

EXCITACIÓN MELANOPSINICA EN CONDICIONES DE ILUMINACION NATURAL Y ARTIFICIAL

MELANOPsin EXCITATION IN CONDITIONS OF NATURAL AND ARTIFICIAL LIGHTING

O. U. Preciado^{a,b}, L.A. Issolio^{a,b}, E. Manzano^{a,b}, E. Colombo^{a,b}, P.A. Barrionuevo^{a,*}

^a Instituto de Investigación en Luz, Ambiente y Visión - CONICET - Universidad Nacional de Tucumán. Av. Independencia 1800 – (T4002BLR) San Miguel de Tucumán – Tucumán - Argentina

^b Departamento de Luminotecnia, Luz y Visión, Facultad de Ciencias Exactas y Tecnología, Universidad Nacional de Tucumán. Av. Independencia 1800 – (T4002BLR) San Miguel de Tucumán – Tucumán - Argentina

Recibido: 12/03/18; aceptado: 22/05/18

Biological adaptation to sunlight evolved over millions of years. The intrusion of artificial lighting has disrupted this relationship with the natural environment. On the other hand the discovery of the photopigment melanopsin poses new challenges for lighting designers. We studied melanopsin excitation under natural and artificial illumination considering aging of intraocular media. Our results showed that artificial illuminants systematically produced lower melanopsin excitation than environmental natural daylight conditions. This reduction is small for LED and Fluorescent illuminants when compared with natural illuminant D50, which could resemble horizon light, but it is much more important when compared with CIE natural illuminants that can roughly reproduce a clear sunny daylight and overcast sky conditions.

Keywords: melanopsin, lighting, aging.

La adaptación biológica a la luz solar ha evolucionado durante millones de años. La aparición de iluminación artificial ha trastornado esta relación con el ambiente natural. Por otro lado, el descubrimiento del fotopigmento melanopsina plantea nuevos desafíos a diseñadores en iluminación. En este trabajo estudiamos la excitación melanopsínica ante iluminación artificial y natural considerando el envejecimiento de los medios intraoculares. Nuestros resultados muestran que iluminantes artificiales sistemáticamente producen menor excitación melanopsínica que condiciones de luz día de ambientes naturales. Esta reducción es pequeña para iluminantes LED y fluorescentes cuando se comparan con el iluminante natural D50, que puede asemejarse la luz en el horizonte, pero es mucho más importante cuando se compara con iluminantes naturales de la CIE que aproximadamente reproducen la luz en un día soleado o con cielo nublado.

Palabras clave: melanopsina, iluminación, edad.

I. INTRODUCTION

Optical radiation (light) is sensed by our eyes and transduced to neuronal signal, which is processed by our brain to allow vision but also to regulate other processes inherent to our behavior and quality of life. This transduction is achieved by cells called photoreceptors. Two of these photoreceptor types are rods and cones, located in the outer retina. They express the photopigments Rhodopsin and Cone-opsins, respectively, for phototransduction. Rods allow vision in very dim situations for example under star and moonlight and they are predominantly located in the periphery. Cones, instead, work in bright environments allowing color vision. They are faster than, but not as sensitive as, rods. Cones are more densely located in the fovea, allowing detail vision.

Rods and cones were thought the only photoreceptors available to sense light in mammals for a long time; therefore the body of knowledge about light processing in humans was founded on this assumption. As a result lighting regulations were written under the same belief. However at the beginning of this century the existence of a different photopigment was reported in humans [1]. This photopigment is called Melanopsin and it is expressed by a small group of cells in the retina called ipRGCs (intrinsically photosensitive retinal ganglion cells), which is other type of photoreceptor located in the inner retina. The peak wavelength of melanopsin spectral function is 482 nm (Fig. 1). ipRGCs were discovered some years later; first in rodents [2,3], and then in humans [4]. These cells innervate the supraquiasmatic nucleus (SCN) for synchronization of the circadian clock to the solar day

*upreciado@herrera.unt.edu.ar

[5], the olivary pretectal nucleus (OPN) for pupillary control [6], the lateral habenula (LHb) and medial amygdala (MA) to control mood and learning process [7], and the lateral geniculate nucleus (LGN) for visual processing [4], among other regions. Besides intrinsic phototransduction, ipRGCs receives input from rods and cones through intermediate retinal cells [4,8]. In this way ipRGCs can codify a vast range of light levels.

