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Supplemental Information

Distinct Contributions of Rod, Cone, and Melanopsin Photoreceptors to Encoding Irradiance

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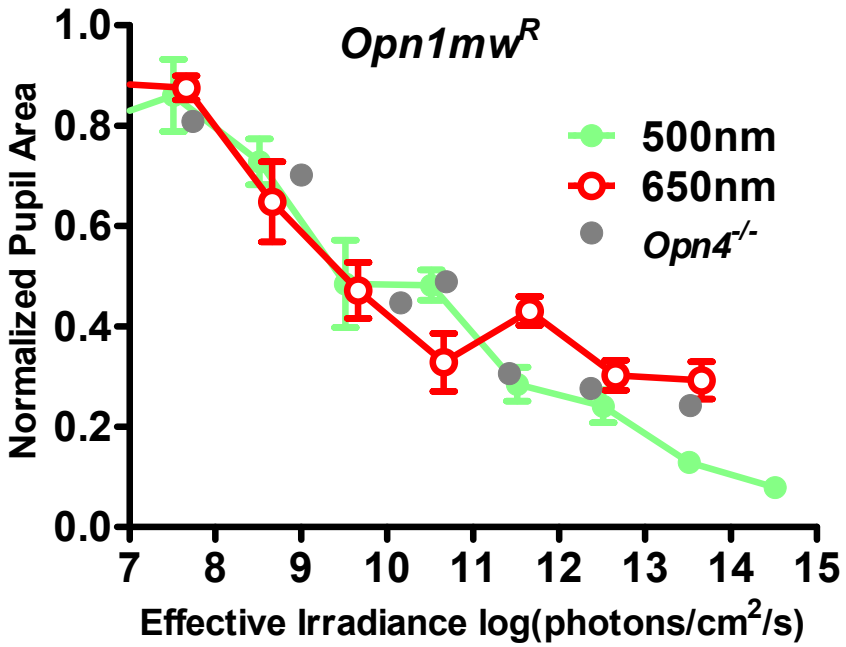


Figure S1: The response of *Opn1mw*^{R} mice to 650nm but not 500nm light (mean±SEM n=4-12 replotted from Figure 2) is qualitatively similar to historical data from *Opn4*$^{-/-}$ animals (mean response for n=5-8 mice replotted from Lucas *et al (*2003) Diminished pupillary light reflex at high irradiances in melanopsin-knockout mice. **Science** 299:245-247).

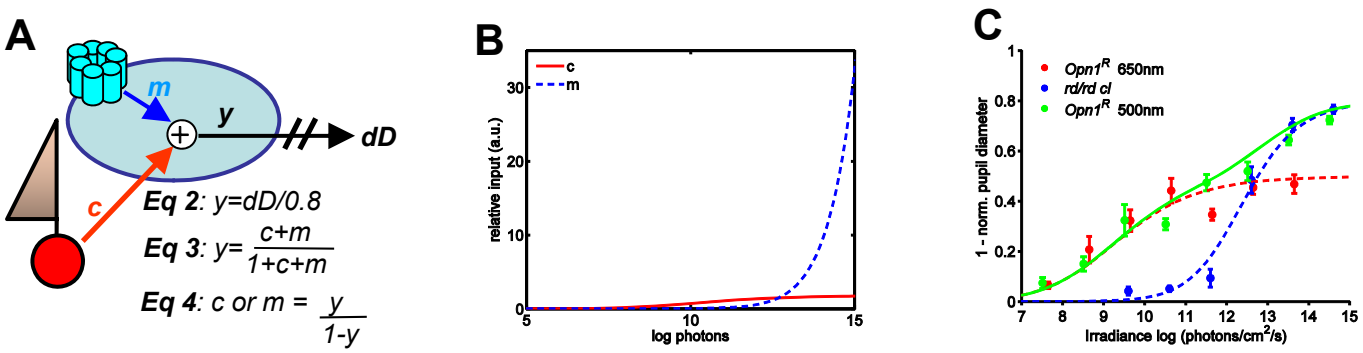


Figure S2: A quantitative model for integrating cone and melanopsin signals within irradiance detection pathways. We set out to determine a method by which the irradiance response relationship for *Opn1mw*^{R} at 500nm (to which both cones and melanopsin contribute) could be approximated from the responses driven by cones (approximated by the *Opn1mw*^{R} 650nm response) or melanopsin (*rd/rd cl)* alone. We started, by fitting the data in Figure 2B (i.e. after normalising for red cone sensitivity) and C with a monotonic function of the form:

$$D(I) = D_{min} + (1\text{-}D_{min}) \frac{I_0^n}{I_0^n + I^n} \qquad \text{Eq. (1)}$$

