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Review

A Review of Silent Substitution Devices for Melanopsin Stimulation in Humans

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Abstract: One way to study the specific response of the non-visual melanopsin photoreceptors of the human eye is to silence the response of cones and rods. Melanopsin photoreceptors (ipRGC), highlighted in the early 2000s, are intimately linked to the circadian rhythm and therefore to our sleep and wakefulness. Rest and sleep regulation, health and cognitive functions are all linked to ipRGC and play an important role in work and human relationships. Thus, we believe that the study of ipRGC responses is important.We searched and reviewed scientific articles describing instrumentation dedicated to these studies. PubMed lists more than 90,000 articles created since the year 2000 that contain the word circadian but only 252 with silent substitution. In relation to melanopsin, we found 39 relevant articles from which only 11 give a device description for humans, which is incomplete in most cases. We did not find any consensus for light intensity description, melanopsin contrast, sequences of melanopsin light stimulation and optical setup to expose the retina to the light.

Keywords: melanopsin; silent substitution; ipRGC; LED; vision; retina; black-metamer.

1. Introduction

Inside the eye, light is captured and interpreted by the rod and cone photoreceptive system for image perception. At the beginning of the 21st century, a non-visual photoreceptive system was identified in mammalian eyes [1,2]. Its base unit is an intrinsically photosensitive retinal ganglion cell that uses the photopigment melanopsin (ipRGC, pRGC, mRGC or simply melanopsin). It was later discovered that it also plays a role in spatial vision [3,4]. The temporal properties of ipRGC signaling are distinct from those of rods and cones, including longer latency and sustained signaling after light offset [5,6]. The sensitivity of melanopsin overlaps those of S, M and L cones and its peak lies at 490 nm [7] (see Figure 1).

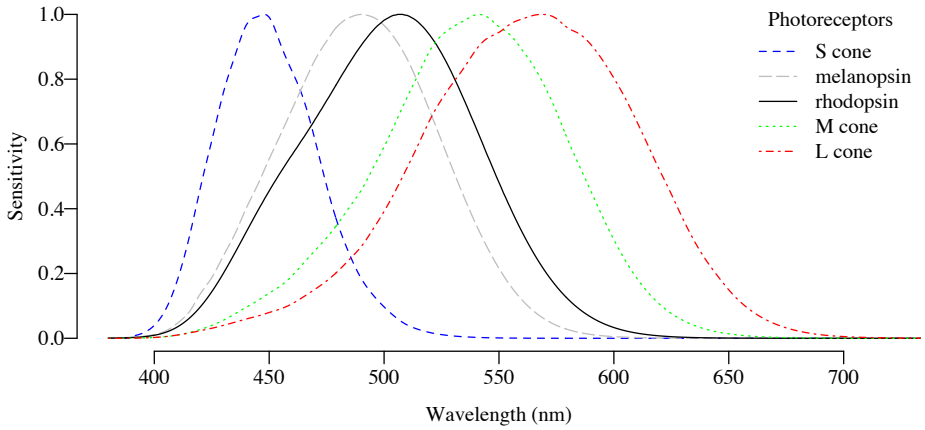

Figure 1. Photoreceptors sensitivity as given by the toolbox of the International Commission on Illumination (CIE) [7].

This system is optimized for signaling temporal changes in irradiance [8] and contributes to a diverse range of non-visual responses to light such as the circadian rhythm [9], health [10–12], pupillary light response [13,14], regulation of rest-activity states and sleep [15], cognitive functions in humans [16], regulation states of rest or sleep and suppression of melatonin [17], which in turn influences alertness, mood-related behavior and cognitive functions [18,19]. It is therefore important to better understand the links between ipRGC and all these important biological functions.

Some studies used so-called blue light to study sleep quality [20] in patients with glaucoma [21,22], age-related macular degeneration [23,24], dark adaptation [25] or choroidal blood flow [26]. Other studies used black metamers, which exhibit identical tristimulus but different ipRGC stimulation. Silent substitution is a technique able to generate black metamers [27] and can be used to study the influences of ipRGC on different biological functions.

1.1. Silent Substitution Technique

Silent substitution [28] is a technique to excite photoreceptors of the eye in a way in which only one type of photoreceptor is modulated, the others remaining at the same level of stimulation (silent). In order to provide such photo stimulation, at least four different primaries, i.e., different spectral power distributions in the visible range, are necessary for photopic stimulation and at least five are necessary for mesopic stimulation.