Biological adaptation to the daily solar cycle and to sunlight evolved over millions of years. The intrusion of artificial lighting has disrupted this relationship with the natural environment. Today, people in cities spend several hours per day in front of computers or using smart phones. Artificial lighting is used during night time hours to enlarge working or recreational time, but also during daytime, light coming from artificial luminaries is preferred to solar radiation for illumination purposes. In some way we are prepared for natural conditions that do not exist anymore [9].

Lighting specifications is given in photometric units. Photometry is the measurement of light and it is based on the photopic luminous efficiency function $V(\lambda)$ [10]. This function represents the human visual effectiveness and it results of the added combination of two types of cone signals. $V(\lambda)$ weights and defines a region of the electromagnetic spectrum with a peak wavelength at 555 nm (Fig. 1). This function is adequate when cones are active and rods saturated, typically diurnal light levels (photopic vision). In lower light levels, for example under night road lighting, both rods and cones are active (mesopic vision), and for even dimmer light levels cones are not sensitive and vision is only supported by rods (scotopic vision). Considering that scotopic spectral sensitivity function [$V'(\lambda)$] is shifted towards shorter wavelengths than $V(\lambda)$ (Fig. 1), in low light levels photopic units could not reflect the actual visual perception. For this reason from the lighting engineering was proposed the so-called S/P ratio, which is the relation of scotopic units by photopic units. The S/P ratio is a simple and convenient way to characterize light sources since it combines the $V(\lambda)$ and $V'(\lambda)$, which represent the extreme situations of a photometric system proposed for mesopic lighting conditions [11]. The international commission on illumination (CIE) encourages the use of light sources with high S/P ratios such as metal halides and LED's, because human have a higher visual sensitivity to short wavelength light in the mesopic range [12]. However, it has recently been shown that the involvement of other factors such as intraocular transmittance and reflectance of road surfaces produce that light sources with a low S/P ratio are more efficient than expected in reaching the retina of the human eye [13].

The discovery of melanopsin impose a new challenge to lighting regulations since it has been shown that artificial light produce lower melanopsin excitation than natural lighting for the same photopic value [14]. Light affects health, it suppress melatonin production, constricts pupil, increase hearth rate and body temperature, etc. In this way light can be considered as a drug [15]. In similar way to the use of S/P ratios, in this

work we proposed to use the relation Mel/P (melanopic/photopic) to study melanopsin excitation under natural and artificial illumination considering the aging of intraocular media.

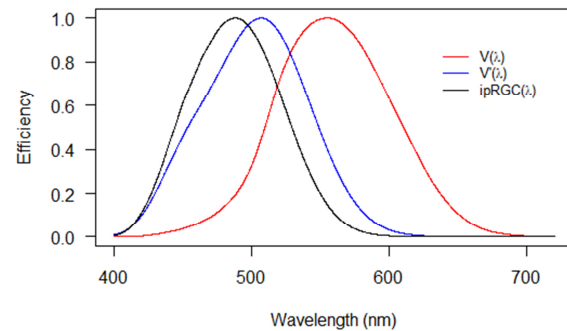


Figure 1. Spectral efficiency functions of CIE 1924 $V(\lambda)$ for photopic vision, CIE 1954 $V'(\lambda)$ for scotopic vision and ipRGC(λ) for melanopsin excitation.

II. METHODS

SPDs database of the light sources

The spectra power distribution (SPD) database used in this work was composed by the 25 illuminants of the CIE D Series representing natural daylight (Figs. 2A and 2B); the CIE Standard Illuminant A that represents typical tungsten filament lighting (Fig. 2A); 27 SPDs of fluorescent light (FL) sources (Fig. 2C) [16]; six SPDs of LED light sources (Fig. 2D); five from Philips Lumileds Lighting Company, USA and one measured by the authors; five SPDs of MH light sources (Fig. 2E): four were obtained from the website database called LSPDD: Lamp Spectral Power Distribution Database (www.lspdd.com) and one more measured by the authors; finally, 2 SPDs of HPS light sources (Fig. 2F), one was taken again from the LSPDD database and one measured by the authors. All SPDs illuminants were considered from 400 nm to 720 nm.

