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Blue Light Stimulates Cognitive Brain Activity in Visually Blind Individuals

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Abstract

Light regulates multiple non-image-forming (or non-visual) circadian, neuroendocrine and neurobehavioral functions, via outputs from intrinsically-photosensitive retinal ganglion cells (ipRGCs). Exposure to light directly enhances alertness and performance, so that light is an important regulator of wakefulness and cognition. The roles of rods, cones and ipRGCs in the impact of light on cognitive brain functions remain unclear, however. A small percentage of blind individuals retain non-image-forming photoreception and offer a unique opportunity to investigate light impacts in the absence of conscious vision, presumably through ipRGCs. Here, we show that three such patients were able to choose non-randomly about the presence of light despite their complete lack of sight. Furthermore, 2s of blue light modified EEG activity when administered simultaneously to auditory stimulations. fMRI further showed that, during an auditory working memory task, less than a minute of blue light triggered the recruitment of supplemental prefrontal and thalamic brain regions involved in alertness and cognition regulation, as well as key areas of

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the default mode network. These results, which have to be considered as a proof of concept, show that non-image-forming photoreception triggers some awareness for light and can have a more rapid impact on human cognition than previously understood, if brain processing is actively engaged. Furthermore, light stimulates higher cognitive brain activity, independently of vision, and engages supplemental brain areas to perform an ongoing cognitive process. To our knowledge, our results constitute the first indication that ipRGC signaling may rapidly affect fundamental cerebral organization, so that it could potentially participate to the regulation of numerous aspects of human brain function.

Keywords

Biological rhythms and sleep; Functional MRI; EEG; Attention: Auditory; Memory: Working memory

INTRODUCTION

Light is essential for the regulation of numerous circadian, neuroendocrine and neurobehavioral functions, sometimes termed non-visual or non-image-forming responses, via outputs from intrinsically-photosensitive retinal ganglion cells (ipRGCs) (Hatori et al., 2008; Schmidt et al., 2011). These ipRGCs represent a recently discovered novel class of retinal photoreceptors (in addition to rods and cones), which express the photopigment melanopsin, are maximally sensitive to short wavelength blue light (~480nm) and project broadly throughout the brain. Importantly, light exposure can directly enhance alertness and performance during multiple cognitive tasks, with a greater efficiency for short wavelength light, so that light is an essential regulator of wakefulness and cognition (Chellappa et al., 2011a; Lockley et al., 2006). The brain mechanisms involved in the stimulant effect of light on cognitive function are only starting to be elucidated, however (Vandewalle et al., 2009).

Human neuroimaging has demonstrated that ocular light exposure acutely modulates attentional, executive and emotional brain responses to auditory tasks (Perrin et al., 2004; Vandewalle et al., 2011; Vandewalle et al., 2006; Vandewalle et al., 2007a; Vandewalle et al., 2009; Vandewalle et al., 2007b; Vandewalle et al., 2010). These studies identified the hypothalamus, the thalamus pulvinar, and the brainstem locus coeruleus as likely subcortical interfaces mediating the non-image-forming impact of light on the cortical areas involved in the ongoing cognitive process, particularly in prefrontal and parietal cortices. IpRGCs are likely to be the primary photoreceptors mediating these effects as, compared to other wavelengths, blue light is more effective in inducing sustained modulations of brain responses. A direct demonstration of the implications of a non-image-forming photoreception system in the light-induced modification of cognitive brain activity is still lacking, however, since prior neuroimaging data were acquired in fully sighted participants (Allen et al., 2011; Dkhissi-Benyahya et al., 2007; Lall et al., 2010).

Nearly two decades ago, it was discovered that retinal light exposure could suppress melatonin and entrain circadian rhythms in some blind people, despite a complete absence of conscious light perception (Czeisler et al., 1995). Later research confirmed this finding and established that light could also induce circadian phase resetting and slow-onset pupil

constriction in this small percentage of totally visually blind individuals with outer retina degeneration, but presumably with an intact ganglion cell layer (Klerman et al., 2002; Zaidi et al., 2007). These responses are similar to the circadian and pupil responses to light observed in rodents lacking functional rods and cones, which are mediated through ipRGC photoreception (Hattar et al., 2003; Lucas et al., 1999; Panda et al., 2003). In addition, in one of these individuals, prolonged exposure to light improved subjective and objective electroencephalogram (EEG) correlates of alertness, as well as performance during a simple auditory psychomotor vigilance task (PVT) (Zaidi et al., 2007). This non-image-forming impact of light is likely to arise from ipRGCs because i) these cells are known to be preserved in individuals with outer retinal degeneration (Hannibal et al., 2004); ii) ophthalmologic examination confirmed atrophy of the retinal pigment epithelium and found no detectable functional responses from rods and cones (Zaidi et al., 2007); iii) light effects were more pronounced using 460–480 nm monochromatic light as compared to other wavelengths (Zaidi et al., 2007); and iv) recent data show that the dynamics of pupillary constriction in a blind human with outer retinal degeneration was compatible with the exclusive involvement of ipRGCs (Gooley et al., 2012). Blind individuals with preserved non-image-forming photoreception might also exhibit some non-conscious awareness of light. In a previous report, despite complete visual blindness, one participant was able to choose successfully when a light exposure was presented in a two-alternative forced choice task, but only when ~480 nm blue monochromatic light, and not other wavelengths, were administered (Zaidi et al., 2007). These rare individuals - only 9 have been identified to date worldwide - therefore offer a unique opportunity to investigate the selective impact of light on cognitive brain functions in the absence of conscious vision, presumably solely via ipRGCs intrinsic light sensitivity.

In the present study, we aimed to confirm and extend the finding that non-conscious awareness of light is apparent in visually blind participants who retain non-imaging forming responses to light. In addition, we used a traditional EEG protocol, adapted from the multisensory integration literature, to investigate whether brief exposure (up to 2 seconds) to high intensity blue light could modify EEG activity in these participants while they performed an auditory cognitive task. Finally, we used functional magnetic resonance imaging (fMRI) to test whether exposure to high intensity blue light for less than a minute modulated cognitive brain responses to an auditory task and to identify neural correlates of this light-induced modulation.

METHODS

Participants

Three totally visually blind individuals, with complete loss of sight and no conscious light perception, participated in this study (60–67 years; 1 female; Table 1 for detailed characteristics). They provided written informed consent and all experiments were approved by the Comité mixte d'éthique de la recherche du Regroupement Neuroimagerie/Québec. All three had previously completed studies that established that they retained a light-induced melatonin suppression response despite the absence of conscious vision [published for two of them (Klerman et al., 2002; Zaidi et al., 2007)]. Participant 1 had had pupil muscle

damages during lens removal surgery for cataract problems and did not respond to a standard pen-light examination. Participant 2 exhibited pupil constriction if the pen-light exposure was continued for up to 10 seconds. Participant 3 had no clearly distinguishable pupil. In previous visits to Boston, a fundoscopic examination in participants 1 and 2 confirmed atrophy of the retinal pigment epithelium, with thinning of retinal vessels and bone spicule pigmentation. These findings were confirmed on several occasions by different ophthalmologists who examined the patient independently. Visual acuity tests previously performed in Boston indicated no light perception in either eye of the 3 participants. Standard visually evoked EEG potential (VEP) procedures were also previously administered to participants 1 and 2, and a standard electroretinogram (ERG) procedure was previously administered to participant 3. No classical visual responses were detected in any tests. Finally, questionnaire scores indicated that participants 2 and 3 reported, respectively, relatively poor sleep quality and high propensity to fall asleep during the day (Table 1).

