Rest-activity cycles and melatonin phase angle of circadian entrainment in people without cone-mediated vision

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Abstract

Light is strong *zeitgeber* to the human circadian system, entraining internal rhythms in physiology and behaviour to the external world. This is mediated by the melanopsin-expressing intrinsically photosensitive retinal ganglion cells (ipRGCs), which sense light in addition to the classical photoreceptors, the cones and rods. Circadian responses depend on light intensity, with exposure to brighter light leading to bigger circadian phase shifts and melatonin suppression. In congenital achromatopsia (prevalence 1 in 30,000 to 50,000 people), the cone system is non-functional, resulting in light avoidance and photophobia at light levels which are tolerable and habitual to individuals with a normal, trichromatic retina. Here, we examined chronotype and self-reported sleep, actigraphy-derived rest-activity cycles and increases melatonin in the evening in a group of genetically confirmed congenital achromats. We found normal rest-activity patterns in all participants, and normal melatonin phase angles of entrainment in 2/3 of our participants. Our results suggest that a functional cone system and exposure to daytime light intensities are not necessary for regular behavioural and hormonal entrainment. This may point to a compensation mechanism in circadian photoreception, which in conjunction with non-photic *zeitgebers*, ensures synchronisation of activity to the external world.

Significance statement

Rhythms in physiology and behaviour are synchronised to the external cycle of light exposure. This is mediated by the retinohypothalamic tract, which connects the photoreceptors in the eye with the “circadian pacemaker” in our brain, the suprachiasmatic nucleus. What happens to our circadian rhythm when we lack the cone photoreceptors in the eye that enable us to see in daylight? We examined this question in a group of rare congenital achromats. Our work reveals that normal rhythms in rest and activity, and production of hormones, does not require a functional cone system.

Main text

Introduction

Light exposure at even moderate intensities at night attenuates the production of the hormone melatonin and shifts circadian rhythms in physiology and behaviour (1, 2). Light acts as a *zeitgeber*, enabling synchronisation, or entrainment, of the circadian clock to the periodic changes in ambient light levels (3). Generally, brighter light has a stronger *zeitgeber* strength, thus providing a more powerful input drive to the circadian timing system (3, 4). Circadian phase shifting follows a sigmoidal dose-response curve (5, 6), with half-maximum responses occurring between 80 and 160 lux for fluorescent white light (5).

These non-visual effects of light on the circadian clock are mediated by the retinohypothalamic pathway, which is largely driven by the intrinsically photosensitive retinal ganglion cells (ipRGCs) expressing the photopigment melanopsin (1, 7, 8). The ipRGCs are 'non-classical' photoreceptors signalling environmental light intensity independent of the 'classical' retinal photoreceptors, the cones and the rods (Fig. **1*a***). The range at which these photoreceptors are active differ between photoreceptor classes, with cones and ipRGCs responding to moderate to bright light (photopic light levels). Rods, expressing rhodopsin, on the other hand, are 3-4 orders of magnitude more sensitive and signal light at dim and dark light, scotopic light levels, and are saturate at photopic light levels (9, 10). Importantly, however, cones, rods and ipRGCs have a broad and overlapping wavelength tuning, or spectral sensitivity, determined by the photopigments they contain (Fig. **1*b***). This means that light exposure in most circumstances will activate all photoreceptors.

The normal trichromatic retina (Fig. **1*a***) contains three classes of cones – the short [S]-, medium [M]- and long [L]-wavelength sensitive cones –, the rods, and the ipRGCs. In *congenital autosomal recessive achromatopsia* (ACHM), also called *rod monochromacy* (estimated prevalence 1 in 30,000-50,000 people (11, 12)), the cone photoreceptors are non- or dysfunctional. In most cases this is due to mutations in the genes *CNGA3*, *CNGB3*, *GNAT2*, *PDE6H*, and *PDE6C* affecting different aspects of the phototransduction process in cone cells (13) (Fig. **1*c, d***). Rarely, mutations in *ATF6* have been shown to also cause ACHM.

As the cones are sensitive to moderate to bright lights and responsible for vision of colour, motion and spatial details at daylight light levels, patients with congenital ACHM lack functional photoreception in the upper range of typical day light exposures. This leads to strong visual discomfort, glare and light aversion (14). Quantitatively, achromats are hypersensitive to light (15), with corneal photosensitivity thresholds being 100 to 1000 times lower than for healthy controls (~3 lux (0.5 *log* lux) for achromats vs. ~1500 (3.2 *log* lux) for healthy controls (16, 17)). To be able to cope with typical, in particular daytime light levels, management of congenital ACHM includes tinted filter glasses (18-21).

Standard circadian theory predicts that lack of exposure to bright light would reduce the strength of light as *zeitgeber* (3, 4), prompting the question: If congenital achromats are not exposed to bright light levels, is their circadian photoentrainment disrupted? While aspects of rod-mediated vision have been examined, the question of non-classical photoreception in congenital ACHM has to our knowledge not yet received scientific attention. The authoritative book on vision in congenital ACHM does not contain any discussions on non-visual effects of light (14), not least because it predated the discovery of melanopsin. A 1992 *New York Times* article on congenital ACHM stated that “[m]any with the disorder are proud night owls, who love going out after dark” (22), suggesting at least anecdotal evidence for an adjustment of the circadian system in congenital achromats. A publication by *The Achromatopsia Network* suggests that many achromats prefer timing of outdoor and recreational activities to the “magical time of twilight” (23).

Previously, it has been shown that in some individuals who are functionally blind, the melatonin-suppressive effect of light is preserved (24-26), likely due to a functioning melanopsin ipRGC system even in the absence of cone and rod function. Direct evidence for a functional preservation of melanopsin-mediated ipRGC function has also been found in other conditions (e.g. Leber congenital amaurosis, (27)). Importantly, however, these individuals do not necessarily experience the severe discomfort reaction to light that congenital achromats do and therefore may indeed be exposed to much more daytime light levels than achromats.

Here, we specifically examined the circadian phenotype and photoreception in a group of genetically confirmed retinal achromats (n=7, age range 30-72 years), employing a

range of instruments to arrive at a first picture of how sleep and circadian rhythms are affected by a lack of daylight vision.

Results

Common spectral filters prescribed for management of congenital achromatopsia also reduce melanopsin-mediated ipRGC activation. In line with a recently developed framework to examine how spectral filters modify visual and non-visual parameters (28), we first examined how commonly used filters affect rod and melanopsin-expressing ipRGC responses to light. The spectral sensitivities of melanopsin and rods overlap substantially, with their maximum spectral sensitivities only separated by 10-15 nm (Figure **1*d***) and are highly correlated (Pearson's r=0.946, $p<0.001$). This suggests that filters reducing rod activation would also reduce ipRGC activation. We put this hypothesis to the test and examined how two common spectral filters prescribed in congenital ACHM – F540 and F90 – change the signals of rods and ipRGCs (Fig **2*b***). We considered an exhaustive sample of illumination spectra in the real world, corresponding to an approximation of the typical "spectral diet" of such people, ranging from daylights representative of different solar angles, to artificial light source such as fluorescent, incandescent, and LED lights (29).

