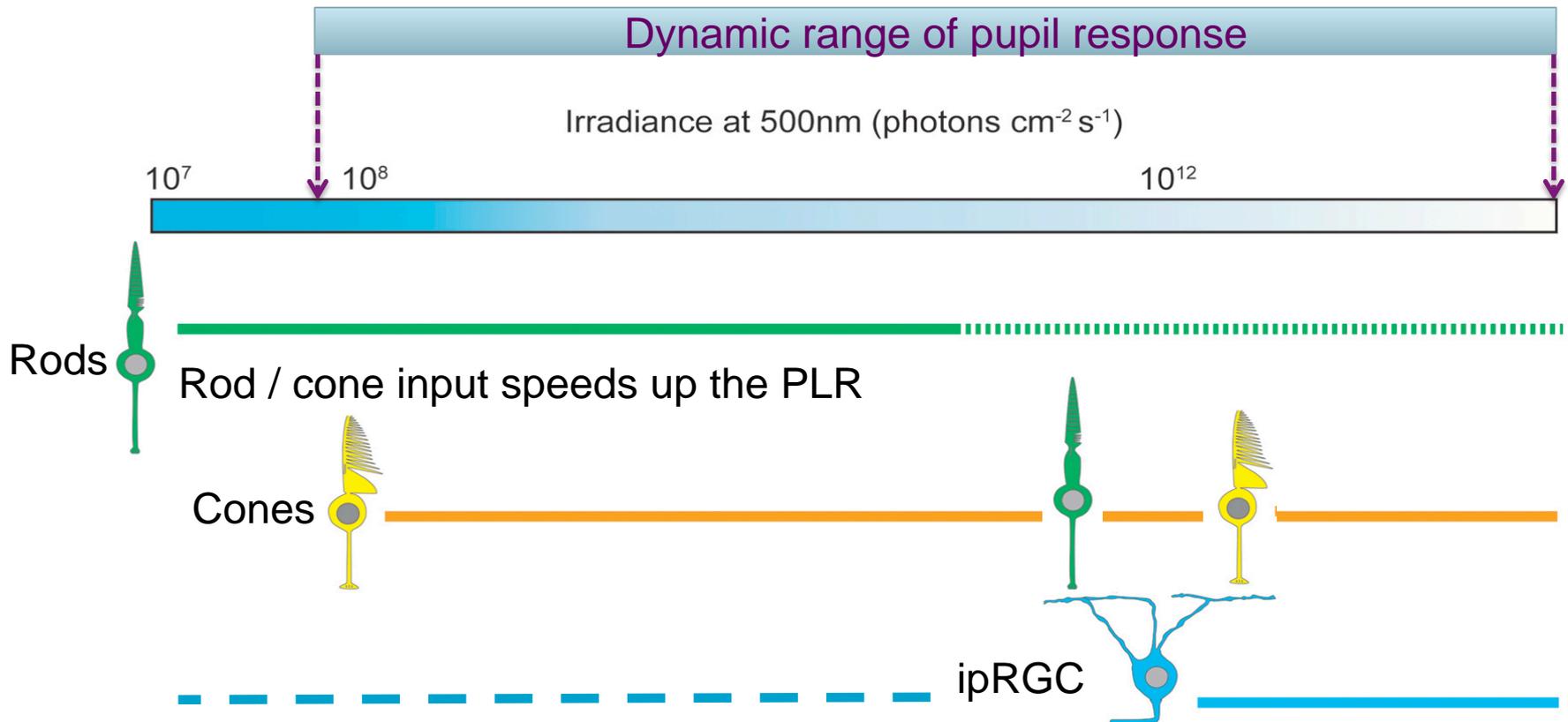
- Vision is often referred to as:
  - “Non-image forming” vision
  - “Image-forming” vision
- Non-image forming vision
  - Circadian physiology (photoentrainment, pineal melatonin, body temperature).
  - Pupillary light reflex (PLR)
  - Light perception
- Image-forming vision
  - Brightness, acuity, contrast, motion
  - Spatial and temporal dimensions

# Genetic ablation of cells expressing melanopsin causes severe deficits in phase shifting and PLR



To physically remove melanopsin cells, the attenuated diphtheria toxin A subunit was introduced into the mouse melanopsin gene locus. In mice homozygous for this mutation (aDTA/aDTA), there was a severe reduction in target innervation in subcortical brain regions, an abolition of circadian photoentrainment and only a 40% pupil constriction at the highest irradiances.

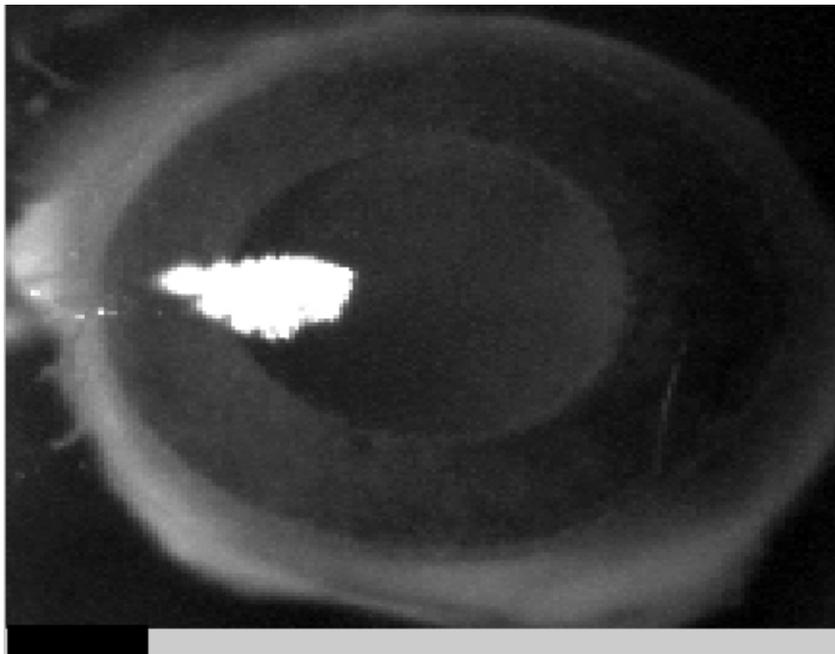
# Melanopsin sustains the PLR at high irradiance but ipRGCs signal over a wide dynamic range due to rod/cone input



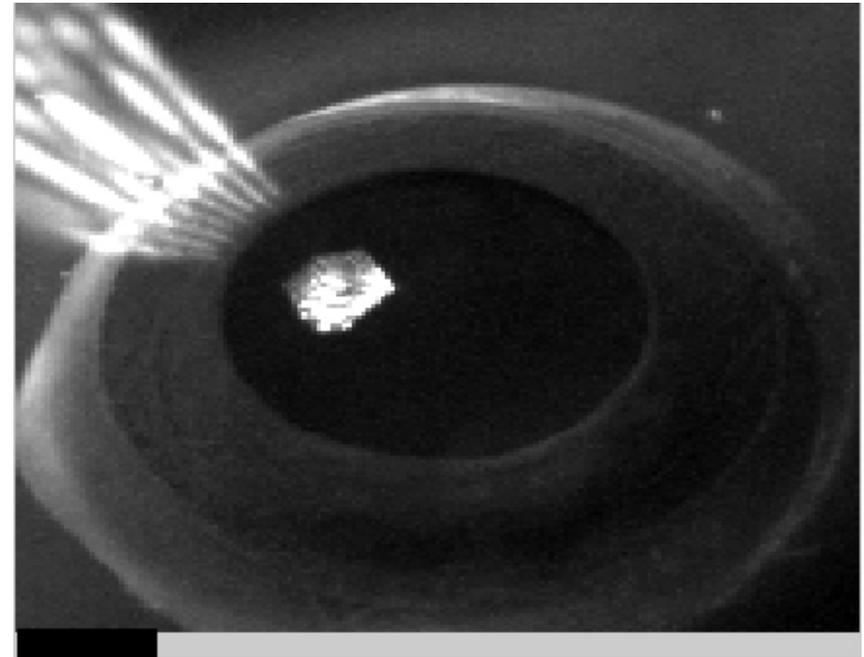
# In rodents, melanopsin maintains pupil constriction independently of the brain!



Adult Wildtype mouse



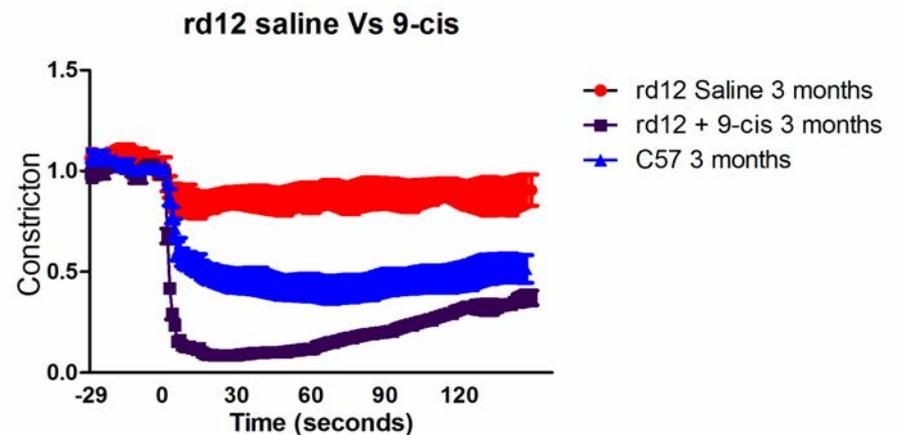
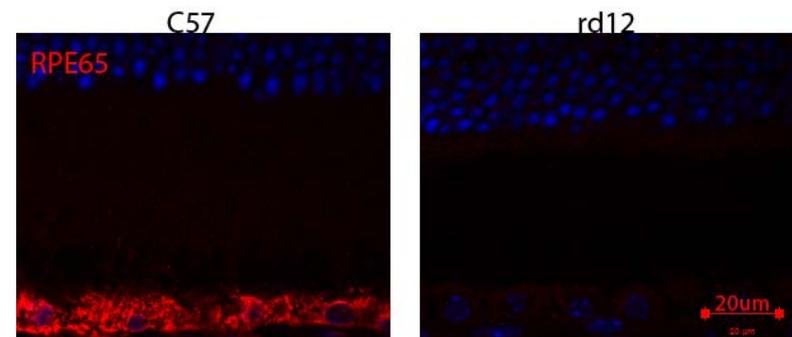
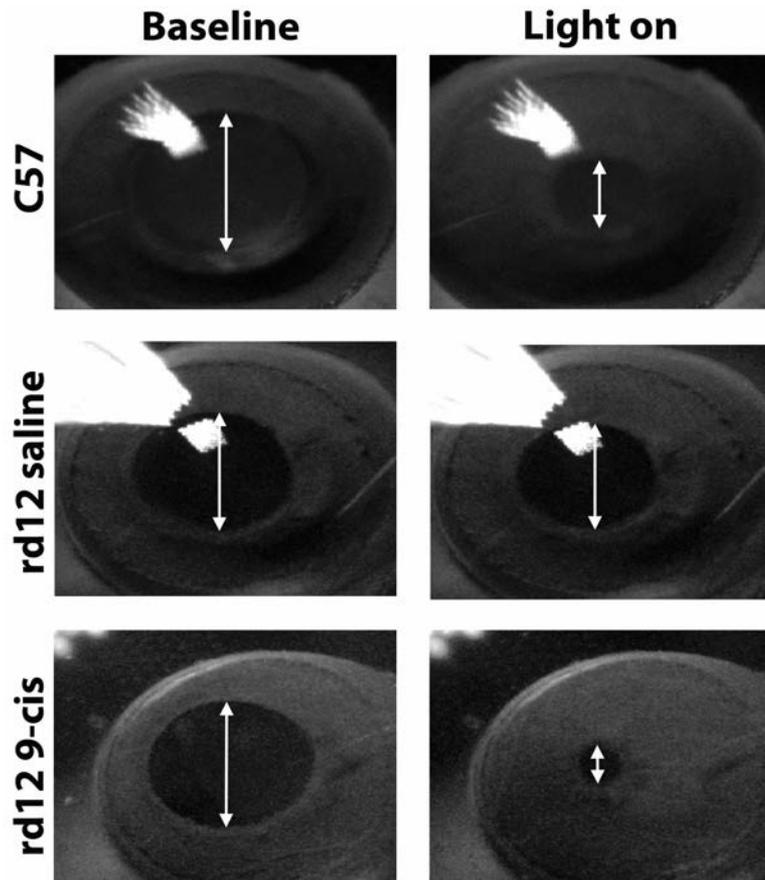
*Opn4*<sup>-/-</sup> mouse



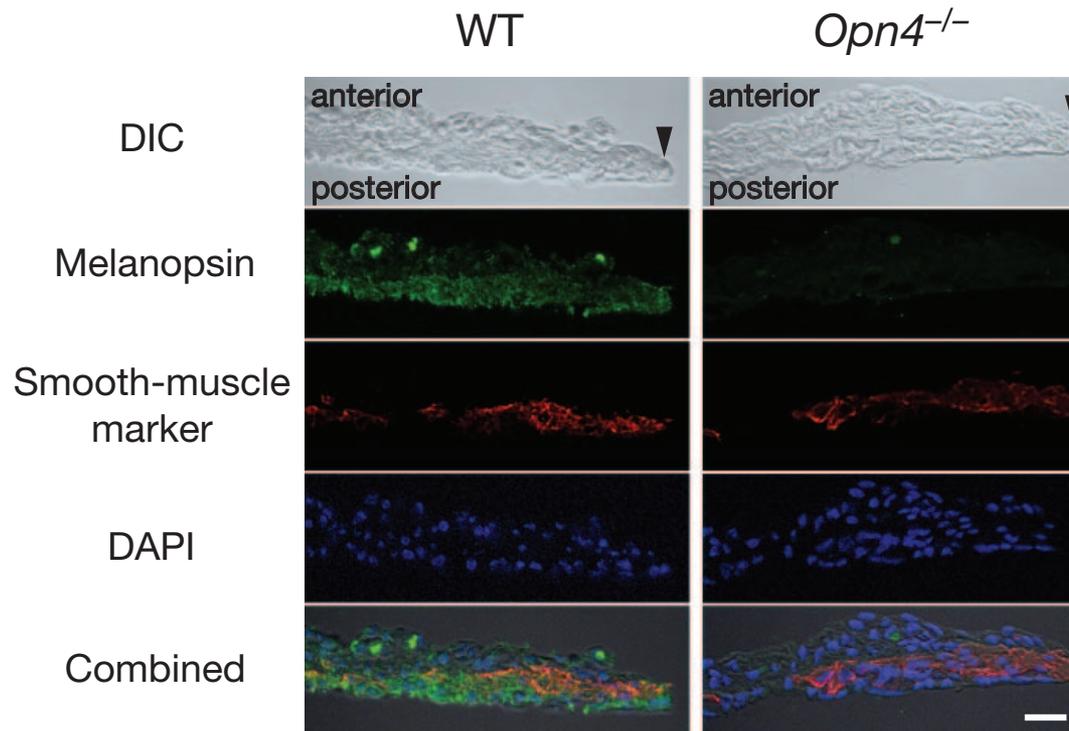
The intrinsic PLR (iPLR) is slower than the conventional PLR.

