Irradiance encoding in the suprachiasmatic nuclei by rod and cone photoreceptors

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ABSTRACT **Light information is transmitted to the central clock of the suprachiasmatic nuclei (SCN) for daily synchronization to the external solar cycle. Essential for synchronization is the capacity of SCN neurons to respond in a sustained and irradiance-dependent manner to light. Melanopsin has been considered to mediate this photosensory task of irradiance detection. By contrast, the contribution of the classical photoreceptors in irradiance encoding is less clear. Here we investigate the role of classical photoreceptors by *in vivo* electrophysiological responses in freely moving animals to specific wavelengths of light (UV, λ_{max} 365 nm; blue, λ_{max} 467 nm; and green, λ_{max} 505 nm) in both melanopsin-deficient (*Opn4*$^{-/-}$) mice and mice lacking rods and cones (*rd/rd cl*). Short- and long-wavelength light induced sustained irradiance-dependent responses in congenic wild-type mice (+19.6%). Unexpectedly, sustained responses to light persisted in *Opn4*$^{-/-}$ mice (+18.4%). These results provide unambiguous evidence that classical photoreceptors can transmit irradiance information to the SCN. In addition, at light intensities that would stimulate rod and cone photoreceptors, the SCN of *rd/rd cl* mice showed greatly reduced sustained responses to light (+7.8%). Collectively, our data demonstrate a role for classical photoreceptors in illuminance detection by the SCN.—van Diepen, H. C., Ramkisoensing, A., Peirson, S. N., Foster, R. G., Meijer, J. H. Irradiance encoding in the suprachiasmatic nuclei by rod and cone photoreceptors. *FASEB J.* 27, 4204–4212 (2013). www.fasebj.org**

Key Words: circadian · melanopsin · light · electrophysiology

The mammalian circadian pacemaker is located in the suprachiasmatic nucleus (SCN) and coordinates daily cycles in behavior and physiology. Light is the primary cue for synchronization (entrainment) of the SCN such that dawn and dusk adjusts the endogenous period of SCN neurons to the 24-h external cycle. Light information is detected in the retina by classical rod-and-cone photoreceptors and by a specialized subset of intrinsically photosensitive retinal ganglion cells (ipRGCs) that express the photopigment melanopsin. Photic information is then transmitted to the SCNs *via* the retinohypothalamic tract (RHT; ref. 1). In addition to their direct photosensitivity, the ipRGCs receive an indirect light input from rod and cone photoreceptors (2–5), and by this route the classical photoreceptors also signal light information to the circadian system (6).

In response to retinal illumination, light-responsive SCN neurons show an increase in electrical impulse frequency (7–12). Typically, SCN responses to light consist of two components, a fast transient at the on- and off-set of the light pulse, and a sustained component that depends on the irradiance level of the light (8, 13). These two response components have been variously linked to differential melanopsin and rod/cone inputs, with sustained responses thought to arise from melanopsin and the transients from the rods and cones (9, 11, 12). However, several studies have suggested a role for the rods and cones in circadian entrainment, melatonin suppression and SCN electrical activity (7, 9, 12, 14–21). While ultraviolet (UV) light *via* the stimulation of UV-sensitive (UVS) cones has been shown to drive sustained responses in SCN neurons (19), light in the visible range failed to drive this sustained response in opsin 4 (melanopsin)-deficient (*Opn4*$^{-/-}$) mice (11). These findings are difficult to reconcile with behavioral data showing that light in the visible range can still drive illuminance detection in mice lacking melanopsin (15, 20, 21). For example,

Abbreviations: ANOVA, analysis of variance; CT, circadian time; ipRGC, intrinsically photosensitive retinal ganglion cell; MWS, middle-wavelength sensitive; Opn4, opsin 4 (melanopsin); RHT, retinohypothalamic tract; SCN, suprachiasmatic nucleus; UV, ultraviolet; UVS, ultraviolet sensitive

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illuminance-dependent period lengthening still occurs in *Opn4*$^{-/-}$ mice (20, 22).

In this study we explored the effect of both short and long wavelengths of light on SCN electrical activity in wild-type (*Opn4*$^{+/+}$) mice, *Opn4*$^{-/-}$ mice, and mice lacking rods and cones (*rd/rd cl*). Electrical activity recordings from the SCN of freely moving mice show an acute irradiance-dependent firing of SCN neurons on UV (λ_{max} 365 nm), blue (λ_{max} 467 nm), and green (λ_{max} 505 nm) light exposure. These responses are sustained for the full duration of the stimulus. This sustained response was unaffected by the loss of melanopsin. Recordings in *rd/rd cl* mice showed intensity-dependent sustained responses in the SCN, but these were attenuated at lower irradiances across the sensitivity range of the rods and cones. Our findings provide strong evidence that classical photoreceptors, along with melanopsin, encode tonic irradiance information to the SCN.

MATERIALS AND METHODS

Ethics approval

All animal experiments were approved by the Animal Experiments Ethics Committee of the Leiden University Medical Center (Leiden, The Netherlands).

Animals

Adult male *Opn4*$^{+/+}$ (n=4) and *Opn4*$^{-/-}$ (n=8) mice on a C57Bl/6 × 129 background (aged 3–9 mo) and male *rd/rd cl* (n=4) and wild-type (n=4) mice on a C3H background (aged 3–9 mo) were used for *in vivo* recordings of multiunit activity from the SCN. Mice were housed individually, and food and water were available *ad libitum*. Ambient room temperature was maintained at 20 ± 2°C.