As predicted from the strong overlap and correlation of rod and melanopsin spectral sensitivities, we confirm that rod and ipRGC signals are strongly correlated in real-world light exposures (Fig. **2*a***). Indeed, the relationship between rod activation (expressed as scotopic luminance) and ipRGC activation (melanopic irradiance) at the same *photopic* luminance (reflecting a weighted sum of L and M cones) can be described by a linear curve (Fig. **2*a***, inset). Importantly, the spread of scotopic luminance and melanopic radiance at pegged photopic luminance reflects the fact that different light sources may affect rods and melanopsin-expressing ipRGCs differently when the photopic luminance is the same (30). When these spectra are seen through the F540 and F90 filters, respectively, the reduction of rod and ipRGC activation is highly correlated. This confirms that filters commonly prescribed for the management of congenital ACHM also reduce melanopic light exposure.

Visual discomfort in a congenital achromat. It is well known that congenital ACHM is characterised by photophobia and photoaversion. In one participant who visited the laboratory in Basel, we had the opportunity to examine physiological responses to artificial light stimuli. In Newtonian viewing conditions, this participant was exposed to a wide-field, spatially homogenous and unarticulated light stimulus (inner diameter 11°, outer diameter 58°; Fig. **3*b***). After 20 minutes of dark adaptation, we presented two spectral stimuli (448±18 nm, *xy* chromaticity: [0.16, 0.03]; 597±75 nm [0.57, 0.42]; Fig. **3*a***) in ascending and then descending radiance. We measured the pupil size under these illumination conditions (Fig. **3*c, e***). We found normal pupil constriction with increasing irradiance levels, confirming a previous study (31). Using the time-averaged pupil size aggregated across the entire duration of the exposure at that radiance, we found stronger pupil constriction in the ascending compared to the descending series. This is due to an overshoot of the pupil constriction in response to stimulus increments relative to pupil dilation in response to stimulus decrements. Additionally, we asked the participant to rate the visual discomfort in response to the different radiances on a subjective 1-10 scale (Fig. **3*d, f***). We found a monotonic relationship between irradiance and visual discomfort. As we only took one rating per ascending and descending series, and per spectral stimulus, our conclusions are somewhat limited.

Survey-estimated chronotype and sleep. We asked participants to complete the Pittsburgh Sleep Quality Index (PSQI) (32), the Epworth Sleepiness Scale (ESS) (33), the Morningness-Eveningness Questionnaire (MEQ) (34), the Munich Chronotype Questionnaire (MCTQ) (35), the NEI Visual Function Questionnaire (25 items, NEI-VFQ-25) (36), and the Visual Light Sensitivity Questionnaire-8 (VLSQ-8) (37). All results are listed in Table **1**. For the PSQI, assessing sleep quality over the previous four weeks, we found a range of 3 to 12 (median±IQR: 7±3.5**)**. Only two volunteers scored below the conventional cut-off of 5. Only one participant was found to have excessive daytime sleepiness according to the ESS (median±IQR: 6±3). We found a range of MEQ values between 24 and 49 (median±IQR: 40±13), with one definitive evening type, two moderate evening types, and three neutral types. Using the MCTQ, we found a median±IQR mid-sleep MSF_{sc} (mid-sleep on free days) on ~4:00±0.55, corresponding to intermediate/slightly late chronotypes (38). In aggregate, the survey instruments may indicate a slight tendency to late chronotypes. Unexpectedly, we

found low scores on the NEI-VFQ-25 (composite score median±IQR 33.15±4), and high sensitivity to light on the VLSQ-8 (median±IQR: 27±4).

Congenital achromats have normal behavioural entrainment to the 24-hour cycle. Of our seven participants, six participants completed a three-week long assessment during which they wore actigraphy watches and completed a sleep diary, but were not instructed to follow any particular sleep-wake schedule. We found regular rest-activity cycles in all individuals (Fig. **4**). We subjected the actigraphy data to a Lomb-Scargle periodogram analysis (Figs. **5*a***, **6**), finding that the rest-activity patterns are periodic with a period length of 24 hours. Additionally, we assessed regularity (intra-daily stability; IS: 0.65±0.05 [mean+1SD]), fragmentation (intra-daily variability; IV: 0.80±0.02) and amplitude (relative amplitude; RA; 0.37±0.08) of the participants' activity rhythms (39), indicating moderate non-parametric rhythmicity.

Social jetlag (40) refers to the phenomenon that participants go to bed and wake up later on the weekends. Do achromats experience social jet lag? By drawing on self-reported bed and wake-up times, we found that most of our participants display on average a delay shift in their wake-up times on weekends (Fig. **6**) of approximately one hour difference in wake-up time on the weekend (median±IQR: 1.12±1.26), but a smaller nominal difference in bed-time (median±IQR: 0.17±0.30).

Congenital achromats have normal hormonal secretion profiles. While our actigraphy results strongly point to preserved normal diurnal rhythms in activity and behaviour, these data themselves do not establish that this is due to a preserved circadian rhythm. For example, it is conceivable that the behavioural entrainment can be attributed to non-photic *zeitgebers* (e.g. alarm clocks as a simple example). To rule out this possibility, we examined the secretion of melatonin during the evening hours in a modified at-home DLMO protocol (41). In brief, participants collected saliva every 30 minutes from five to one hour prior, to one hour after their habitual bedtime. Samples were refrigerated and shipped to us for biochemical assays (radioimmunoassay for melatonin). In four of six participants who participated in this study, we found a clear rise in melatonin levels (Fig. **7**).

In one participant (s005), we failed to detect an increase in melatonin levels. This specific individual habitually takes a beta-blocker for hypertension, which are known to affect melatonin secretion (42-44). In another individual (s006), who entered the

laboratory for five sessions, we did not find a clear dynamic increase in melatonin (Fig. **7**), and no relationship between light levels and melatonin secretion. This individual is also habitually taking beta-blockers, and had the latest MSF_{sc}, so our melatonin assessment might have been too early. Behaviourally, however, both individuals had normal entrainment.

In the participants who showed a clear DLMO, the phase angle of entrainment ranges between ~3 hours to ~45 minutes prior to habitual bedtime. This range is within the normal limits for the phase angle (45).

Discussion

Overall, our results suggest that, in the population of achromats we examined, both rest-activity cycles and phase angles of entrainment are normal, despite a non-functional cone system. In people with a trichromatic retina, entrainment to a 24 hour cycle (but not lower or higher period lengths) in people with normal vision can be supported by dim light at around 1.5 lux (46). Some laboratory studies have also found that the threshold for melatonin suppression is rather low (47), though the overwhelming evidence suggests that moderate light exposure is necessary to produce an appreciable effect. Importantly, sensitivity to evening light is regulated by the preceding light exposure (48, 49). Outdoor light exposure is also systematically related to chronotype (50). In animal models, rods have been found to contribute to phase shifting responses (51), thereby effectively extending the range at which light can contribute to circadian photoentrainment. Our results show that a functional cone system is not necessary for normal cycles in behaviour and physiology.

