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# Cerebral neural correlates of differential melanopic photic stimulation in humans

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ABSTRACT

Photic stimulation of rods, cones and intrinsically photosensitive melanopsin-containing retinal ganglion cells (ipRGCs) mediates non-visual light responses, including entrainment of circadian rhythms and pupillary light reflex. Unlike visual responses to photic stimulation, the cerebral correlates of non-visual light responses in humans remains elusive. In this study, we used functional magnetic resonance imaging (fMRI) in 14 healthy young participants, to localize cerebral regions which are differentially activated by metameric light that gave rise to different levels of melanopic excitation. Mean blood oxygen-level dependent (BOLD) responses disclosed bilateral activation of the frontal eye fields during exposure to light geared towards melanopsin. Furthermore, multivariate pattern analyses showed distinct bilateral pattern activity in the inferior temporal gyri and the caudate nuclei. Taken together, our findings suggest that melanopsin-based photoreception activates a cerebral network including frontal regions, classically involved in attention and ocular motor responses.

## Introduction

In addition to the classical rod and cone photoreceptors in the outer retina, a small subset of retinal ganglion cells contain the photopigment melanopsin and respond directly to light (Berson, 2003; Dacey et al., 2005; Gooley et al., 2001; LeGates et al., 2014; Provencio et al., 1999). These intrinsically photosensitive retinal ganglion cells (ipRGCs) respond preferentially to short wavelength blue light, but also integrate neural signals originating from rods and cones, before projecting to various cerebral structures implicated in non-visual photic responses such as the pupillary light reflex and circadian entrainment (Lucas et al., 2014). Recent studies suggest that ipRGCs may contribute to luminance differentiation in blind patients without functional rods and cones (Zaidi et al., 2007) and brightness detection in healthy individuals (Brown et al., 2012). Although the response properties of ipRGCs and their efferent projections have been thoroughly investigated in animal models (Hatori and Panda, 2010; LeGates et al., 2014), little is known regarding their cerebral correlates in humans. This is mainly due to methodological limitations *in vivo* and the complexity of identifying brain activations specifically mediated by the intrinsic (melanopic) stimulation of ipRGCs from those originating from classical visual photoreceptors (Vandewalle et al., 2013; 2009; 2007b). To date, a handful of studies have used narrow-bandwidth light stimuli and functional magnetic resonance imaging (fMRI) to investigate non-visual light-induced cerebral activations in humans (Chellappa et al., 2014; Perrin et al., 2004; Vandewalle et al., 2007b; 2007a). For example, using complex tasks such as working memory task, both Chellappa et al. (2014) and Vandewalle et al. (2007b) showed that executive regions may be susceptible to wavelength-dependent influence of light exposure. These studies, however, could not fully isolate melanopsin-dependent cerebral activations, because the light stimuli that were used presumably activated both ipRGCs and visual photoreceptors. In order to isolate the intrinsic photo-responses of ipRGCs, we designed two perceptually similar white lights (i.e., metameric light stimuli) with different levels of melanopic excitation and similar stimulation of cones (Estévez and Spekreijse, 1982; Estévez

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and Spekreuse, 1974; Viénot and Brettel, 2014). By combining individually tailored metameric light stimulation and fMRI, we explored the cerebral correlates of ipRGCs photic activation in healthy individuals.

## Methods

### *Participants*

Seventeen healthy participants (18–35 years) took part in a study that included both psychophysical and fMRI assessments. All participants were free of any neurological, psychiatric or sleep disorders and were not on any medication; they all had normal visual functions and normal color vision assessed by Ishihara's test for Color-Blindness. Participants were instructed to maintain a regular sleep cycle a week before the experiment and refrain from consuming alcohol and caffeine 24 h prior to the experiment. Also, according to a follow-up report, none of the participants reported having any sleep issues. The experiments conducted complied with the Helsinki tenets for biomedical research and were approved by the institutional review boards at the National University of Singapore and Singapore Health Services. All participants gave written informed consent prior to the experiments. Three participants were excluded from data analysis: one participant with excessive head movements and two participants with corrupted data. Fourteen participants (5 males, 2 left handed, 1 smoker, mean age ± SD was 24.7 ± 4.5 years, average BMI was 20.2 ± 2.3.) were included in the final analysis. Participants were scanned during daytime (i.e. 9 am to 12 pm & 2–5 pm, $n$= 5) and nighttime (i.e. 7–10 pm, $n$=9).

### *Metameric lights generation*

#### *LED projector and calibration*

A light projector comprising 60 light-emitting-diodes (LEDs) of 7 color types was used in the current study (Desire D60, Electronic Theatre Controls, Middleton, Wisconsin, U.S.). The spectral radiance of the lights transmitted was measured on a colorless diffuser (Canson, Annonay, France), which was also used in our later psychophysical and fMRI experiments. The measurements were obtained by a JETI specbos 1211 UV spectroradiometer (JETI, Jena, Germany) (see (Viénot et al., 2012) for calibration details).