Where D is the normalized pupil diameter, calculated from the normalized pupil area and used because it is thought to have a simpler relationship with irradiance than does area. We then applied these data to a simple conceptual model (**A**) in which mRGCs encode irradiance as a parameter (*y*) that is the product of intrinsic (melanopsin; *m*) and extrinsic (cone; *c*) inputs. For simplicity, we assume a linear relationship between *y* and the degree of constriction (dD; defined as 1 – *D*), and a nominal value of 1 for the magnitude of *y* required to drive a saturating constriction (normalized area ~0.03, corresponding to dD=0.8). Thus *y* can be estimated as *y*=dD/0.8 (Eq. 2) at any irradiance/wavelength from the data presented in Figure 2A. To account for the notable experimental observation that the melanopsin alone can drive a saturating pupil constriction, *y* was modeled with a minimal Hill function:

$$y = \frac{x}{1+x} = \frac{c+m}{1+c+m} \qquad \text{Eq. (3)}$$

Eq. (3) can be rewritten for *x* as a function of *y* as follows.

$$x = \frac{y}{1-y} \qquad \text{Eq. (4)}$$

B. Therefore, by using Eq. (4), cone input (*c*) is estimated from the mRGC signal (*y* = *dD*/0.8) of *Opn1mw*^{R} 650nm, for which melanopsin input (*m*) is 0, while *m* is estimated from *y* of *rd/rd cl* 500nm, for which *c* is 0. **C.** When applied to Eq. 3 these estimates of *c* and *m* provided a good approximation of experimentally defined pupil responses for *Opn1mw*^{R} at 500nm (at which both cone and melanopsin are functional). The large discrepancy in the magnitude of *c* and *m* at higher irradiances (**B**) implies that the ability of melanopsin to influence mRGC activity over these timeframes far exceeds that of cones.