Behavioural light avoidance and use of filters that reduce retinal illuminance may lead to chronic modification of the “spectral diet” (52) of congenital achromats. What is the effect of this chronic modification? The human visual system is delicately adaptable (53). This is easily demonstrated during aging, where the lens in the eye yellows, increasing the extent of short-wavelength filtering and thereby affecting melanopsin-mediated responses to light (7). A previous study examining the spectral sensitivity of melatonin suppression indicated that in older individuals, there is a compensation

mechanism (54). A similar mechanism might tune the sensitivity of circadian photoreception to the range of available light intensities in the environment.

Retinal light exposure and subsequent non-visual responses to light may be modulated by the pupil (55), although the dynamic range is somewhat limited (from 2 to 9 mm, only ~1.2 log units). In general, the pupil response to light in achromats is not sluggish (31) as one would predicted from a melanopsin-mediated system (27, 56). Some congenital achromats exhibit a paradoxical pupillary response: a constriction in pupil size at a reduction in stimulus radiance (57-59). Under photopic conditions, the pupil area of the achromat is about 70% larger than that of healthy control eyes (60), suggesting that counterintuitively, retinal illuminance is higher in congenital achromats.

The circadian system is also sensitive to nonphotic *zeitgebers* (61), including the behavioural sleep-wake cycle, physical exercise, meal times, as well as social contact. These, in conjunction to an adapted sensitivity of circadian photoreception, may explain our results.

Materials and Methods

Participants. We recruited participants through advertisements targeted to ACHM patients via the Achromatopsie Selbsthilfeverein e.V., a self-help organisation of achromats, and Retina Suisse. A total of ten patients responded to our adverts and agreed to participate. Of these, nine patients completed the surveys, six completed the observational period and five the at-home melatonin assessment. One participant completed the melatonin assessment in the laboratory. Here, we only consider data from the seven participants with genetic confirmation of autosomal recessive ACHM (Table **2**). All participants underwent remote psychiatric examination by the study physician using the MINI-DIPS-OA instrument (62), none revealing clinical psychiatric problems.

Saliva and melatonin assays. Saliva samples were collected at home (five individuals) or in the laboratory (one individual) using Sarstedt salivettes. In the at-home DLMO assessment, participants were instructed to refrigerate the samples immediately, and ship them using express shipping methods. Upon arrival in the laboratory, the samples were centrifuged and frozen at 20°. These were then either transferred for analysis to the local laboratory or shipped on dry ice to Groningen (Chrono@Work, Groningen, Netherlands), for determination of melatonin concentrations using a direct double-antibody radioimmunoassay (RIA; Bühlmann Laboratories AG, Allschwil, Switzerland).

Genetic confirmation. All participants included in the final analysis were all genetically confirmed achromats. Five of these were *CNGB3*-associated ACHM patients, while two of them carried mutations in the *CNGA3* gene. Of the six participants who participated in the observational study and the melatonin assessment, five were *CNGB3*-ACHM patients and one was a *CNGA3*-ACHM patient. Genetic confirmation (Table 2) was performed by the Institute for Ophthalmic Research, Centre for Ophthalmology, University of Tübingen, Germany in a research setting.

Surveys. All survey data were collected and managed using REDCap electronic data capture tools hosted at the University of Basel (63, 64).

Actigraphy. Condor ActTrust, Condor, São Paolo, Brasil. From the 21-day protocol, we included the time period from 12:00 on Day 2 to 12:00 on Day 20. We analysed

actigraphy as follows: We estimated the periodicity of the actimetry data using the Lomb-Scargle periodogram. Further, we fit a sum-of-sinusoid to the data with MATLAB's Curve Fitting Toolbox using non-linear least squares (Mathworks, Natick, MA). We incorporated the fundamental frequency (corresponding to a period length of 24 hours) and the second harmonic (corresponding to a period length of 12 hours). Finally, Using the pyActigraphy package (65), standard non-parametric analyses of actigraphy-derived activity cycles (39) were implemented.

Pupillometry and discomfort assessment. We examined the pupil size in one individual at five light levels using one LED with peak emission at 449 nm (FWHM 20 nm), and another LED with peak emission at 599 nm (FWHM 81 nm).

At-home and in-laboratory DLMO assessment. DLMO timing was identified using the Hockey Stick software (v2.4) (66).

Ethical approval. Ethical approval for this study was granted from the Ethikkommision Nordwest- und Zentralschweiz (EKNZ), no. 2018-02335. The genetic analysis in a research setting was approved by the ethics committee of the University of Tübingen, no. 116/2015BO2.

Acknowledgements

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306 # Figures and Tables

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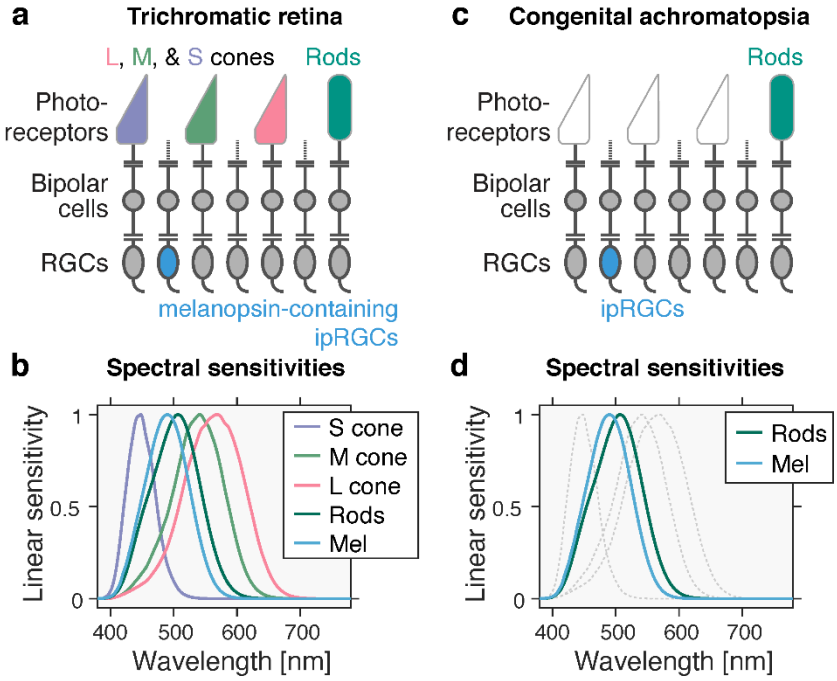

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309 **Figure 1.** ***Photoreceptors in the trichromatic and achromatic human retina. a***
310 Schematic diagram of the normal, trichromatic human retina containing three classes
311 of cones – long [L]- , medium [M]- - and short [S]-wavelength-sensitive cones –, rods,
312 and the intrinsically photosensitive retinal ganglion cells (ipRGCs) expressing the
313 photopigment melanopsin. ***b*** Spectral sensitivities of the photoreceptors in the
314 trichromatic retina, showing the overlapping *in vivo* wavelength sensitivity for the S
315 (λ_{max} = 440 nm), M (λ_{max} = 541 nm), and L (λ_{max} = 459 nm) cones, the rods (λ_{max} = 507
316 nm), and melanopsin (λ_{max} = 490 nm). Spectral sensitivities shown here assume a 32-
317 year old observer and include pre-receptoral filtering (67). ***c*** Schematic diagram of the
318 retina of a congenital achromat, missing functional cones, thereby only containing rods
319 and ipRGCs. ***d*** Spectral sensitivities of the photoreceptors in the achromat retina. Faint
320 dashed lines corresponding to the L, M and S spectral sensitivities are given for
321 reference only.