In vivo electrophysiology

Surgical procedures were performed under deep anesthesia (ketamine, 100 mg/kg; xylazine, 20 mg/kg; atropine 1 mg/kg). A tripolar stainless steel electrode (Plastics One, Roanoke, VA, USA) was implanted using a stereotactic instrument (Stoelting, Wood Dale, IL, USA). Two twisted polyimide-insulated electrodes aimed at the SCN were used for differential recording, and a third uncoated electrode was placed in the cortex as a reference. The electrode was implanted under a 5° angle using the following coordinates: 0.61 mm lateral from Bregma and 5.38 ventral to the dura. After a week of recovery, animals were placed in custom-designed recording chamber and were connected to a counterbalanced swivel recording system that allowed the animal to move freely. The electrical signal was amplified and bandwidth filtered (0.5–5 kHz); window discriminators were used to count digital pulses in 2-s epochs (CircaV1.9 custom-ade software). Data was stored for offline analysis.

Animals were exposed to monochromatic light using a custom-designed sphere. The diameter of the sphere was 30 cm, and it was coated with high-reflectance paint (barium sulfate; WRC-680; Labsphere Inc., North Sutton, NH, USA) to obtain uniform illumination levels. At the top of the sphere, an opening was created for the connecting swivel system. Around the opening at the top of the sphere, high-power UV (λ_{max} 365 nm, NCSU033B; Nichia, Tokyo, Japan), blue (λ_{max} 467 nm, XML-PB01-0023; Luxeon Rebel; Philips, Amsterdam, The Netherlands), and green (λ_{max} 505 nm, Luxeon Rebel; Philips) LEDs were positioned, with a baffle to prevent the animal from looking directly at the light source. The wavelength of light and its irradiance were measured using a calibrated spectrometer (AvaSpec2048; Avantes, Apeldoorn, The Netherlands). Using these parameters, the actual amount of photons was calculated. Irradiance levels were manually regulated, and the timing of the light pulses was computer controlled. To investigate the response characteristics (*i.e.*, transient and sustained components), mice were exposed to light pulses of various durations (100 and 300 s) and intensities (ranging from 9 to 13 log quanta/cm^2/s). These durations are similar to or exceed those used in other studies to characterize SCN responses (7, 9, 11, 12). All light pulses were applied between circadian time (CT) 14 and CT18, which corresponds with the middle of the subjective night, the phase at which light pulses induce phase shifts. Animals were housed in constant darkness for 1–3 d prior to the recording of the light responses. For the determination of the response characteristics, mean changes in SCN electrical activity were compared to baseline discharge levels. The baseline level was defined as the average firing rate during the last 100 s before lights on, and the sustained level of SCN electrical activity was defined as the average discharge rate during the entire duration of the light pulse, excluding the first 25 s because of the transient response.

To determine the response latency of the SCN discharge rate in response to a light pulse at a high resolution, the electrophysiological signals of the SCN neurons were digitized at 25 kHz using Spike2 hardware and software (Cambridge Electronic Design, Cambridge, UK) and stored for offline analysis. Analysis was performed as described previously (19). The digitized recordings were imported into MATLAB as waveform data, including data from light and movement sensors, using parts of the sigTOOL SON Library. Imported waveform data were triggered at fixed voltage amplitude settings, and time and amplitude of these action potentials were used for analysis. To investigate the latency of the neuronal response to light onset, digitized action potentials were counted in 0.01-s bins. For a detailed analysis of population activity, a baseline recording (100 s before light pulse) was used to create a spike amplitude histogram. On the basis of this amplitude histogram, thresholds were set in such a way that the average number of counts within each threshold window was equal. Threshold windows were nonoverlapping and started above noise level. Action potentials were counted within each step of set thresholds for the remainder of the recording.

Histology

At the end of each recording, animals were euthanized using CO_2, and a small electrolytic current was passed through the electrode to mark the recording site. Brain tissue was collected and immersed in a fixative solution containing potassium ferrocyanide to stain the recording site blue. The brain was sectioned coronally and stained with cresyl violet for microscopic reconstruction of the recording site (**Fig. 1*A***).

Statistical analysis

Statistical analysis was performed using SPSS Statistics 17 (PASW; SPSS Inc., Chicago, IL, USA). Two-tailed Student's t tests or 1-way analysis of variance (ANOVA), followed by a Bonferroni's *post hoc* test, were used to test significant differ-