We consider a set $[i]$ of wavelengths in the visible range, usually between 380 and 780 nm. With $S_p(\lambda_i)$ being the sensitivity of the photoreceptor p and $L_q(\lambda)$ the irradiance of the qth primary light expressed in W/m^2. The photoreceptor stimulation is given by $\vec{PS} = P2C \cdot \vec{LR}$, where $\vec{LR}$ is the luminance ratio of the light source and $P2C$ is a matrix whose elements are given by $P2C_{pq} = K_p \cdot S_p(\lambda_i) \cdot L_q(\lambda_i)$. The factor $\vec{K}$ converts watt into lumen and is now explicitly given by CIE in their toolbox [7]. By inverting the matrix $P2C$, it is possible to define the required irradiance in order to get a defined retinal stimulation, i.e., $\vec{LR} = P2C^{-1} \cdot \vec{PS}$.

The maximum max and minimum min of the luminous radiation for ipRGC during an experiment define either the Weber $C_W = \frac{\max - \min}{\max}$ or the Michelson $C_M = \frac{\max - \min}{\max + \min}$ contrast.

1.2. Light Sources

Any given stimulation is either produced by the homogeneous mixing of n primary with $n \geq 4$ for photopic stimulation and $n \geq 5$ for mesopic stimulation (scotopic stimulation is not considered here). To modulate the light spectrum, the superposition of different light sources with given bandwidth is used. The available technologies and the type of light field restrict the possible choices for light generation for the retinal stimulation represented by $\vec{PS}$.

For each article, the type and number of different light sources with their central wavelength and spectral bandwidth were considered. We also searched information with reference to the ipRGC sensitivity curve used.

1.3. Optical Setup for Silent Substitution

Three types of light field to stimulate ipRGC are possible: (1) one homogeneous field (Ganzfeld), (2) homogeneous zones such as the annulus and (3) pixel images where each zone or pixel has its own generated spectrum.

The light is brought to the retina either by a natural (Newtonian) view or through a retinal projection (Maxwellian view). In a Newtonian view, the pupil size and thus retinal illuminance cannot be controlled. In the Maxwellian view, the exit pupil of the device defines the entrance pupil of the eye and thus retinal illuminance can be controlled. However, there is an intermediate view where the subject looks at the screen (Newtonian view) but an artificial pupil controls the retinal illuminance and thus limits the field of view.

The relevant illumination units are lm/m^2 or lux either on the object seen or at the cornea for Newtonian observation views and troland (Td) for a Maxwellian view. Conversion from one unit to another is only possible if relevant geometry and spectra are given.

2. Materials and Methods

We specifically looked at the scientific publications using the silent substitution method for studying ipRGC.

2.1. Publication Search

We first restricted our search to PubMed, then we also searched on the International Society for Optics and Photonics (SPIE) and Institute of Electrical and Electronics Engineers (IEEE) databases. We looked exclusively for the following words, alone or in combination of two with the command AND:(1) silent substitution, (2) melanopsin and (3) circadian, and the abbreviations using RGC (4) ipRGC for intrinsically photosensitive RGC, (5) pRGC for photosensitive RGC and (6) mRGC for melanopsin RGC.

Occurrences of these words were found using Google Ngram [29], which looks within its database of books published between 1800 and 2012, and Google Trends [30] worldwide, which looks at the queries run on its search engine, but only since 2004.

2.2. Device Description

We searched for publications describing optical devices used for exciting ipRGC photoreceptors. Then, we classified them by the technique used to produce light, the range of illuminance and contrasts, the measured biological signal and finally the technical and electronic descriptions.

3. Results

3.1. Publications Search

Google books with Ngram searched all its digitalized books for occurrences of the words between 1800 and 2019 (Figure 2a and Table 1 last column (Ngram)). It found similar occurrences for melanopsin, ipRGC, pRGC and mRGC.The retinal ganglion cell is the most cited during these years and silent substitution is regularly cited. Melanopsin appears around 1995 and was cited 90,550 times between 2000 and May 14th, 2020.

The yearly means of the monthly relative query trends on Google of the selected words from 2004 to 2019 (Figure 2b) show that pRGC is searched for more than melanopsin even if we consider that the

word pRGC is included in ipRGC. However, Ngram shows that ipRGC appears in printed documents more often than pRGC and mRGC.The other query words are rarely used.

