A technique to ensure correct color stimulation by functional MRI to study in vivo the human melanopsin ganglion cells system

Andrea Siniscalco[1], Caterina Tonon[2,3], Micaela Mitolo[3,4], Claudia Testa[5], Marco Gaiani[6], Maurizio Rossi[1]

[1]Department of Design, Politecnico di Milano, Italy, andrea.siniscalco@polimi.it, maurizio.rossi@polimi.it,

[2]Department of Biomedical and Neuromotor Sciences, University of Bologna, Bologna, Italy, caterina.tonon@unibo.it,

[3]Functional and Molecular Neuroimaging Unit, IRCCS Institute of Neurological Sciences of Bologna, Bologna, Italy

[4]Department of Medicine and Surgery, University of Parma, Parma, Italy, micaela.mitolo@unipr.it

[5]Department of Physics and Astronomy, University of Bologna, Bologna, Italy, claudia.testa@unibo.it

[6]Department of Architecture, Alma Mater Studiorum - Università di Bologna, Italy, marco.gaiani@unibo.it.

Corresponding author: Andrea Siniscalco (andrea.siniscalco@polimi.it)

ABSTRACT

This paper describes a methodology to achieve correct light radiation coloring for stimulating intrinsically photosensitive melanopsin retinal ganglion cells. Indeed, it has been shown that light is capable of causing a response from the master circadian pacemaker located in the suprachiasmatic nucleus of the hypothalamus. A study was conducted in an experimental set-up using a high field clinic Magnetic Resonance scanner equipped with a stereoscopic viewer capable of projecting specific wavelengths to stimulate melanopsin retinal system. Subjects were monitored by acquiring Functional Magnetic Resonance Imaging, observing the response in subcortical (i.e., hypothalamus) and limbic areas (i.e., amygdala) and in some cortical areas primarily related to alertness. The spectral radiation emitted by the viewer was measured with laboratory instruments, and some considerations were also made on its possible influence at the level of the circadian cycle.

KEYWORDS Brain, Light treatment, Color stimulation, Functional Magnetic Resonance Imaging, Spectral measurements, Circadian rhythms

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1. Introduction

Melanopsin (Opn4) containing retinal ganglion cells (mRGCs) in humans are a subset - about 0.5-1% - of RCG, the output neurons whose axons form the optic nerves (Hattar *et al.*, 2002) and represent the third class of photoreceptor discovered in 2000 (Provencio *et al.*, 2000). The mRGCs act as an intrinsically photosensitive system contributing to image and mainly non-image-forming (NIF) visual circuits (Hattar *et al.*, 2002).

The broad spectrum of mRGCs functions includes the synchronization of the biological clock with the light-dark cycle (circadian rhythm photoentrainment), mediated by their projections to the master circadian pacemaker of the mammalian brain, located in the suprachiasmatic nucleus (SCN) of the hypothalamus, and the pupillary light reflex through the projection to the olivary pretectal nucleus (OPN) (La Morgia, Carelli and Carbonelli, 2018). More recently, several studies have pointed to the role of mRGCs in regulating the effect of light in several behavioral and physiological functions such as sleep, cognitive functions- learning or memory - and mood (LeGates, Fernandez and Hattar, 2014).

The functional integrity of the circadian regulatory network, partially dependent on melanopsin cells integrity, is crucial for well-being and health (Mure, 2021). Its dysregulation may contribute to sleep, neurodegenerative, and seasonal affective disorders (Mure, 2021). The alteration of the circadian rhythm can occur late, called owl disorder, or early, called lark disorder (Phillips, 2009). Throughout an individual's life, it is pretty common for him/her to be more owlish when young, highly active in the evening but with late morning awakenings, while in old age, they become larks, with fatigue just after sunset and early morning awakenings. The dysregulation of the circadian cycle can cause migraine (van Oosterhout *et al.*, 2018), headaches (Pringsheim, 2002), irritability (Evans and Davidson, 2013), seasonal depression (Rosenthal, 2006), immune system deficiencies (Christoffersson *et al.*, 2014), chronic fatigue (Bonsall and Harrington, 2013), obesity and diabetes mellitus (Cedernaes, Schiöth and Benedict, 2015). It has also been hypothesized that there is an increased likelihood of developing certain cancers as a result of the alteration of the circadian cycle that affects the production of various hormones and the efficiency of the immune system (Stevens and Rea, 2001; Schernhammer *et al.*, 2013; Yadav, Verma and Singh, 2017; Malik *et al.*, 2022).