Spectral transmittance of human eye

In 2012, CIE published “A Computerized Approach to Transmission and Absorption Characteristics of the Human Eye” [17]. The spectral transmittance curves for people between 20 and 70 were calculated from this technical report (Fig. 3).

There is a significant decrease in spectral transmittance with age. The most evident change occurs at the blue end of the spectrum where, for example, at 500 nm, the transmittance of a human eye of 60 years is 25% less than the transmittance of an eye of 20 years and, 48% less at 450 nm (Fig. 3).

The age-related reduction in spectral transmittance is the main responsible of the changes in the spectral luminous efficiency function with age [18]. When the current standard luminous efficiency function $V(\lambda)$ was developed, implicitly, a certain spectral transmittance of the eye has already been considered. $V(\lambda)$ was derived with data from 52 observers, most of them young people: 20 observers (20-29yr), 21 observers (30-39yr),

9 observers (40-49yr) and 2 observers (50-59yr) [19]. The average age of the observers was 33 which could be considered as the reference age for the spectral transmittance.

the human eye. For the age of 20, $T_\lambda = 1$ along the entire spectrum. The melanopsin excitation was obtained from the melanopsin spectral sensitivity function (Fig. 1) [21]. Normalization of melanopsin

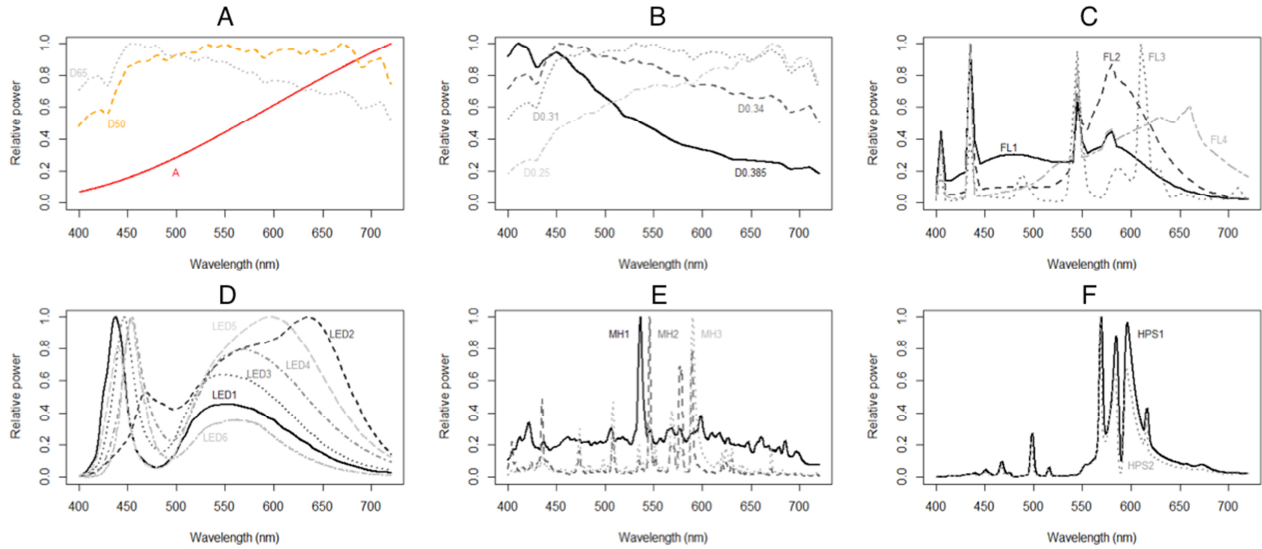


Figure 2. Spectral power distributions used in this study. A) Standard CIE illuminants. B) Some natural CIE daylight illuminants identified with their x-value in the CIE 1931 chromaticity diagram [20]. C) Representative sample of fluorescent illuminants. D) LED illuminants. E) Representative sample of MH illuminants. F) HPS illuminants.