Xu et al., Nature (2011) 479: 67-73  
 Semo et al., Experimental eye research (2014) 119: 8-18  
 Vugler et al., Neuroscience (2015) 286 60-78

# The iPLR is deficient in rd12 mice which lack the RPE65 protein

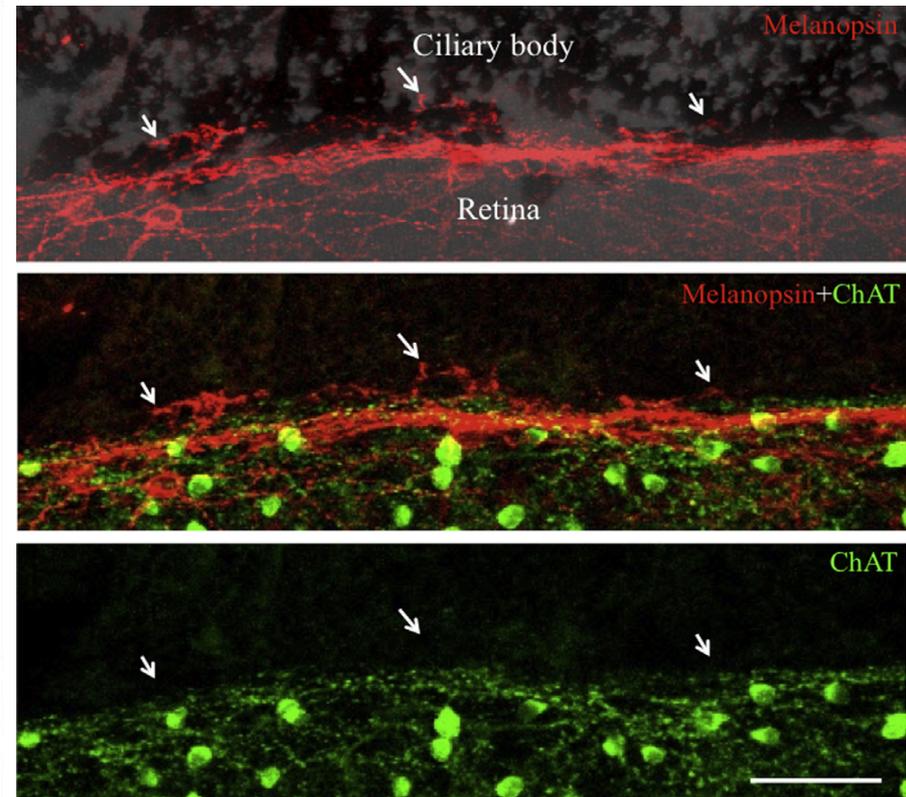
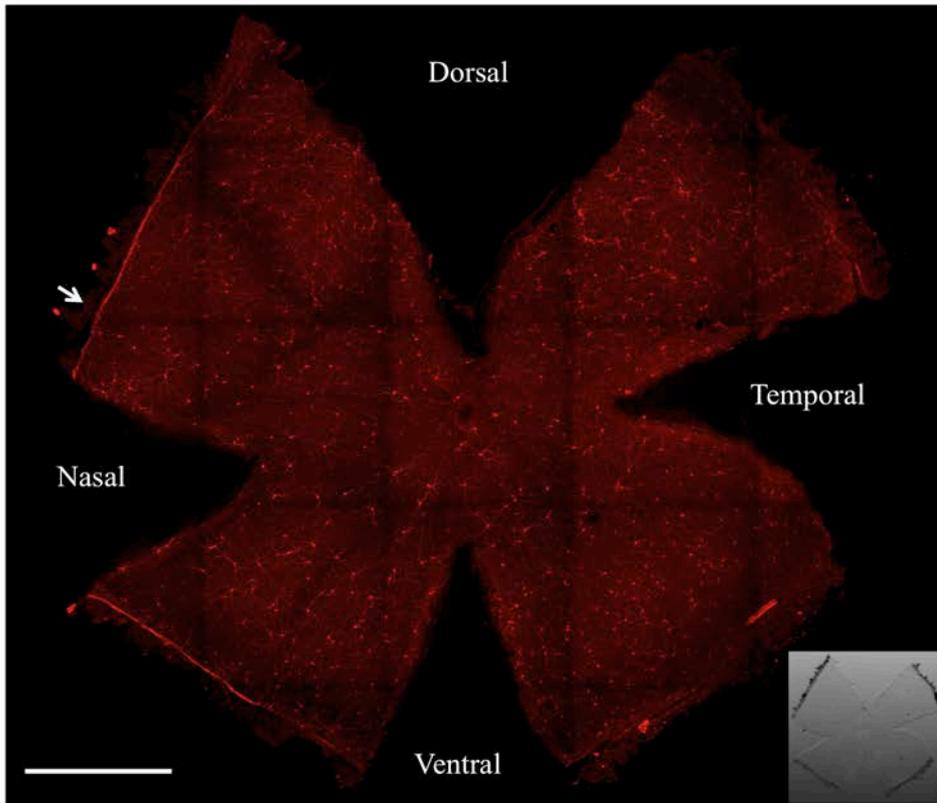


# The intrinsic PLR (iPLR) is thought to involve melanopsin in the iris



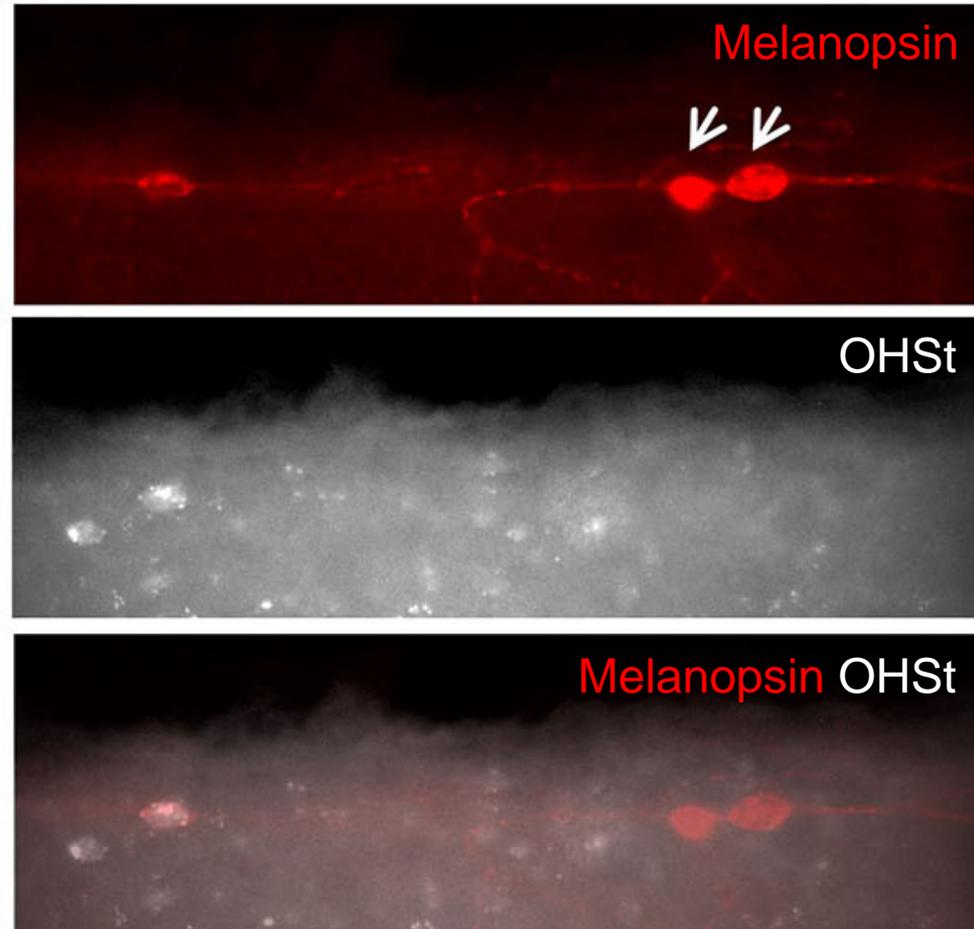
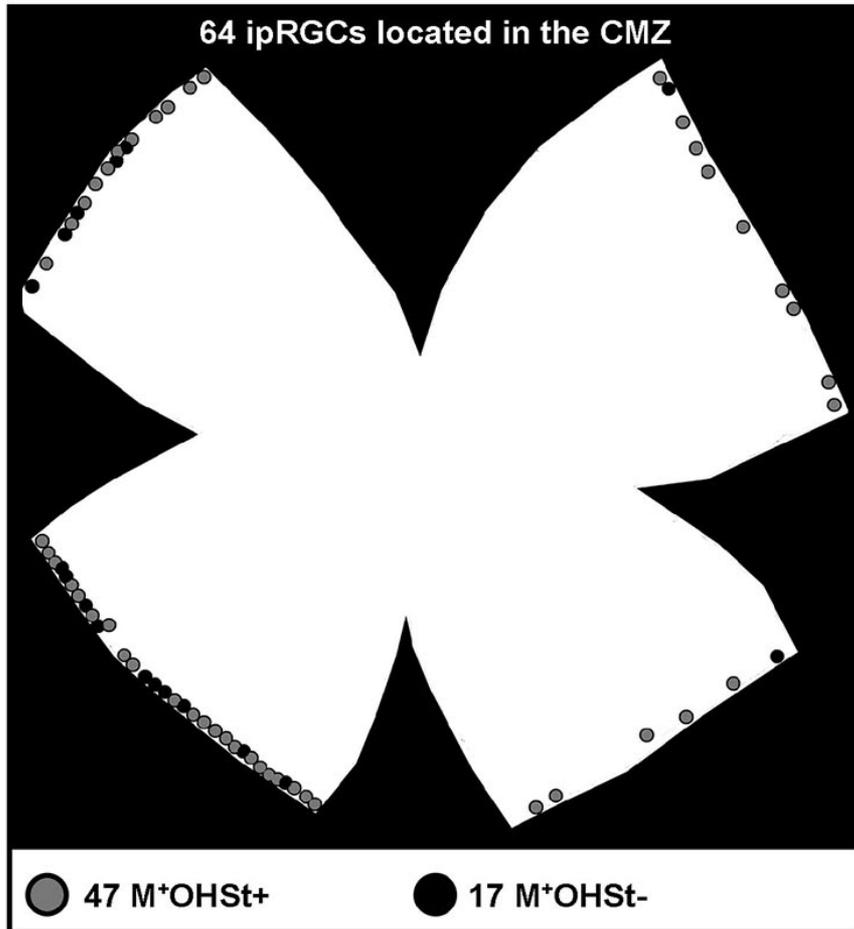
Iris sphincter muscle of the albino mouse eye  
 Stained for melanopsin and smooth muscle actin  
 (Xu et al., Nature (2011) 479: 67-73)

# In mice, ipRGCs at edge of retina also send small projections into the ciliary body...



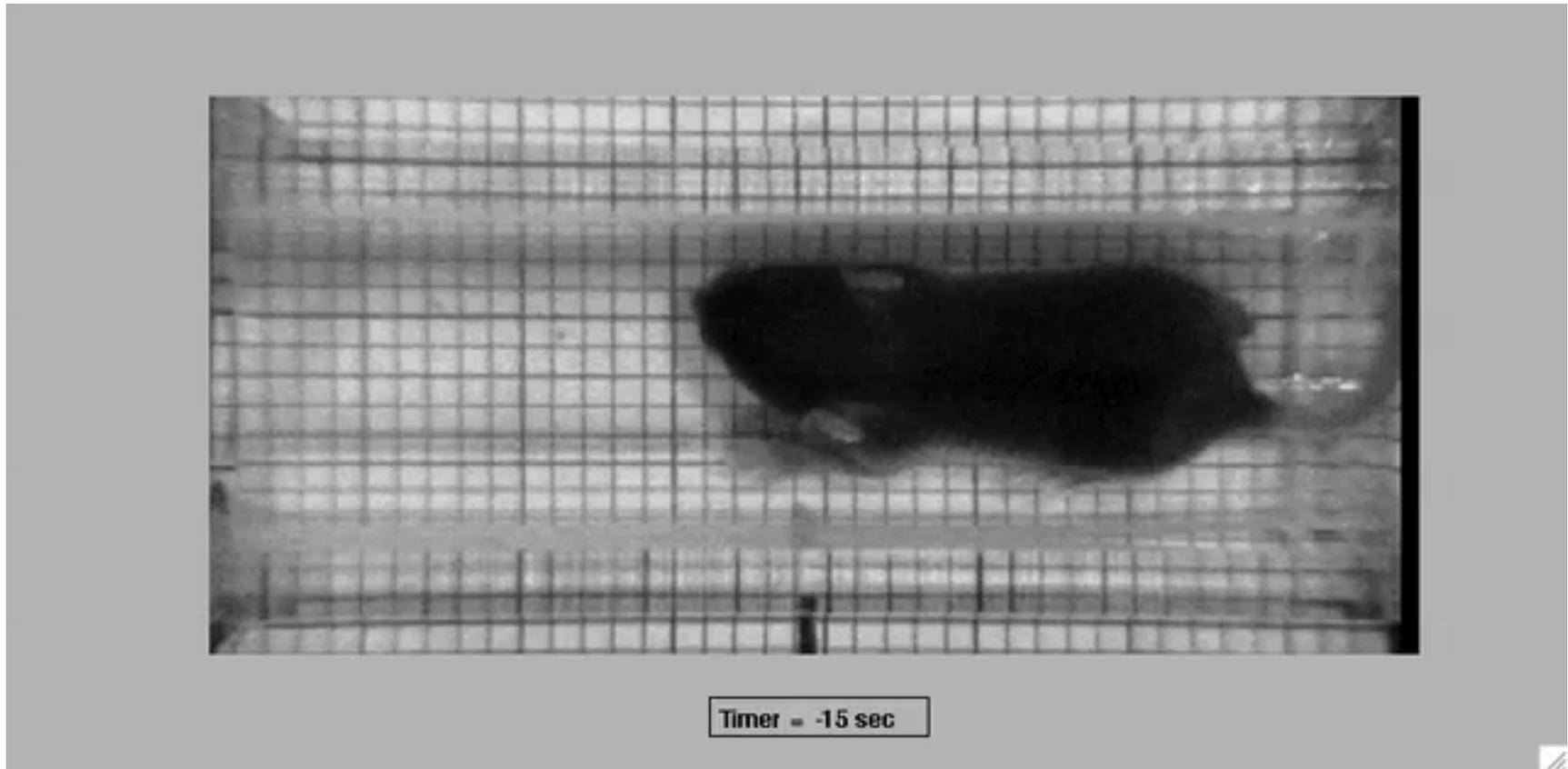
Melanopsin is also expressed at low levels in the ciliary body

# At the ciliary marginal zone (CMZ) ~20% of ipRGCs lack an axon to the brain



Demonstrated by application of the retrograde tracer OHSt to the severed optic nerve in wildtype pigmented mice.