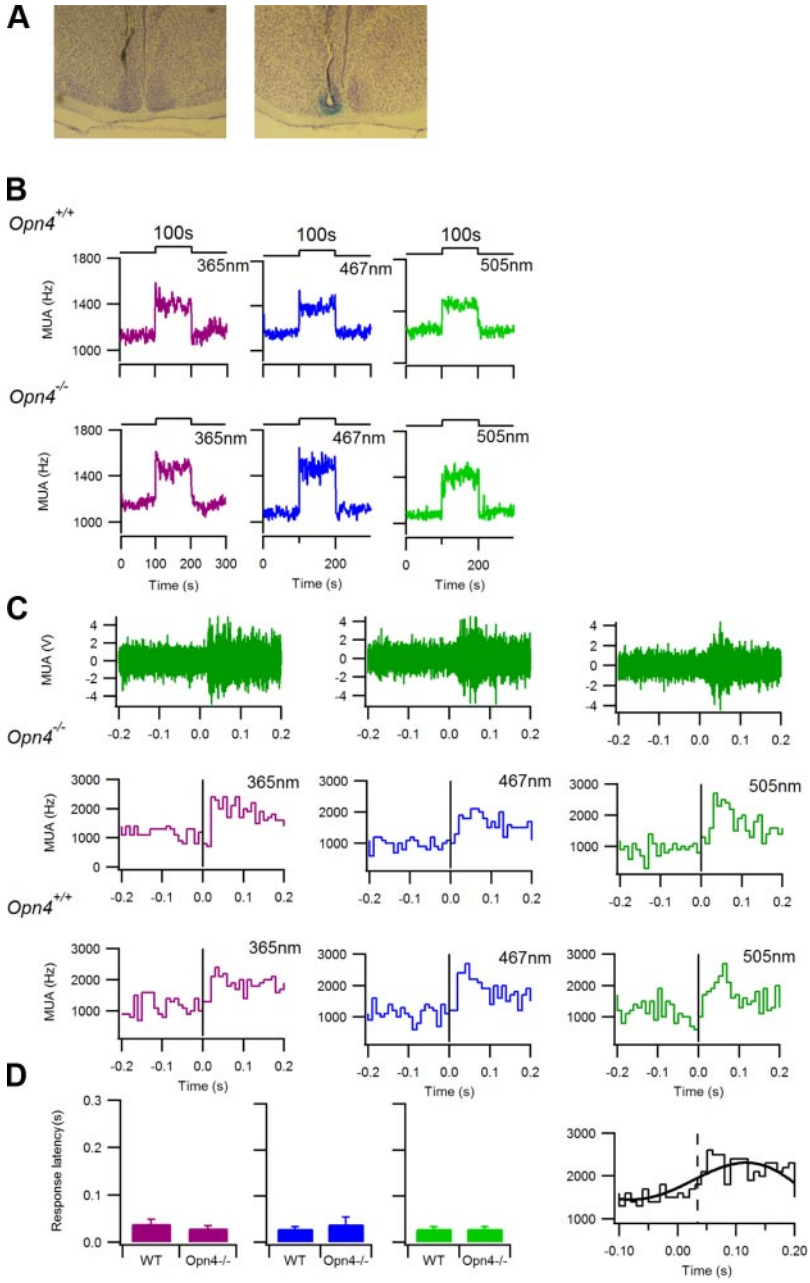


Figure 1. *A*) An example of a coronal slice of the mouse brain with the SCN right above the optic chiasm at the base of the hypothalamus (left panel). The location of the electrode can be verified by the blue spot, which is marked using an electrolytic current (right panel). *B*) SCN electrical activity responses to various wavelengths of light in freely moving melanopsin-deficient ($Opn4^{-/-}$) mice and their wild-type ($Opn4^{+/+}$) littermates. Graphs show representative SCN multiunit activity (MUA) traces in response to a 100-s UV (λ_{max} 365 nm), blue (λ_{max} 467 nm), and green (λ_{max} 505 nm) light pulse. Light pulses are indicated above the graphs. Bin size is 1 s. SCN electrical activity shows a sustained response during exposure to UV, blue, and green light in both $Opn4^{-/-}$ (+18.4%) and $Opn4^{+/+}$ (+19.6%) mice. *C*) Response latencies to UV (λ_{max} 365 nm), blue (λ_{max} 467 nm), and green (λ_{max} 505 nm) light exposure in $Opn4^{-/-}$ and $Opn4^{+/+}$mice. Time of lights on is indicated by the vertical line in each graph. Top graphs show a corresponding oscilloscope trace of SCN electrical activity; counts are shown in the bottom graphs. Bin size is 0.01 s. SCN firing rate is increased in both melanopsin-deficient and wild-type mice, with a response latency of 0.04 s to the 3 wavelengths of light. *D*) Histograms showing mean ± SEM response latencies of $Opn4^{-/-}$ and $Opn4^{+/+}$ mice in response to 3 wavelengths of light. Right panel is an example of a multiunit activity trace through which a polynomial fit is plotted. This fit was used to calculate the half maximum values. The dashed line indicates the half maximum value in the representative example. Half maximum values were used to determine the response latencies.

ences between groups. Values were considered significant at values of $P < 0.05$.

RESULTS

We determined the effect of UV (λ_{max} 365 nm), blue (λ_{max} 467 nm) and green (λ_{max} 505 nm) light on electrical activity of populations of SCN neurons in freely moving mice. Wild-type mice (n=8), possessing melanopsin ipRGCs, UVS cones (λ_{max} 360 nm), rods (λ_{max} 498 nm), and middle-wavelength-sensitive (MWS) cones (λ_{max} 508 nm) showed a robust increase in SCN electrical discharge, with a transient overshoot in firing at lights on and a sustained elevation in electrical activity throughout light exposure, in response to 100 s of monochromatic light (Fig. 1). We found a sustained increase in SCN electrical activity in response to light exposure up to 30 min. At lights off, a fast drop in electrical discharge rates was observed to levels that were transiently below baseline level. No differences in light-response characteristics of transient and sustained responses were found between responses to 365-, 467-, or 505-nm light (Fig. 1*B*).