However, Sagawa and Takahashi, after measured spectral luminous efficiency functions for 91 observers of different ages from 11 to 78, showed that the spectral luminous efficiency function for age 25 fitted best to $V(\lambda)$ [18]. For that reason, in this work the spectral transmittance for the age of 20 has been considered as the reference (100%) and spectral transmittances relative to this transmittance were calculated for the other ages.

Calculation of the Mel/P ratios

The following procedure was carried out to calculate the Mel/P ratios for all possible combinations of light source and age:

1. The photopic and “melanopic” illuminances (E_V and E_{Mel} , respectively) were calculated in the wavelength interval 400 nm to 720 nm from:

$$E_V = 683 \int_{400}^{720} (SF \cdot E_{e,\lambda}) \cdot V(\lambda) \cdot T_\lambda d\lambda$$

$$E_{Mel} = \int_{400}^{720} (SF \cdot E_{e,\lambda}) \cdot ipRGC_N(\lambda) \cdot T_\lambda d\lambda$$

where $E_{e,\lambda}$ is the SPD of the light source or spectral irradiance; SF is a scale factor that affects $E_{e,\lambda}$ in order to always reach the same photopic illuminance with all light sources. T_λ is the relative spectral transmittance of

function $[ipRGC_N(\lambda)]$ was achieved such that for an equal-energy spectrum light at $E_V = 1$ phot. lx and $T_\lambda = 1$, the $E_{Mel} = 1$ mel lx [22].

2. The Mel/P ratio of the light source reaching the retina, $Mel/P_{light\ source}$ would be:

$$Mel/P_{light\ source} = \frac{E_{Mel}}{E_V}$$

The normalization in the melanopsin function of a light source implies that the values obtained for the ratio E_{Mel}/E_V are arbitrary and only provide a relative information, as when they are presented related to other light sources.

All these calculations were made using the R language program [23].

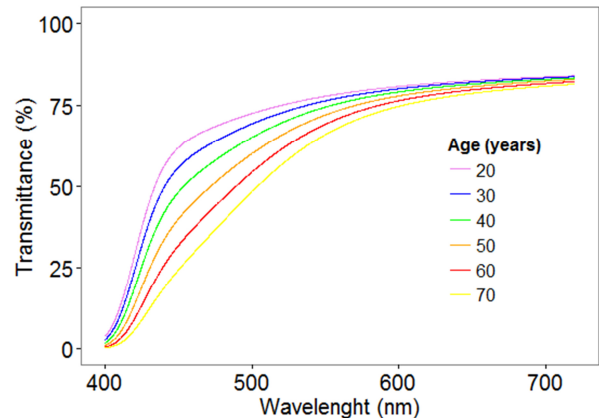


Figure 3. Human eye transmittance considering aging factor.

III. RESULTS

Figure 4 contains averaged values of the Mel/P ratio for artificial and natural illuminants weighted by the intraocular media, therefore this ratio represents the

actual light reaching the retina of a standard observer at different ages. For all illuminants Mel/P ratio decreased with aging. Natural and artificial Mel/P ratios get slightly closer with age. With respect to artificial light sources, LED technology produces higher Mel/P ratios than FL, MH, illuminant A (incandescent), and HPS technologies. However it should be considered that LED variability, due to lamps with different correlated color temperature (CCT, Fig. 2D), overlaps data from mercury illuminants (MH and FL).

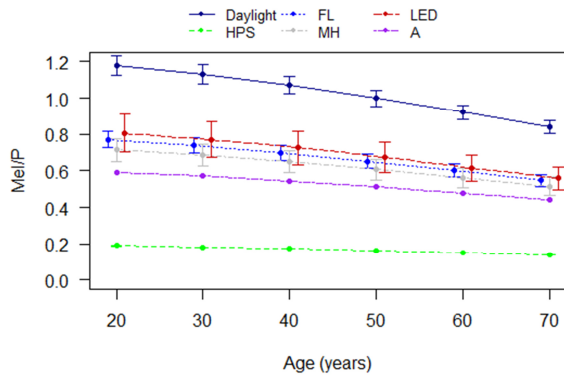


Figure 4. Averaged Mel/P ratios of natural and artificial illuminants. Error bars represent the standard error of the mean.