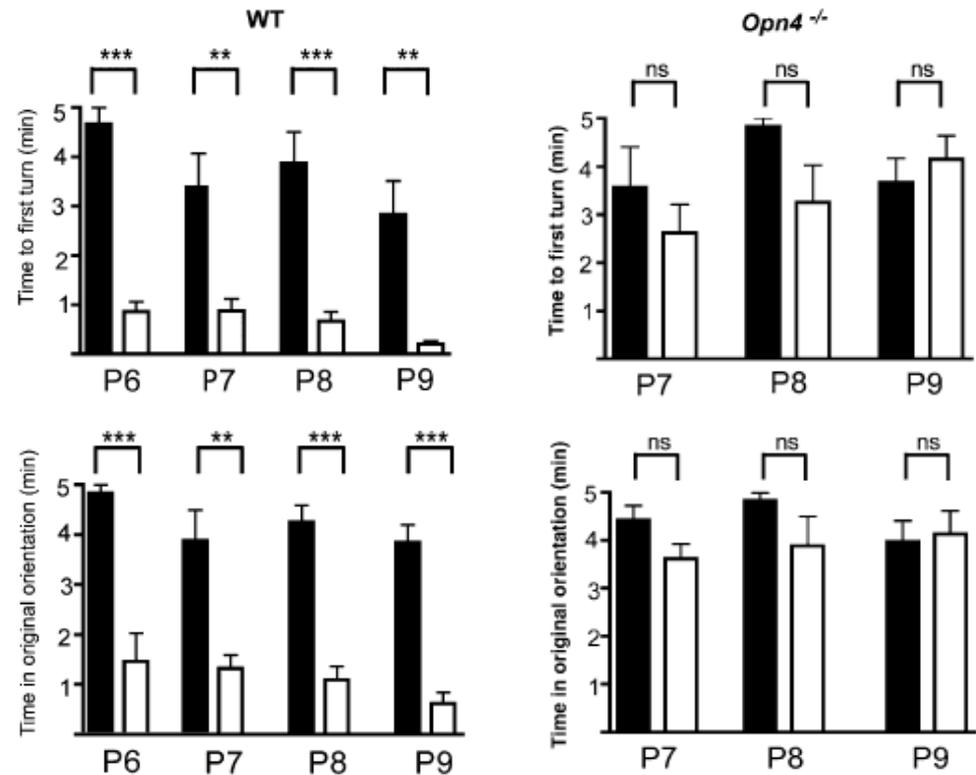
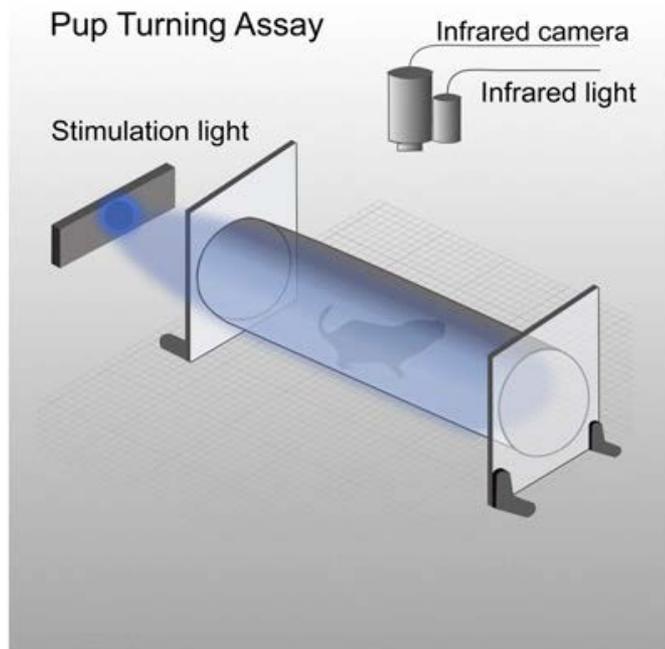
# A role for melanopsin in light perception ?



They used a turning assay to study light avoidance behaviour in neonatal mice (P6-P9)

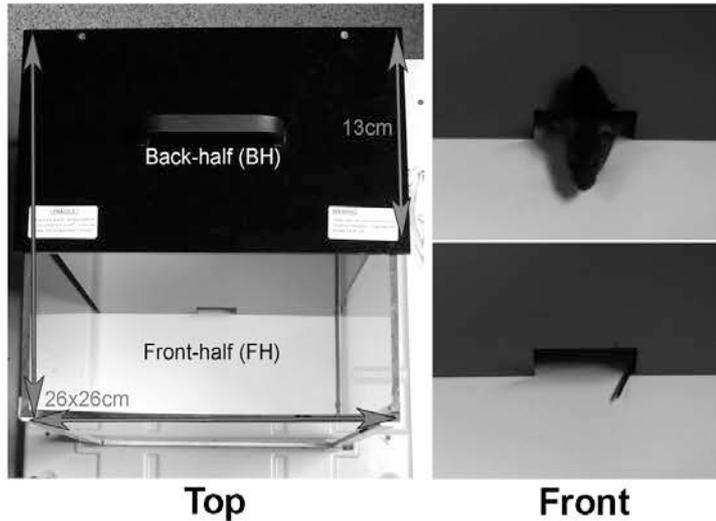
(Johnson et al., *PNAS* (2010) 107(40) 17374-8 )

# Melanopsin is required for light avoidance behaviour in neonatal mice



Neonatal mice were exposed to blue light (open bars) or darkness (black bars). In wildtype mice, the latency to their first turn in the experimental tube was significantly reduced by light exposure, as was the time spent in their original orientation. This was not the case for *OPN4<sup>-/-</sup>* mice. \*\*\*  $P < 0.001$ , \*\*  $P < 0.01$ , ns = non significant.

# ipRGCs drive behavioural light aversion in adult mice

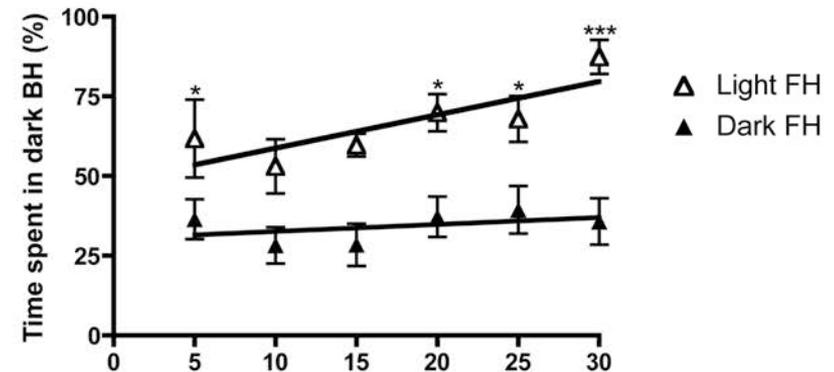


**30-minute trial, 1300 lux light**

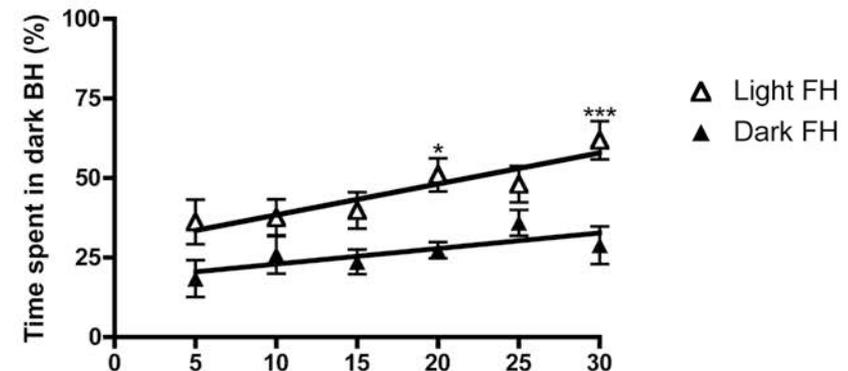
We used *rd/rd cl* mice (lacking rods and cones) to show that ipRGCs can mediate light aversion behaviour in adult mice. Interestingly, light aversion behaviour gets stronger as the trial progresses in *rd/rd cl* mice. *Opn4<sup>-/-</sup>* mice retain an aversion to light without the potentiation over time (not shown)

Semo et al., PLoS One. (2010) 5(11), e15009

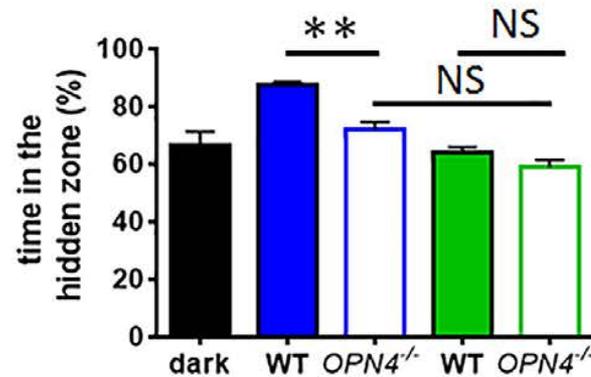
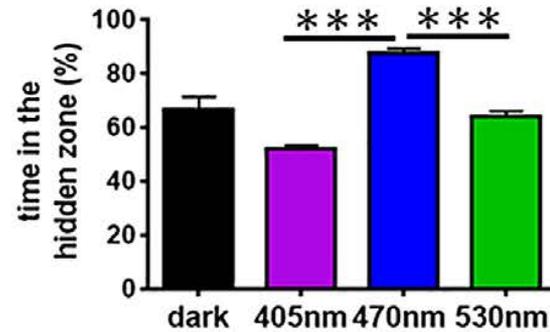
Wildtype mice



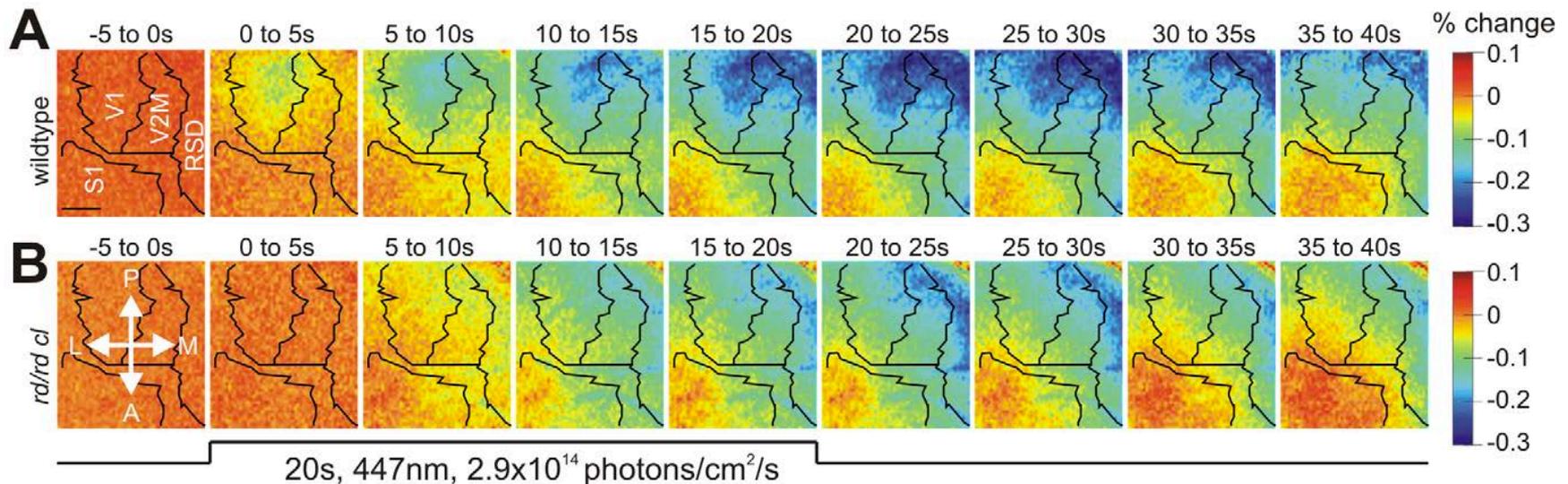
*rd/rd cl* mice



# Blue light drives behavioural light aversion in adult mice

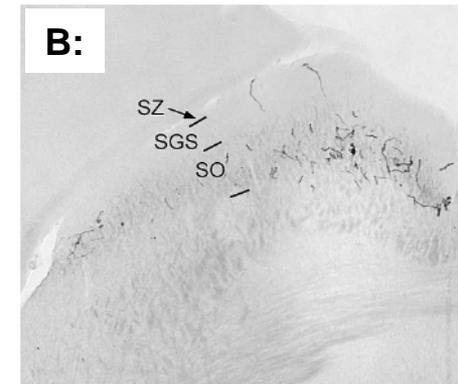
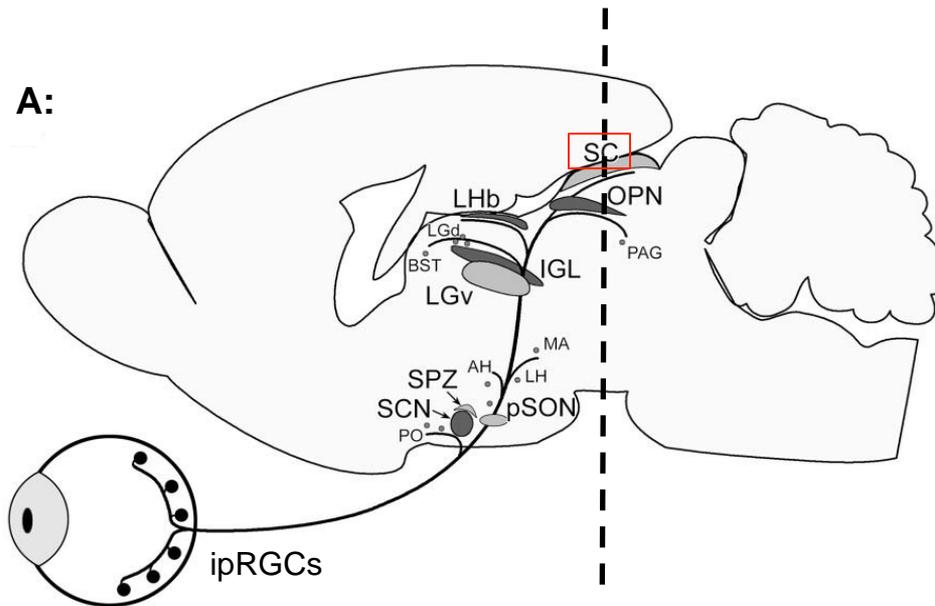


# Intrinsic optical imaging reveals the dynamics of ipRGC-driven cortical activation in *rd/rd cl* mice



Intrinsic optical imaging signals from the visual cortex of wildtype and *rd/rd cl* mice exposed to a 20 second pulse of bright blue light. The time course before (-5 0)s and after light exposure is shown across the top. Blue indicates regions of most intense neural activity. Note that the activity begins in V1 and spreads across to retrosplenial visual cortex (RSD) in wildtype (WT) mice, while activation appears more slowly in *rd/rd cl* mice. Cortical activation in *rd/rd cl* mice has also been demonstrated using functional anatomy (c-fos staining).