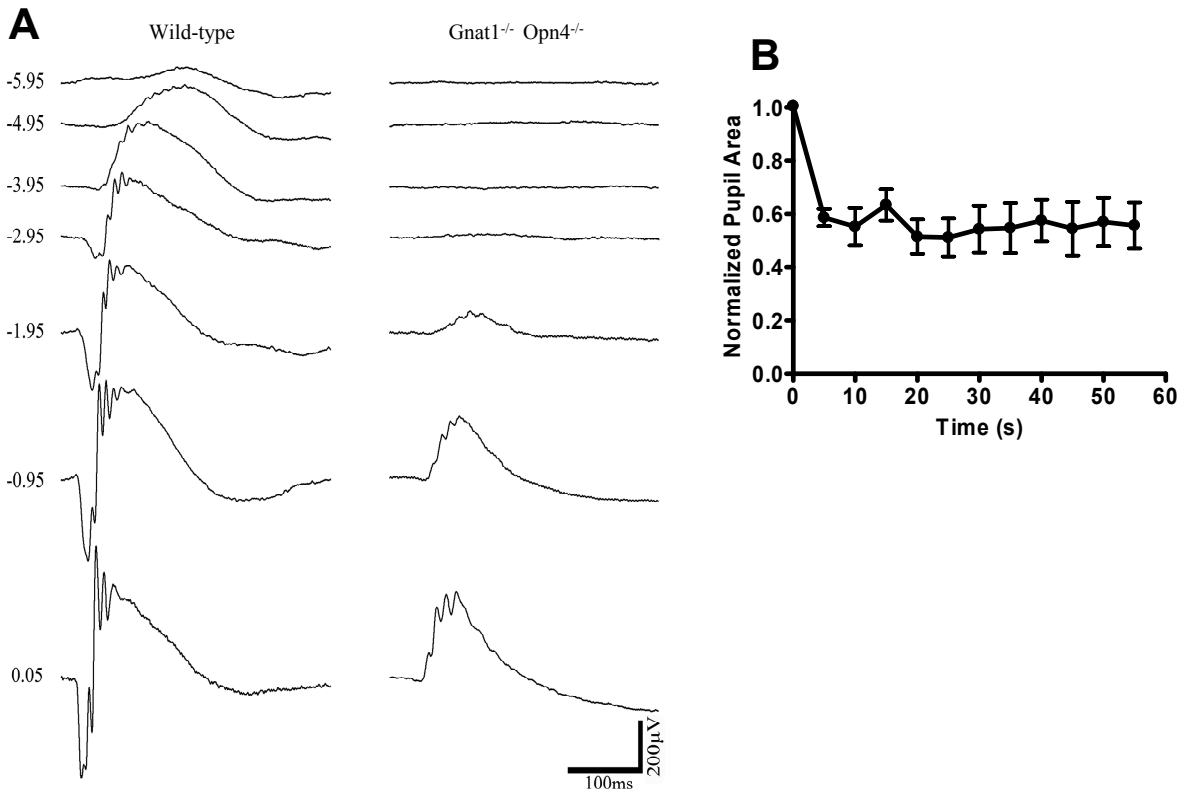
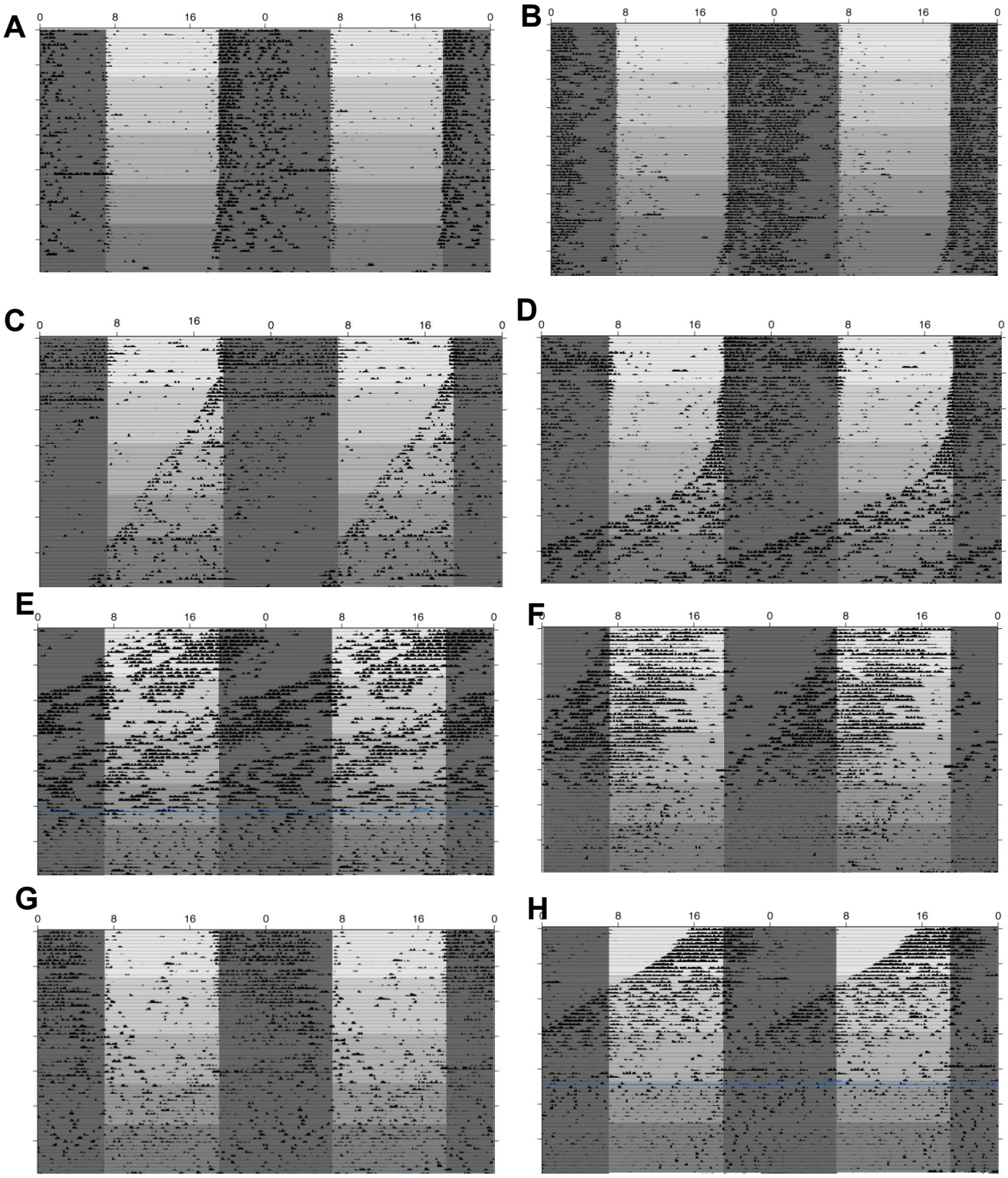