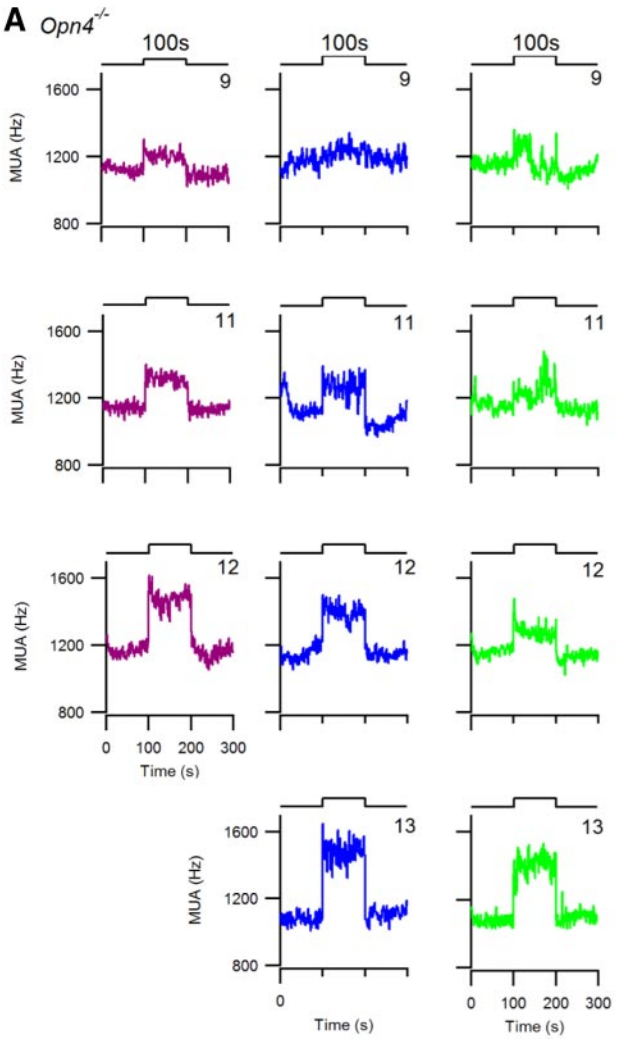
The possible contribution of rods and cones to the sustained response was investigated by determining whether the sustained component was affected by the absence of melanopsin in $Opn4^{-/-}$ mice (n=8). In response to 100 s of monochromatic UV light at 365 nm, SCN electrical discharge showed a tonic firing rate that was consistent with our previous findings (19). Surprisingly, exposure to longer wavelengths of light (467- and 505-nm stimuli) also elicited a sustained

response for the full duration of the light presentation. The kinetics of the light response were similar to those of wild-type mice and contained both phasic and tonic components (Fig. 1*A*). Response latencies in *Opn4*$^{-/-}$ mice to all wavelengths of light were in the range of 30–40 ms (n=8; Fig. 1*C, D*). The latencies were defined as the half maximum of a fitted polynomial function. These data show that tonic activation of SCN cells to 365-, 467-, and 505-nm light can all occur independently of melanopsin, indicating that rods and/or cones can induce a sustained response at the level of the SCN.

To investigate the sensitivity to different wavelengths of light, we undertook irradiance response measurements in *Opn4*$^{-/-}$ mice. Mice were exposed to 100 s of light of different irradiances ranging over 4 or 5 log units (9–13 log photons/cm^2/s). *Opn4*$^{-/-}$ mice and congenic wild-type mice showed a sustained response that was irradiance dependent (**Fig. 2**).

In *Opn4*$^{-/-}$ mice, both rods and MWS cones would be strongly stimulated by 467- and 505-nm stimuli. Only a small increase in SCN electrical activity was observed in response to the lowest irradiances (9–11 log photons/cm^2/s). At this irradiance, rod photoreceptors should be fully stimulated (23) and able to induce a maximal change in SCN electrical activity. However, at high irradiances (12–13 log photons/cm^2/s), the increase in SCN electrical activity was significantly larger compared to the sustained response at low rod-mediated irradiances (P=0.01, 1-way ANOVA).

To determine the possible contribution of classical photoreceptors to SCN light responses, we undertook recordings in *rd/rd cl* mice lacking both rods and UVS and MWS cones. SCN firing frequencies were increased in *rd/rd cl* mice (n=4) in response to 300-s light pulses of 365, 467, and 505 nm (**Fig. 3*A***). Because of the slow response kinetics, pulses with a duration of 300 s were used to make sure we could observe the full response repertoire. Typically, the light-activated SCN discharge started with a transient overshoot at lights on, followed by a relatively small sustained response, which was significantly smaller than that observed in wild-type animals (p=0.02, independent samples t test). No transient off inhibition was present at the end of light exposure; rather, SCN electrical discharge rate slowly returned to baseline after termination of light exposure. The *rd/rd cl* mice showed a response latency of 200 ms (n=4 animals; Fig. 3*B*) in reaction to light pulses of 365, 467, and 505 nm. The latencies were significant longer than the response latencies of 30–40 ms seen in