All three participants had been declared totally blind for at least 10 years, but the cause and duration of sight loss differed (Table 1). Participants maintained a regular sleep schedule for 7 days prior to travelling to Montréal (verified using sleep logs). They remained on their home time zone for their entire stay in Montréal and were allowed to go outside when not performing an experiment. No pupil dilator was administered for any of the experiments described below.

EEG protocol

On two consecutive days, participants arrived in the laboratory 6.5h after wake time and were blindfolded for 1h before the first recording was initiated. All recordings were conducted in a dark and sound attenuated Faraday room while participants sat with their head on a chin rest with their eyes 6cm away from the center of a 21×11 cm diffusion glass, including ultraviolet and infrared filters, behind which a 48 blue light emitting diode (LED) array was placed (peak 465nm; Full Width at Half Maximum – FWHM: 27nm; spectrum assessed with LightspeX [GretagMacbeth, NY], see Supplemental Figure S4). Irradiance at eye level was high and set at $414 \mu\text{W}/\text{cm}^2/\text{s}$ (PM100D, Thorlabs, NJ) which corresponds to 9.7×10^{14} photons/cm²/s. The light device produced no perceptible sounds or temperature change. Throughout the EEG protocol, participants' 'gaze' was monitored with an infrared camera to ensure that their eyes remained open. Presentation (Neurobehavioral Systems Inc., Albany, NY) was used to produce auditory stimuli, control the LED array and record keyboard responses. Auditory stimuli were transmitted to the participant via headphones (EarTone3A, Etymotic Research, Elk Grove Village, IL). Volume was set to an individual comfortable level before the task was initiated.

Two Alternative Forced-Choice task (2AFC)—At the start of the EEG protocol, and after being blindfolded for at least 1h, participants were asked to report whether the high irradiance blue LED array was on during the first or second half of a 4, 10 or 20s interval in a two-alternative forced-choice task (2AFC). Each trial started with the auditory instruction “start” and ended with the auditory instruction “end”. A high-pitched sound (500ms, 1000Hz) indicated the middle of the trial. The participant gave his/her response at the end of each trial both orally and by pressing a key. Blue light was pseudo-randomly turned on for

10s in the first or second half of the trial. Each trial duration was tested in a separate session which included 40 trials delivered to both eyes simultaneously. The 4 and 10 s trial duration were tested on the same day while the 20 s trial duration was acquired the preceding or following day. Cumulative binomial statistics on discrimination were carried out to determine whether responses were significantly different from random choices.

Visually Evoked Potential—Participants were instructed to keep their eyes wide open in front of the LED array while blinking as little as possible. Given the small sample size and their visual blindness, the number of trials was high (800) so that slight EEG evoked responses could be detected. Flashes of blue light (duration 500ms; inter-stimuli interval: from 1600 to 1800 ms) were delivered to the participant in four blocks of 200 stimuli (2 blocks per experimental day).

Bimodal psychovigilance task (PVT)—Visual stimuli consisted of 500ms and 2s duration of blue light. Auditory stimuli consisted of 150ms pink noise bursts (90% normalized peak value, plateau time 90ms, rise/fall time 5ms). For bimodal stimuli, auditory stimuli were administered for the last 150ms of the visual stimuli (i.e. 350ms or 1850ms after light onset). Participants were asked to respond as fast as possible to each sound by pressing a keyboard with their right hand. Given the limited number of subjects, a high number of trials were recorded. Participants completed 5 blocks of each conditions (500ms and 2s), and each block included 300 trials equally divided between each stimuli types (100 sound alone, 100 light alone, 100 bimodal) for a total of 500 trials per stimulus type. Inter-stimuli interval randomly ranged from 1200 to 2600ms (mean: 1900ms) in the 500ms condition and from 1200 to 1600ms (mean: 1400ms) in the 2s condition. Participants 2 and 3 started with the 500ms condition, while participant 1 started with the 2s condition.

EEG recordings and analyses—During the VEP and bimodal PVT protocols, EEG was recorded from 40 Ag/AgCl electrodes cap (Neurosoft, Sterling Inc., VA), placed according to the extended International 10/20 system, including a ground electrode. All electrodes were referred to both mastoids and impedance was maintained <5 kOhms. EEG and electro-oculogram (EOG) were digitized at 1000Hz, high-pass filtered at 0.1Hz, low-pass filtered at 100Hz and averaged offline.

Trials with artifacts at electrode sites of interest (Cz and Oz), or eye blinks (vertical eye movements >100 μ V) were manually excluded. EEG time series of 600ms, with 100ms pre-auditory-stimulus, were edited off-line using BrainVision Analyzer (Brain products, Gilching, Germany) following 3 steps: 1) data filtering (0.1–35Hz), 2) data epoching 3) baseline corrections. For trials with light stimuli alone of the bimodal PVT protocol, the last 150ms of the light pulse were considered as post-auditory stimulus for comparison with trial with auditory stimulations (i.e. as if a sound had been produced). Edited EEG time series of each trial for each participant and for each condition were exported to Matlab 7.1 (MathWorks Inc., MA) for analyses. For the VEP recording, trials were averaged and displayed in Supplemental Figure S1. Since no responses could be isolated, no statistical analyses were performed. For the bimodal PVT, as classically performed in EEG experiments investigating multisensory integration (Giard & Peronnet, 1999; Molholm et al., 2002), Event Related Potentials (ERP) from the auditory-alone and visual-alone conditions

were summed for statistical comparison with the ERP response to the simultaneous audiovisual condition.

Given the limited sample size, conservative single subject analyses was undertaken. We used a nonparametric Monte Carlo permutation approach, in order to find the time points, in each individual separately, with significant differences between the ERPs obtained in the bimodal condition and the sum of the ERPs obtained in the two unimodal conditions. For each time points, bimodal trials and the sum of the two unimodal trials were grouped in a single set which was random partitioned 500 times between 2 conditions. T-statistics was computed for each partition to construct a histogram of t-statistic distribution. We finally tested for each time point whether the proportion of t-test of our permutations that was above the t-test of our original conditions. This proportion is the Monte Carlo significance probability, which constitutes the p-value. To further control for false positives, we only considered significant differences present for 10 consecutive time-points (~10ms at a sampling rate of 1024Hz), because the likelihood of getting 10 false positives in a row is considerably low, even if these time-points are non-independent (Giard & Peronnet, 1999).

FMRI protocol

Participants arrived at the laboratory 2.5h before habitual sleep time. A structural image of the brain was acquired and participants were familiarized with the MR setting. Participants were then blindfolded for 2h prior to the fMRI runs, which started 30 minutes after habitual sleep onset time. Light was produced by a quartz halogen white light source (PL950, Dolan-Jenner Industries, Boxborough, MA), filtered by narrow interference band-pass filter (Edmund Optic, Barrington, NJ) to produce blue monochromatic light (peak 480nm; FWHM: 13nm; spectrum assessed with LightspeX; Supplemental Figure S4). Light was transmitted by a metal-free optic fiber to diffusers (glasses frames mounted with 7 by 9 cm uniform diffusing glass placed 2cm away from the eye). Blue light irradiance at eye level was set at $81 \mu\text{W}/\text{cm}^2$ (1.95×10^{14} photons/cm²/s). The light device produced no perceptible sounds or temperature change.

2-back task—Stimuli consisted of nine English monosyllabic consonants (duration: 500ms; Inter-stimulus-interval: 2000ms), produced using COGENT 2000 (<http://www.vislab.ucl.ac.uk/cogent.php>), implemented in MATLAB, and transmitted to the participants using MR CONTROL amplifier and headphones (MR Confon, Germany). The first run was preceded by a short session during which volunteers set volume level. For each auditory stimulus, volunteers were requested to state whether or not it was identical to the stimulus presented 2 stimuli earlier, by pressing buttons with their right hand on an MR compatible keypad. Series of stimuli were constructed with ~30 % hits so that difficulty was similar in all blocks, were presented only once and were randomly assigned to a task block. Participants were trained to the task for 30min in the afternoon preceding fMRI acquisition and performance was > 88% in all three participants (Supplemental results; Supplemental Figure S3A, upper panel).