Studies conducted in vitro and animal models have demonstrated that the spectral sensitivity of the mRGCs ranges from 446 to 483 nm, corresponding to "blue light" (Mure, 2021).

2. Functional Magnetic Resonance Imaging to investigate the response of mRGCs

To address *in vivo* the role of melanopsin expressed by retinal ganglion cells in humans, isolating visual and NIF functions in humans is challenging.

The specific pattern of activation/deactivation in brain regions involved in cognitive functions has been demonstrated in healthy subjects by using different paradigms of monochromatic light stimulation administrated by ad hoc devices integrated into functional magnetic resonance imaging technology (Vandewalle *et al.*, 2007).

Functional Magnetic Resonance Imaging (fMRI) is an advanced in-vivo metabolic MRI technique able to achieve unique insight into brain activity and network connectivity. Introduced at the beginning of the nineties, fMRI (Bandettini *et al.*, 1992; Kwong *et al.*, 1992; Ogawa *et al.*, 1992; Kwong, 2012) can give an indirect measure of brain activity during the administration of specific stimuli without the injection of any intravenous contrast agent. This technique is used in clinical practice for the presurgical planning of lesions in eloquent regions (Castellano *et al.*, 2017) and the field of cognitive neuroscience. Vandewalle and colleagues (2009) reviewed PET and functional MR studies demonstrating that the experimental setting of light exposure - primarily its wavelength, intensity, and duration - modulate brain responses to cognitive tasks administrated via auditory (not visual) system.

Specifically, these responses were observed in subcortical (i.e., hypothalamus) and limbic areas (i.e., amygdala), as well as in some cortical areas mostly related to alertness (i.e., frontal regions) (Vandewalle, Maquet and Dijk, 2009). Moreover, Evangelisti and colleagues (2020) also explored in Leber's Hereditary Optic Neuropathy (LHON) the mRGCs' contribution to light-driven visual and cognitive brain responses. In these disorders, optic nerve atrophy occurs consequent to retinal ganglion cells (RGCs) degeneration in the inner retina, while mRGCs are relatively spared. Authors found higher occipital activation in response to blue vs. red stimulation and larger brain responses over the lateral prefrontal cortex in LHON under blue vs. red light (Evangelisti *et al.*, 2021).

Most recently, other studies demonstrated age-related loss of optic nerve axons and specifically mRGC loss in postmortem Alzheimer's Disease (AD) patients associated with Aβ deposition. These results support the concept that mRGCs degeneration contributes to circadian rhythm dysfunction in Alzheimer's Disease (AD) (La Morgia *et al.*, 2016; Ortuño-Lizarán *et al.*, 2018);

however, other studies with specific fMRI protocols are strongly needed to confirm this evidence in vivo. Considering the key role of mRGCs on circadian rhythms and sleep, this system of intrinsically photosensitive mRGCs represents a potential target for therapeutic exploitation using bright light. Although the absence of randomized controlled trials in this field, a recent systematic review demonstrated that Bright Light Treatment (BLT) is a promising intervention in patients affected by dementia, specifically in Alzheimer's Disease (AD), and does not have significant adverse effects (Mitolo *et al.*, 2018).

3. Instrumentation Specifications

In the setup of the present study, light is safely conveyed via a purpose-built 3D-printed stereoscopic visor.

The visualization device consists of a binocular head-mounted display (HMD) (NordicNeuroLab) featuring a 28.6° horizontal x 20.3° vertical field of view. This device is designed to provide high-resolution images to the subject lying down on the MRI scanner bed (thanks to the material used and the length of the cable), both for patient comfort and for visual task-based functional imaging applications.

Fig. 1. The MR system compatible stereoscopic visor has two OLED displays and integrated eye-tracking cameras to both real-time visual monitor and record direction of gaze and pupil diameter.

Displays consist of dual SVGA active-matrix OLED microdisplays produced by eMagin (*eMagin*, 2023) and presenting a resolution of 800x600 pixels @85Hz. The displays viewing area is 12.78 x 9 mm, the contrast ratio ≥300:1, uniformity is > 85%, and White Luminance Maximum (Color) ≥ 140 cd/m^2 (front luminance) for SVGA 60Hz VESA mode. The sRGB color space is fully covered.