As we stated in the Introduction section, our visual system has evolved to respond to solar radiation. Therefore one could expect that the artificial illuminants mimic the behavior of natural lights. Following this rationale and since the value of the Mel/P ratio is not informative by itself, because it is not relating two magnitudes producing visual sensations (as the S/P ratio), we decided to normalize the results of artificial illuminants with respect to three natural illuminants. We choose the standard illuminants D65, D50 and illuminant D0.25 (CIE 1931 [20] x -value = 0.25; CCT = 24770K), which roughly could represent three environmental conditions of the day: the overcast sky, the horizon light, and a clear sunny day, respectively [22,24,25].

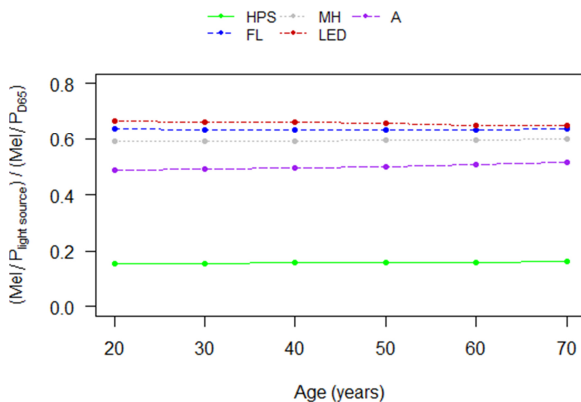


Figure 5. Normalization of Mel/P ratios of artificial illuminants with respect to the Mel/P ratio of CIE D65 daylight corresponding to overcast sky.

Results of this normalization are shown in Figure 5 for D65 daylight, Figure 6 for D50 daylight and Figure 7 for D0.25. If the artificial illuminants present similar behavior than natural illuminants the data points should be close to one in the Y-axis. In this way we can analyze the performance of artificial illuminants with respect to natural illuminants in terms of melanopsin excitation.

When compared with D65 illuminant (overcast sky), LED illuminants had slightly better performance, than MH and FL lamps. Lower values were obtained for incandescent lamp (A illuminant). Instead HPS technology showed much lower responses. All illuminants showed values lower than 0.7, which means that the melanopsin excitation with these illuminants is lower than the melanopsin excitation produced by D65 daylight.

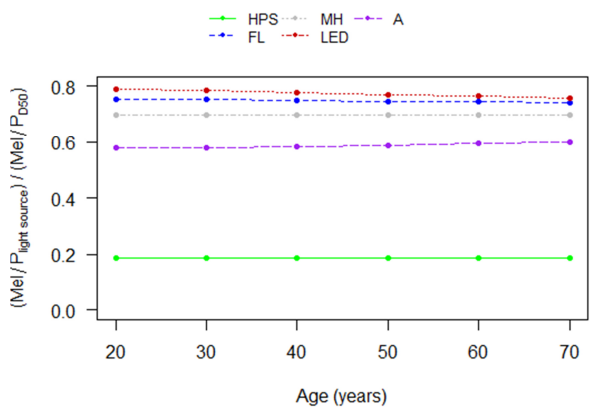


Figure 6. Normalization of Mel/P ratios of artificial illuminants with respect to the Mel/P ratio of CIE D50 daylight, corresponding to horizon light.

When compared with D50 illuminant (Horizon light), similar results to D65 data were obtained. However, higher values of this ratio were obtained for all artificial illuminants, with LED and FL technologies reaching almost 0.8. Therefore, melanopsin excitation produced by LED and fluorescent lamps are closer to melanopsin excitation produced by horizon light (D50 illuminant) than overcast sky (D65 illuminant).