Participants performed 20 task blocks which lasted 55s and contained 22 auditory stimuli. Participants performed the task alternatively in complete darkness (<0.01 lux) or while

exposed to blue light, in which case the light was turned on and off at the beginning and end of a task block (and therefore exposure to light also lasted 55s). Ten blocks included blue light and 10 blocks included darkness. Blocks were separated by 13-to-22s episodes of rest in darkness (<0.01 lux; mean duration: 17.6s). The first block for Subject 1 and 2 included blue light, while for subject 3 the first block was conducted in darkness.

Technical issues—For technical reasons, the initial fMRI data obtained in participant 3 could not be used. Therefore, the following evening, this participant performed the identical fMRI protocol, with the exception that he was allowed to sleep later than usual the night preceding acquisitions (equal sleep duration but bedtime and waketime were delayed by 1.5h) to prevent sleep deprivation from the previous testing night.

fMRI data acquisition—Functional MRI time series were acquired using a 3T MR scanner (TIM-TRIO, Siemens, Germany). Multislice T2*-weighted fMRI images were obtained with a gradient echo-planar sequence using axial slice orientation (32 slices; voxel size: 3.4×3.4×3 mm³ with 30% of gap; matrix size 64×64×32; repetition time: 2000ms; echo time: 30ms; flip angle: 90°). Structural brain images consisted of a T1-weighted 3D MPRAGE (repetition time: 7.92ms, echo time: 2.4ms, time of inversion: 910ms, flip angle: 15°, field of view: 256×224mm², matrix size: 256×224, voxel size: 1×1×1mm³).

fMRI data analyses—Data were analyzed using Statistical Parametric Mapping (SPM8 - <http://www.fil.ion.ucl.ac.uk/spm>). They were realigned, coregistered and spatially normalized using the “new_segment” and “dartel” toolboxes of SPM8 (which includes smoothing). Due to the limited sample size, random effects analyses accounting for inter-subject variability were not carried out, and the analysis consisted in a single fixed-effects step including all three participants. This approach guaranties that any significant differences obtained in our analyses are valid within our sample of 3 subjects but implies it cannot yet be generalized to the entire population of totally blind individuals with preserved non-image-forming photoreception. Changes in brain responses were estimated using a general linear model, in which task blocks were modeled using boxcar functions, convolved with a canonical hemodynamic response function, and its first and second derivatives. Light onsets were modeled using stick function (“event”), convolved with a canonical hemodynamic response function and its first and second derivatives. A parametric modulation was added to each regressor to track any linear change of the amplitude of brain responses across time. Regressors modeled separately task block performed under blue light exposure and in complete darkness. Movement parameters derived from functional volumes realignment were considered as covariates of no interest. High-pass filtering was implemented in the matrix design using a cut-off period of 256s to remove low frequency drifts from the time series. Serial correlations in the fMRI signal were estimated using autoregressive (order 1) plus white noise model and restricted maximum likelihood algorithm. The contrasts of interest compared task blocks performed under blue light and in darkness. Statistical inferences on the t-statistics maps resulting from contrasts of interest were performed at a threshold of p=0.05 after conservative correction for multiple comparisons computed on the *entire* brain volume (Family Wise Error method).

RESULTS

We conducted classical visual evoked potential procedures and administered 800 500ms flashes of high intensity blue light to each participant (465nm ; 9.7×10^{14} photons/cm²/s). No evoked response was detected at the occipital Oz location, where visual responses are expected to be the greatest, nor at any other electrode location (Supplemental Figure S1). This finding confirms a lack of image-forming light perception in either eye.

Non-conscious awareness of light is present in visually blind individuals

We investigated whether the blind participants could ‘detect’ the presence of high intensity blue light (465nm ; 9.7×10^{14} photons/cm²/s) by performing a two-alternative forced-choice task (2AFC) (Zaidi et al., 2007). In addition, we varied the length of the light stimulus to examine whether the ability to detect blue light was duration-dependent. In three separate sessions, participants were given forty of either 4s, 10s and 20s trials randomized for whether the first or second half of the trial included blue light exposure (i.e., 2s, 5s or 10s of light), the other half consisting of darkness. Although the participants were hesitant about reporting the presence of visual stimuli that they were not conscious of, their selections were not random (Figure 1).

Responses from Participant 1 were extremely accurate (95%) irrespective of the interval duration ($p < 10^{-8}$). The accuracy of Participant 2’s responses appeared to be duration-dependent with a linear increase from random choices for 2s light exposures ($p = 0.44$), to 65% accuracy for 5s exposures ($p = 0.04$) and 80% accuracy for 10s exposures ($p < 0.001$). Participant 3 made random choices for 2s light exposures ($p = 0.44$), and 5s exposure, even though a tendency for significant detection was found for the later exposure duration (62.5%; $p = 0.08$). When exposures were increased to 10s (half of a 20s epoch), his performance was poor (30%) but significantly different from a random choice ($p = 0.008$) suggesting some non-conscious awareness of the stimulation. Overall, the results indicate that at least two of the three participants could detect the presence of light during the 2AFC task, despite a complete lack of classical photoreception as measured by standard ophthalmological techniques.

Two-second blue light exposure modulates EEG activity if administered simultaneously to the processing of auditory stimulations in visually blind individuals

We investigated whether brief high intensity blue light exposures (465nm ; 9.7×10^{14} photons/cm²/s) could modify EEG activity while participants were required to respond as fast as possible to randomly occurring auditory stimuli in a PVT, which probes the ability to maintain sustained attention (Dinges & Powell, 1985). The goal was to investigate if a non-consciously perceived flash of blue light could influence the EEG brain response of another (auditory) sensory modality. A similar approach is commonly used to investigate audio-visual multisensory integration in sighted individuals, in which simultaneous presentation of an auditory and a visual input produces brain integration, resulting in a greater response to simultaneous visual and auditory stimulation than the sum of the responses to each stimulation modality alone (Driver & Noesselt, 2008).

Light and auditory stimuli were administered either alone or simultaneously, in which case auditory stimuli were produced during the last 150ms of the 500ms light exposure so that light extinction and the termination of the sound coincided. We also administered 2s stimuli to explore the duration-dependent impact of light, in which case auditory stimuli were also produced during the last 150ms of the exposure.

No EEG response was evoked by 500ms or 2s blue light exposures if they were administered alone (Figure 2, blue lines), confirming the results of the visually evoked potential test and the EEG time-frequency analysis during the 2AFC test. Reaction times to the PVT task were not affected by 500ms or 2s light exposures ($p>0.1$; Supplemental Figure S2). Importantly, however, analysis procedures developed for investigation of multisensory integration mechanisms (Giard & Peronnet, 1999) showed that activity evoked by the processing of the auditory stimuli was reliably affected by simultaneous 2s light pulses in all three participants. As depicted on figure 2 (right panels), at Cz, where auditory responses are expected to be the greatest, auditory evoked responses simultaneous to light administration were significantly different not only from responses evoked by auditory stimuli alone and by light alone, but also from the sum of responses to sound and light alone (Figure 2). The amplitude of the response at Cz increased continuously between 183ms and 371ms and between 411ms and 434ms post-stimuli for participant 1; between 119ms and 150ms, and between 209ms and 226ms for participant 2; and between 137ms and 163ms for participant 3. In contrast, the 500ms blue light exposure had only a marginal impact on auditory evoked responses in participant 2 (Figure 2, left panels), with significant amplitude increase between 189ms and 206ms.