# Light aversion may involve ipRGC input to both the Superior Colliculus and visual cortex



Superior Colliculus (SC)

The Superior Colliculus (SC) is a specialised region of the dorsal midbrain which co-ordinates basic movements / behaviour. as shown in the sagittal section (A), ipRGCs project to the SC. The image in B is a coronal section through the SC of a melanopsin-reporter mouse (*Melanopsin<sup>tau-LacZ/+</sup>*).

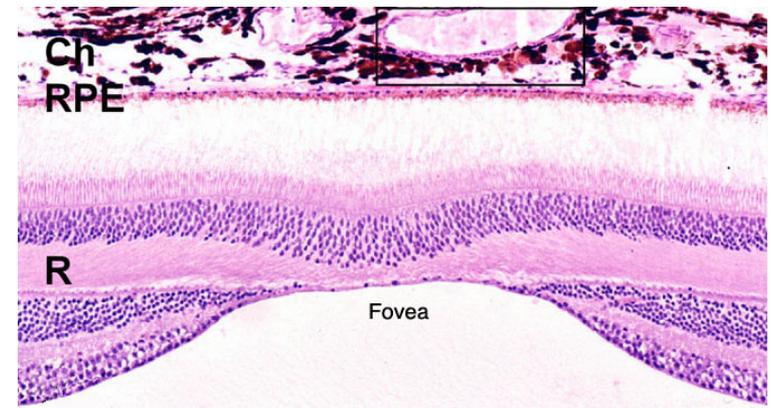
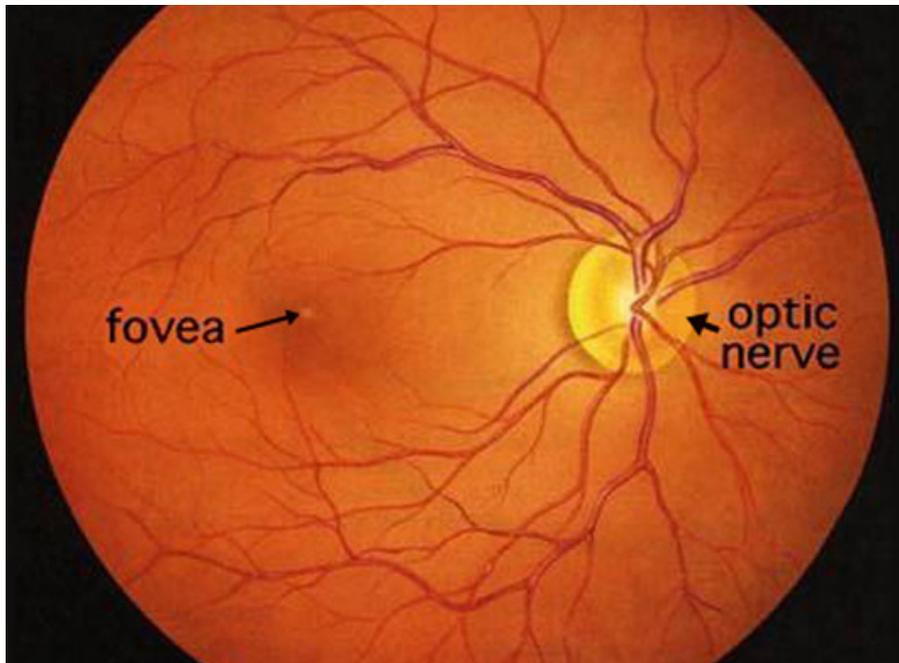
It has been reported that lesions to the SC in P5 neonatal rats can prevent the light avoidance response (Routtenberg et al., *Developmental Psychobiology* (1978) 11(5): 469-478).

However, light aversion behaviour is also impaired in adult rats with lesions to visual cortex (Altman, *Am J. Physiol* (1962) 202: 1208-1210).

# A role for melanopsin in the conscious perception of light in humans?

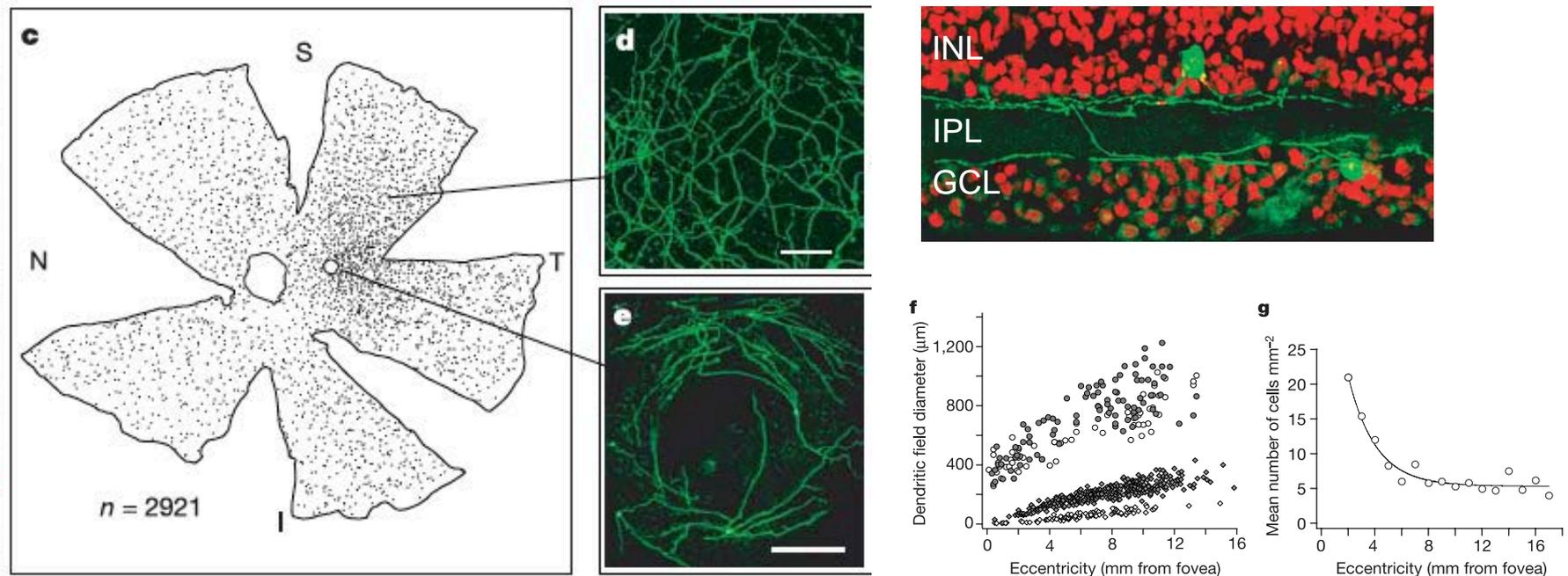
- Three main studies to date:
  - ipRGCs project to the dLGN in primates (Dacey et al., Nature (2005) 433(17) 749-54).
  - Humans lacking rods / cones can perceive blue light (Zaidi et al., Current Biology (2007) 17 2122-28) and experience photophobia (Nosedá et al., Nature Neuro. (2010) 13(2) 239-45).
  - Psychophysical tests in human subjects suggest a role for melanopsin in brightness discrimination (Brown et al., Current Biology (2012) 22, 1-8).
- Does melanopsin contribute to image-forming vision?
  - Acuity? Contrast? Brightness? Motion?

# Evidence for a role of melanopsin in image-forming vision: Anatomy



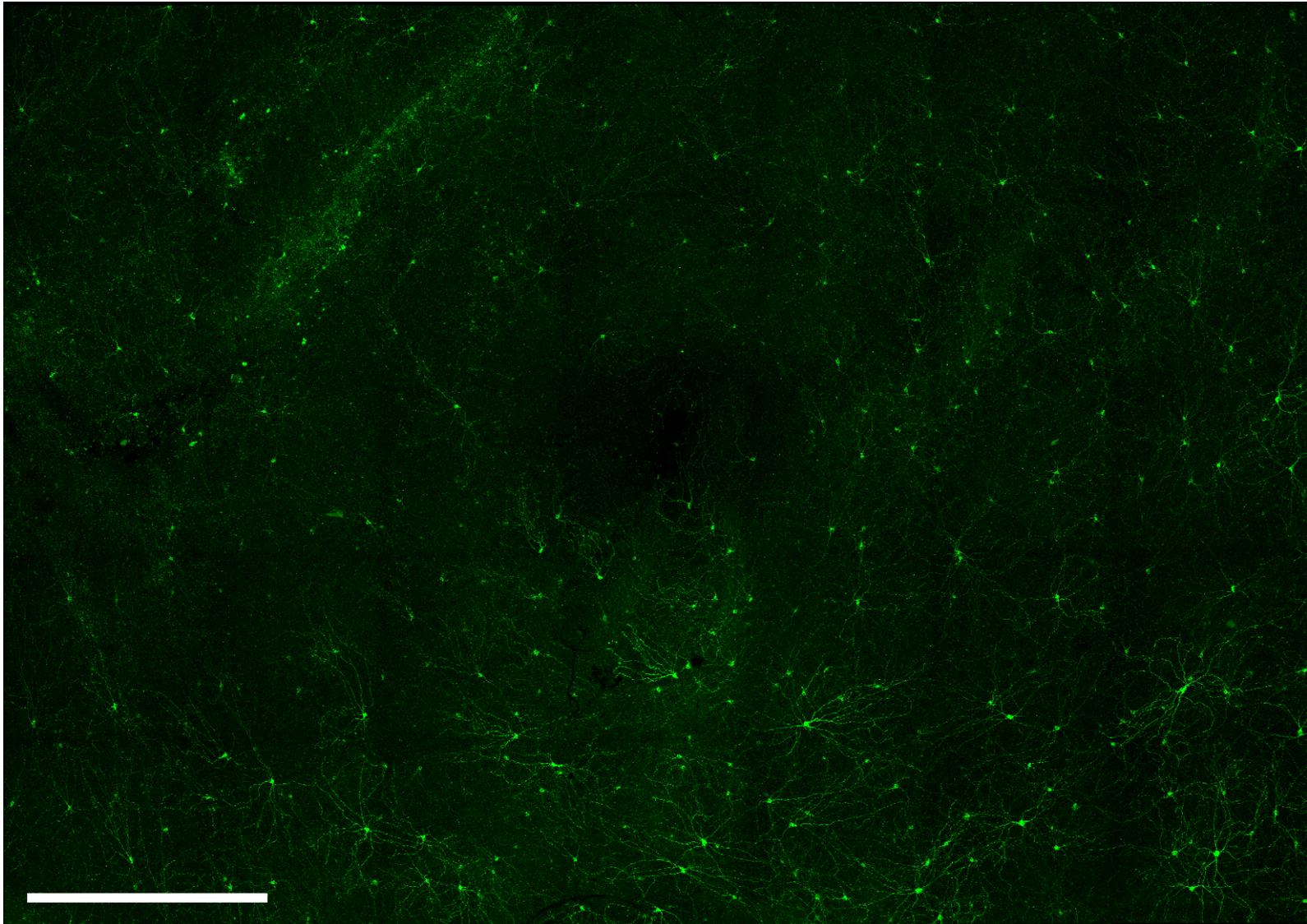
The fovea of the retina, as viewed through the ophthalmoscope and in a histological section.

# In primates, ipRGCs increase in density towards the fovea and project to dLGN



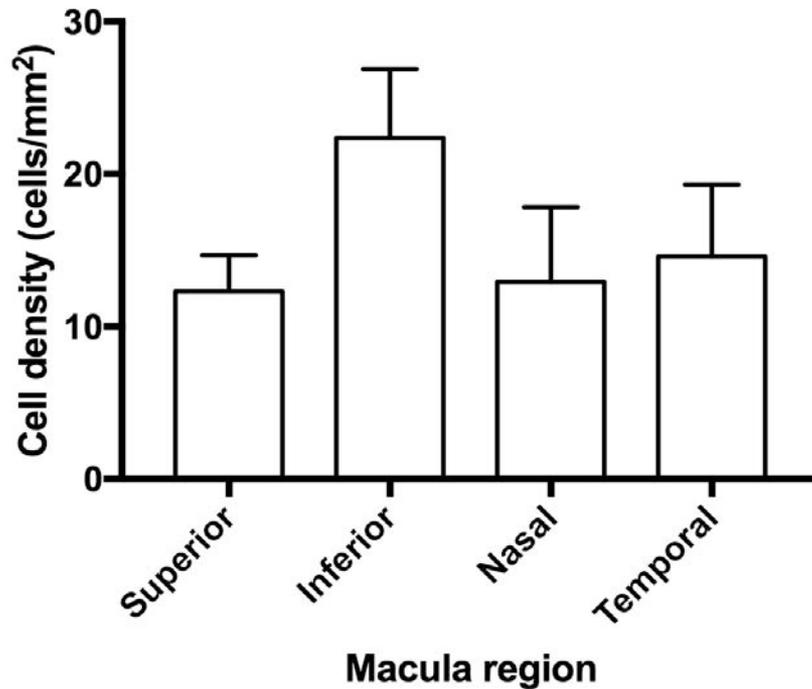
A study by Dacey and colleagues has shown that intrinsically photosensitive melanopsin positive ganglion cells project to the dLGN and are strongly activated by rods and cones. The receptive field of these cells displays colour opponency (S-OFF, L+M-ON). In contrast to rodents, the primate retina has a high percentage (40%) of melanopsin ganglion cells displaced to the inner nuclear layer (INL).