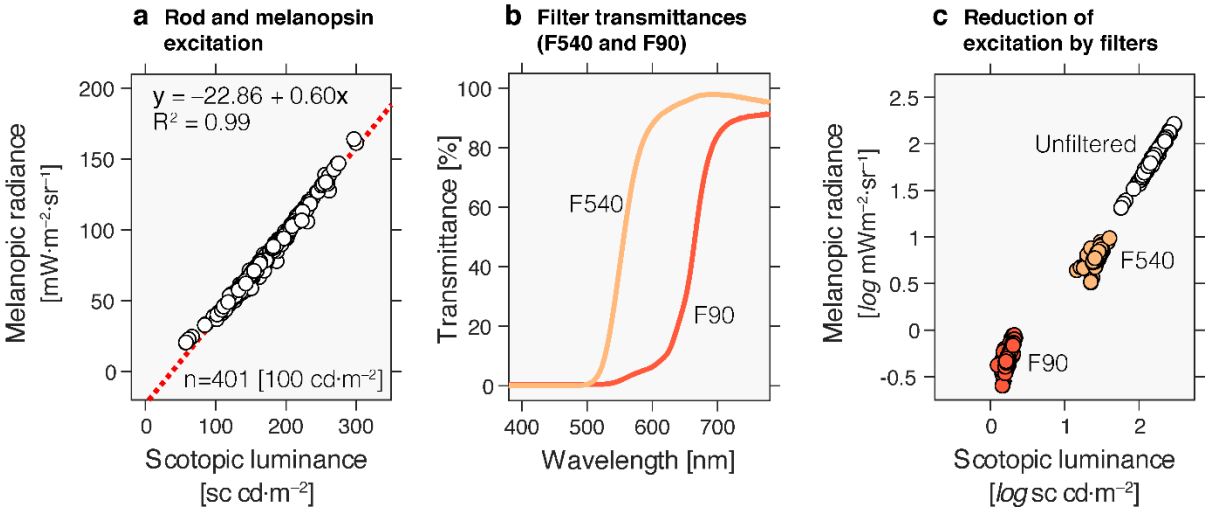

Figure 2. ***Spectral filters affect rod and melanopsin signals in the achromatic retina.*** ***a*** Simulation of rod and melanopsin-expressing ipRGC signals under wide range of spectral conditions. We simulated the distribution of rod signals (expressed as scotopic luminance) and ipRGC signals (expressed as melanopic radiance) while pegging the photopic luminance to match 100 cd/m^2 for 401 spectra representing a wide variety of light sources, including daylight, fluorescent and LED light (29). Under these conditions, rod and melanopsin expressing ipRGC signals are highly correlated and linear with each other, not least owing to the small spectral separation of the rod and ipRGC spectral sensitivities. ***b*** Transmittances of F540 and F90 tinted filter glasses, which are commonly prescribed in congenital ACHM. ***c*** Simulation of rod and ipRGC signals under the two filters (F90 and F540). The filters reduce both rod and ipRGC radiance by approximately 1 and 2 log units, on average.

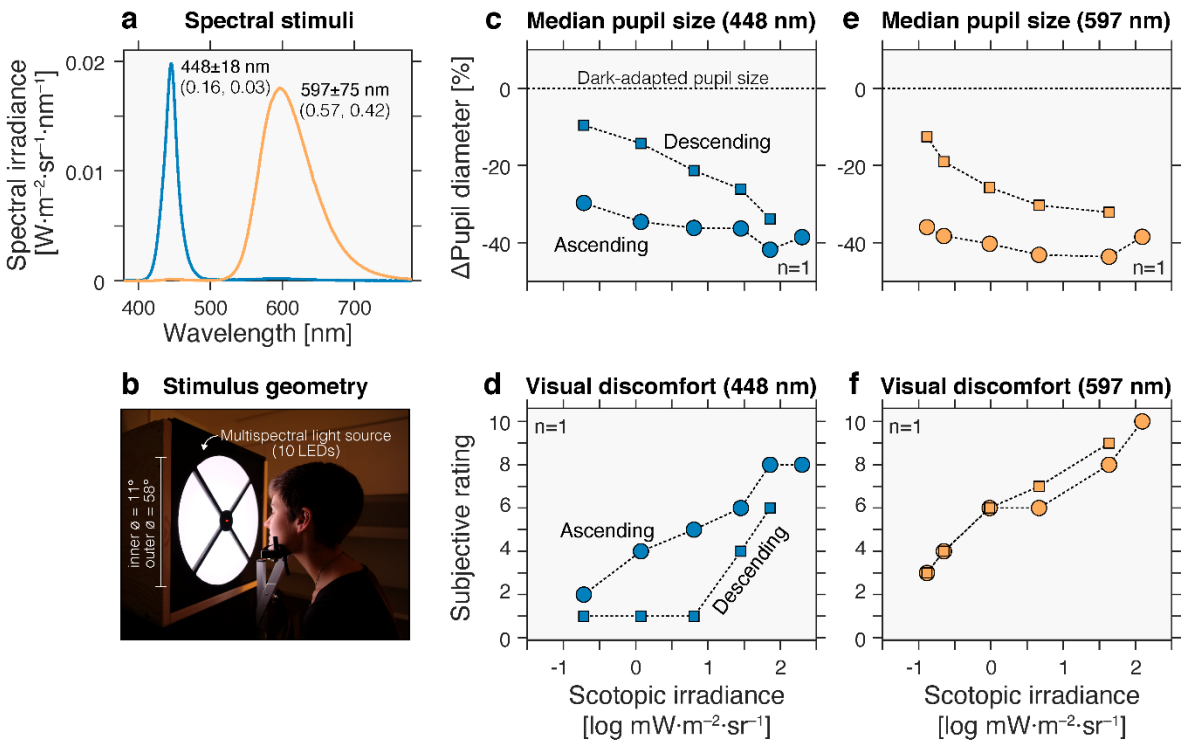

Figure 3. ***Visual discomfort and pupil responses in a congenital achromat (n=1).*** ***a*** Irradiance spectra of stimuli used, corresponding to a blue appearing and orange appearing light (to a trichromat). ***b*** Overview of stimulus geometry. Light emitted from a 10-primary tuneable LED-based light source was back-projected on a plexiglass surface, which the participant viewed in free-viewing conditions. ***c*** Pupil responses (relative to dark-adapted pupil size) in response to a series of ascending 448 nm stimuli, increasing in irradiance (squares), or to a series of descending light stimuli, decreasing in irradiance (circles). ***d*** Visual discomfort ratings in response to a series of ascending 448 nm stimuli, increasing in irradiance (squares), or to a series of descending light stimuli, decreasing in irradiance (circles). ***e*** Pupil responses (relative to dark-adapted pupil size) in response to a series of ascending 597 nm stimuli, increasing in irradiance (squares), or to a series of descending light stimuli, decreasing in irradiance (circles). ***f*** Visual discomfort ratings in response to a series of ascending 597 nm stimuli, increasing in irradiance (squares), or to a series of descending light stimuli, decreasing in irradiance (circles).