Symbol	Parameter	Min	Typ.	Max.
CIE White	X	0,270	0,320	0,370
	Y	0,290	0,340	0,380
CIE Red	X	0,565	0,574	-
	Y	0,338	0,347	0,360
CIE Green	X	0,240	0,300	0,340
	Y	0,450	0,500	-
CIE Blue	X	0	0,168	0,200
	Y	0	0,158	0,200

Tab. 1. CIE white point and primaries coordinates.

The visual stimulus is enabled using images coded as TIFF file format, 24-bit RGB color in the Apple Display P3 color space. As a result, it minimizes most of the downsides of the sRGB color space, the most used today.

x_R	y_R	x_G	y_G	x_B	y_B
0,680	0,320	0,265	0,690	0,150	0,060

Tab. 2. Coordinates of the primary used.

The Display P3 color space is 26% larger than the tiny sRGB color space, and it can accurately reproduce vivid colors, such as yellow cadmium and, mainly in our case, blue cobalt, clipped in the sRGB color space. It can be viewed almost entirely on most medium-high-end smartphones and totally on professional monitors such as the Apple XDR. This color space is a variant of the DCI-P3 color space using the D65 illuminant instead of the D50 and a gamma of 2.2, as in the sRGB color space.

These changes allow a more consistent workflow and visualization for devices supporting only the sRGB color space colors because the area of the sRGB color space is fully covered by the Display P3 color space.

The hardware image pipeline is consistent with this choice. First, a PC enables it with a graphic card Nvidia GeForce RTX 2060, a performance-segment graphics card launched in 2019 that guarantees resolutions of up

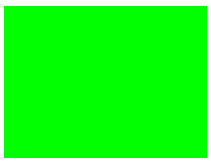
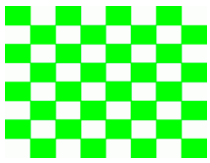
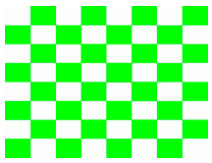
to 4K 12-bit HDR at 144Hz on two monitors. Then the signal is handled on a Brain Product Trigger Box to be sent to the displays via a 6 meters long optical fiber cable exploiting a 16-bit connection.

4. Spectral measurements

Spectral measurements to evaluate the visible radiation emitted by the visor have been done to get feedback on the radiation that will reach the patient's visual system.

Sample images were chosen for the measurement and projected into the eyepieces of the visor.

The eyepiece was immobilized on a plane, and the measurements were conducted by eliminating the presence of stray light by covering the entire setup with blackout sheets.

		
Ch_blue_all	Ch1_blue (left)	Ch2_blue (right)
		
Ch_green_all	Ch1_green (left)	Ch2_green (right)
		
Ch_red_all	Ch1_red (left)	Ch2_red (right)

Tab. 3. Images were projected in the binoculars during the measurements. The full-colored samples (Ch_blue_all, Ch_green_all to Ch_red_all) were measured in both the eyepieces, while the remaining ones (the chessboards) left and right were both measured, but results are separated due to the different patterns.

The spectral data measured on each sample are:

- Tristimulus values from CIE1931 (X, Y, and Z).
- CIE 1931 color coordinates (x, y).
- CIE UCS 1960 color coordinates (u, v).
- CIE UCS 1976 color coordinates (u', v').
- Spectral radiance in the range 380 - 780 nm, with a step of two nanometers from which the total radiance value is obtained.

The instrument used is a PotoResearch SpectraScan PR701s with a standard MS55 objective, making it possible to measure the spectral radiance at a solid angle with an aperture of 0.5°. The measurements were made after the instrument's 10' heating period to favor its thermal stabilization. The ambient temperature was about 25°C.

Wavelength range	380-780 nm
Aperture	1/2°
Luminance accuracy	±2% referred to NIST with standard illuminant at 2856 K
Luminance precision	The standard deviation of repeated measurements over a 30' period is less than 0.1% when the instrument is operating under normal operating conditions
Colorimetric accuracy for standard illu-minant CIE A	CIE 1931 x±,0015 y±,001
Color precision	±,005 for CIE 1931 x, y by measuring the standard illuminant CIE A
Polarization error	>=5% when measuring 100% linearly polarized sources
Digital resolution	65535:1 (16 bits)
Integration time	From 25 ms to 60000 ms

Tab. 4. Technical characteristics of the SpectraScan PR 701s spectroradiometer.