Less than a minute of blue light exposure stimulates fMRI brain responses in visually blind individuals

We then employed an fMRI procedure based on previous studies in sighted individuals (Vandewalle et al., 2011; Vandewalle et al., 2007b). Participants performed blocks of an auditory 2-back task alternatively in complete darkness or while exposed to high intensity blue light for 55s (480nm; 1.95×10^{14} photons/cm²/s). This task requires updating, maintenance and comparing of information in working memory, in addition to attention and auditory processing (Cohen et al., 1997). Similar to our prior findings in sighted participants (Vandewalle et al., 2011; Vandewalle et al., 2007b), the performance of the task was not affected by short exposures to blue light ($p>0.25$; Supplemental Figure S3A) and confirms that differences in brain activation are not biased by behavioural differences.

Statistical analyses of fMRI data showed that brain activity was significantly increased under blue light exposure in numerous brain areas, as compared to complete darkness (blue > dark). These areas include the ventrolateral prefrontal cortex (VLPFC), medial prefrontal cortex (MPFC), precuneus (PREC), anterior cingulate cortex (ACC) and the dorso-posterior thalamus in the dorsal pulvinar (PULV) (Figure 3, Table 2). Most of these areas are known to be involved in cognitive processes associated to the n-back task (Collette et al., 2006) and we have previously reported light-induced activity modulations in many of them in sighted participants while performing the same auditory task (Vandewalle et al., 2011; Vandewalle et al., 2007a; Vandewalle et al., 2009; Vandewalle et al., 2007b). In addition, blue light

exposure increased brain activity in several regions of occipital cortex relative to darkness, including the inferior and superior occipital gyri (IOG and SOG), and the calcarine sulcus (CALC). In all these brain areas affected by the light condition, average activity estimates showed that responses to the task under blue light were superior compared to darkness (Figure 3). Individual activity estimates are also depicted in Figure 3 (right graphs) and shows that activity was higher under blue light as compared to darkness in each participant, but that the magnitude of the difference between the light conditions could differ substantially. Importantly, in each participant, no brain responses to the task were significantly greater in darkness compared to during blue light exposure. Furthermore, analyses examining transient brain activity associated with light onsets, to which rods and cones and the visual system are particularly responsive (Allen et al., 2011; Brown et al., 2010), yielded no significant results.

Brain responses to the task performed in darkness only exhibited a classical pattern of activations typically observed with an n-back task, with fronto-parietal recruitment, left lateralized in the case of a verbal task (Supplemental Figure S3B and Supplemental Table S1) (Cohen et al., 1997; Collette et al., 2006). Interestingly, the significant relative increases in brain responses induced by blue light did not appear to be located in areas that were significantly recruited by the task when performed in darkness. To test this assumption formally, we created a broad map of the brain areas recruited by the task in darkness in our sample, even marginally so (i.e. we displayed results of the brain responses associated to the task in darkness at a very permissive statistical threshold of $p < 0.05$ *uncorrected* for multiple comparisons), and used it to mask out the brain areas affected by blue light exposure. This procedure revealed that only 4 clusters found to be significant when comparing directly blue light and darkness presented a marginal overlap with the brain areas at least slightly engaged in the task in complete darkness (see # in Table 2), while all others significant clusters showed no overlap. These results indicate that not only did blue light significantly increase brain responses when directly compare to darkness, but it did so mainly in areas not significantly engaged in the task during darkness.

Activity estimates showed that in many of these areas, the effect of blue light administration, compared to darkness, was due to a reduction in the deactivation observed in the darkness condition (Figure 3c–g, l). Most of these areas, and particularly the anterior cingulate and medial prefrontal cortex, are part of the so called “default mode network”, which constitutes a set of coupled areas that are generally disengaged relative to quiet passive wakefulness to perform an active process (Buckner et al., 2008; Raichle et al., 2001). We therefore tested whether the significant differential effect of blue light versus darkness was located in areas showing significant deactivation, or disengagement, when the task was performed in darkness. Deactivations in response to the task while in darkness are displayed in red on figure 4 and correspond to a typical map of the default mode network (Buckner et al., 2008; Raichle et al., 2001), including areas of the medial prefrontal cortex, anterior, posterior and retrosplenial cingulate and inferior parietal cortex (see Supplemental Table S1 for detailed list of brain regions). Widespread overlaps between these deactivations and locations of the significant impacts of blue light as compared with darkness were observed within the

anterior cingulate and medial prefrontal cortex, demonstrating that blue light prevented the deactivations observed in complete darkness in these parts of the brain (Figure 4).

DISCUSSION

These results show that, in the absence of conscious sight, but when non-image-forming photoreception is retained, light is able to modulate activity in the pulvinar, precuneus, anterior cingulate cortex, occipital cortex, as well as in the prefrontal cortex, including in medial prefrontal areas of the default mode network. The results also indicate that, in our sample, administration of as little as 2 seconds of high intensity blue light is able to modulate brain activity, as shown by modification of auditory evoked EEG responses during a sustained attention task, but only when the brain is actively processing the acoustic stimulations. The data further confirm and extend the finding that rudimentary ‘non-conscious awareness’ of light can occur in the absence of conscious vision if non-image-forming photoreception is preserved.

A first conclusion is that the impact of light on auditory cognitive brain responses previously reported in sighted participants (Vandewalle et al., 2011; Vandewalle et al., 2009; Vandewalle et al., 2010) involved non-image-forming photoreception and cannot be attributed solely to conscious vision. Given the limited sample of three participants (although they represent a third of the population of such individuals identified to date), our EEG and fMRI results have to be considered as a “proof of concept” rather than a fine-grained identification of the brain mechanisms involved in the non-image-forming impact of light on cognition. Inter-individual variability is reflected in the fMRI and EEG results, but also in the forced choice task. The fact that we obtained significant results in so few cases, however, is remarkable since the onset and etiology of blindness differed substantially between participants. It is even more remarkable when considering the reduction in retinal illumination due to age-related decrease in pupil size and increase in lens yellowing, which in combination may lead on average to a 0.6 log unit reduction in retinal illumination in a 60 to 70-year old (Turner et al., 2010).

Participants had no conscious light perception and no visually evoked EEG responses to 800 flashes of light, and ophthalmologic examinations found no indication of residual rod or cone function. While we cannot physically confirm the absence of functional rod and cone photoreceptors in these blind patients, the results of the EEG, fMRI and light ‘non-conscious awareness’ responses to blue light are consistent with the hypothesis that ipRGCs are the primary photoreceptors mediating these effects. At the irradiance we employed, the ipRGC intrinsic response, which is typically sluggish in the absence of functional rods and cones, can be detected in rodents in about a second and this is the case even when assuming a putative 1.2 log unit age-related reduction in retinal illumination (i.e. doubled compared to normal aging) (Berson et al., 2002; Dacey et al., 2005; Lall et al., 2010). The temporal dynamics of the melanopsin response may also explain why only 2s but not 500ms of light reliably modulated auditory-evoked EEG responses. Melanopsin primary involvement is further supported by our recent demonstration that the dynamics, wavelength sensitivity and sensitivity threshold of the pupillary light reflex in participant 2 of the present study matched mice lacking functional rods and cones (Gooley et al., 2012; Lucas et al., 2001).

Our data constitute the first direct indication that non-image forming photoreception alone can mediate light-induced modulation of fMRI and EEG brain activity while performing a cognitive task and is the clearest evidence that these effects can be mediated through ipRGCs. Future studies will be able to capitalize on these initial results to isolate and quantify the contribution of non-image-forming photoreception and of ipRGCs, using additional wavelengths and different light levels in blind and sighted control individuals.