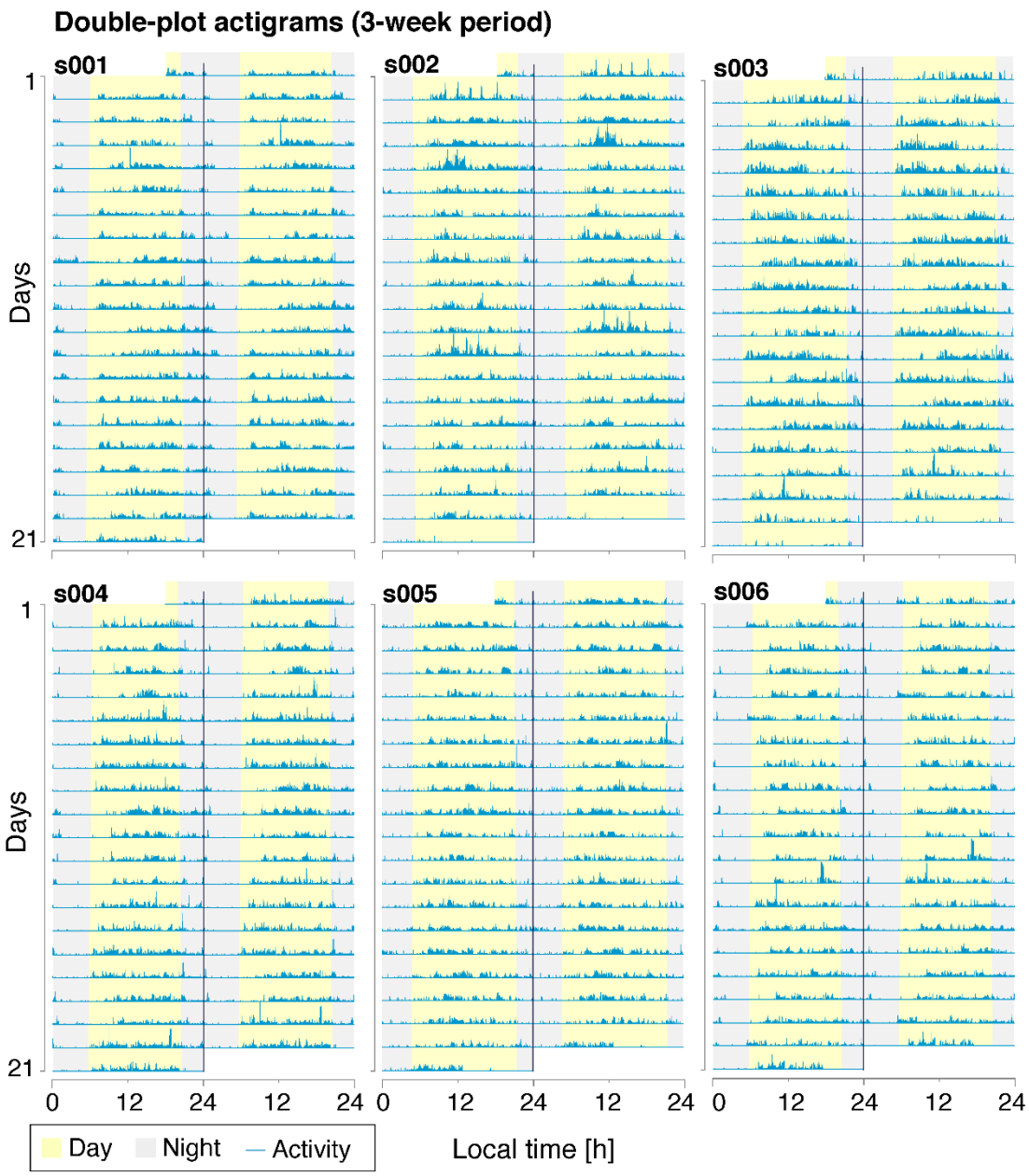

Figure 4. ***Wrist-referenced actigraphy shows regularity in activity in a group of six achromats (n=6)***. Data shown across the three weeks of observation. Participants were not instructed to follow a particular rest-activity pattern. Actigrams are shown as double-plots with the x-axis spanning a period of two consecutive days. Shading for day and night is taken from sunrise and sunset times at these chronological dates.

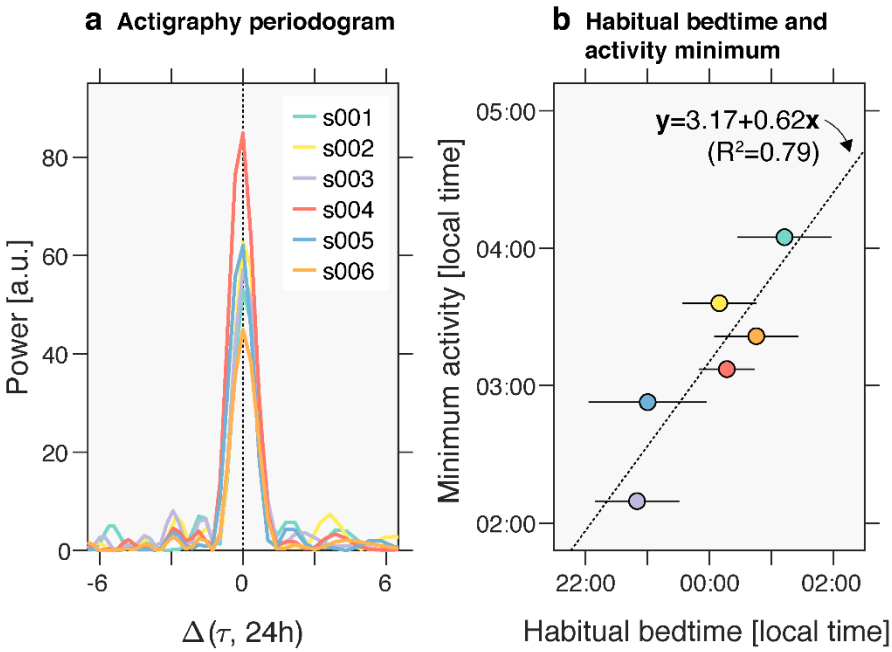

Figure 5. ***Periodic rest-activity cycles (τ=24 hours) and habitual bedtime in six congenital achromats (n=6)***. ***a*** Lomb-Scargle periodogram of actigraphy-derived activity, shown in terms of the deviation from a 24-hour cycle. ***b*** Linear relationship between the habitual bedtime (obtained from sleep diary data) with the actigraphy-derived activity minimum (based on fit to fundamental and harmonic).