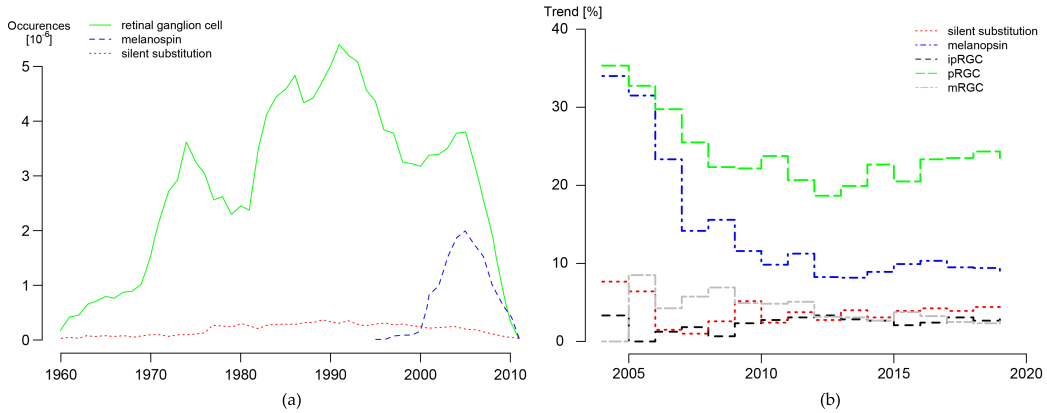

Figure 2. (**a**) Occurrences of selected words within the digitalized books of Google published between 1960 and 2019. (**b**) Yearly means of the monthly relative trends of the selected words from 2004 to 2019. The maximum of 100% occurs in March 2004 for *ipRGC*.

Table 1 shows the number of papers containing one or a combination of two words from the previous list. The "term circadian" yields the largest hit and is the earliest to be cited (in 1945).

Table 1. Number of articles found on PubMed on the 14th of May, 2020 with the keywords and their combinations. The diagonal shows the result for a single term. The last column gives the 10^{-9} occurrence of the word within the books digitalized by Google (Ngram) as of 2019.

	silent substitution	melanopsin	ipRGC	pRGC	mRGC	circadian	Ngram (2019)
silent substitution	252	20	5	0	2	6	0.9
melanopsin		1018	151	10	21	527	72.1
ipRGC			194	0	1	102	2.8
pRGC				25	0	9	1.8
mRGC					68	15	0.2
circadian						90550	1665.0

Considering the PubMed search (Table 1), taking out duplicates and articles not related to melanopsin, (mRGC also means "Mas-related G protein-coupled receptor and medullary ray glomerular counting"), a total of 26 articles remained. We found 2 other articles from SPIE and 1 from IEEE.One reference could not be retrieved. While reading these articles, we found 11 new articles, some describing the instrument used in a study but not found with our keywords. Thus, there were 40 articles left to review. These articles were written by a total of 101 authors; 81 wrote about human experiments and 27 about animal experiments, meaning that some wrote about both.

From these 40 articles related to melanopsin and silent substitution, 2 were reviews, 13 were referring to previous device descriptions, 2 gave results but no information about the device used, 2 were describing general illumination and 7 were describing devices exclusively for mice, among which 5 used silent substitution for screen projection or environmental lighting. Finally, 3 articles could not be retrieved.

We were left with a grand total of 11 different original devices presented by 41 authors. All came from different journals except 2 from the Journal of the Optical Society of America A (JOSA A). The 2019 impact factors of these journals are between 4.57 and 1.86 with a mean of 3.56.

Interestingly, one device was used to silence ipRGC for Purkinje-tree perception [31].

3.2. Devices Description

Table 2 summarizes most of the following results. Sensitivity of melanopsin was assumed to be the same as S-cone sensitivity but shifted to 480 nm [32,33] or 482 nm [34]. One article used silent substitution to carefully study the trichromacy theory [34].

Eight devices generate homogeneous fields by using either a digital spectral light modulator [33] or a set of four LEDs [35–39], five LEDs [32,40,41] or six LEDs [34,42]. Only one device is able to generate two homogeneous fields [38]. Descriptions of two devices able to generate images for stimulating ipRGC [41,42] were found.

Projector-based devices have a luminance resolution of only 8 bits, but homogeneous fields can reach a resolution of 16 bits [35].

No devices use coherent light sources and no publication gave information about the reason for their choice of primaries and corresponding bandwidth.

The most common units for light are cd/m^2 or lux. Troland is only used in two cases. Only one paper [35] gives us the correspondence between lux, troland and photon/m2/s.

To get the modulated light into the eye, the Newtonian view is the most common [31,32,35,36,41, 42]. For the Maxwellian view, an artificial pupil is used to control the retinal illuminance [33,38–40], except for one device that uses a small instrumental pupil [37]. In one case [34] it was not clear if and how they controlled the amount of light on the retina.