4.1. *Spectral measurement results*

The colorimetric values detected are shown in table 5, while the graphs shown in figure 2 have been created from the spectral radiance values for the wavelengths considered. In abscissa, the wavelengths are reported, while in ordinate, it is possible to observe the radiance values expressed in W/sr/m^2.

4.2. *Evaluations on the circadian response*

In addition to the in vivo observations on the brain's reaction to light stimulation, with the measured spectral radiance values, it is possible to consider the possibility that the light produced by the visor may influence the circadian cycle.

It is now known that the factors regulating the circadian system are very different from that of the human visual system (Rossi, 2019). In the retina-hypothalamus tract, numerous non-image-forming channels interact with the

biological clock in the supra-chiasmatic nucleus (SCN) of the hypothalamus in the brain. The normal circadian cycle is generated by the SCN and is synchronized thanks to the succession of local light/dark cycles. These cycles are essential for the sustenance of life. Their aberrant behavior can lead to numerous problems, such as obesity, fatigue (Reiter *et al.*, 2012), and breast cancer (Davis, Mirick and Stevens, 2001).

As expected, however, the human spectral sensitivity for the circadian system is significantly different from that of the visual system. For example, the visual system refers to a Gaussian-like sensitivity curve commonly known as Vλ, which peaks at 555 nm, while the spectral sensitivity curve relative to the circadian system (Cλ) appears to peak, according to many of the studies conducted, a value of 460 nm.

Sample												
Name ocular	Ch1_blue (left)	Ch1_green left)	Ch1_red (left)	Ch_blue_all (left)	Ch_green_all (left)	Ch_red_all (left)	Ch_red_all (right)	Ch_green_all (right)	Ch_blue_all (right)	Ch2_blue (right)	Ch2_green (right)	Ch2_red (right)
X	16,690	21,000	22,200	4,236	12,130	13,760	14,770	13,360	4,338	16,940	21,190	22,490
Y	18,500	26,940	20,290	4,498	19,490	8,625	9,001	22,130	4,597	18,900	27,570	21,410
Z	18,080	14,570	10,970	11,670	7,432	1,763	1,557	8,365	13,120	18,180	15,190	11,930
x	0,3134	0,3360	0,4153	0,2076	0,3107	0,5698	0,5831	0,3045	0,1967	0,3136	0,3313	0,4028
y	0,3473	0,4310	0,3795	0,2205	0,4990	0,3572	0,3555	0,5047	0,2085	0,3499	0,4311	0,3835
u	0,1917	0,1792	0,2471	0,1588	0,1485	0,3708	0,3824	0,1442	0,1541	0,1908	0,1765	0,2370
v	0,3186	0,3448	0,3387	0,2529	0,3579	0,3487	0,3497	0,3585	0,2449	0,3195	0,3444	0,3386
u'	0,1917	0,1792	0,2471	0,1588	0,1485	0,3708	0,3824	0,1442	0,1541	0,1908	0,1765	0,2370
v'	0,4779	0,5172	0,5080	0,3793	0,5368	0,5230	0,5245	0,5377	0,3673	0,4792	0,5166	0,5079
Total radiance [W/sr/m²]	0,06553	0,07774	0,06681	0,02424	0,04863	0,03087	0,03235	0,05487	0,02604	0,06609	0,07954	0,06957

Tab. 5. The table shows the colorimetric coordinates for all the measured samples (columns). The first three rows of the data are the tristimulus values from CIE1931 (X, Y, and Z), following the CIE 1931 color coordinates (x, y), the CIE UCS 1960 color coordinates (u, v), the CIE UCS color coordinates 1976 (u', v') and the total radiance (W/sr/m²).