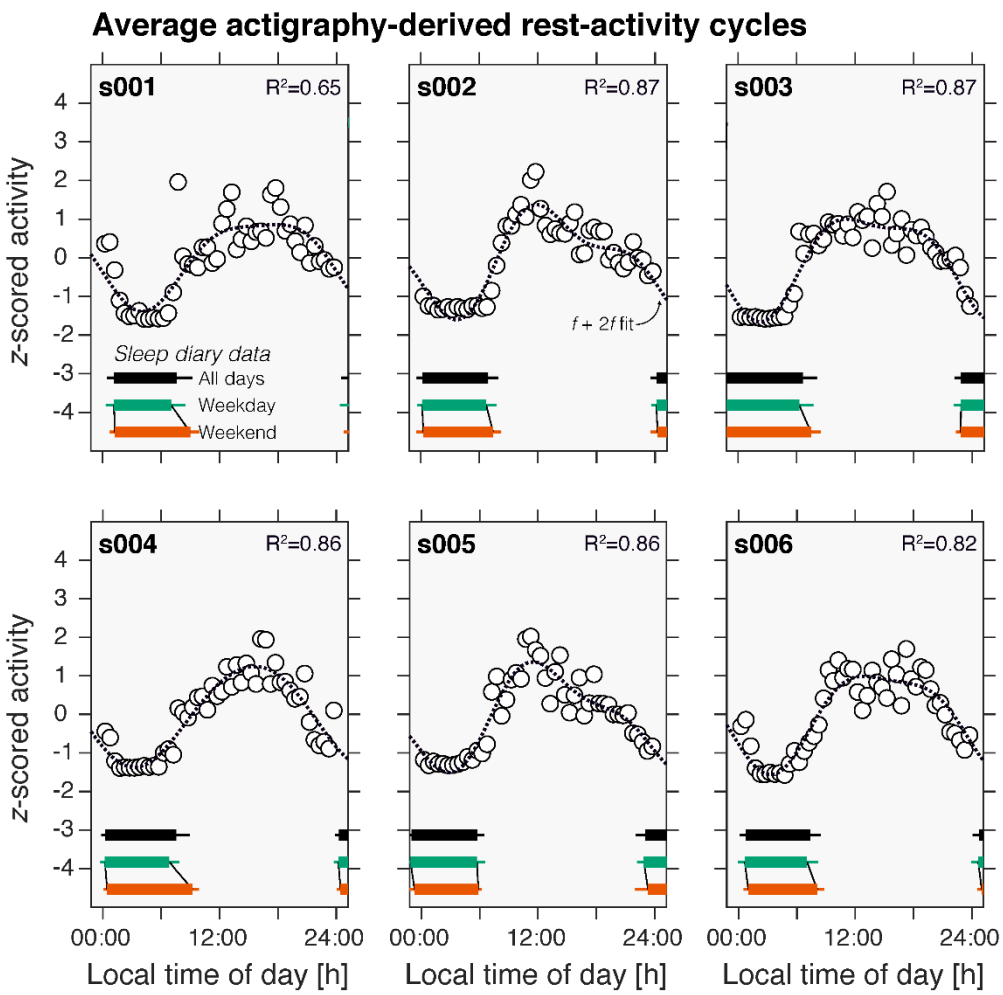

Figure 6. ***Average activity as a function of time of day in six congenital achromats (n=6).*** Data from the 21-day observational period were collapsed across days within time of day to yield the average time-of-day activity curves. Data were fitted using a sine-cosine f+2f fit, where f=1/(24.0 hours). All participants show a strong diurnal activity rhythm which can be characterised by a sinusoidal fit (range of R^2 values: 0.65-0.87*)*. *Insets*: Average (mean±1 SD, horizontal error bars shown on one side only) bed and wake-up times across the 21-day observational period across all days (black), or aggregated by weekday (green; Monday–Friday), and by weekend (red; Saturday and Sunday).

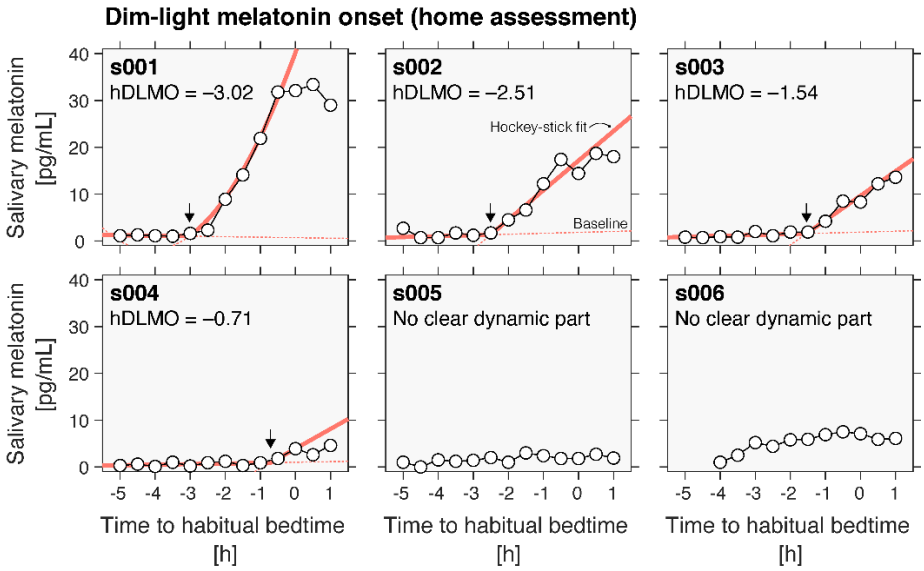

Figure 7. ***Normal melatonin phase angles of entrainment in four congenital achromats (n=4; total of 6 tested).*** Dim-light melatonin onset profiles as a function of habitual bedtime, assessed in an at-home measurement protocol using saliva collection. Saliva samples were assayed using radioimmunoassay (see details in text) and DLMO timing was extracted using Hockey Stick software (66). Two of the six participants did not show a clear dynamic rise in their melatonin profiles.

Table 1. ***Survey results and participation in study elements.***

	Sleep		**Chronotype**		**Visual function**		**Participation**		
Subject	PSQI (32)	ESS (33)	MEQ (34)	MSF_{sc} (35)	NEI-VFQ-25 (Composite Score) (36)	VLSQ-8 total (37)	Survey	At-home DLMO assessment	Laboratory DLMO assessment
s001	9	44	6	4.50	33.15	31	✓	✓	
s002	3	40	2	3.99	32.83	19	✓	✓	
s003	12	46	6	3.60	36.67	27	✓	✓	
s004	7	49	9	4.09	27.00	28	✓	✓	
s005	7	24	8	3.88	35.19	26	✓	✓	
s006	5	31	14	5.00	33.61	26	✓		✓
s007	10	33	6	2.00	27.96	31	✓		
Median	**7**	**40**	**6**	**3.99**	**33.15**	**27**	n=7	n=5	n=1
IQR	**4**	**13**	**3**	**0.56**	**4.00**	**4**			