Figure S3: Electroretinogram and pupillary responses in *Opn4*$^{-/-}$ *Gnat1*$^{-/-}$ mice. **A.** Representative flash ERG traces from dark adapted *Opn4*$^{-/-}$ *Gnat1*$^{-/-}$ (right) and wild type (left) mice reveal selective loss of dim (<-2.95 log W/m^2; <-0.48 log cd-s/m^2) but not bright light responses in the transgenic animals confirming the presence of cone but not rod pathways. Figures to the left are stimulus irradiance in log W/m^2. Electroretinography was undertaken under ketamine (70mg/kg) and xylazine (7mg/kg) anaesthesia as previously described (Barnard et al., 2006). Mice were long-term dark adapted (>12hr) prior to recording scotopic ERGs using 15ms ganzfled flash stimuli (Xe arc source; Cairn Research Ltd., Faversham, UK) were applied at corneal irradiances in the range -5.95 to 0.05 log W/m^2 (-3.48 to 0.05 log cd-s/m^2) Inter-stimulus interval (ISI) was 1.5s at the dimmest intensities and was increased, proportionally with irradiance, to 30s at the brightest intensities. Similarly, the number of repetitions decreased from 30 to 6 as the stimulus intensity was increased. Signals were band-pass filtered 0.5 to 200Hz, and digitized at a sampling rate 2kHz. **B**. The ability of cones to drive NIF responses is confirmed by a marked and sustained reduction in pupil size (mean±SEM; n=4) to a 60s 480nm light stimulus (5.7x10^{14} photons/cm^2/sec; switched on at time 0) in dark adapted *Opn4*$^{-/-}$ *Gnat1*$^{-/-}$ mice.