Previous studies in sighted individuals (Cajochen et al., 2000; Lockley et al., 2006) and a study in which participant 2 took part (Zaidi et al., 2007), reported light-induced improvement in alertness, objectively measured by modifications in the power spectrum of waking EEG. These improvements were observed using exposures lasting several hours while participants were quietly awake, suggesting that the non-image-forming impact of light on cognitive brain function requires prolonged exposures. Similarly, significant changes in cognitive performance in sighted subjects have only been reported after 30 minutes of illumination (Cajochen et al., 2011; Chellappa et al., 2011b; Lockley et al., 2006). In the present study, and consistent with previous fMRI studies in sighted participants (Vandewalle et al., 2011; Vandewalle et al., 2007b; Vandewalle et al., 2010), changes in brain responses were observed using only 55s exposure. In addition, modifications of the EEG were detected if the auditory responses were scheduled simultaneously with as little as 2s of exposure to light if the brain was simultaneously processing auditory information. Our data therefore demonstrate that the non-image-forming impact of light can affect cortical activity within a few seconds if brain processing is actively engaged. This finding constitutes the first indication that it is crucial to differentiate the effects of light while at rest or engaged in a cognitive process when characterizing the dynamics of the non-image-forming influence of light on brain function. In addition, our findings suggest that the bimodal PVT protocol, together with pupillary constriction test (when applicable) (Gooley et al., 2012) could constitute a simple means to assess whether non-image-forming photoreception is retained in a blind totally individual, for example prior to elective enucleation.

Most brain areas that showed a light-induced increase in activation in fMRI were not recruited by the task at all when it was performed in complete darkness, supporting a general increase of brain responsiveness leading to the significant recruitment of supplementary brain areas. Data previously acquired in sighted participants and in participant 2 of the present study, showed that reaction times in an auditory PVT or in executive tasks are improved under prolonged night time blue or blue-enriched light exposure (Cajochen et al., 2011; Chellappa et al., 2011b; Lockley et al., 2006; Zaidi et al., 2007). It is therefore plausible that the recruitment of supplemental brain areas under blue light exposure we report here precedes and causes significant behavioral changes, such as reaction time improvements. In addition, light induced-modulations were found in higher order ventrolateral prefrontal areas, considered to be at the top of the hierarchy of cognitive control (Koechlin & Hyafil, 2007). Light can therefore not only allow the engagement of supplemental areas, but it does so in areas known to be involved in “higher-level” processes. This finding clearly demonstrates that the non-image-forming photic input pathway is as a strong regulator of human cognition.

Our observation that the influence of light on EEG signal could only be detected when a sound was administered supports the notion that the stimulant impact of light is initiated in subcortical structures (to which the EEG is rather insensitive) and can modulate cortical activity when it is engaged (Vandewalle et al., 2009). Targets of ipRGCs are primarily subcortical include the suprachiasmatic nucleus, ventrolateral preoptic areas, and lateral hypothalamus, which are all involved in circadian and sleep-wake regulation (Schmidt et al., 2011). These hypothalamic structures are intimately connected with several nuclei of the brainstem ascending arousal system, such as the locus coeruleus and dorsal raphe, which regulate cognition and broadly project to the cortex (Saper et al., 2010). Light could therefore have a widespread impact on brain activation via the hypothalamus and brainstem, in which a non-image-forming effect of light has previously been suggested in sighted participants (Perrin et al., 2004; Vandewalle et al., 2007b; Vandewalle et al., 2010). In addition, the pulvinar, which receives indirect retinal light information through the superior colliculus (Danckert & Rossetti, 2005) and has been shown repeatedly to be sensitive to light in studies of sighted individuals performing auditory task (Vandewalle et al., 2011; Vandewalle et al., 2006; Vandewalle et al., 2007a; Vandewalle et al., 2009; Vandewalle et al., 2007b), is also significantly affected by light in the blind participants. This finding further support a primary role of the pulvinar in mediating the broad non-image-forming impact of light on cognition at the subcortical level, possibly through the facilitation of thalamo-cortical information flow. IpRGC output could also directly reach the occipital cortex, as previously shown in rodents (Brown et al., 2010). The occipital cortex is known, however, to be devoted to auditory processes in blind individuals due to brain plasticity phenomenon (Collignon et al., 2011) and was indeed at least slightly engaged in the task in darkness in the present study (see Supplemental Figure S3B and Supplemental Table S1). It therefore remains unclear whether the significant recruitment of occipital areas during light exposure is related to the task or to direct ipRGC light information reaching these areas or both.

Given that light had an impact on another (auditory) modality, the effects we describe might be associated with multisensory integration. The time window of the modulation of the auditory evoked potential by light observed in this study (between 120 and 180ms) appears congruent with several studies reporting multisensory modulations in higher-order regions (Driver & Noesselt, 2008). The contribution of non-image-forming photoreception to multisensory integration is speculative and remains to be thoughtfully investigated, but our data show that the non-image-forming impact of light contributes to a multisensory interplay between exposure to light and auditory processing, involving likely ipRGCs. A site of such interplay could lie within the pulvinar, through which information can transit between areas mainly devoted to different sensory modalities (Cappe et al., 2009). Multisensory interplay could also take place in the superior colliculus, which is one of the most documented structures involved in multisensory integration (Stein & Stanford, 2008) and receives direct inputs from ipRGCs (Hatori & Panda, 2010; Schmidt et al., 2011).

The neurobiological substrate of non-conscious awareness to light remains unclear. Light awareness could be caused by the increased brain responsiveness induced by light exposure mainly through subcortical structure which could be “sensed” by the participants but remains difficult to describe. Multisensory interplays within the pulvinar or superior colliculus could

also be involved. Alternatively, direct ipRGC projections to the lateral geniculate nucleus (LGN) have been demonstrated in rodents and non-human primates (Dacey et al., 2005; Hatori & Panda, 2010; Schmidt et al., 2011) and could also mediate sensitivity to the presence of light. Recent data suggest that, in rodents and, possibly, in sighted humans, these ipRGCs projections contribute to melanopsin-based brightness detection (Brown et al., 2012), a phenomenon which could contribute to non-conscious light awareness in our visually blind participants. Our fMRI protocol did not reveal significant light-induced activity modulations in the superior colliculus or LGN, however.

Remarkably, deactivations within the medial prefrontal cortex and anterior cingulate, which are part of the brain default mode network (Buckner et al., 2008; Raichle et al., 2001), were prevented when blue light was administered. This observation indicates that, in our participants, light prevented the relative disengagement of the medial prefrontal cortex and anterior cingulate that was normally observed when initiating a block of 2 back in darkness. Even though the role of the default mode network is still under intense investigation, two main hypotheses stand out. One postulates that the network serves internal mentation while the other proposes that its role is to maintain minimal resource for monitoring of external environment when the brain is not engaged in an active process (Buckner et al., 2008). In the latter perspective, our results raise the intriguing possibility that, during the performance of a cognitive task, light would triggers the recruitment of supplemental areas in part through the maintenance of activity within the default mode network, so that the known impact of light on sustained attention (Chellappa et al., 2011a; Lockley et al., 2006; Zaidi et al., 2007) would also allow the maintenance of attentional resources to monitor the environment. This theory may underlie how performance during a PVT or other executive task is improved by light exposure (Cajochen et al., 2011; Chellappa et al., 2011b; Lockley et al., 2006; Zaidi et al., 2007).