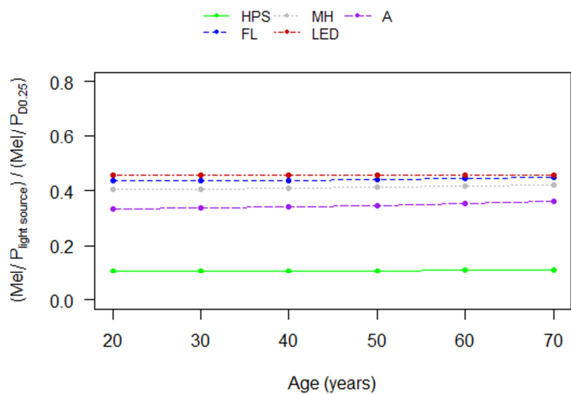


Figure 7. Normalization of Mel/P ratios of artificial illuminants with respect to the Mel/P ratio of a natural illuminant with CIE 1931 x -value = 0.25 (CCT = 24770K), corresponding to a sunny day.

However when normalized with respect to a natural illuminant with high CCT value (high components for short wavelengths), the performance of artificial illuminants is quite poor, lower than 0.5 for all cases (Fig. 7).

Since similar effects on Mel/P ratios with age was found for both natural and artificial illuminants (Fig. 4), no important influence of aging was evident after normalization. For the three comparisons, HPS results were very small compared with LED, FL, MH and A data.

IV. CONCLUSIONS

Our results showed that artificial illuminants systematically produced lower melanopsin excitation than environmental natural daylight conditions considering different aging of ocular media. This reduction is small for LED and Fluorescent illuminants when compared with natural illuminant D50, which could resemble horizon light, but it is much more important when compared with CIE natural illuminants that can roughly reproduce a clear sunny daylight and overcast sky conditions; for all artificial lights tested.

Aging effect produces a decrement of the Mel/P ratio for all illuminants and slightly reduces the difference between natural and artificial illuminants for older standard observers.

Why to replicate ecological melanopsin excitation is important? We live in an era in which lighting has become a public health issue [26]. The overwhelming exposure to artificial lighting pose potential health issues and affects quality of life, considering, for example, the evolved circadian synchronization to natural daylight. Recent work showed that exposure to light favoring circadian entrainment in the morning is associated with shorter sleep onset latency, and exposure during the entire workday was associated with lower depression scores and higher sleep quality, highlighting the importance of daytime light in sleep and health [27].

Distinct from transient rod and cone photoresponses, inherent response of ipRGCs is tonic and regular [4]. This characteristic offers the brain a steady representation of average environmental illumination [28]. The SCN receives inputs exclusively from ipRGCs, since elimination of ipRGCs produce loss of circadian photoentrainment [29,30]. It has been showed that disruption of the natural circadian rhythm (such as that suffered by shift-workers, frequent travelers across time zones, etc) can lead to different health problems such as cardiovascular complications or cancer [31,32]. Also ipRGCs have a key role in insomnia, and season-related disorders [33]. Therefore ipRGCs exerts a major influence on circadian rhythm, which in turn impacts on mental and physical health [14].

Also steady melanopsin photoresponse is used to maintain pupillary constriction [34], which serves as a light adaptation mechanism. The neural circuit of pupillary light reflex is well studied and can be used as

a biomarker to assess the health of the retinal circuit and to detect retinal diseases such as glaucoma [35,36].

It has been shown in behavioral studies that lighting is related with more pleasant mood [see for example: 36]. From physiological studies, a traditional point of view relates mood with circadian rhythmicity. However new studies show evidence of light affecting mood without circadian impairment [7,38]. ipRGCs innervate directly mood-regulating regions such as the amygdala and the lateral Habenula and could affect mood via this pathway. Furthermore ipRGCs axons reach a neural network that mediates light-associated migraines [39] and the ventrolateral preoptic area implicated in sleep induction [40,41].

The above evidence summarized the importance of melanopsin excitation and ipRGCs behavior on health and quality of life. However the concrete circadian, neuroendocrinal, and neurobehavioral consequences of our results cannot be outlined since dependence of these functions on melanopsin excitation level is not totally understood [15]. More basic research is needed to clarify this relationship.