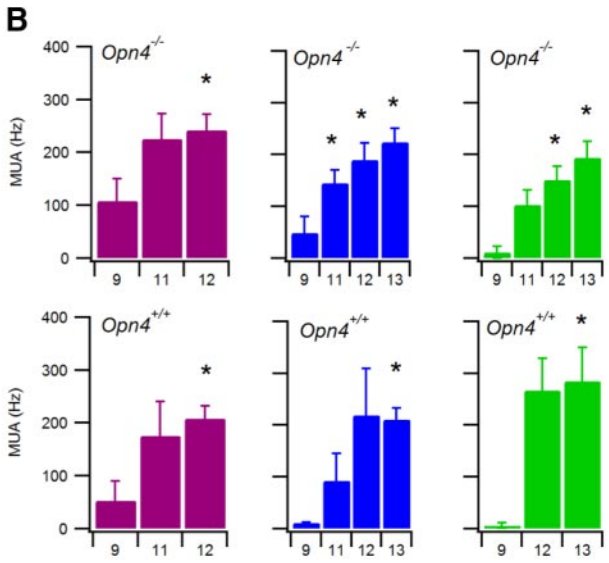


Figure 2. *A*) SCN electrical activity responses to UV (λ_{max} 365 nm), blue (λ_{max} 467 nm), and green (λ_{max} 505 nm) light exposure at 3 different irradiances of light in *Opn4*$^{-/-}$ mice. Mice were exposed to 100 s of light, as indicated in a step diagram above each graph. One representative trace of SCN electrical activity is shown per irradiance, with log quanta per square centimeter per second shown in each graph. Bin size is 1 s. *B)* Histograms showing mean sustained responses in SCN electrical activity as a function of irradiance of UV, blue, and green light in *Opn4*$^{-/-}$ and *Opn4*$^{+/+}$ mice. Graphs show the difference in spike frequency between sustained and baseline levels for each irradiance level (n=4–7/irradiance). Sustained responses to light in SCN electrical activity are increased in an irradiance-dependent manner in both *Opn4*$^{-/-}$ and *Opn4*$^{+/+}$ mice. No difference was detected between UV, blue, and green light. *$P < 0.05$.