The default mode network stands as the most robust brain functional network and its organization appears to be similar in blind and sighted individuals (Buckner et al., 2008; Burton et al., 2004). The functional significance of the impact of light in some of its areas remains to be established but the default mode network constitutes one of the fundamental organization mode of the brain (Buckner et al., 2008). To our knowledge, our results constitute the first indication that non-image-forming signaling, likely through ipRGCs, can rapidly affect basic cerebral organization, so that it could potentially participate in the regulation of numerous aspects of human brain function, in addition to the rapid recruitment of supplemental brain areas to perform an ongoing cognitive process.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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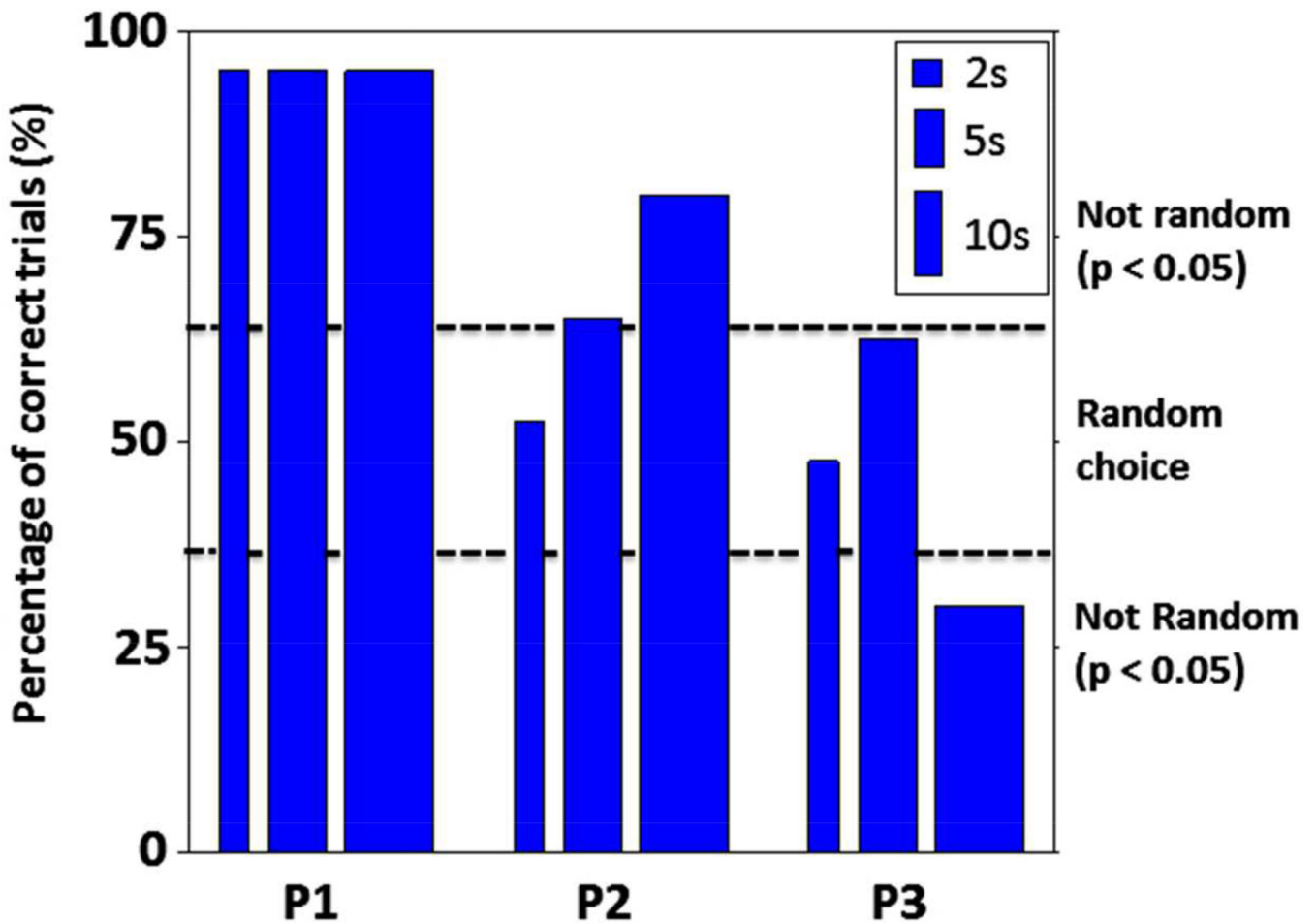


Figure 1. Results of the two-alternative forced choice (2AFC) task

Participants chose whether the light was on or off for 2, 5 or 10 s during the first or second half of a 4, 10 or 20 s trial, respectively. Each type of trial duration was recorded in a separate session which included 40 trials in total. The 4 and 10 s trial duration were tested on the same day while the 20 s trial duration was acquired the preceding or following day. Participant 1 had high accuracy (95%) irrespective of trial duration. Participant 2 exhibited a duration-dependent behavior with a linear increase in accuracy from 52.5% (2s), to 65% (5s) and 80% (10s). Participant 3 also showed a duration-dependent behavior with 47.5% (2s), 62.5% (5s) and 30% (10s) selection accuracies. The latter value indicates that participant 3 stated the incorrect option on 70% of occasions, which is a non-random behavior. *Dashed lines* indicate the limit between random and non-random selections ($p < 0.05$).