388 **Table 2.** ***Genotypes of all participants in this study.***

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Subject	Genotype
s001	CNGB3: NM_019098:c.[1148delC];[1255G>T] NP_061971.3:p.[(T383Ifs*13)];[(E419*)]
s002	CNGB3: NM_019098:c.[1148delC];[1148del] NP_061971.3:p.[(T383Ifs*13)];[(T383Ifs*13)]
s003	CNGB3: NM_019098:c.[1148delC];[1148del] NP_061971.3:p.[(T383Ifs*13)];[(T383Ifs*13)]
s004	CNGB3: NM_019098:c.[1148delC];[1148del] NP_061971.3:p.[(T383Ifs*13)];[(T383Ifs*13)]
s005	CNGA3: NM_001298:c.[458C>T;1585G>A];[1228C>T] NP_001289.1:p.[(T153M);(V529M)];[(R410W)]
s006	CNGB3: NM_019098:c.[1148delC];[1304C>T] NP_061971.3:p.[(T383Ifs*13)];[(S435F)]
s007	CNGA3: NM_001298:c.[830G>A];[1706G>A] NP_001289.1:p.[(R227H)];[(R569H)]

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References

1. C. Blume, C. Garbazza, M. Spitschan, Effects of light on human circadian rhythms, sleep and mood. *Somnologie (Berl)* **23**, 147-156 (2019).
2. J. F. Duffy, C. A. Czeisler, Effect of Light on Human Circadian Physiology. *Sleep Med Clin* **4**, 165-177 (2009).
3. J. F. Duffy, K. P. Wright, Jr., Entrainment of the human circadian system by light. *J Biol Rhythms* **20**, 326-338 (2005).
4. M. E. Jewett, D. B. Forger, R. E. Kronauer, Revised limit cycle oscillator model of human circadian pacemaker. *J Biol Rhythms* **14**, 493-499 (1999).
5. J. M. Zeitzer, D. J. Dijk, R. Kronauer, E. Brown, C. Czeisler, Sensitivity of the human circadian pacemaker to nocturnal light: melatonin phase resetting and suppression. *J Physiol* **526 Pt 3**, 695-702 (2000).
6. J. J. Gooley *et al.*, Spectral responses of the human circadian system depend on the irradiance and duration of exposure to light. *Sci Transl Med* **2**, 31ra33 (2010).
7. M. Spitschan, Melanopsin contributions to non-visual and visual function. *Curr Opin Behav Sci* **30**, 67-72 (2019).
8. T. M. Brown, Melanopic illuminance defines the magnitude of human circadian light responses under a wide range of conditions. *J Pineal Res* 10.1111/jpi.12655, e12655 (2020).
9. D. C. Hood, M. A. Finkelstein, "Sensitivity to light" in Handbook of Perception and Human Performance, K. Boff, L. Kaufman, J. Thomas, Eds. (Wiley, New York, 1986), vol. 1, pp. 5.1-5.66.
10. A. Stockman, L. T. Sharpe, Into the twilight zone: the complexities of mesopic vision and luminous efficiency. *Ophthalmic Physiol Opt* **26**, 225-239 (2006).
11. S. Kohl, C. Hamel, Clinical utility gene card for: Achromatopsia - update 2013. *Eur J Hum Genet* **21** (2013).
12. J. Francois, *Heredity in Ophthalmology* (CV Mosby, St. Louis, 1961).
13. M. H. Remmer, N. Rastogi, M. P. Ranka, E. J. Ceisler, Achromatopsia: a review. *Curr Opin Ophthalmol* **26**, 333-340 (2015).
14. R. F. Hess, L. T. Sharpe, K. Nordby, *Night vision : basic, clinical, and applied aspects* (Cambridge University Press, Cambridge ; New York, 1990), pp. xii, 550 p.
15. J. Aboshiha *et al.*, A Quantitative and Qualitative Exploration of Photoaversion in Achromatopsia. *Invest Ophthalmol Vis Sci* **58**, 3537-3546 (2017).
16. M. C. Aguilar *et al.*, Automated Photosensitivity Assessment in Achromatopsic and Healthy Subjects [ARVO Annual Meeting Abstract]. *Investigative Ophthalmology & Visual Science* **56**, 4313 (2015).
17. M. C. Aguilar *et al.*, Automated instrument designed to determine visual photosensitivity thresholds. *Biomed Opt Express* **9**, 5583-5596 (2018).
18. G. Schwerdtfeger, M. Gräf, Kantenfilterkontaktlinse und Kantenfiltergläser bei Achromatopsie. *Z Prakt Augenheilkd* **15**, 322-328 (1994).
19. M. M. Schornack, W. L. Brown, D. W. Siemsen, The use of tinted contact lenses in the management of achromatopsia. *Optometry* **78**, 17-22 (2007).
20. G. F. Bridgman, The use of restricted spectral range red lenses for the diagnosis and relief of dazzlement of a rod monochromat. *Clinical and Experimental Optometry* **72**, 91-93 (1989).
21. R. S. Young, R. A. Krefman, R. J. Anderson, G. A. Fishman, Two Additional Benefits of Dark Glasses on Rod Vision in Patients with Congenital Achromatopsia. *Optom Vis Sci* **60**, 56-60 (1983).

22. N. Angier (1992) New Clue to Vision: People Whose Glasses Must Be Rose-Colored. in *New York Times*.
23. F. Futterman, Understanding and coping with achromatopsia. (2004).
24. C. A. Czeisler *et al*., Suppression of melatonin secretion in some blind patients by exposure to bright light. *N Engl J Med* **332**, 6-11 (1995).
25. J. T. Hull, C. A. Czeisler, S. W. Lockley, Suppression of Melatonin Secretion in Totally Visually Blind People by Ocular Exposure to White Light: Clinical Characteristics. *Ophthalmology* **125**, 1160-1171 (2018).
26. A. E. Allen, Circadian rhythms in the blind. *Current Opinion in Behavioral Sciences* **30**, 73-79 (2019).
27. J. Charng *et al*., Pupillary Light Reflexes in Severe Photoreceptor Blindness Isolate the Melanopic Component of Intrinsically Photosensitive Retinal Ganglion Cells. *Invest Ophthalmol Vis Sci* **58**, 3215-3224 (2017).
28. M. Spitschan, R. Lazar, E. Yetik, C. Cajochen, No evidence for an S cone contribution to acute neuroendocrine and alerting responses to light. *Curr Biol* **29**, R1297-R1298 (2019).
29. K. W. Houser, M. Wei, A. David, M. R. Krames, X. S. Shen, Review of measures for light-source color rendition and considerations for a two-measure system for characterizing color rendition. *Opt Express* **21**, 10393-10411 (2013).
30. M. Spitschan, Photoreceptor inputs to pupil control. *J Vis* **19**, 5 (2019).
31. L. T. Sharpe, K. van den Berge, L. H. van der Tweel, K. Nordby, The pupillary light reflex in a complete achromat. *Clinical Vision Sciences* **3**, 267-271 (1988).
32. D. J. Buysse, C. F. Reynolds, 3rd, T. H. Monk, S. R. Berman, D. J. Kupfer, The Pittsburgh Sleep Quality Index: a new instrument for psychiatric practice and research. *Psychiatry Res* **28**, 193-213 (1989).
33. M. W. Johns, A new method for measuring daytime sleepiness: the Epworth sleepiness scale. *Sleep* **14**, 540-545 (1991).
34. A. Zavada, M. C. Gordijn, D. G. Beersma, S. Daan, T. Roenneberg, Comparison of the Munich Chronotype Questionnaire with the Horne-Ostberg's Morningness-Eveningness Score. *Chronobiol Int* **22**, 267-278 (2005).
35. J. A. Horne, O. Ostberg, A self-assessment questionnaire to determine morningness-eveningness in human circadian rhythms. *Int J Chronobiol* **4**, 97-110 (1976).
36. C. M. Mangione *et al*., Development of the 25-item National Eye Institute Visual Function Questionnaire. *Arch Ophthalmol* **119**, 1050-1058 (2001).
37. J. D. Verriotto *et al*., New Methods for Quantification of Visual Photosensitivity Threshold and Symptoms. *Transl Vis Sci Technol* **6**, 18 (2017).
38. T. Roenneberg, L. K. Pilz, G. Zerbini, E. C. Winnebeck, Chronotype and Social Jetlag: A (Self-) Critical Review. *Biology (Basel)* **8** (2019).
39. E. J. Van Someren *et al*., Bright light therapy: improved sensitivity to its effects on rest-activity rhythms in Alzheimer patients by application of nonparametric methods. *Chronobiol Int* **16**, 505-518 (1999).
40. M. Wittmann, J. Dinich, M. Merrow, T. Roenneberg, Social jetlag: misalignment of biological and social time. *Chronobiol Int* **23**, 497-509 (2006).
41. H. J. Burgess, J. K. Wyatt, M. Park, L. F. Fogg, Home Circadian Phase Assessments with Measures of Compliance Yield Accurate Dim Light Melatonin Onsets. *Sleep* **38**, 889-897 (2015).
42. J. B. Kostis, R. C. Rosen, Central nervous system effects of beta-adrenergic-blocking drugs: the role of ancillary properties. *Circulation* **75**, 204-212 (1987).