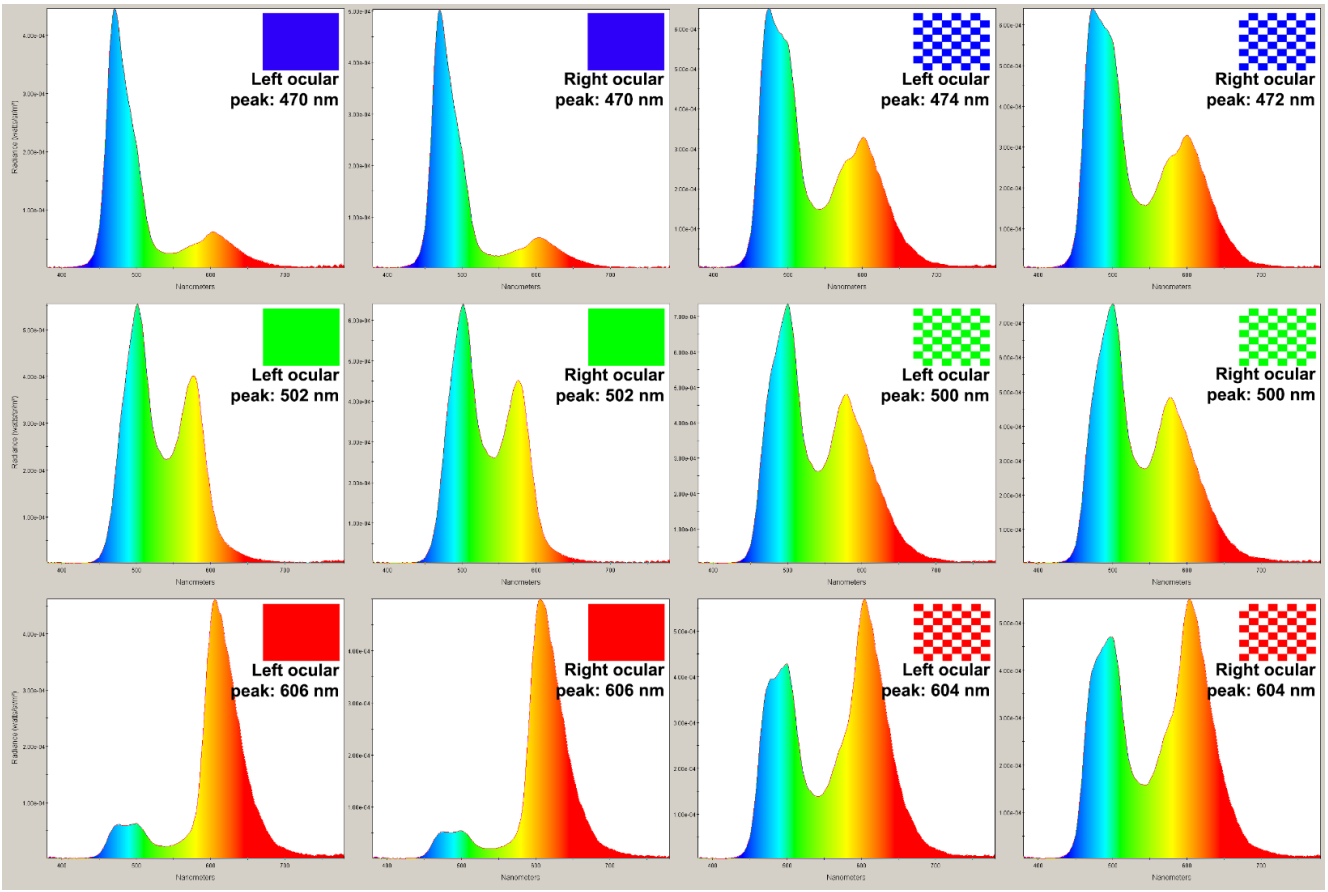


Fig. 2. Cartesian diagrams of the spectral radiances measured for the samples.

The exposure of the visual system to radiation around this wavelength cane reduce the production of melatonin (a hormone linked to the propensity to fall asleep) by the pineal gland.

Over the last twenty years, numerous studies have been conducted that have led to the construction of some models of spectral sensitivity for the human circadian system.

The first two research (Brainard *et al.*, 2001; Thapan, Arendt and Skene, 2001), conducted empirically, paved the way for subsequent studies and numerous discoveries that underline that the regulation of circadian cycles by light is not linear and straightforward.

The photo-transduction of the light into a signal transmitted to the SCN has as central actors the mRGCs, which perform their function thanks to their primary photo-pigment, melanopsin, whose functioning and absorption spectrum (maximum sensitivity at 460 nm) are well known.

Despite the identification of this mechanism, however, it has been demonstrated (Rea, Bullough and Figueiro, 2002) that it is not sufficient to evaluate the spectral sensitivity of a single opsin to predict the circadian response of the system. Indeed, mRGCs are not the only actors in the phototransduction phenomenon. They receive information from other photo-pigments (Hattar *et al.*, 2002) and from rods and cones photoreceptors (Belenky *et al.*, 2003).