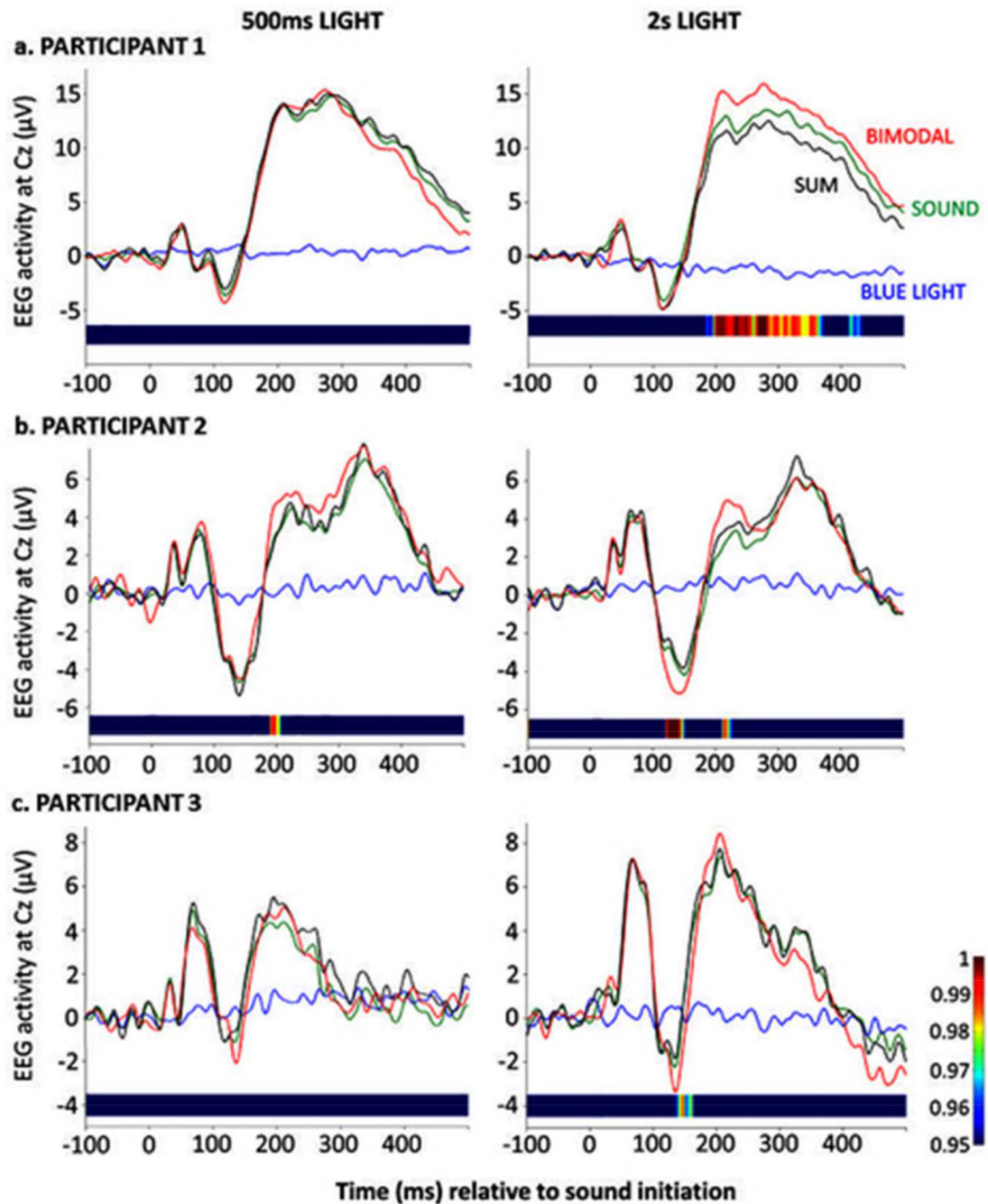


Figure 2. Auditory evoked potentials at the Cz location in the presence or absence of light EEG activity evoked at the Cz location (referred to both mastoids) by an auditory stimuli alone (green), by blue light exposure alone (blue), or light and sound simultaneously (bimodal, red). In order to isolate the modulatory effect of light on auditory evoked potentials, we compared the bimodal trials to the mathematical sum of both the auditory and light alone trials (black line). Horizontal bars correspond to results of the permutation statistics between the bimodal trials and sum of both trials alone (probability values as on the lateral vertical color bar). The difference is significant whenever the color is not dark

blue. In the 500ms condition (*left panels*), each auditory stimulus was preceded by 350ms of light and terminated simultaneously with light extinction, while in the 2s condition (*right panels*), each auditory stimulus was preceded by 1850ms of light and terminated simultaneously with light extinction. In the 2s condition, all three participants showed significant differences between the bimodal trials and the sum of both trials alone, while in the 500ms condition only participant 2 presented marginal (but significant) differences (see text for details).

See Supplemental Figure S2 for detailed behavioral results.

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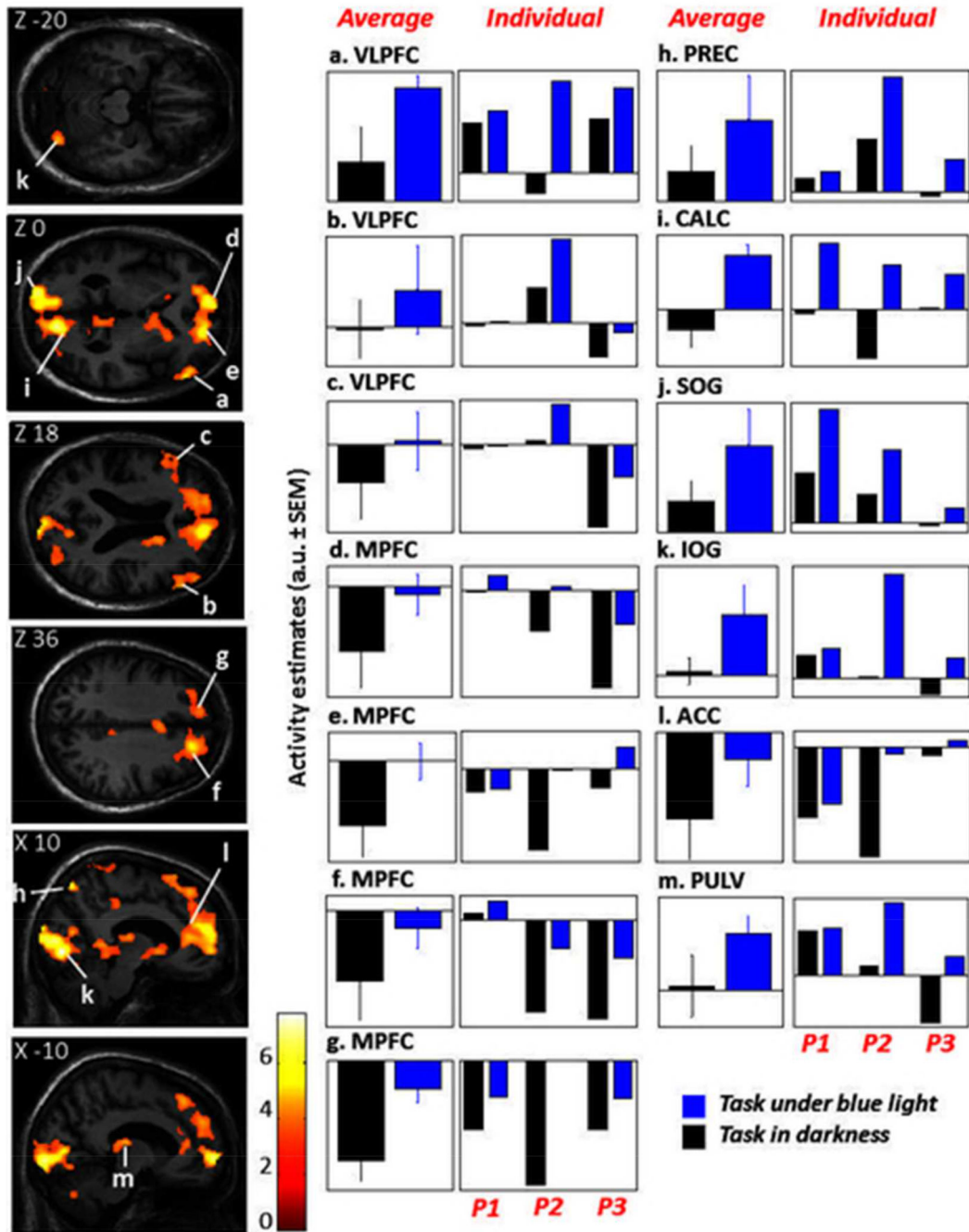


Figure 3. Differences in brain activity between blue light exposure and darkness while performing a 2-back task in fMRI

Brain areas showing significant increases in activity under blue light exposure while performing a 2-back task as compared to darkness.

Left panels. Statistical results ($p < 0.001$ uncorrected) overlaid over the mean structural image of the 3 participants. Vertical color bars (bottom left) refer to T-statistic results.

Right panels. Activity estimates (arbitrary unit – a.u. \pm SEM) in the regions showing significant differences between blue light and darkness episodes ($p < 0.05$ corrected).

Average activity estimates of the three participants (left) are plotted together with the individual activity estimates (right) to show the inter-individual variability of the effects. See Table 2 for names of the brain regions corresponding to the letters and abbreviations. See Supplemental Figure S3 and Supplemental Table S1 for detailed behavioral results, together with main brain responses to the task in darkness.