Table 2. The table is organized for the type of view. L_{res} is the resolution of the luminance expressed in bits. C. obsc. corresponds to the central obscuration. PRJ, projectors; DS, digital spectral integrator; HO, homogeneous field; IM, imaging system; PU, pupil; PURK, Purkinje-tree percept. * converts lux into photon/m^2/s and troland.

Reference	[42]	[41]	[32]	[35]	[36]	[39]	[33]	[40]	[38]	[34]	[37]
View type	Newtonian						Maxwellian				
Pupil type	Normal					Artificial	Artificial			Normal	
Nb primary n	6	5	5	4	4	4	56	5	4	6	4
L_{res} [bit]	8	16	8	-	-	-	-	12	8	12	-
Light source	PRJ	PRJ	LED	LED	LED	LED	DSI	LED	LED	LED	LED
Type of field	IM	IM	HO	HO	HO	HO	HO	HO	2 HO	HO	HO
C. obsc. [○]	0	0	0	0	0	no	5	10.5	-	0	10
FOV [○]	-	-	120	20	26.5	180	27.5	30	-	20	52
Light units	lux	lux	lux	lux*	lux	lux	lux	tr	tr	lux	lux
C_W	117	-	-	53	45	-	300	50	-	-	0
C_M	-	-	1.05	-	18	-	50	25.1	-	-	85
Biosignal	-	PU	PU	PU	ERG	PU	PU	PU	PU	PU	PU

Most of the selected papers describe devices in which pupil size is measured. One device was specifically designed for electroretinogram (ERG) [36] and one did not specify any measurement but described the device extensively [42]. Extending to all 40 papers, 30% measure pupil size, 10% ERG and the remaining 60% measure other biological effects.

The field of view of the devices ranges from 20° to 120° (mean 57°). Only three devices had a central obscuring (5° or 10°) to account for the cone fundamentals.

Half of the publications give either C_W or C_M or both, but there are inconsistencies in the definition used. When not specified, we assumed that the Michelson definition was used, except for one contrast value given as 1.049 [32]. C_M ranges between 18 and 25.1% and Weber contrast between 45 and 300%.

Of the 40 articles, about half give a description of the device used for their experiment. In one case, the paper refers to the device in a conference paper, which says nothing about it.

Considering devices using LEDs, Parry [39] used a commercial device but all others built their own electronic systems based on pulse-width modulation (PWM) using an Arduino [34,40] or other microprocessors [32,35–38].

Five articles [32,35–37,40] give the precision of the PWM ranging from 8 to 16 bits. Among these articles, only one [40] provides extensive information about the electronics in use. However, none of them discuss the effect of the quantification of the current intensity on the silent substitution optical signal. Finally, one publication [42] extensively describes the device producing images with six primaries at a frame rate of 25 Hz.

4. Discussion

Because circadian rhythm and blue light are hot topics, we expected to find many articles on PubMed using our chosen keywords. This was not the case. Moreover, we were surprised to find so few scientific devices and no commercial devices for studying the impact of ipRGC signals, and also that one modified commercial instrument [39] was used. We did not include blue light as keywords, because in such cases the *S*-cone is also stimulated, which leads to other types of experiment.

Compared to the Google searches conducted in September 2020, the monthly popularity of searching for the term "blue light" related to "health" is approximately 4% for the period from 2004 to 2018 and an average of 38% for 2019.

In most of cases, the field of view is homogeneous probably because it is easier to produce, but one device described two concentric homogeneous fields [38]. Recently, a new system [42] able to generate images has been described. This enables experiments targeting the position of the ipRGC within the retina.

Except for three devices [33,41,42], the eight others use LEDs as light sources. The heat produced by LEDs shifts the peak wavelength [43], especially with high-power LEDs that draw a current larger than one ampere. In addition, careful calibrations were performed and heat sinks were used, but none of these articles accurately describe the steps taken to control light emission.

Considering L_{res} as the smallest increase of the luminance in percent, its inverse corresponds to the number of duty cycles of the PWM signal or the number of gray levels of the projectors. The relation between L_{res}, the clock of the controller f_{CPU} and the maximum frequency of the light signal f_{PWM} is $2^{L_{res}} \cdot f_{PWM} = f_{CPU}$. As long as only one channel is driven, an Arduino is able to generate a visual signal up to a temporal frequency of 244 Hz. If n primaries are used, then f_{PWM} must be divided by n. In any case, f_{PWM} must be above the fusion threshold of the photoreceptors.