This is also observable from the discontinuity between 470 and 530 nm of the empirical models of Brainard et al. and Thapan et al. Despite these observations, however, a specific model (Gall, 2004), which ignores these discontinuities, has established its reliability and is still widely considered in the design practices of lighting products that follow the principles of *human-centric lighting*.

For the evaluation of a possible circadian response induced by the stereoscopic visor, the non-linear model proposed by Rea et al. was used (Rea *et al.*, 2012; Figueiro and Rea, 2013), which considers numerous factors, including the transmission of light through the lens of the crystalline lens and the spectral opposition of the blue and yellow channels (Dacey and Packer, 2003).

This non-linear mathematical model results in a quantity called *Circadian Light* (CL_A), which is thought to be normalized so that 1000 CL_A corresponds to 1000 lux emitted by the CIE standard illuminant A (CIE, 1986). This expedient allows to consider light from the point of view of its interaction with NIF channels and applies to all possible spectral radiations. The CL_A value is therefore related to a quantity called *Circadian Stimulus* (CS), which represents the efficacy of CL_A in causing a significant circadian response in terms of inhibition of nocturnal melatonin (Rea *et al.*, 2010).

4.3. *Illuminance measures*

In order to assess whether the visor is capable of provoking a circadian response in terms of CL_A and CS, it was necessary to carry out additional measurements. For the calculation, it is required to have the photopic vertical illuminance value at the height of the cornea produced by the various samples evaluated in the spectral measurements.

Using the same viewer and the same measurement conditions, the vertical illuminance values were measured for each sample shown in table 3.

The instrument used was a Dr. Meter® LX1330B illuminance meter at a measurement distance of 0.5 cm from the viewer lens, and the entire setup was covered with blackout sheets to avoid stray light. The ambient temperature was about 25°C.

Fig. 3. Setup for the illuminance measurements.

The illuminance data and the respective spectral radiance values in the 380-780 nm range, with a step of two nanometers, were entered into two software to calculate CL_A and CS. The tools used were the online spreadsheet *CS Calculator* from Rensselaer Polytechnical Institute in Troy, NY (Rensselaer Polytechnical Institute, 2020) and Osram *Sylvania's LED ColorCalculator* software (OSRAM Sylvania, Inc., 2019). The results are reported in table 6.

Sample	Name (ocular)	Illuminance (lx)	CL_A	CS	Required illuminance (lx) for CS = 0,05
	Ch1_blue (left)	1,1	1,8	0,0021	21
	Ch1_green (left)	1,3	2,6	0,0031	17
	Ch1_red (left)	1,3	2,3	0,0026	20
	Ch_blue_all (left)	0,9	4,4	0,0054	6,5
	Ch_green_all (left)	1,3	2,4	0,0028	19
	Ch_red_all (left)	0,8	0,51	0,0005	52
	Ch_red_all (right)	0,7	0,38	0,0004	64
	Ch_green_all (right)	1	1,9	0,0022	18
	Ch_blue_all (right)	0,9	4,8	0,0061	6,5
	Ch2_blue (right)	1,2	2,0	0,0023	21
	Ch2_green (right)	1,8	3,7	0,0045	17
	Ch2_red (right)	1,5	2,7	0,0032	19

Tab. 6. The table shows the vertical illuminance values at the level of the user's cornea and the Circadian Light (CLA) values, and the effectiveness of the radiation in causing a circadian response (CS). The last column shows the values needed for a CS value of 0.05.

4.4. *Interpretation of the results*

The definition of a working threshold value for CL_A and CS is still debated. This is because many factors can influence the production of melatonin in addition to light stimulation, for example, from subjects' posture (Deacon and Arendt, 1994) to their diet (Peuhkuri, Sihvola and Korpela, 2012), from age-related differences in pre-retinal filtering (Herljevic *et al.*, 2005) to natural fluctuations in melatonin production (Arendt and Skene, 2005).

A study by Figueiro and Rea (Figueiro and Rea, 2013) tried to identify plausible threshold values, taking into account the intrinsic danger of an excessive alteration of circadian cycles, which might also be considered while using devices such as the stereoscopic visor.