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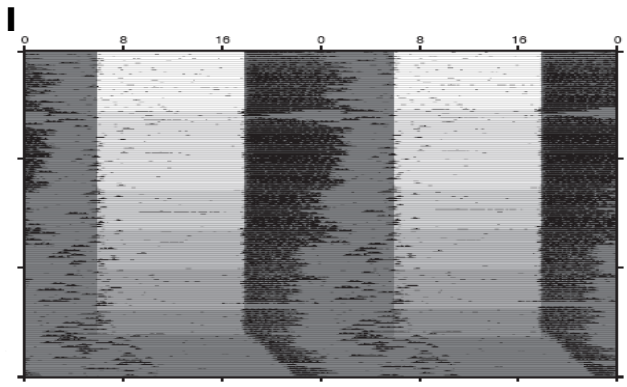
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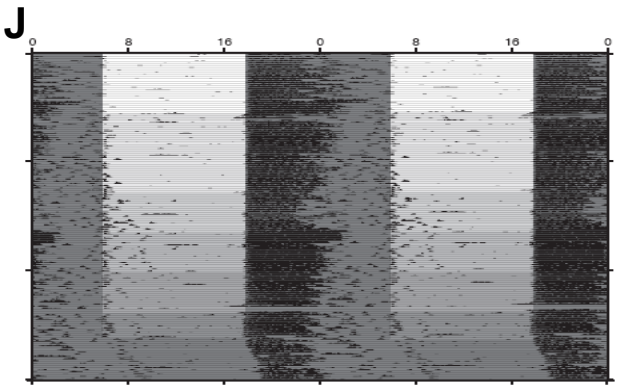
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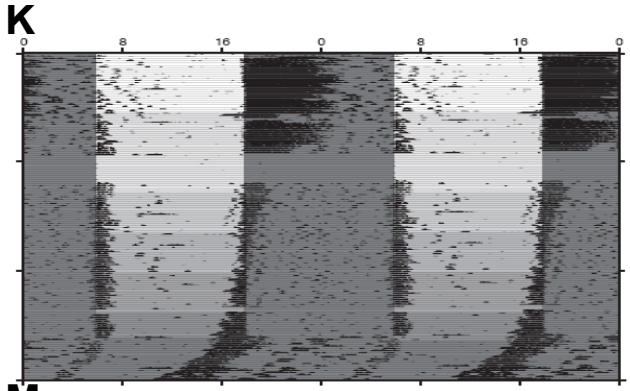
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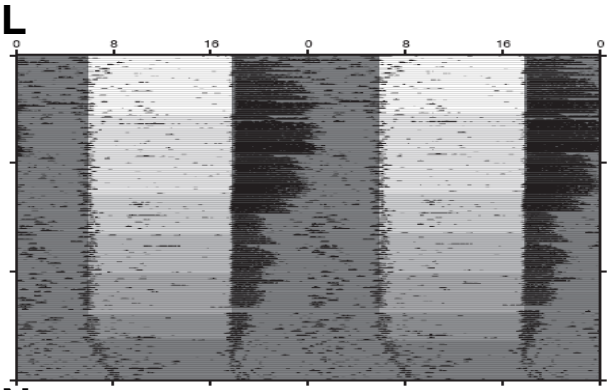
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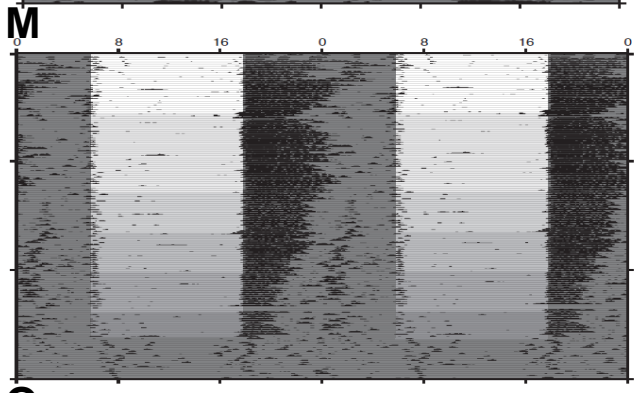
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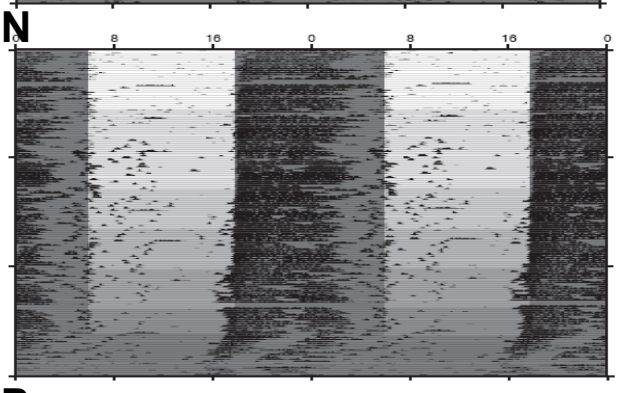
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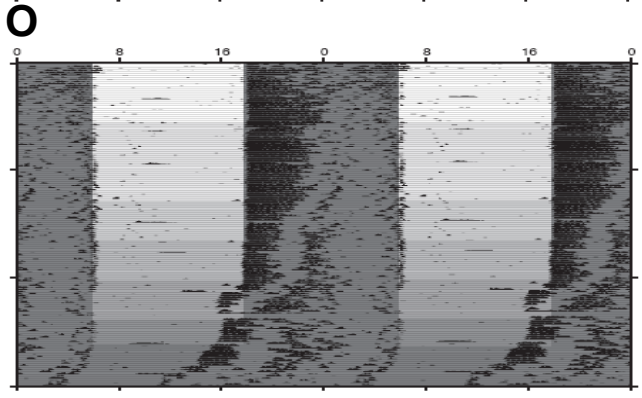
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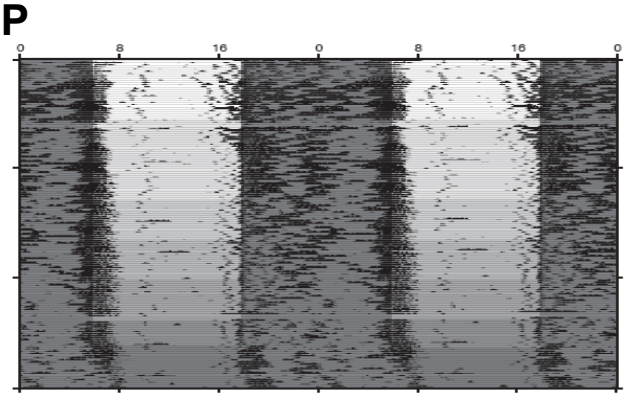
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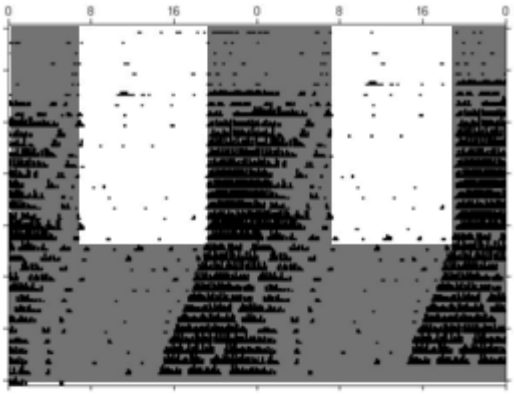
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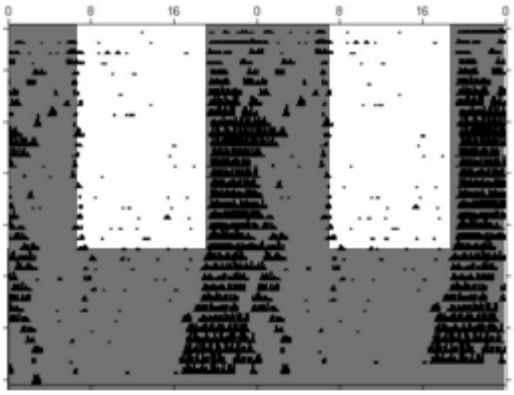
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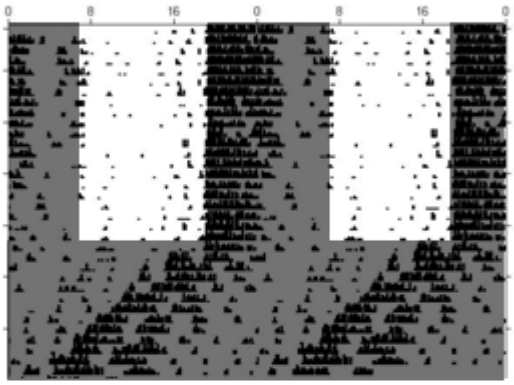
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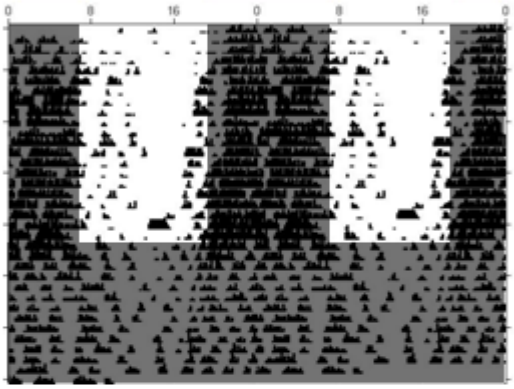
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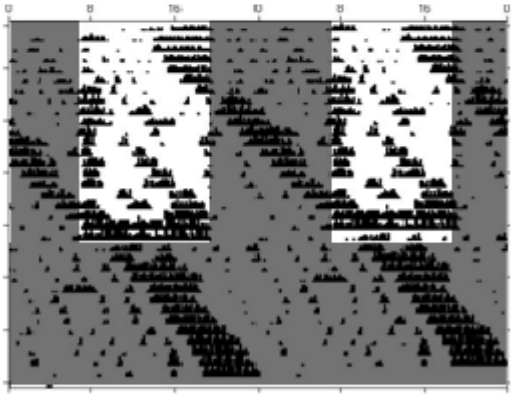