#### *Photic stimulations*

We created a pair of metameric photic stimulations that excited cones similarly but had either a high (HM) or low (LM) melanopic stimulation. These lights were first generated from standard fundamental observers (CIE, 2006; Stockman and Sharpe, 2000; Viénot et al., 2012) and later tailored to each participant in our psychophysical experiment. The mean melanopic excitation difference was 49.9 ± 10.4% (mean ± SD). Please refer to Supplementary Information for light production details. We have also provided a table of HM/LM melanopsin stimulations in arbitrary units for each participant (Table S. 1.).

### *Psychophysical refinement of the photic stimulations*

#### *Design*

The psychophysical experiment was performed in the MRI environment and was designed to tailor the metameric light stimuli for each participant. Participants were exposed to photic stimulation monocularly (left eye) throughout the psychophysical and fMRI experiments, to avoid potential differences in color perception between the two eyes. Their pupils were not dilated with medication. The photic stimuli were projected on a diffuser fixed 5 cm in front of the eyes of the participant (via a headmounted mask). During this procedure, participants were fixating a central fixation area (subtending central 10°), which was designed to reduce the influence of the Maxwell's spot (Horiguchi et al., 2013). Thus, participants could perceive a homogenous light distribution within their whole visual field around the fixation point.

The following two steps were implemented to generate the individually-tailored HM/LM pair. We used flicker photometry to refine the perceptual match of the HM/LM pair in each participant. In this paradigm, the two lights were shown in a successive manner at least 3 times. Each time each light was presented for approximately 3 s with no blank interval in-between the two lights. The initial luminance level of the lights were similar to that of the final light stimuli except that the final light pairs had smaller luminance difference due to 25 Hz photometry adjustment (see below). Participants were instructed to report any perceptual differences (e.g. the 1st light was greener). Based on the participant's feedback the experimenter adjusted the light channels without changing the “blue” channel. This was to ensure sufficient melanopic excitation differences. The color matching procedure stopped when a.) participants reported seeing no color differences between the two lights or b.) participants reported seeing minimal color differences that could not be diminished. Subsequently to this initial step, we created 11 pairs of light with varied luminance level in one of the lights (i.e. ± 5 units alpha value). Participants were then asked to choose the pair with least flickering sensation when presented at 25 Hz (Lennie et al., 1993). We repeated this procedure twice to find the pair that produced minimal or no flickering sensation. Finally, the participants were asked to rate the color differences between the two lights in the finalized HM/LM pair and 27 other equal melanopic stimulation (EM) pairs (see below), without knowing the identities of all the pairs. The EM pairs that had the same ratings as the HM/LM pair were chosen as the controls.

Despite individually tailoring the lights to each observer, residual color perception differences remained between HM and LM metamers. To examine whether this minimal color difference between the metameric HM/LM pair might elicit differential brain activation and confound the results, an additional control experiment was performed. This control experiment was implemented to select pairs of light with equal melanopic stimulation (EM) that simulated the perceived color difference between the HM/LM pair. At least 2 (maximum = 3) EM pairs that had the same mean color difference rating score as that of the HM/LM pair were chosen as our control pairs (mean ratings for HM/LM pairs and EM pairs, respectively: 3.5 vs. 3.5, $p > 0.05$). The two lights in each EM pair had equal luminance and the same melanopic excitation level (Please refer to Supplementary Information for details of the EM pairs). If the control pairs with the same minimal color difference as the HM/LM pair do not reveal any differential brain activation patterns, then it makes the color difference unlikely to have significantly contributed to the observed effect. The spectral radiance of a representative HM/LM and EM pair is shown in Fig. 1.

### *fMRI experiment*

#### *Design*

Scanning was performed using a 3 T Siemens Trio scanner (Siemens, Erlangen, Germany) at Duke-NUS Medical School, Singapore. Functional MRI runs were acquired using a gradient echo-planar imaging sequence (TR=2 s, TE 30 ms, FA 75°, FOV 192×192 mm, 64×64 matrix, 3×3 mm in-plane resolution). Thirty-six slices were collected with a 12-channel head coil (3.8 mm thickness). Slices were oriented roughly parallel to the AC-PC with whole brain covered. A T1-weighted anatomical image was also acquired and later used for co-registration (TR=2.3 s, TI 900 ms, flip angle 9°, BW 240 Hz/pixel, FOV 256×240 mm, 256×256 matrix, 192 slices, 1×1×1 mm). Each participant took part in 12 runs, half of which (HM/LM runs) contained blocks of HM and LM lights and the other half (EM runs) contained blocks of EM lights. Each run contained 9 blocks and followed an alternating *light off – light on* procedure. Each block lasted 30 s with a counterbalanced presentation order of the HM