# Distribution of ipRGCs in the human macula

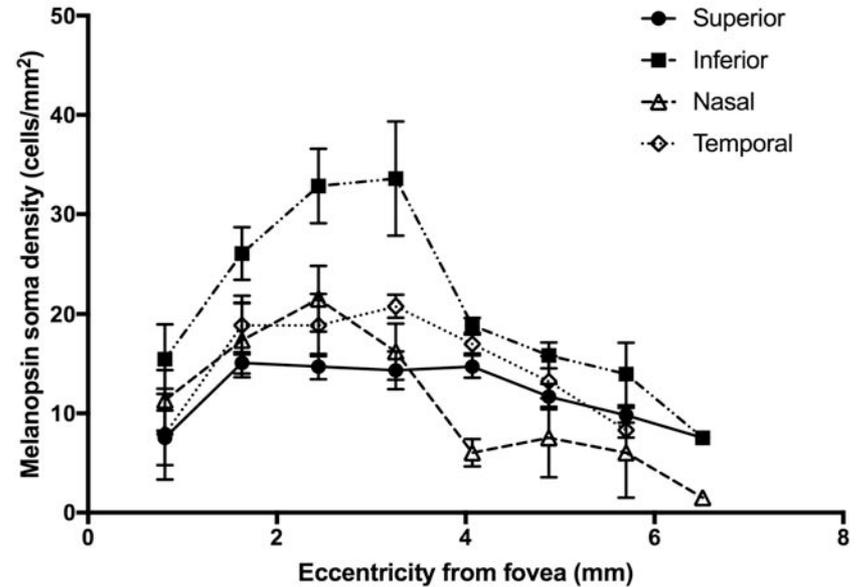


# Distribution of ipRGCs in the human macula

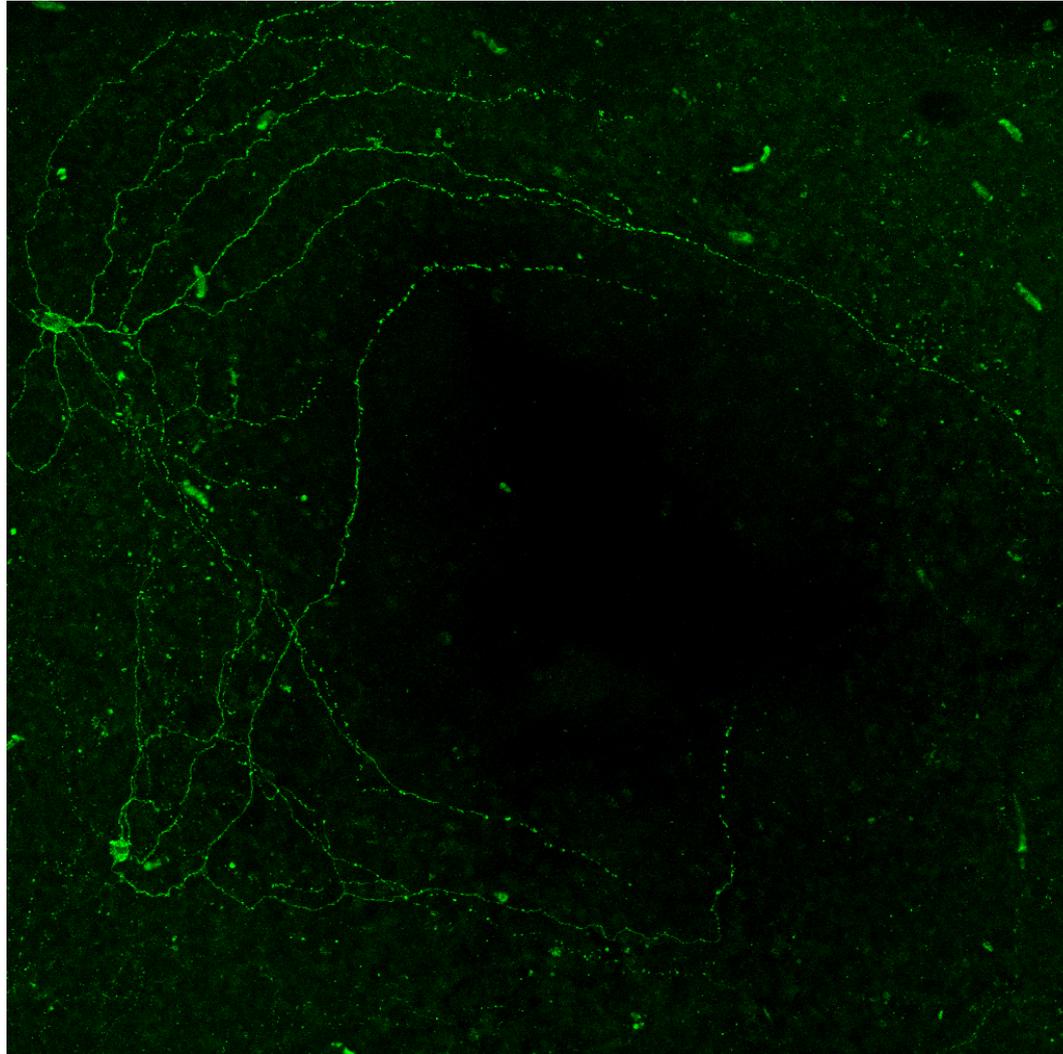
Retinal quadrant



Distance from fovea



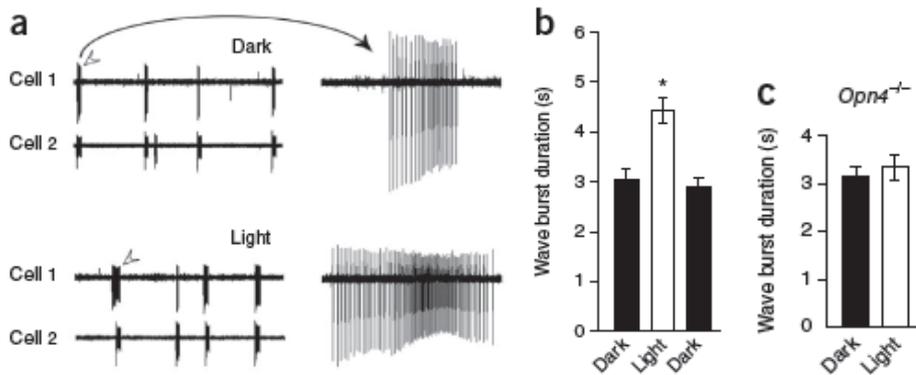
# ipRGCs penetrate the human fovea



## A role for melanopsin in contrast detection?

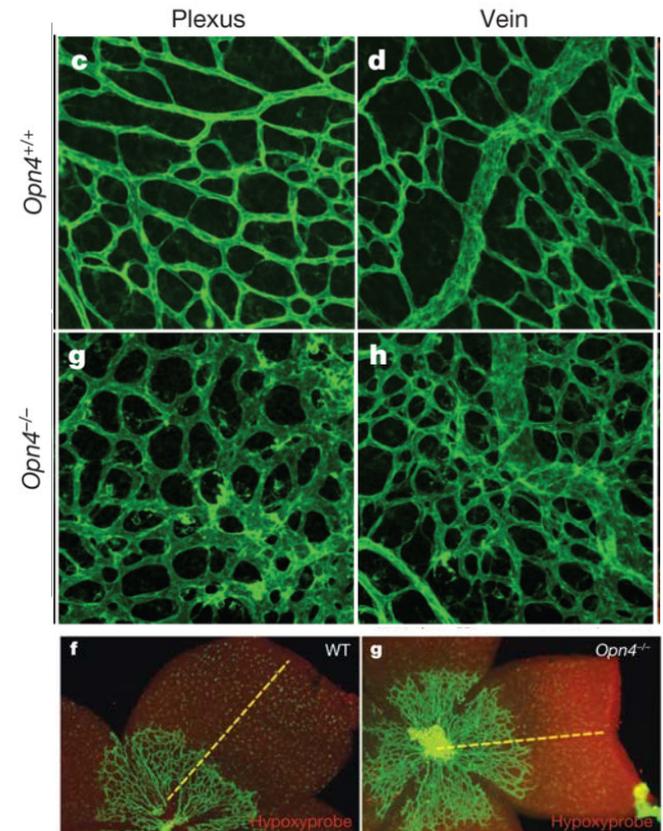
- Melanopsin is found in ON alpha RGCs and has been proposed to contribute to contrast detection
  - These RGCs are sensitive to contrast change signaled by rods/cones
  - *Opn4<sup>-/-</sup>* mice have behavioural deficits in contrast sensitivity
  - Schmidt et al. Neuron (2014) 82: 781-788.
- However, studies looking at visual acuity and contrast sensitivity in *Opn4<sup>-/-</sup>* mice assume that the visual system develops normally in these animals...

# Visual system development is abnormal in melanopsin knockout (*Opn4<sup>-/-</sup>*) mice



Light increases the duration of retinal waves (bursts of spiking activity) in conventional retinal ganglion cells (**a**). This was not the case *Opn4<sup>-/-</sup>* mice (**b**). These mice also have a reduction in the segregation of ipsilateral and contralateral pathways in the retinogeniculate pathway.

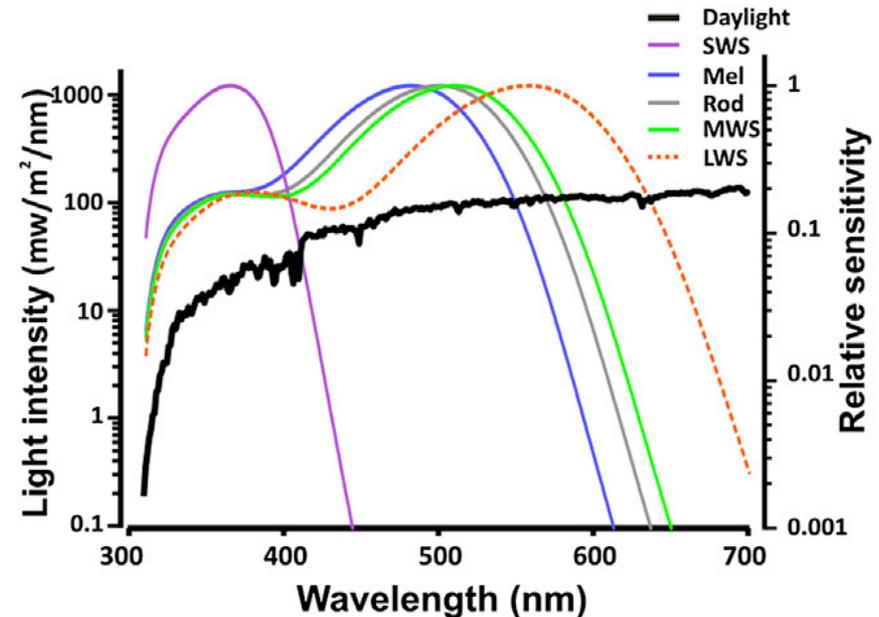
Rena et al., Nature Neuroscience. (2011) 14(7) 827-829).



Vascular abnormalities and hypoxia in the retina of young *Opn4<sup>-/-</sup>* mice. Too many neurons... (Rao et al., Nature (2013) 494(7436) 243-246)

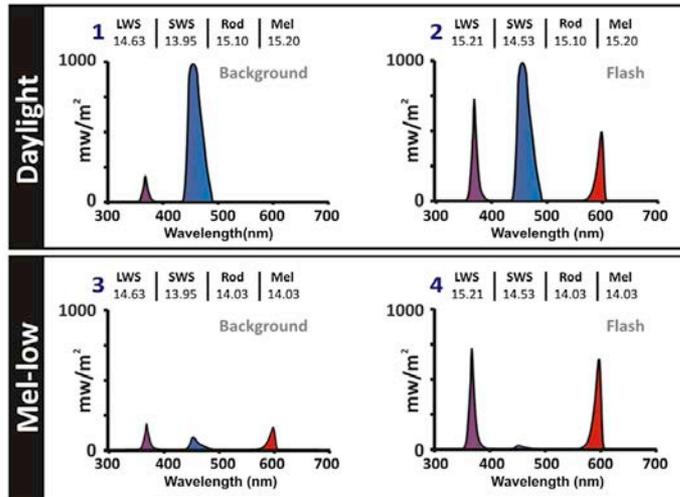
# Selectively stimulating the melanopsin system in mice with an intact retina

Very difficult to stimulate melanopsin without stimulating the green cones in normal mice. So, to make this possible, Rob Lucas' group in Manchester use red cone knock in mice (*Opn1mw<sup>R</sup>*), where the mouse green cones express the human L-opsin (action spectrum shifted to the right and therefore enables a greater separation between melanopsin and green cone activation in mice).



This method was first used to demonstrate melanopsin-mediated brightness discrimination in intact mice (Brown et al., *Current Biology* (2012) 22, 1-8).

# Melanopsin-driven light adaptation in mice

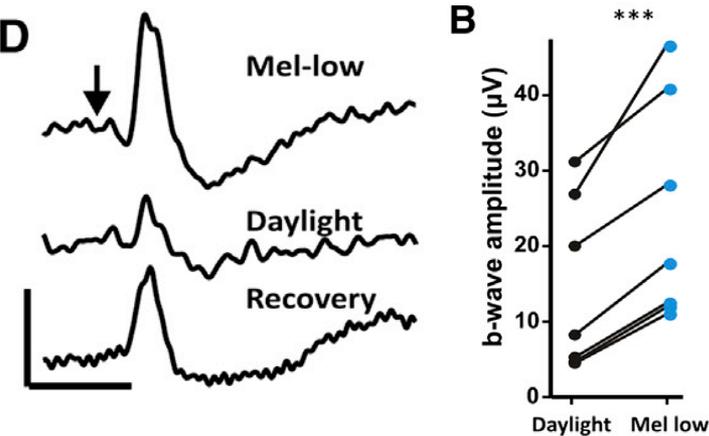


The *Opn1mw<sup>R</sup>* mouse was used to compare retinal and thalamic responses to stimuli with spectral compositions either enriched (daylight) or depleted (Mel-low) in wavelengths to excite the melanopsin system.

They found adaptation in the ERG (**D&B**) under daylight conditions and strong evidence for an increase in feature selectivity of dLGN neurons using multi-electrode recordings from this structure.

In the dLGN, the neurons preferred finer spatial patterns under daylight conditions. These conditions also tuned direction sensitive neurons to faster motion. So, increasing the level of melanopsin stimulation changes the feature detection of visual circuits in both spatial and temporal dimensions.