43. K. Brismar, B. Hylander, K. Eliasson, S. Rossner, L. Wetterberg, Melatonin secretion related to side-effects of beta-blockers from the central nervous system. *Acta Med Scand* **223**, 525-530 (1988).
44. F. A. Scheer *et al.*, Repeated melatonin supplementation improves sleep in hypertensive patients treated with beta-blockers: a randomized controlled trial. *Sleep* **35**, 1395-1402 (2012).
45. C. I. Eastman, V. A. Tomaka, S. J. Crowley, Circadian rhythms of European and African-Americans after a large delay of sleep as in jet lag and night work. *Sci Rep* **6**, 36716 (2016).
46. K. P. Wright, Jr., R. J. Hughes, R. E. Kronauer, D. J. Dijk, C. A. Czeisler, Intrinsic near-24-h pacemaker period determines limits of circadian entrainment to a weak synchronizer in humans. *Proc Natl Acad Sci U S A* **98**, 14027-14032 (2001).
47. G. V. Vartanian *et al.*, Melatonin Suppression by Light in Humans Is More Sensitive Than Previously Reported. *J Biol Rhythms* **30**, 351-354 (2015).
48. A. M. Chang, F. A. Scheer, C. A. Czeisler, The human circadian system adapts to prior photic history. *J Physiol* **589**, 1095-1102 (2011).
49. K. A. Smith, M. W. Schoen, C. A. Czeisler, Adaptation of human pineal melatonin suppression by recent photic history. *J Clin Endocrinol Metab* **89**, 3610-3614 (2004).
50. T. Roenneberg, T. Kantermann, M. Juda, C. Vetter, K. V. Allebrandt, "Light and the human circadian clock" in Handb Exp Pharmacol, A. Kramer, M. Merrow, Eds. (2013), 10.1007/978-3-642-25950-0_13, pp. 311-331.
51. H. Calligaro *et al.*, Rods contribute to the light-induced phase shift of the retinal clock in mammals. *PLoS Biol* **17**, e2006211 (2019).
52. F. S. Webler, M. Spitschan, R. G. Foster, M. Andersen, S. N. Peirson, What is the 'spectral diet' of humans? *Curr Opin Behav Sci* **30**, 80-86 (2019).
53. M. A. Webster, Visual Adaptation. *Annu Rev Vis Sci* **1**, 547-567 (2015).
54. R. P. Najjar *et al.*, Aging of non-visual spectral sensitivity to light in humans: compensatory mechanisms? *PLoS One* **9**, e85837 (2014).
55. J. R. Gaddy, M. D. Rollag, G. C. Brainard, Pupil size regulation of threshold of light-induced melatonin suppression. *J Clin Endocrinol Metab* **77**, 1398-1401 (1993).
56. M. Spitschan, S. Jain, D. H. Brainard, G. K. Aguirre, Opponent melanopsin and S-cone signals in the human pupillary light response. *Proc Natl Acad Sci U S A* **111**, 15568-15572 (2014).
57. G. J. Ben Simon, F. A. Abraham, S. Melamed, Pingelapese achromatopsia: correlation between paradoxical pupillary response and clinical features. *Br J Ophthalmol* **88**, 223-225 (2004).
58. J. T. Flynn, E. Kazarian, M. Barricks, Paradoxical pupil in congenital achromatopsia. *Int Ophthalmol* **3**, 91-96 (1981).
59. M. J. Price, H. S. Thompson, G. F. Judisch, J. J. Corbett, Pupillary constriction to darkness. *Br J Ophthalmol* **69**, 205-211 (1985).
60. E. Alexandridis, E. Dodt, Pupillenlichtreflexe und Pupillenweite einer Stäbchenmonochromatin. *Albrecht von Graefes Archiv für Klinische und Experimentelle Ophthalmologie* **173**, 153-161 (1967).
61. R. E. Mistlberger, D. J. Skene, Nonphotic entrainment in humans? *J Biol Rhythms* **20**, 339-352 (2005).
62. J. Margraf, J. C. Cwik, V. Pflug, S. Schneider, Strukturierte klinische Interviews zur Erfassung psychischer Störungen über die Lebensspanne. *Zeitschrift für Klinische Psychologie und Psychotherapie* **46**, 176-186 (2017).

63. P. A. Harris *et al*., Research electronic data capture (REDCap)--a metadata-driven methodology and workflow process for providing translational research informatics support. *J Biomed Inform* **42**, 377-381 (2009).
64. P. A. Harris *et al*., The REDCap consortium: Building an international community of software platform partners. *J Biomed Inform* **95**, 103208 (2019).
65. G. Hammad, M. Reyt (2020) pyActigraphy (feature/actrust branch, commit 598ef80). (https://github.com/ghammad/pyActigraphy).
66. K. V. Danilenko, E. G. Verevkin, V. S. Antyufeev, A. Wirz-Justice, C. Cajochen, The hockey-stick method to estimate evening dim light melatonin onset (DLMO) in humans. *Chronobiol Int* **31**, 349-355 (2014).
67. CIE (2018) CIE S 026/E:2018: CIE System for Metrology of Optical Radiation for ipRGC-Influenced Responses to Light. (CIE Central Bureau, Vienna, Austria).