We decided to avoid comparison with natural daylight representing times of the day with low optical radiation, since intrinsic melanopsin threshold is probably at least one-log unit higher than cone threshold [2,4,42], probably in the high mesopic range [43]. It means that our analysis is important for conditions where artificial illuminants provide photopic or high mesopic light levels as indoor lighting or outdoor urban illumination. Furthermore considering the prevalence of artificial lighting in offices and closed environments, our analysis suggests that indoor workers during daytime would experience substantially lower melanopsin excitations compared with outdoor daylight for most of artificial light sources, in agreement with previous analysis [14]. This analysis is relevant since research on lighting effects on mood and behavior has focused on light at night but investigations considering daytime hours are scarce.

It is important to note that some few specific illuminants from LED technology (data not show) can produce similar melanopsin excitation than D65 and D50 daylights, but this is not the general case and our conclusions were based on consideration of different illuminant technologies; therefore we averaged artificial illuminants for each technology.

V. REFERENCES

- 1 - I. Provencio, I. R. Rodriguez, G. Jiang, W. P. Hayes, E. F. Moreira, and M. D. Rollag, *J. Neurosci.* **20**, 600 (2000).
- 2 - D. M. Berson, F. A. Dunn, and M. Takao, *Science* **295**, 1070 (2002).
- 3 - S. Hattar, H.-W. Liao, M. Takao, D. M. Berson, and K.-W. Yau, *Science* **295**, 1065 (2002).
- 4 - D. M. Dacey, H.-W. Liao, B. B. Peterson, F. R. Robinson, V. C. Smith, J. Pokorny, K.-W. Yau, and P. D. Gamlin, *Nature* **433**, 749 (2005).
- 5 - S. Hattar, R. J. Lucas, N. Mrosovsky, S. Thompson, R. H. Douglas, M. W. Hankins, J. Lem, M. Biel, F. Hofmann, R. G. Foster, and K.-W. Yau, *Nature* **424**, 75 (2003).