As already pointed out [44], the lack of consistent and adequate methods of quantifying light is a problem when comparing studies. Confusion between irradiance at the level of the cornea and excitance of the light source often occurs and is not always clearly stated. This means that conversion between lux and troland is impossible in most of the cases.

We did not take into account the few publications proposing lighting for the environment because spectrum light entering the eye is strongly dependent on the reflecting surface around the subject.

Because no projectors on the market have more than three colors, the superposition of two images is needed and spectrum selection must be performed that gives between 4 to 6 different primaries. Luminance resolution for projectors is limited to 8 bits. If the Newtonian view is adopted [41,42], the amount of light entering the eye is not controlled. Only a Maxwellian view with a small instrumental pupil would allow that control, but would also increase the retinal illuminance by a manifold.

In the case of a homogeneous field, three types of optical set-up were found (see Figure 3).

A) A Ganzfeld with clever light mixing where the eye of the subject is close to the light source, eventually with an artificial pupil to control the retinal illumination. Because of the lack of space, pupil diameter changes are measured on the contralateral eye.

B_N) One or more homogenized light sources with a field stop observed with a magnifier and eventually an artificial pupil to control the retinal illumination. Due to a lack of space, pupil diameter changes are also measured on the contralateral eye.

B_M) One homogeneous pupil light source illuminating an object plane with stops and projected into the eye of the subject, so that retinal illumination is controlled by the exit pupil of the

instrument. In that case, more space is available and pupil diameter changes can be measured on the excited eye.

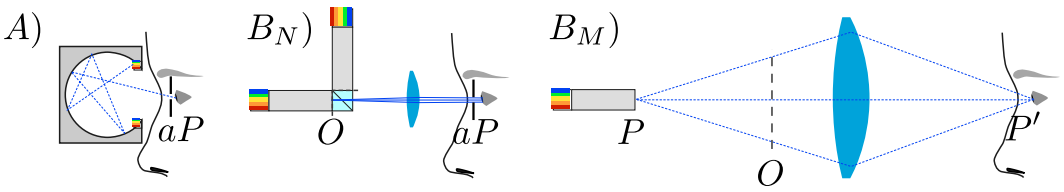

Figure 3. (**A**) Light sources around the exit hole of a small Ganzfeld are mixed and scattered back to an eventual artificial pupil (*aP*) before the eye. (**B_N**) Le light is mixed within a pipe with a field stop at its end (*O*) and combined with another similar light source. The field stop is then observed through an ocular. (**B_M**) A mixed light source output (*P*) is imaged into the eye pupil (*P*′) and the front lens has a very large numerical aperture to allow a large field of view, and the eyes observe an image of *O*.

All authors were aware that the cones' fundamentals close to the fovea are different than they are further away, and most experiments tried to accommodate that fact. For the Ganzfeld, the field of view (FOV) could be large but it was reduced by the artificial pupil.

The contrast between two black metamers, which exhibit the same tristimulus, is an important factor for the experiment; only half of the publications mention it and only three of them use both C_W and C_M.

The 2020 α-opic toolbox and User Guide [7] were recently updated by the International Commission on Illumination (CIE) to enable calculations and conversions of quantities related to ipRGC-influenced responses to light. This means that the relative melanopsin sensitivity and standardized *K* values are now freely accessible, which was not the case before.

5. Conclusions

Silent substitution to study ipRGC remains a difficult task because many physical parameters related to the device, and not obvious physiological parameters related to the vision, must be controlled and taken into account. Even with 1018 articles found to contain the word "melanopsin", the number of devices to study ipRGC without crosstalk with cones is small. Apart from a few exceptions, the descriptions of the devices are incomplete and these cannot be replicated. Almost all device descriptions failed to give unit conversion details, the watt to lumen conversion factor $\vec{K}$ or the device type used for the luminance measurement. Finally, only a single modified commercial device is used, suggesting the very small size of the market for such research devices.

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Abbreviations

The following abbreviations are used in this manuscript:

CIE	International Commission on Illumination
ERG	Electroretinogram
FOV	Field of view
IEEE	Institute of Electrical and Electronics Engineers
ipRGC	intrinsically photosensitive retinal ganglion cells
JOSA A	Journal of the Optical Society of America A
L	L-cone for long (red)
LED	Light emitting diode
M	M-cone for middle (green)
mRGC	melanopsin retinal ganglion cells

PWM Pulse width modulation
S S-cone for short (blue)
SPIE International society for optics and photonics

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