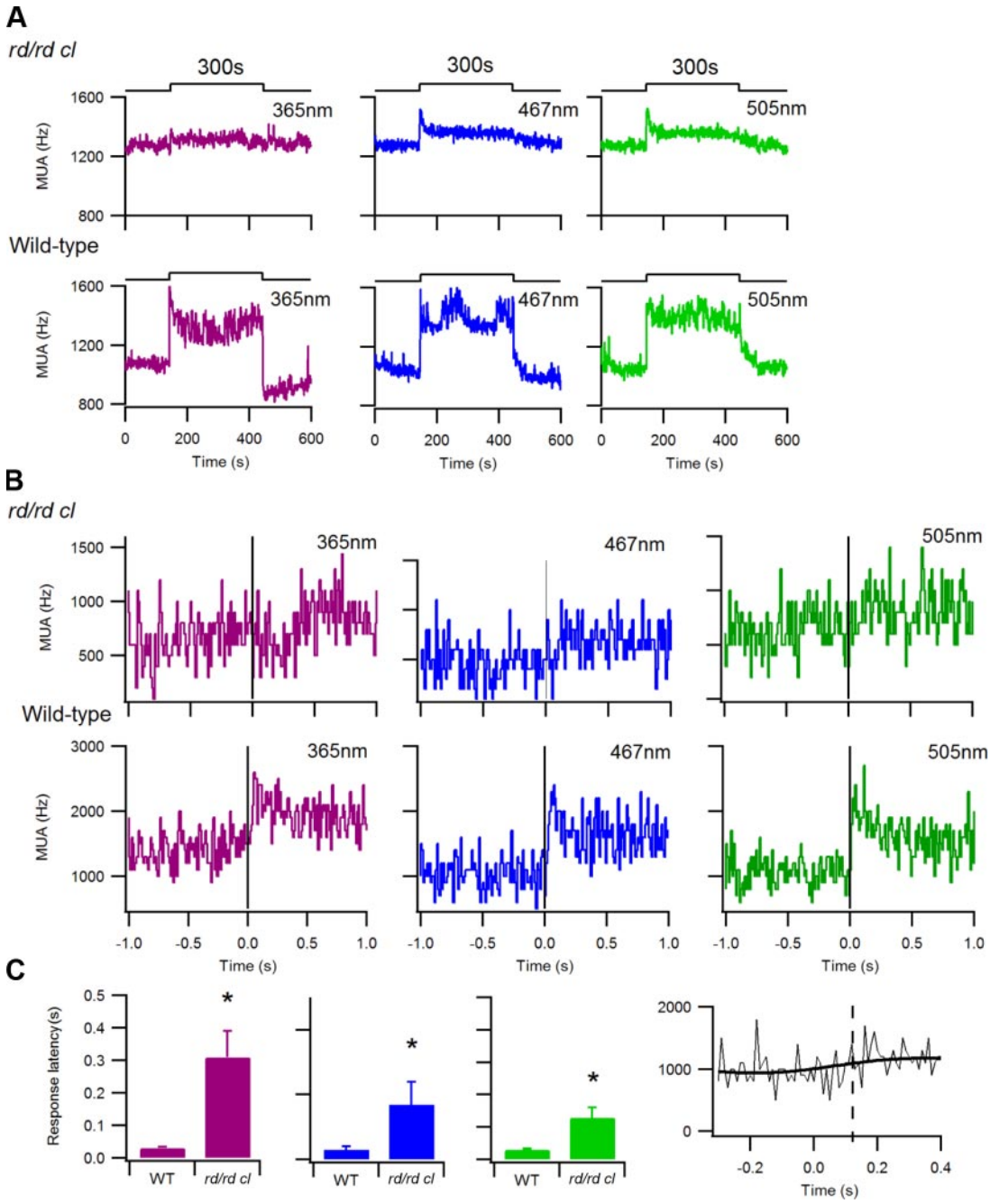


Figure 3. *A*) Representative traces of multiunit activity (MUA) in response to a 5-min light pulse in *rd/rd cl* mice and their wild-type littermates. Mice were exposed to UV (λ_{max} 365 nm), blue (λ_{max} 467 nm), and green (λ_{max} 505 nm) light. *rd/rd cl* mice show a reduced sustained response to UV (+2.3% increase), blue (+12.5%), and green (+8.9%) light and do not show an off-response. SCN electrical activity slowly returns to baseline after light exposure. *B*) Response latencies to UV (λ_{max} 365 nm), blue (λ_{max} 467 nm), and green (λ_{max} 505 nm) light exposure in *rd/rd cl* and wild-type mice. Time of lights on is indicated by the vertical line in each graph. Bin size is 0.01 s. SCN firing rate is increased in both *rd/rd cl* and wild-type mice, with a response latency of 0.04 s in wild-type mice and of 0.20 s in *rd/rd cl* mice. *C*) Histograms showing mean ± SEM response latencies in *rd/rd cl* and wild-type mice in response to 3 wavelengths of light. Right panel is an example of a multiunit activity trace through which a polynomial fit is plotted. This fit was used to calculate the half maximum values. The dashed line indicates the half maximum value in the representative example. Half maximum values were used to determine the response latencies. Note the different scale on the *x* axis compared to panel *B*. $^*P < 0.05$.

congenic wild-type mice ($P<0.001$, independent samples *t* test; Fig. 3*C*).

To investigate the sensitivity of *rd/rd cl* mice ($n=4$) to various wavelengths of light, these mice were exposed to 300 s of light at 3 different wavelengths (365, 467, and 505 nm), ranging from low to high irradiances. While a sustained response to 365-nm light was detected at the highest irradiance (~12 log photons/cm^2/s, $P=0.22$, 1-way ANOVA; **Fig. 4**), no light response was detected at lower irradiances. Only the

highest irradiances of 467- and 505-nm stimuli induced a significant sustained response in SCN electrical activity levels ($P=0.04$, 1-way ANOVA). Transient responses were also detected at the highest light intensities and showed a longer latency compared to wild-type congenics.

DISCUSSION

Our findings show that the SCN exhibits sustained firing levels in the absence of melanopsin, and across a broad range of wavelengths. Sustained increases in electrical discharge were observed during exposure to UV, blue, and green light for the entire duration of the light pulse from the SCN of wild-type and *Opn4*$^{-/-}$ mice. These findings suggest strongly that classical photoreceptors can induce tonic responses to light at the level of the SCN. We undertook recordings from *rd/rd cl* mice lacking all rods and cones and found an attenuation of the sustained response across all wavelengths at irradiances that were sufficient to fully stimulate classical photoreceptors. Our findings are consistent with behavioral experiments that have shown preserved tonic responses to light on circadian period in rod-only mice (15); attenuated phase-shifting responses in mice lacking MWS-cones (14, 16); and tonic responses to light recorded from retinal ganglion cells in the absence of melanopsin (24).

Origin of sustained responses in the SCN

In vivo electrophysiological recordings in the SCN showed that UV light (365 nm) can induce sustained light responses within the SCN, consistent with our previous studies (19). The current results show that green (505 nm) and blue (467 nm) light pulses can also elicit transient and sustained responses to light within the SCN, fully mimicking the effect of white light on the SCN. Remarkably, in the absence of melanopsin, the full response characteristics were maintained. This finding was unexpected, as the ability to drive a tonic/sustained response is generally assumed to be the property of melanopsin-based phototransduction (9, 11, 12, 25). In contrast to the present study, Mure *et al.* (11) failed to detect sustained responses to light in mice lacking melanopsin. This difference might arise because Mure *et al.* (11) recorded from the SCN of anesthetized animals and at a different time of day, zeitgeber time (ZT) 4 to ZT12 *vs.* CT14 to CT18 in the present study. In agreement with our findings, direct recordings from illuminated ipRGCs of the macaque monkey have been shown to elicit sustained responses at irradiances considered too low to drive melanopsin phototransduction (2). In addition, recordings from mice lacking melanopsin (*Opn4*$^{-/-}$) show that ipRGCs retain the ability to respond to light in a sustained manner (24). These findings, with the data we present here, all strongly suggest that rods and cones can mediate sustained responses to light from SCN neurons. Furthermore, recent findings indicate that short-wavelength light triggers sustained responses in electrical activity within the PON and SCN (19, 26). The present findings show that illuminance detection by the SCN occurs across a broad spectral range (UV to visible) and can be mediated by both melanopsin and the classical photoreceptors.

We also examined light-induced electrical responses in the SCN of *rd/rd cl* mice with melanopsin ipRGCs, but lacking rods and UVS and MWS cones, and observed attenuated sustained responses to UV, blue, and green light. The light response of *rd/rd cl* mice was characterized by an absence of the fast transient component, as well as by a reduction in the sustained discharge. In response to low-light irradiances (9 log/quanta/cm^2), no light responses were observed, consistent with the relative insensitivity of melanopsin-based ipRGCs (3, 25, 27). At higher irradiances (>12 log/quanta/cm^2) an intensity-dependent increase in the sustained response was observed. In agreement with Wong *et al.* (3, 24), these responses were "sluggish" and slow, even for the moderate irradiances, and all transients were lost. Also, in agreement with direct recordings from ipRGcs (3, 24), we failed to detect responses to low levels of light in the SCN in the absence of classical photoreceptors.