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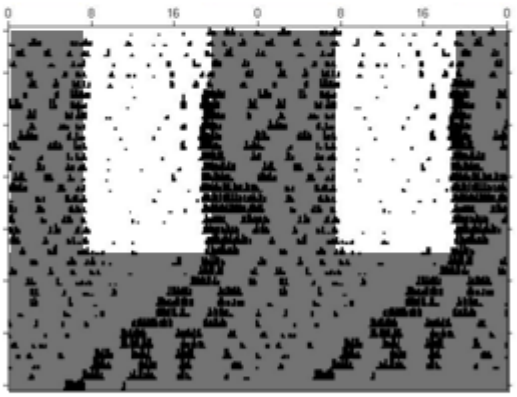
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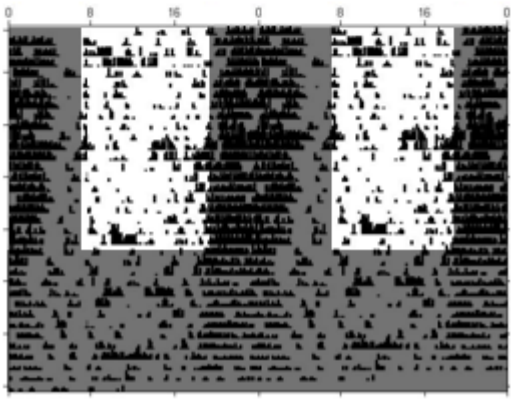
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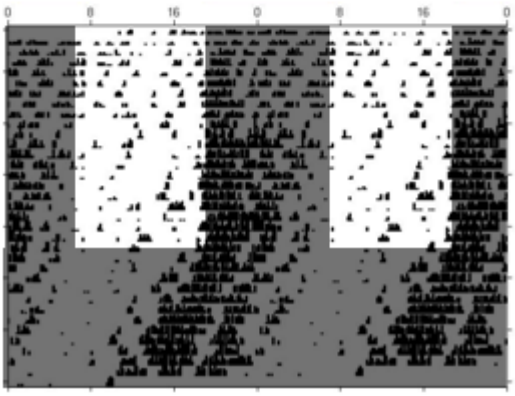
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Figure S4: *Opn4*$^{-/-}$ *Gnat1*$^{-/-}$ mice show inconsistent circadian photoentrainment. Representative double plotted actograms from wild type (**A**&**B**) and *Opn4*$^{-/-}$ *Gnat1*$^{-/-}$ (**C**-**H**) mice exposed to a 12h:12h LD cycle (dark phase shown as intense background shading) in which the irradiance of the light phase started at 235μW/cm^2 (fluorescent white source) and was reduced by a factor of 10 at two weekly intervals (depicted as increases in shading). At 235μW/cm^2 half (3/6) of *Opn4*$^{-/-}$ *Gnat1*$^{-/-}$ mice showed clear free-run (period<24h), and only 1/6 entrained down to 23.5μW/cm^2. By contrast, all wild type mice entrained to as little as 0.235μW/cm^2. Similar records from *Opn4*$^{-/-}$ (**I**-**M**) confirm that mice of this genotype show similar entrainment as wild types (**N**-**P**) at a range of irradiances from 235-0.0235μW/cm^2 (irradiance reduced by at times indicated by increases in shading x10 until finally released into DD) indicating that this was not a reflection of melanopsin