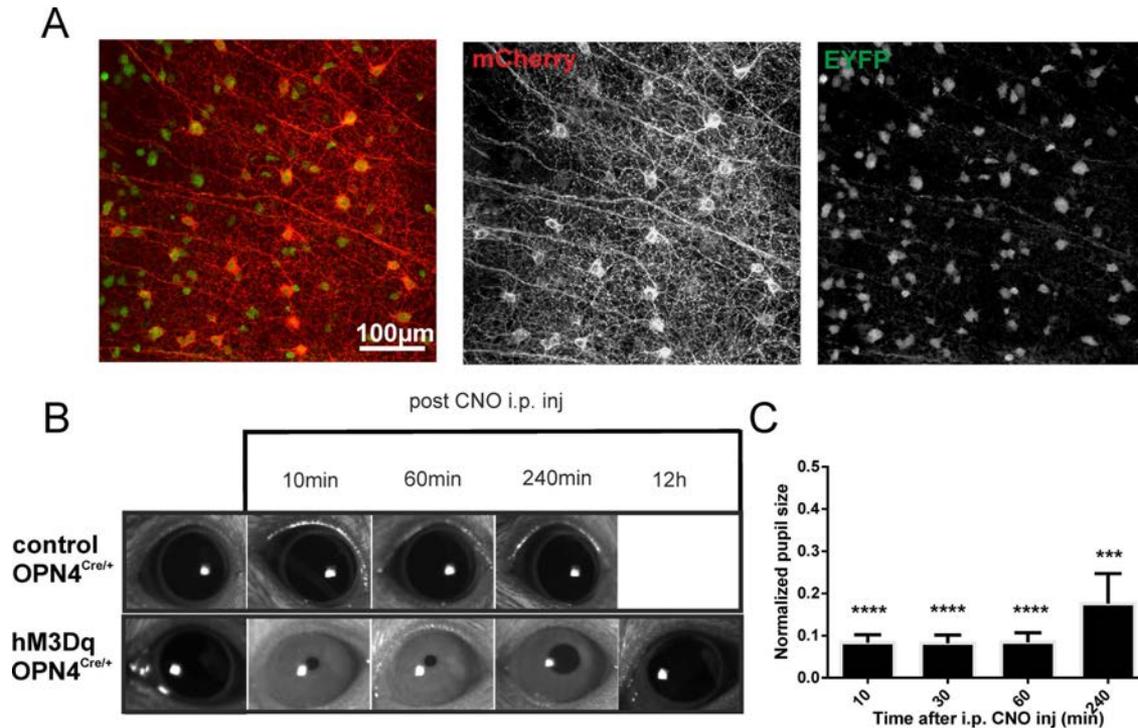
**“Melanopsin works like a photographer's light meter, providing an independent measure of irradiance to determine optimal settings for visual circuits.”**



Melanopsin stimulation adapts the cone ERG in mice (200 x 1Hz, 50ms with Background illumination). Allen et al., Current Biology (2014) 24: 1-10

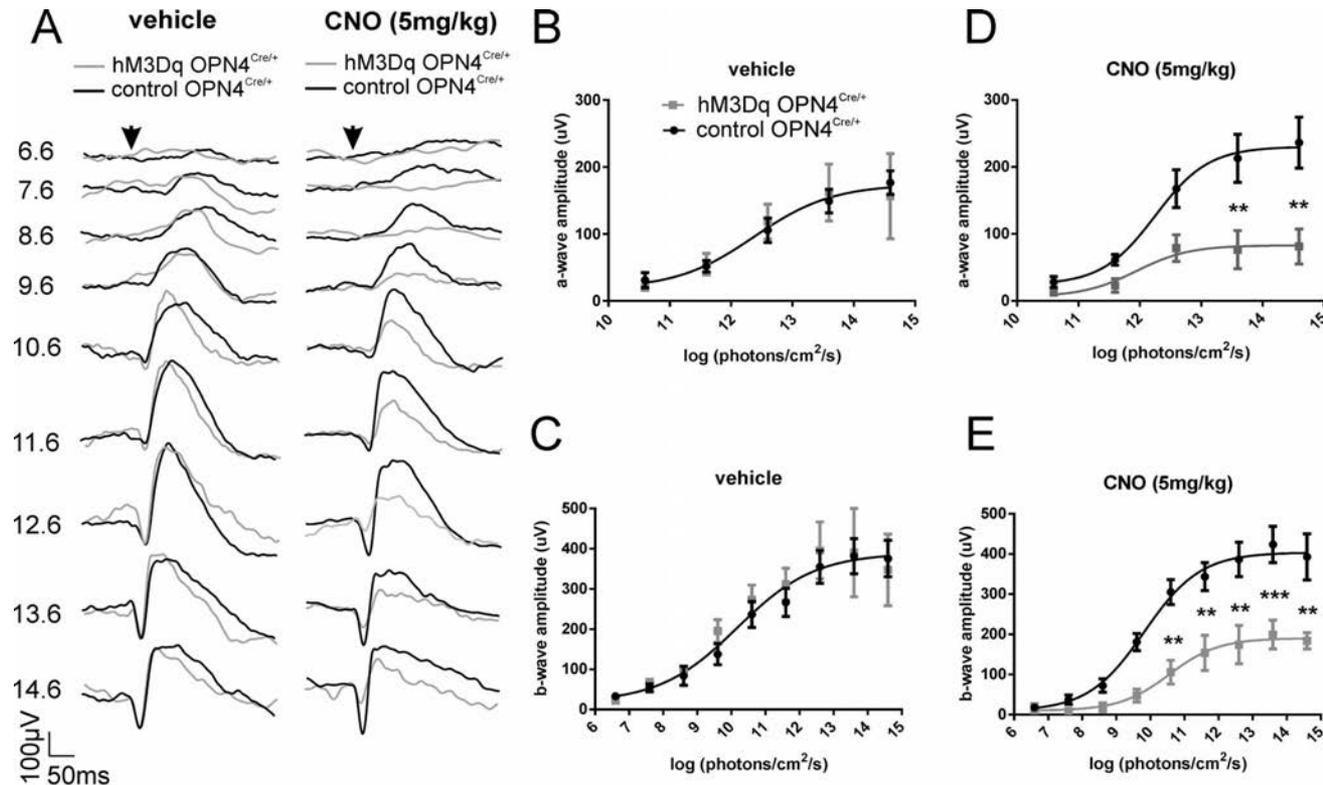
At least part of this melanopsin-driven light adaptation may happen in the retina...

# Chemogenetic activation of ipRGCs



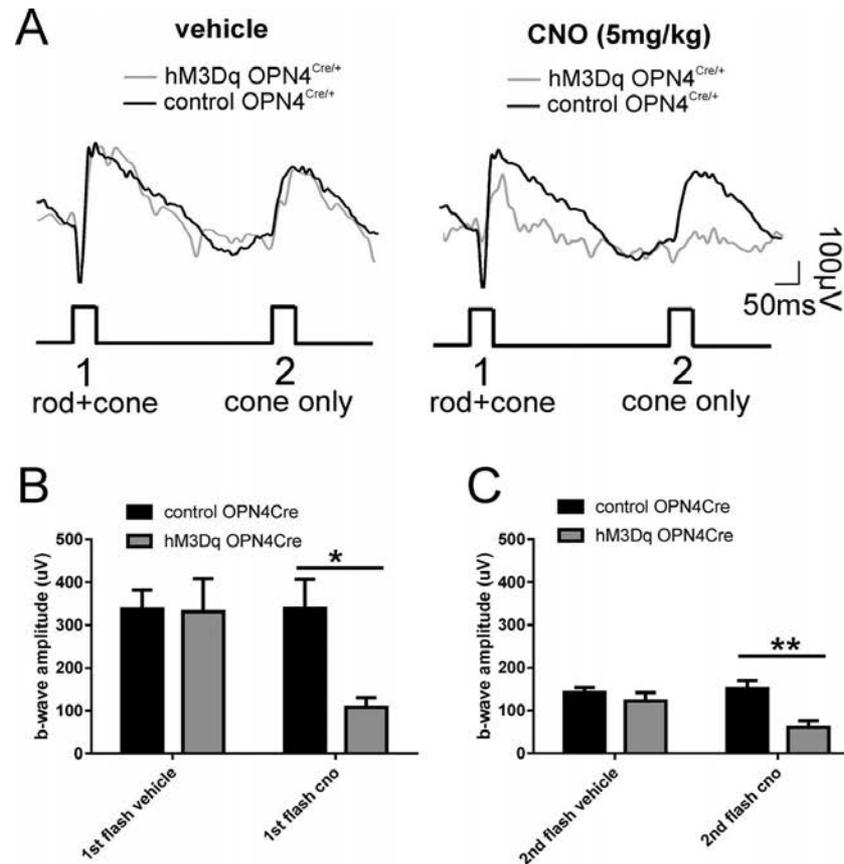
The ipRGCs in OPN4-CRE mice can be selectively transfected by intravitreal eye injections of an AAV2 viral vector encoding the hM3Dq Gq-coupled receptor (which depolarises cells upon activation). The ipRGCs can then be selectively activated by intraperitoneal injections of Clozapine *N*-Oxide (CNO) in the dark. The images in **A** show Melanopsin expressing ipRGCs (green) transfected with hM3Dq (visualised in red using the mCherry reporter). In the dark, application of CNO causes sustained pupil constriction in conscious mice (B-C).

# Chemogenetic activation of ipRGCs - Retina



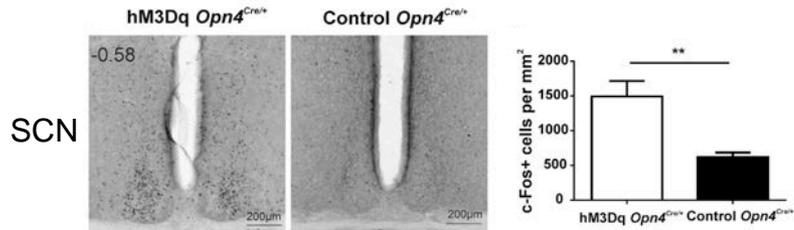
ipRGC activation by CNO injection in hM3Dq OPN4-Cre mice reduces the amplitude of a and b-waves in the scotopic (dark-adapted) electroretinogram (ERG).

# Chemogenetic activation of ipRGCs - Retina

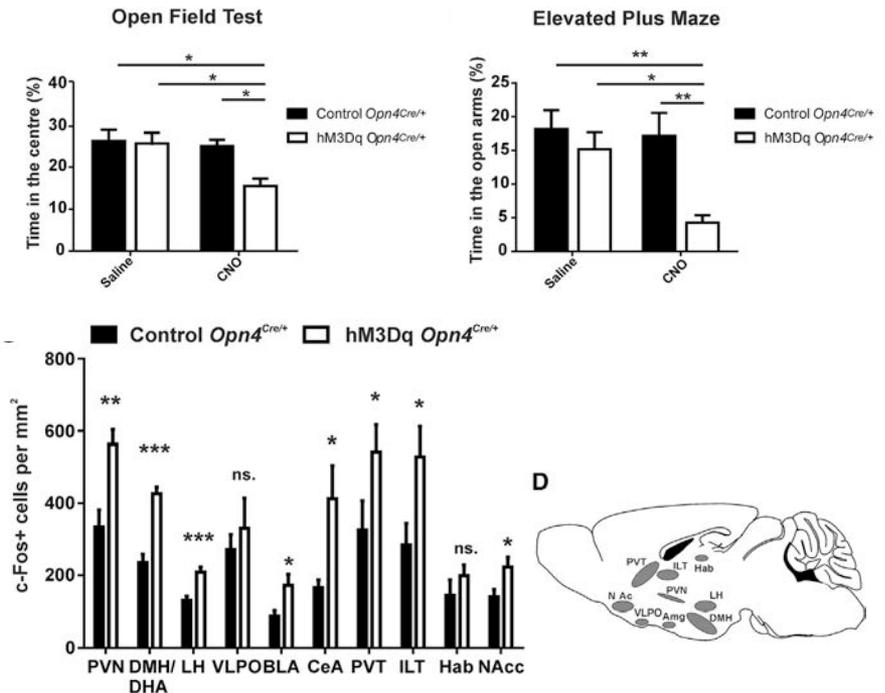
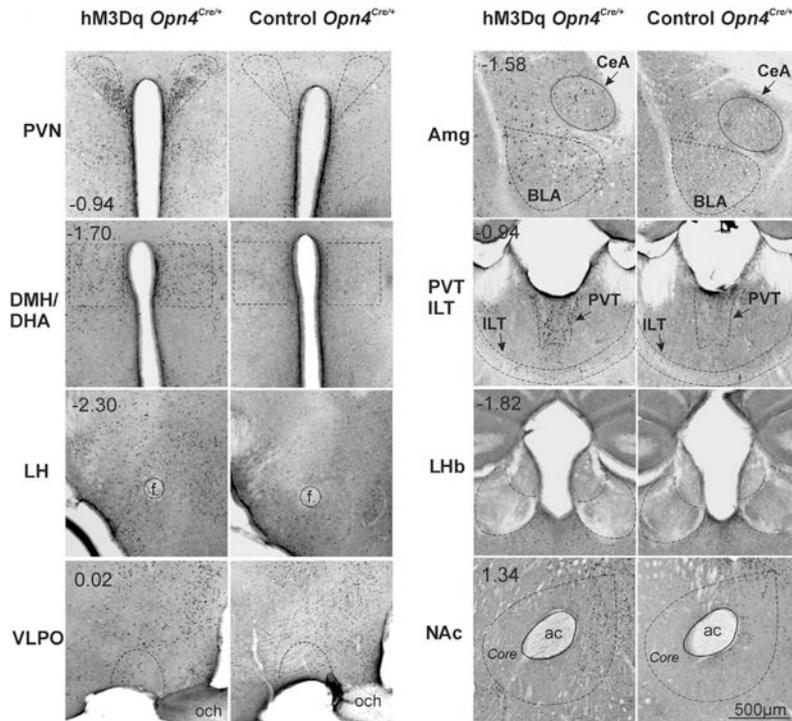


Using the paired flash technique, both rod and cone responses are suppressed by ipRGC activity induced by CNO injections. There is also evidence of reduced amacrine cell activity, with suppression of oscillatory potentials (not shown).