Origin of transient on and off responses

We observed transient "on-excitation" and "off-inhibition" in response to UV, blue, and green light in both wild-type and melanopsin-deficient mice. The presence of these responses in the absence of melanopsin indicates a role for rods and/or cones in evoking the transient on-excitation and off-inhibition. Interestingly, the off-inhibition following light exposure was not present in mice lacking rods and cones, which suggests a role for classical photoreceptors in mediating this response. The on-excitation was still present in *rd/rd cl* mice, but the reaction time of SCN electrical activity in response to light in these mice was 200 ms (Fig. 2*B*). This finding is consistent with melanopsin-mediated response latencies measured from ipRGCs reported previously (>300 ms to minutes; refs. 2, 25, 28) and differs from both rod-mediated (150 ms) and cone-mediated (30–60 ms) response latencies (2, 3). The response onset latency for all wavelengths of light in wild-type as well as in melanopsin-deficient mice has a very short duration of 30–40 ms (Fig. 2*A*). This similarity to cone-mediated response latencies supports the conclusion that cones can mediate broad-spectrum light input to the SCN at high light intensities.

Key features of the response properties of SCN neurons correlate well with the effects of light on circadian behavior. For example, mice possessing rods but lacking cones and melanopsin have been shown to entrain to both low- and high-light-intensity

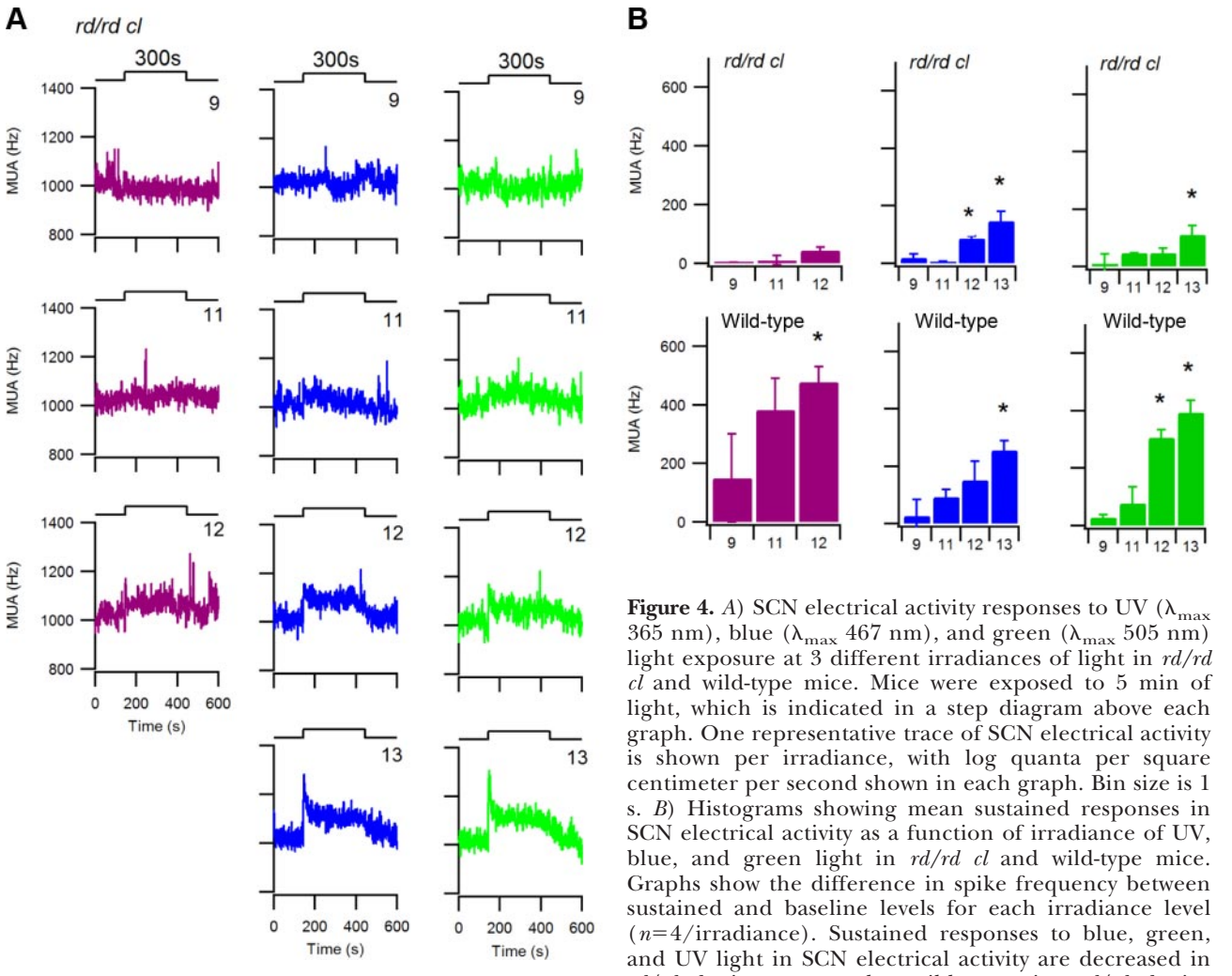


Figure 4. *A*) SCN electrical activity responses to UV (λ_{max} 365 nm), blue (λ_{max} 467 nm), and green (λ_{max} 505 nm) light exposure at 3 different irradiances of light in *rd/rd cl* and wild-type mice. Mice were exposed to 5 min of light, which is indicated in a step diagram above each graph. One representative trace of SCN electrical activity is shown per irradiance, with log quanta per square centimeter per second shown in each graph. Bin size is 1 s. *B*) Histograms showing mean sustained responses in SCN electrical activity as a function of irradiance of UV, blue, and green light in *rd/rd cl* and wild-type mice. Graphs show the difference in spike frequency between sustained and baseline levels for each irradiance level (n=4/irradiance). Sustained responses to blue, green, and UV light in SCN electrical activity are decreased in *rd/rd cl* mice compared to wild-type mice. *rd/rd cl* mice only respond to high light intensities of UV light. Both *rd/rd cl* and wild-type mice show irradiance-dependent effects of green and blue light. *$P < 0.05$.