The study presented the illuminance values for specific lighting sources necessary to obtain a circadian response of 0.05, 0.1, and 0.15 CS. This illumination was applied to the subjects' corneas using LEDs mounted on specially designed glasses. The subjects, who followed a specific preparation protocol, were subjected to light radiation for one hour. Through a blood sample before and after exposure to light, it was possible to observe the inhibition of melatonin production for different illuminance levels and different spectral components. For example, a CS value of 0.05 corresponds to a 5% reduction in melatonin in the bloodstream.

Observing the results obtained from the measurements at the IRCCS Institute of Neurological Sciences, Bellaria Hospital, we can assert that, although the data obtained by the software are in line with the circadian sensitivity curves of the cited studies, the illumination produced on the cornea by the stereoscopic visor is too low to cause a significant circadian reaction even in the hypothesis of exposure to radiation for one hour.

The last column in Table 6 shows the illuminance values/hour, which would be necessary for each sample to obtain a 5% reduction in melatonin in the bloodstream.

It is safe to say that the visor, used during daytime at the actual conditions, can be used for research purposes without causing shifts in the circadian cycle.

5. Further possible investigation

It has been shown (Glickman *et al.*, 2003) that the retinal ability to lead to the inhibition of melatonin is not uniform over the entire area covered by photoreceptors. The lower part gave blood melatonin inhibition results equal to those obtained on the whole retina, suggesting that the upper part is less sensitive to radiation regarding NIF processes. It is still unclear whether this difference is due to melanopsin in the mRGCs or the different concentrations of S-cones on the retinal carpet.

Since the measured stereoscopic viewer is equipped with OLED screens capable of generating different images, this could allow us to investigate, using appropriate levels of illuminance, the aspects related to the different sensitivity of the photoreceptors on the retina. For example, it would be possible to observe how the different spectral compositions of light can influence these differences.

6. Conflict of interest declaration

All authors wish to state that no financial or personal interests have affected the objectivity of this study and that no conflicts of interest exist.

7. Funding source declaration

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8. Short biography of the authors

Andrea Siniscalco - MSc in Design in 2002 and Ph.D. in 2007 in lighting fixture design. Since 2003, he has collaborated with the Lab Luce - Department of Design - Politecnico di Milano. Since 2008, he has been teaching lighting (design theory and CAD methods) as an adjunct professor at the School of Design - Politecnico di Milano. Deputy Director of the Masters in Lighting Design & Technology. Vice President of the GdC-Associazione Italiana Colore.

Caterina Tonon. MD, PhD. - Full Professor of Clinical Biochemistry and Molecular Biology, Neurologist, and Director of the Functional and Molecular Neuroimaging Unit, multidisciplinary team within the IRCCS Institute of the Neurological Sciences of Bologna. Her scientific activity is devoted to the implementation of advanced Magnetic Resonance Imaging techniques for clinical and research purposes.

Micaela Mitolo – Neuropsichologist, completed her PhD in the field of Neuroscience at University of Padua spending a period, as Visiting Ph.D., at University College London and University California San Diego. She is currently a Researcher at University of Parma and working at the IRCCS Institute of the Neurological Sciences of Bologna, exploring the neuroimaging correlated of clinical and neuropsychological impairments in neurodegenerative and neuro-oncology patients.

Claudia Testa - Professor of Physics at University of Bologna. She is a medical physicist with experience in acquisition and analysis of neuroimaging data. Her expertise concerns multiparametric data analysis for neurological disorders. Her work is also on the effect of light on brain activity at different wavelengths.

Marco Gaiani - Full Professor of Architectural Representation at University of Bologna, Dept. of Architecture, past Director of the INDACO Dept. of the Politecnico of Milano and DAPT Dept. of University of Bologna. A specialist in 3D computer imaging, modeling, and visualization for Heritage and architecture, he was one the first developers/user of laser scanning and automatic photogrammetry technologies in the Heritage field.

Maurizio Rossi - MSc, PhD. Full professor at Politecnico di Milano is the chair of the Lab. Luce, the Master in Lighting Design & Technology director, and member of the Ph.D. Design faculty. He directed 25 research-financed projects on topics related to light and color. 2012-18 he was the President of the GdC-Associazione Italiana Colore. Since 2018 member of the Executive Committee of AIC-International Color Association. Since 2022 vice-president of the AIC (president-elect 2024-25). Since 2021 he is member of the board of directors of the SID (Società Italiana Design).

Licensing terms

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