# Chemogenetic activation of ipRGCs - Brain

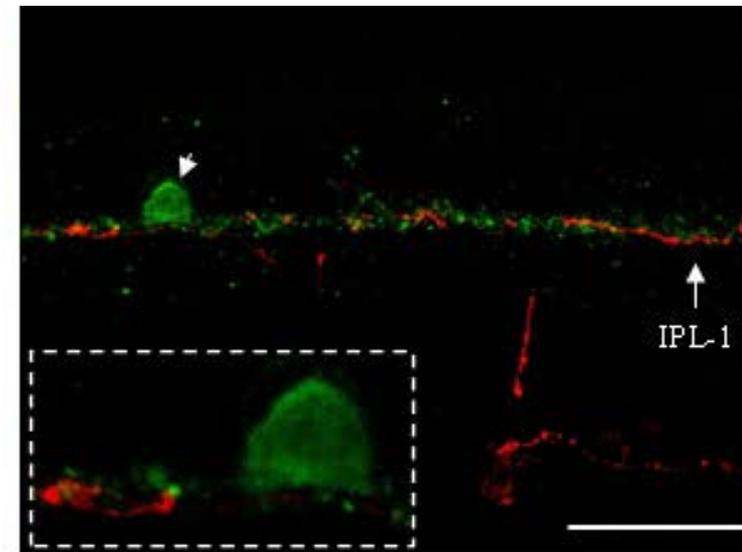


Chemogenetic activation of the hM3Dq receptor by CNO in the dark induces neural activity (as measured by c-Fos staining) in the SCN of hypothalamus and other brain regions associated with arousal / anxiety. This is also indicated by behavioural data from open field and elevated plus maze tests.

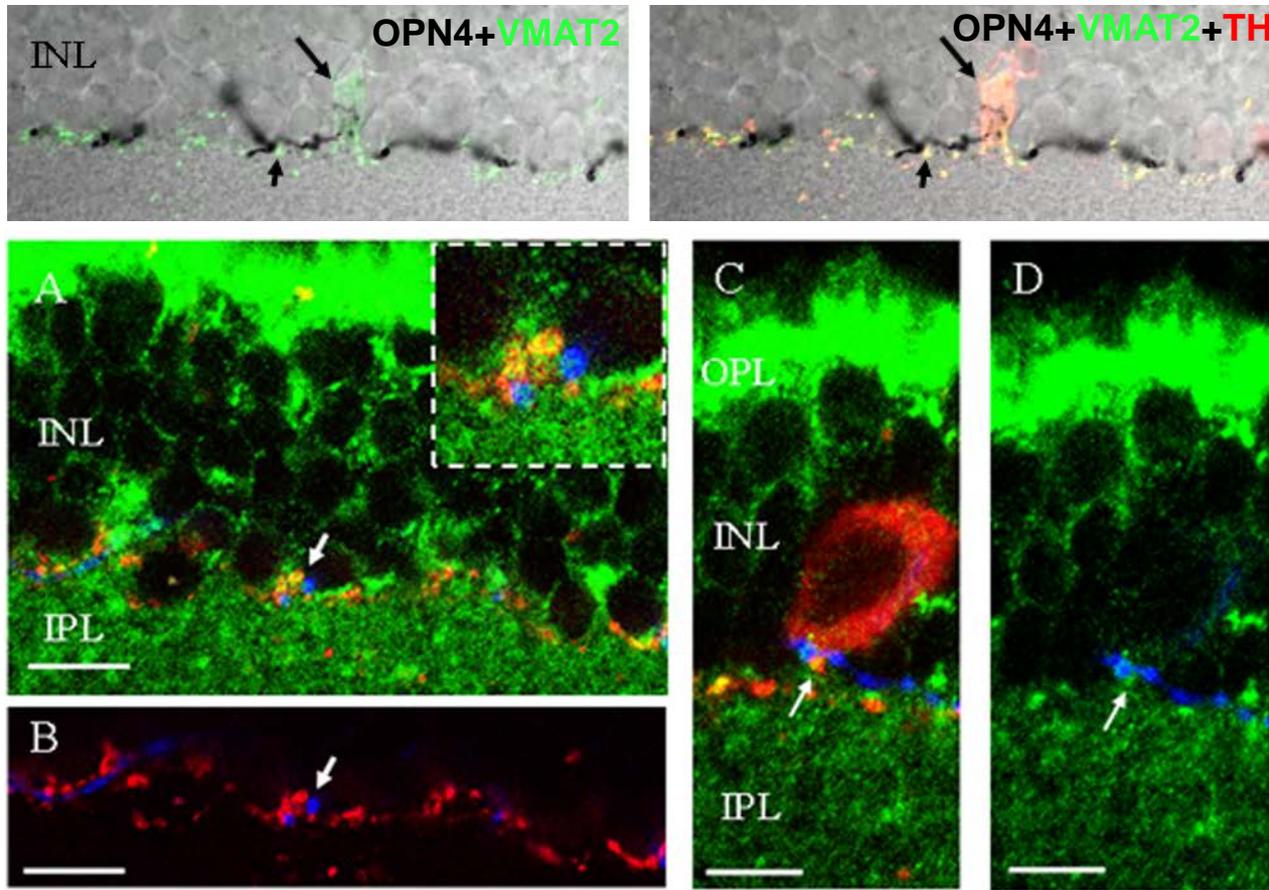


# How could melanopsin influence the surrounding retinal circuits?

- ipRGCs signal to other retinal neurons via gap junctions
  - (Sekaran et al., *Current Biol.* (2003) 13, 1290-1298)
  - ipRGCs are electrically coupled to GABAergic amacrine cells in the RGC layer (Muller et al., *J. Comp. Neurol.* (2010) 518, 4813-4824)
- ipRGCs contact retinal dopamine neurons (Vugler, 2005)
  - Type of inter-plexiform neuron
  - In the retina, dopamine acts to light-adapt retinal circuitry and enhance visual acuity / contrast detection.
  - Retinal dopamine neurons traditionally thought to be driven by cone input...

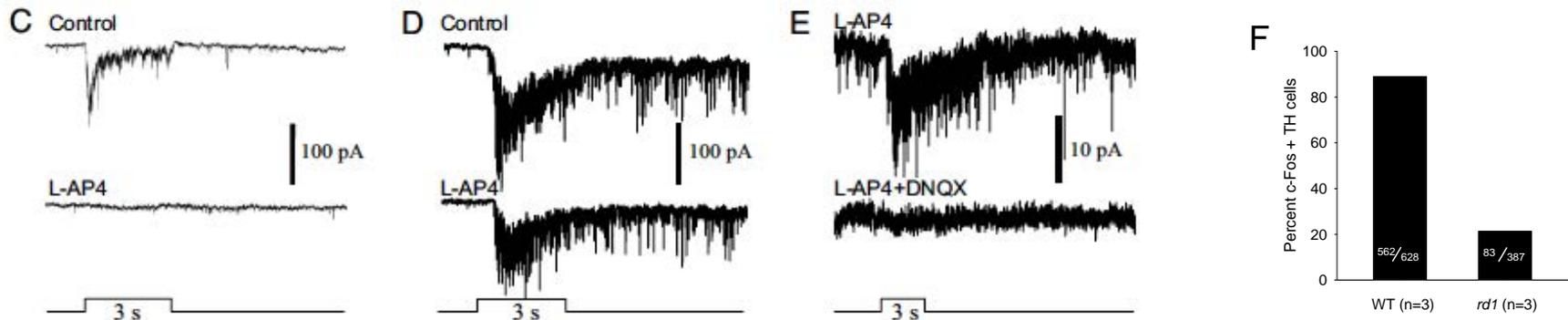


# Dopamine neurons appeared largely presynaptic to ipRGCs



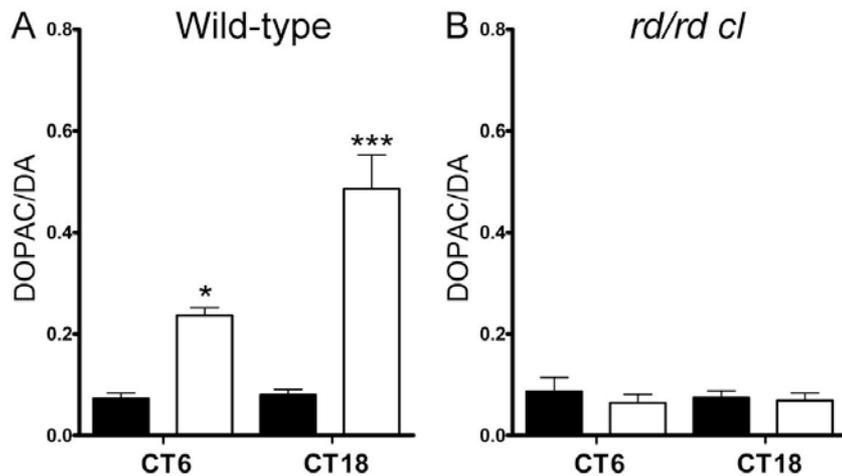
A-D: Tyrosine hydroxylase (red), melanopsin (blue), SNAP25 (green)

# Electrophysiological evidence suggests “retrograde” intra-retinal signaling to dopamine neurons

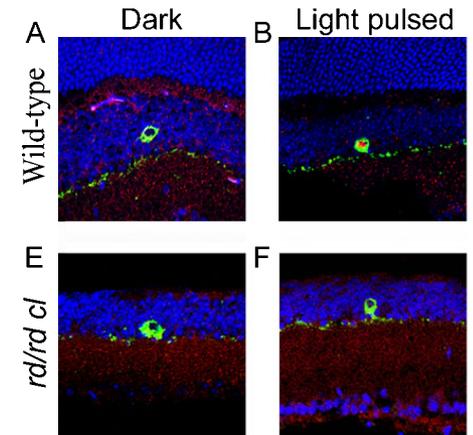
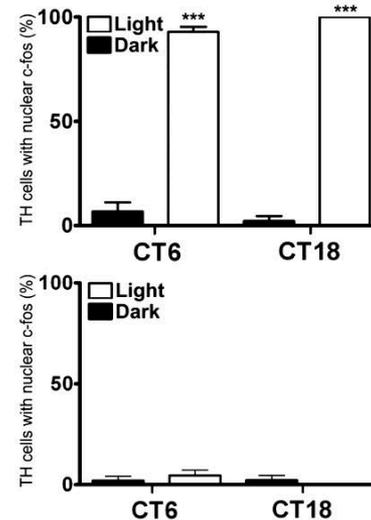


Two types of electrophysiological response found in dopamine neurons: transient (C) and sustained (D and E). The transient response elicited by a 3 second bright light stimulus can be abolished by application of 75 $\mu$ M L-AP4 (blocks signals from rods/cones). The sustained response is resistant to L-AP4 but abolished by co-application of 40 $\mu$ M DNQX (E). This implies that signaling from ipRGCs to dopamine neurons in the inner retina occurs and is mediated by AMPA/kainate-type glutamate receptors. They also reported that ~20% of dopamine (TH) cells are activated by light in both wildtype mice treated with L-AP4 and *rd* mice lacking rods (F).

# However, light stimulation fails to elevate dopamine turnover in mice lacking rods & cones

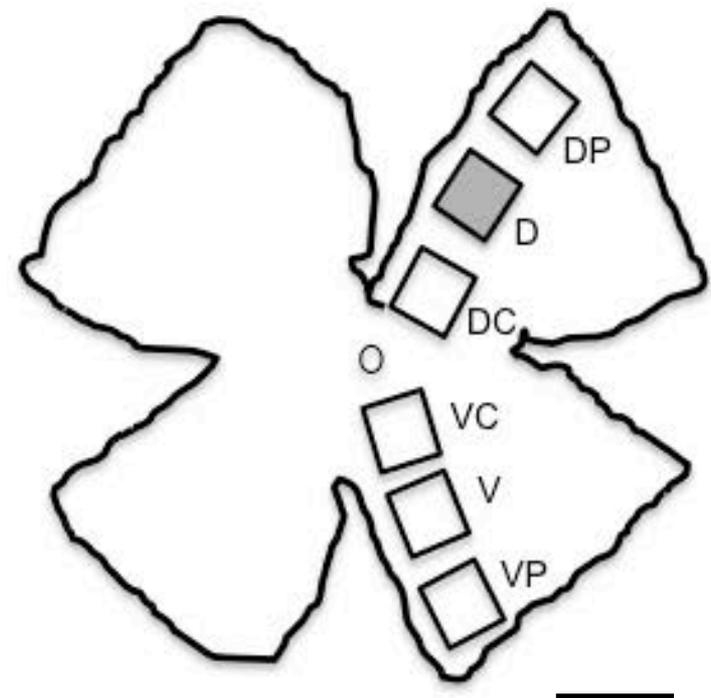
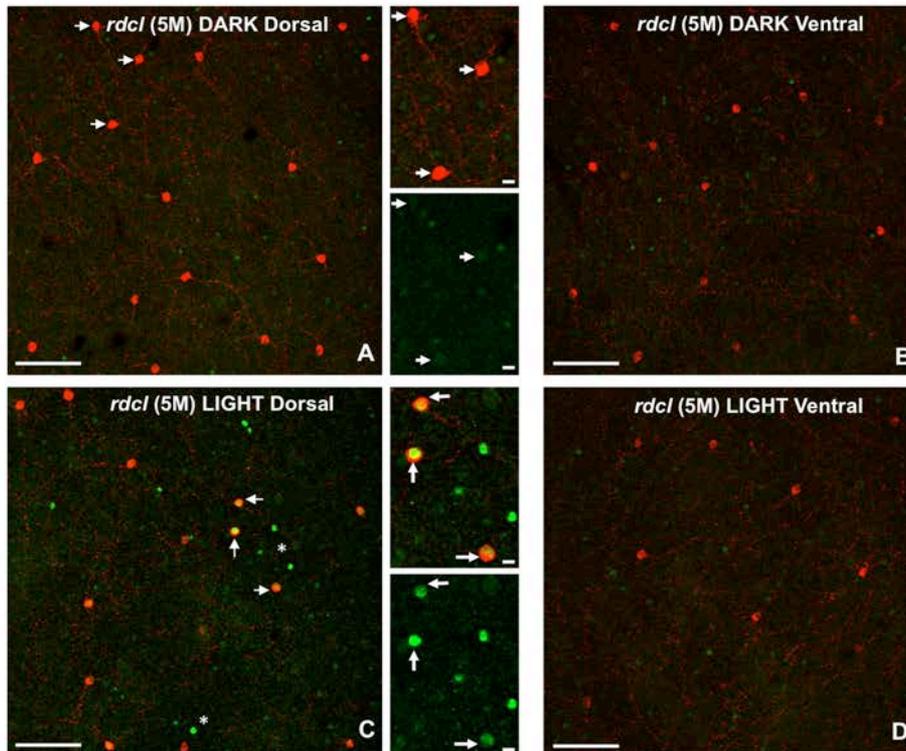


HPLC analysis showed no significant effect of light on dopamine turnover in mice lacking rods and cones (*rd/rd cl*).