light-dark cycles, suggesting that rods alone can mediate photic entrainment (15). These findings raise the question of whether rods can drive tonic responses within the SCN. In this study, we observed small increments in SCN firing frequency in response to light intensities (9–11 log photons/cm^2/s), which should have been sufficient to maximally stimulate the rods. These responses were sustained and are, therefore, attributable to full stimulation of the rods and/or partial stimulation of the cones. Sustained responses significantly increased at higher light intensities compared to low light intensities, which are expected to have saturated the rods. These results suggest that cones can contribute to the sustained response of the SCN at higher light intensities. The observed short response latencies are also in accordance with cone activation, as well as the reduction in phase-shifting magnitude in the absence of MWS cones (9, 14). By contrast, cone-only mice show poor entrainment to light-dark cycles (18), which is difficult to reconcile with a contribution of cones to the sustained response of the SCN. It is possible that cone-only mice have deficiencies that have occurred developmentally in retinal information processing, or, alternatively, that the sustained cone driven discharge in the SCN is not sufficient for triggering functional entrainment pathways. This remains to be elucidated.

The discovery that the photic regulation of circadian behavior (29), melatonin suppression (30), and sleep induction (31) can all persist in the absence of rods and cones does not of course preclude a role for these photoreceptors in non-image-forming responses to light in the intact retina. Behavioral studies in rodents have shown that the circadian system is very sensitive to small changes in light-intensity, such that small variations in light intensity can be sufficient for entrainment (15, 18, 32, 33). Thus, activation of only a subset of retinal photoreceptors may be sufficient for photic entrainment. Collectively, the studies published to date show a mixed and complex arrangement of photoreceptor inputs involved in irradiance detection. The relative contribution of melanopsin, rods, and cones remains to be determined, and is most likely dependent on the intensity and wavelength composition of the light source.

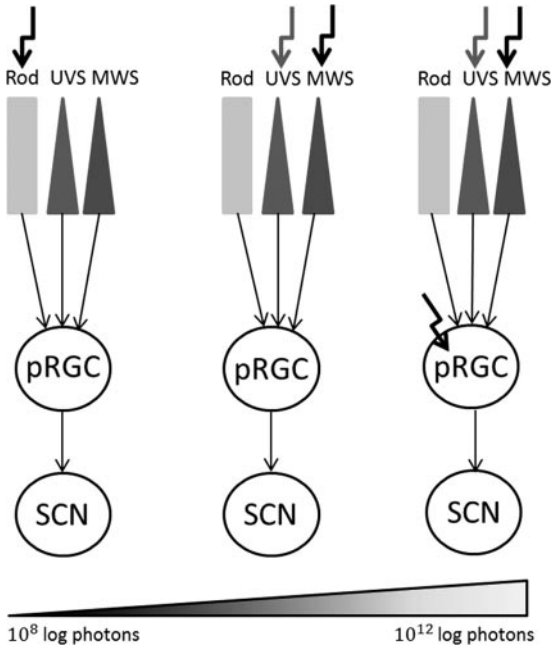


Figure 5. Schematic representation of the involvement of various photoreceptors in the photic input to the circadian system across a range of light intensities. Light intensities are depicted below the figure. Arrows depict light input; gray arrows indicate UV light, and black arrows indicate long-wavelength light. Our data suggest that, at high irradiances, cones can play a role in irradiance detection. Classical photoreceptor input to the SCN is most likely *via* the pRGCs. Even when these cells lack intrinsic photosensitivity (*Opn4*$^{-/-}$), rod and cone inputs to these cells may be capable of mediating normal SCN responses.

CONCLUSIONS

An increasing body of evidence shows an important contribution of classical photoreceptors to circadian entrainment (14, 15, 18, 19), pupil constriction (26, 34) and SCN neuronal responses to light (7, 9, 11, 19, 20). Both rods (15, 18) and cones (14, 16, 19) were suggested to be important for the circadian system, and our present findings offer a mechanism through which classical photoreceptors regulate the SCN (**Fig. 5**). We suggest that at lower to intermediate irradiances, classical photoreceptors play a role in transmitting light information to the SCN. While we cannot distinguish between the relative contribution of rods and cones, it seems that both rods and cones are involved. At low light intensities, only rods are stimulated, and we observed clear tonic responses in the SCN. An increment in light intensity to levels that should have saturated the rods led to a further increment in light-response levels, presumably mediated by cones. Our studies make clear that melanopsin is not required for sustained responses to light at the level of the SCN and suggest an advanced level of functional redundancy in the irradiance detection capacity of the SCN. FJ

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Note added in proof: While the present article was in production, Weng *et al.* (35) was published. This paper characterizes the mechanisms *via* which rod and cone input may influence pRGC signals at the level of the retina, and provides complementary data relating to the role of classical photoreceptors in irradiance encoding.

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