- 6 - R. J. Lucas, S. Hattar, M. Takao, D. M. Berson, R. G. Foster, and K.-W. Yau, *Science* **299**, 245 (2003).
- 7 - T. A. LeGates, C. M. Altimus, H. Wang, H.-K. Lee, S. Yang, H. Zhao, A. Kirkwood, E. T. Weber, and S. Hattar, *Nature* **491**, 594 (2012).
- 8 - P. R. Jusuf, S. C. S. Lee, J. Hannibal, and U. Grünert, *Eur. J. Neurosci.* **26**, 2906 (2007).
- 9 - M. C. Moore-Ede and M. Moore-Ede, *The 24 Hour Society : The Risks, Costs and Challenges of a World That Never Stops* (London : Piatkus ; New York : distributed in the U.S. by Addison-Wesley, 1993).
- 10 - CIE, *Commission Internationale de l'Eclairage Proceedings, 1924* (Cambridge University Press, Cambridge, 1926).
- 11 - M. S. Rea, *Value Metrics for Better Lighting* (SPIE Press Bellingham, WA, 2013).
- 12 - Commission International de L'Eclairage, *Recommended System for Mesopic Photometry Based on Visual Performance* (CIE, Vienna, Austria, 2010).
- 13 - O. U. Preciado and E. R. Manzano, *Light. Res. Technol.* 1477153517718227 (2017).
- 14 - D. Cao and P. A. Barrionuevo, *J. Solid State Light.* **2**, 1 (2015).
- 15 - R. J. Lucas, S. N. Peirson, D. M. Berson, T. M. Brown, H. M. Cooper, C. A. Czeisler, M. G. Figueiro, P. D. Gamlin, S. W. Lockley, J. B. O'Hagan, L. L. A. Price, I. Provencio, D. J. Skene, and G. C. Brainard, *Trends Neurosci.* **37**, 1 (2014).
- 16 - J. M. M. Linhares and S. M. C. Nascimento, *J. Opt. Soc. Am. A Opt. Image Sci. Vis.* **29**, A174 (2012).
- 17 - CIE, *A Computerized Approach to Transmission and Absorption Characteristics of the Human Eye* (Commission Internationale de l'Eclairage, CIE Central Bureau, 2012).
- 18 - K. Sagawa and Y. Takahashi, *JOSA A* **18**, 2659 (2001).
- 19 - K. S. Gibson and E. Tyndall, *Sci Pap. Bur Stand* **19**, 131 (1923).
- 20 - C. CIE, Camb. Univ. Press Camb. (1932).
- 21 - J. al Enezi, V. Revell, T. Brown, J. Wynne, L. Schlangen, and R. Lucas, *J. Biol. Rhythms* **26**, 314 (2011).
- 22 - P. A. Barrionuevo and D. Cao, *J. Opt. Soc. Am. A* **31**, A131 (2014).
- 23 - R Core Team, (2013).
- 24 - G. Wyszecki and W. S. Stiles, *Color Science: Concepts and Methods, Quantitative Data and Formulae. 2000* (Wiley-Interscience, New York, n.d.).
- 25 - L. Thorington, *Ann. N. Y. Acad. Sci.* **453**, 28 (1985).
- 26 - S. M. Pauley, *Med. Hypotheses* **63**, 588 (2004).
- 27 - M. G. Figueiro, B. Steverson, J. Heerwagen, K. Kampschroer, C. M. Hunter, K. Gonzales, B. Plitnick, and M. S. Rea, *Sleep Health* **3**, 204 (2017).
- 28 - D. M. Berson, in *New Vis. Neurosci.*, edited by J. S. Werner and L. M. Chalupa (The MIT Press, Cambridge, MA, 2014), pp. 183–196.
- 29 - A. D. Güler, C. M. Altimus, J. L. Ecker, and S. Hattar, *Cold Spring Harb. Symp. Quant. Biol.* **72**, 509 (2007).
- 30 - D. Göz, K. Studholme, D. A. Lappi, M. D. Rollag, I. Provencio, and L. P. Morin, *PLOS ONE* **3**, e3153 (2008).
- 31 - B. Lemmer, *Pharmacol. Ther.* **111**, 629 (2006).
- 32 - R. G. Stevens, G. C. Brainard, D. E. Blask, S. W. Lockley, and M. E. Motta, *CA. Cancer J. Clin.* **64**, 207 (2014).
- 33 - T. A. LeGates, D. C. Fernandez, and S. Hattar, *Nat. Rev. Neurosci.* **15**, 443 (2014).
- 34 - P. D. Gamlin, D. H. McDougal, J. Pokorny, V. C. Smith, K.-W. Yau, and D. M. Dacey, *Vision Res.* **47**, 946 (2007).
- 35 - L. Kankipati, C. A. Girkin, and P. D. Gamlin, *Investig. Ophthalmology Vis. Sci.* **52**, 2287 (2011).
- 36 - P. Adhikari, A. J. Zele, R. Thomas, and B. Feigl, *Sci. Rep.* **6**, 33373 (2016).
- 37 - J. A. Veitch, G. R. Newsham, P. R. Boyce, and C. C. Jones, *Light. Res. Technol.* **40**, 133 (2008).
- 38 - L. Lazzarini Ospri, G. Prusky, and Samer Hattar, *Annu. Rev. Neurosci.* **40**, 539 (2017).
- 39 - R. Nosedá, V. Kainz, M. Jakubowski, J. J. Gooley, C. B. Saper, K. Digre, and R. Burstein, *Nat. Neurosci.* **13**, 239 (2010).
- 40 - C. M. Altimus, A. D. Güler, K. L. Villa, D. S. McNeill, T. A. LeGates, and S. Hattar, *Proc. Natl. Acad. Sci.* **105**, 19998 (2008).
- 41 - J. W. Tsai, J. Hannibal, G. Hagiwara, D. Colas, E. Ruppert, N. F. Ruby, H. C. Heller, P. Franken, and P. Bourgin, *PLOS Biol.* **7**, e1000125 (2009).
- 42 - M. T. H. Do, S. H. Kang, T. Xue, H. Zhong, H.-W. Liao, D. E. Bergles, and K.-W. Yau, *Nature* **457**, 281 (2009).
- 43 - P. A. Barrionuevo, N. Nicandro, J. J. McAnany, A. J. Zele, P. Gamlin, and D. Cao, *Invest. Ophthalmol. Vis. Sci.* **55**, 719 (2014).