loss. To determine whether *Opn4*$^{-/-}$ *Gnat1*$^{-/-}$ entrainment would be more normal at higher irradiances, we subjected a further group of *Opn4*$^{-/-}$ *Gnat1*$^{-/-}$ mice to a 12:12 LD cycle at an irradiance of 1mW/cm^2. Double-plotted actograms (periods of darkness indicated by shading) confirm that wild types (**Q** & **R**, representative of 4 such records) entrain well to this cycle until released into free-run in constant darkness. By contrast, even at this highest light intensity only a minority of *Opn4*$^{-/-}$ *Gnat1*$^{-/-}$ mice (**S**-**X**) showed clear 'wild type-like' entrainment with an appropriate phase angle (**V**, **X**). The remainder free-ran (**U**), entrained with a strong positive phase angle (**S**) or exhibited $t \approx 24$h, making an assessment of entrainment difficult (**T**&**W**). These findings are thus consistent with the conclusion drawn from *Opn1mw*^{R} experiments that while cones do provide light input to the clock, their ability to support entrainment is limited.

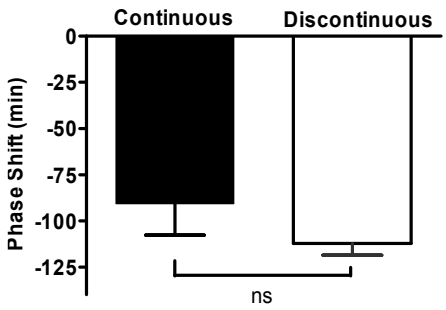

Figure S5: *rd/rd cl* mice show equivalent phase shifts (mean±SEM; n=4; paired t-test $p>0.05$) to 1.5×10^{13} photons/cm^2/sec of 500nm presented either as a single 15min (continuous) stimulus (filled bar) or as 15x1min (discontinuous) pulses spread over 43min (empty bar).