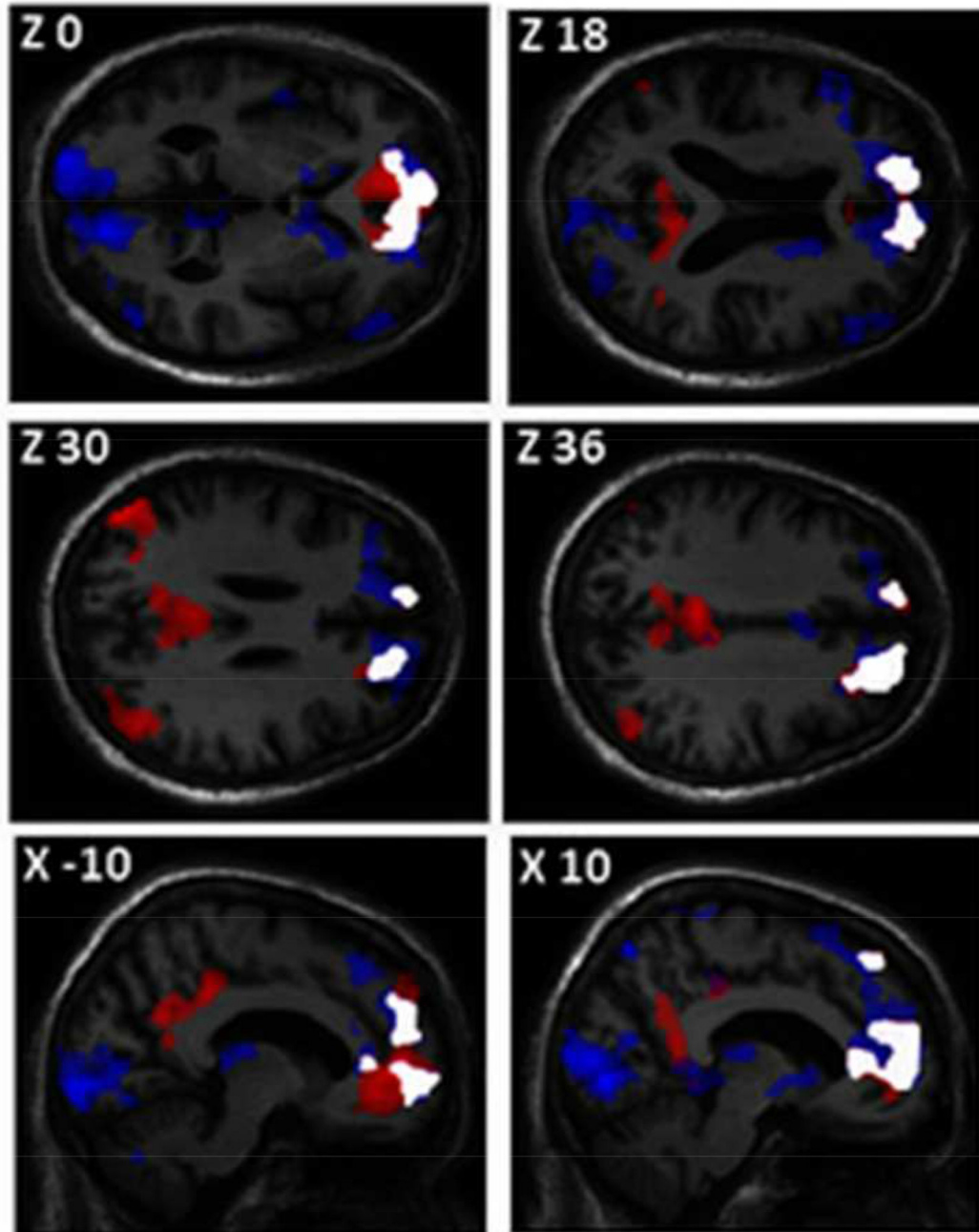


Figure 4. Blue light exposure prevents deactivation within medial prefrontal areas of the default mode network during the performance of an auditory 2 back task

Significant deactivations, as compared to baseline, in response 2-back task during darkness are displayed in **red**, while significant differences between blocks of task performed under blue light and in complete darkness are in **blue** (blue light > darkness -corresponding to results displayed in figure 3). Overlays are displayed in **white** and indicate that a significant relative increase in brain response induced by blue light is located in areas showing a significant deactivation in darkness.

See Supplemental Table S1 for a complete list of significant deactivations in responses to the task in darkness.

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Table 1

Participants characteristics

	Participant 1	Participant 2	Participant 3
Age	67	60	66
Sex	Female	Male	Male
Body Mass index (BMI)	27.3	30.0	27.4
Cause of blindness	Retinitis Pigmentosa	Retinitis Pigmentosa	Retinopathy of Prematurity
Years of total blindness	10	25	66
Chronotype (Horne & Ostberg, 1976)	62 - Moderate morning type	72 - Moderate morning type	68 - Moderate morning type
Anxiety (from 0 = lowest, to 63 = highest) (Beck et al., 1988)	5	0	2
Mood (from 0 = best, to 63 = worst) (Steer et al., 1997)	0	0	0
Sleep disturbances (from 0 = lowest, to 21 = highest) (Buysse et al., 1989)	3	10	4
Daytime propensity to fall asleep (Johns, 1991)	1	4	16
Laterality	Right handed	Right handed	Right handed
Time zone difference with Montreal	-1h	0h	-3h
Cigarette consumption	No	No	No
Medication	None	None	Low dose of blood pressure medication
Years of education	17	12	12
Alcohol consumption (units/week)	<1	<1	0
Caffeine consumption (cups/day)	2	5	2
Last confirmation of light – induced melatonin suppression	Yes, in 2008	Yes, in 2006	Yes, in 2002
Previous standard ERG examination (no detectable signal)	No	No	Yes, in 1994
Previous standard VEP examination (no detectable signal)	Yes, in 2008	Yes, in 2006	No
Pupil response to prolonged light exposure (> 5s)	No	Yes	No
Participated in a published study	No	Yes (Zaidi et al., 2007)	Yes (Klerman et al., 2002)

CHRONOTYPE was assessed by the Horne-Östberg Questionnaire (Horne & Ostberg, 1976); *ANXIETY LEVEL* was measured on the 21 item Beck Anxiety Inventory (Beck et al., 1988); *MOOD* was assessed using the 21 item Beck Depression Inventory II (Steer et al., 1997); *SLEEP DISTURBANCE* was determined by the Pittsburgh Sleep Quality Index Questionnaire (Buysse et al., 1989); *DAYTIME PROPENSITY TO FALL ASLEEP* during daytime non-stimulating situations was assessed by the Epworth Sleepiness Scale (Johns, 1991). *ERG*: electroretinogram; *VEP*: visually evoked EEG potential.

Table 2

Significant differences between brain responses to an auditory 2-back task performed under blue light exposure and in darkness.

	Side	X, Y, Z	Z	P _{corrected} Value*
Blue light > Darkness				
Ventrolateral prefrontal/frontopolar cortex	R	28 52 6	4.72	0.032
Ventrolateral prefrontal cortex (VLPFC) [a,b,c]	R #	58 38 0	5.32	0.002
	R	54 44 14	5.05	0.007
	L	-56 30 14	4.61	0.049
Medial prefrontal cortex (MPFC) [d,e,f,g]	L	-12 60 -2	6.88	< 0.001
	R	10 54 18	6.19	< 0.001
	L	-12 50 30	6.05	< 0.001
	R	18 40 36	5.22	0.003
	R	18 54 0	4.76	0.026
	R	16 38 50	4.74	0.029
	L	-18 56 16	4.71	0.033
Precuneus (PREC) [h]	R #	8 -74 58	6.59	< 0.001
Calcarine sulcus (CALC) [i]	R	10 -82 -4	7.73	< 0.001
Superior occipital gyrus (SOG) [j]	L #	-18 -98 -4	7.71	< 0.001
Inferior occipital gyrus (IOG) [k]	R #	32 -80 -22	5.03	< 0.008
Anterior cingulate cortex (ACC) [l]	R	10 38 10	4.78	0.024
Thalamus (pulvinar) (PULV) [m]	L	-10 -20 12	4.64	0.044
Darkness > Blue light				
No significant voxel				

Letters between [] correspond to letters of figure 3.

* p-values corrected for multiple comparisons over the entire brain volume (Family Wise Error approach).

#. in these areas, an additional analyses showed cluster size of the significant difference was slightly reduced after applying an exclusive mask (p=0.05 uncorrected for multiple comparisons) for the brain response to the task blocks performed in complete darkness. All other clusters remained unaffected after mask application, supporting that blue light induces the significant recruitment of additional brain regions.