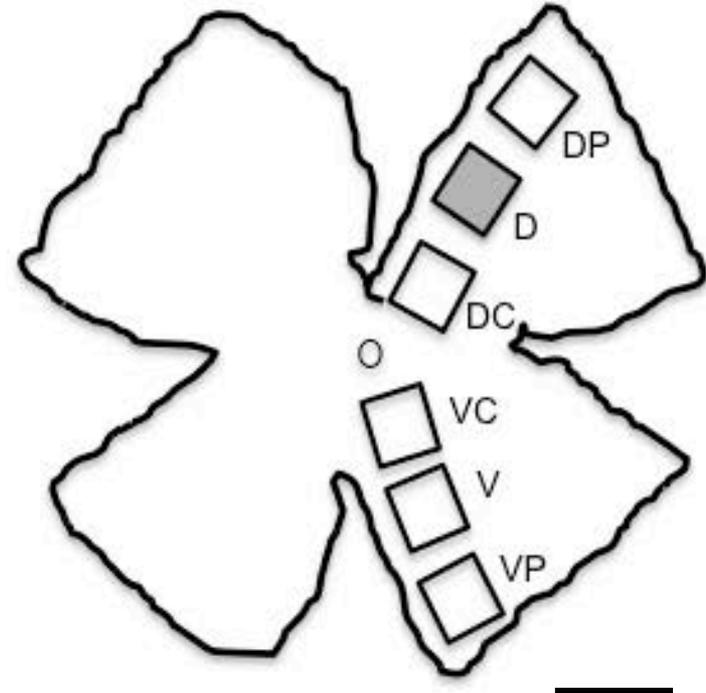
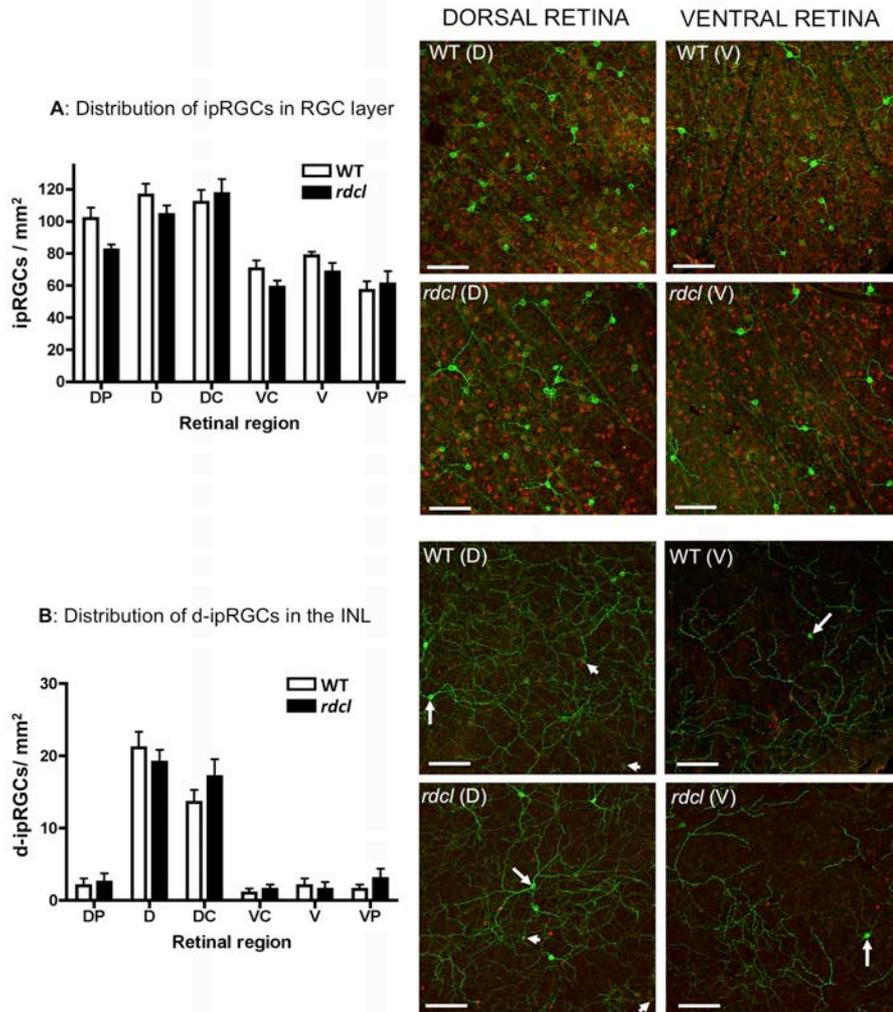


Functional anatomy using light-driven c-fos (red) in dopamine cells (green) showed no activation in mice lacking rods and cones (*rd/rd cl*).

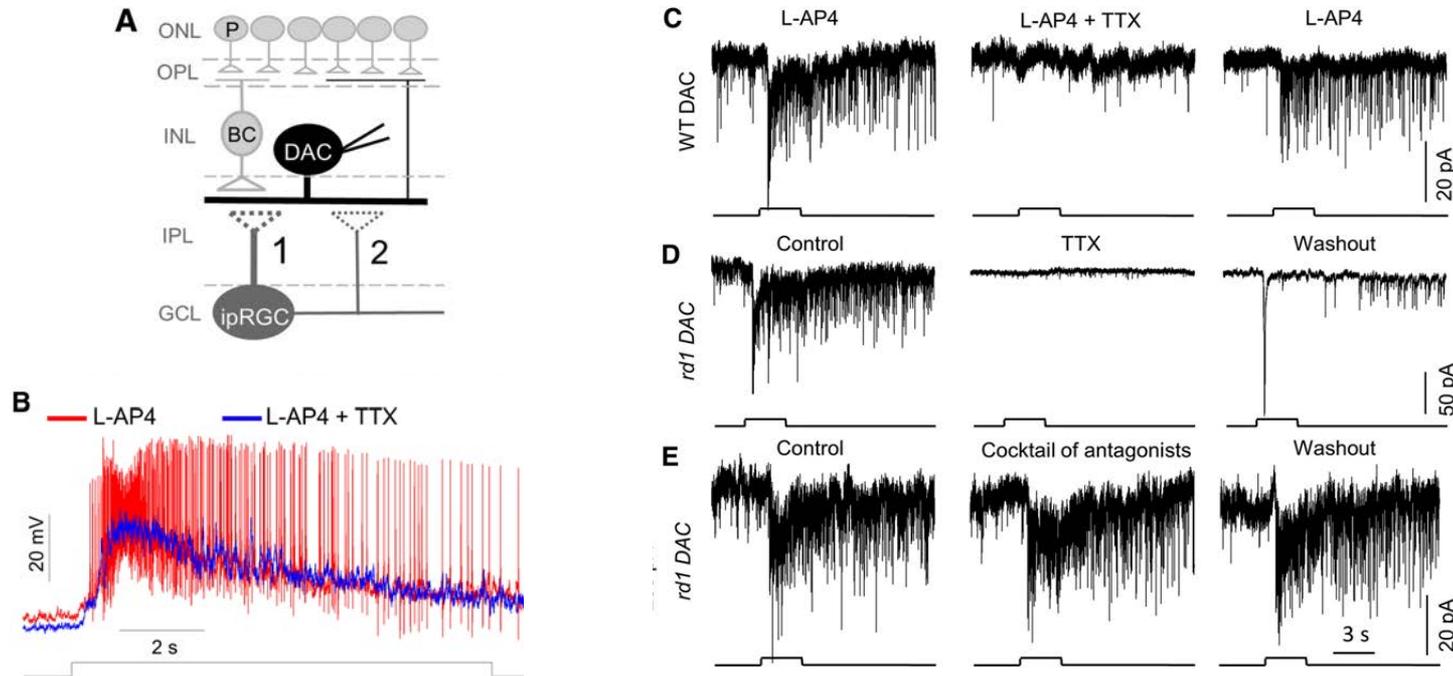
# Retrograde intra-retinal signaling in mice lacking rods and cones occurs in a discrete region of dorsal retina



# Retrograde intra-retinal signaling appears strongest where ipRGC density is highest

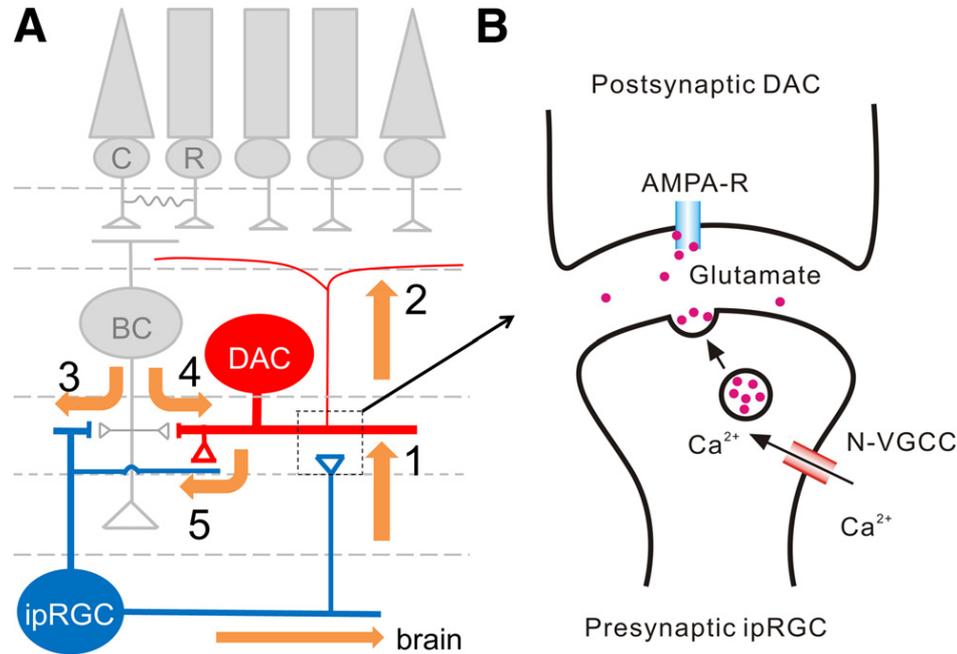


# Intra-retinal retrograde signaling via ipRGC axon collaterals



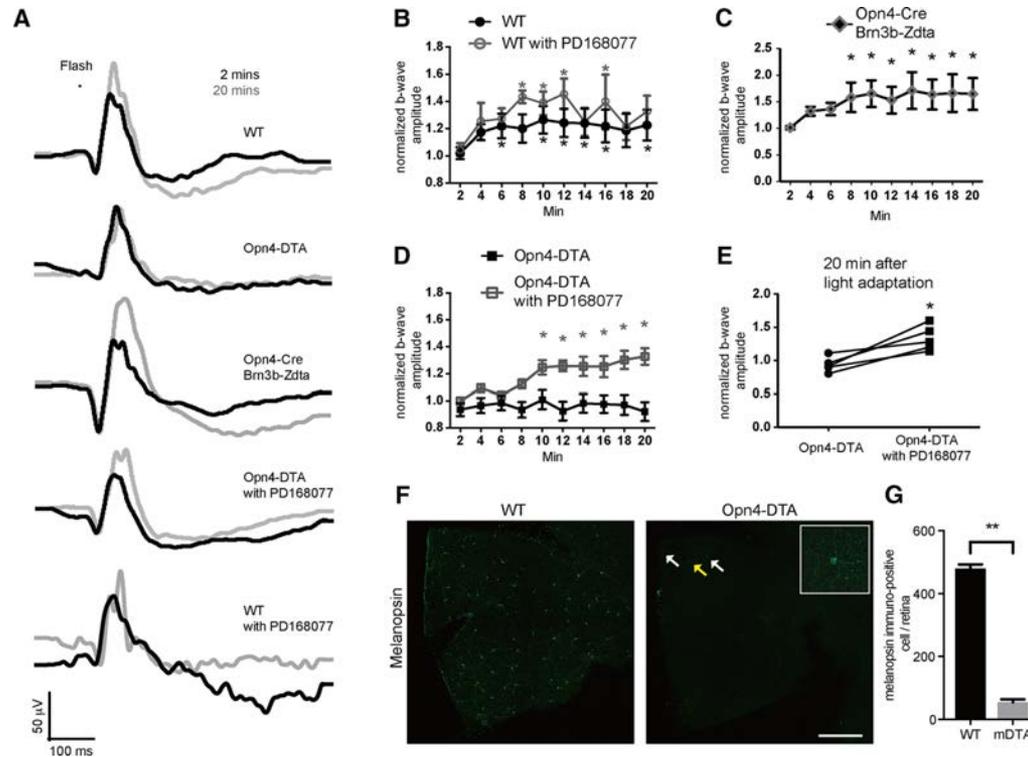
Evidence for action potential transmission from ipRGCs to dopaminergic amacrine cells (DACs) **A**. Potential route of transmission: dendrodenritic (1) or axon collateral mediated (2). **B**. TTX removes action potentials from the ipRGC response but does not inhibit the light-Induced graded potential of ipRGCs. **C-D**, The intracellular sodium channel blocker QX314 was included in the pipette solution when recording from DACs to ensure that the voltage gated Na<sup>+</sup> channels were already blocked within DACs prior to extracellular TTX application. **E**. Cocktail of GABA/Glycine receptor antagonists added In **E** to discount potential polysynaptic signaling from ipRGCs, via wide-field amacrine cells.

# Intra-retinal retrograde signaling via ipRGC axon collaterals



Proposed neural pathways and synaptic mechanism underlying the ipRGC influence on light adaptation. **A:** Route 1, ipRGCs signal via axon collaterals to the dopaminergic amacrine cells (DAC), which then signal to outer retina via interplexiform axons. Routes 3 and 4 are proposed feedback pathways from rods / cones. Route 5 represents negative feedback from DACs back onto ipRGCs, whereby rod/cone signals could suppress ipRGC activity. **B** The proposed signaling mechanism involves N-type voltage gated calcium channels (N-VGCC) and AMPA type glutamate receptors.

# Dopamine mediates the effect of ipRGCs on the light-adapted ERG



M1 ipRGCs modulate the light-adapted ERG b-wave via dopamine D4 receptors. Opn4-DTA mice have genetic ablation of ipRGCs, while Opn4-Cre Brn3b Zdta mice retain Brn3b negative ipRGCs (M1 ipRGCs). PD168077 is an agonist of D4 receptors.

## Summary:

- ipRGCs signal irradiance information to the brain
  - Integrating rod/cone signals with their own intrinsic light response
  - Their intrinsic light response is driven by melanopsin (Opn4)
- ipRGCs are a heterogeneous population of cells
  - In terms of morphology, physiology and connectivity to the brain.
- Melanopsin may support image-forming vision during daylight hours
  - By acting like a photographer's light meter, providing an independent measure of irradiance to light-adapt visual circuits.
  - Enhancing feature selectivity in both spatial & temporal dimensions.
  - This appears to occur at least in part in the retina (dopamine).
- **Melanopsin is involved in visual system development**
  - **This should be considered when interpreting data from studies relying upon *Opn4*<sup>-/-</sup> mice alone.**