The Effect Of Extraocular Light On Brain Activity

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Silmän ulkopuolelle kohdistetun valon on havaittu vaikuttavan lintujen, kalojen ja nisäkkäiden aivoihin. Tunnetuin vaikutus kohdistuu vuodenaikojen mukaiseen käyttäytymisen säätelyyn, mutta silmän ulkopuolisen valon on havaittu vaikuttavan myös muihin toimintoihin. Myös ihmisillä on todettu valoa aistivia soluja silmien ulkopuolella, mutta näiden vaikutusmekanismeista ei ole paljoa tutkimustietoa.

Tutkiaksemme, onko silmän ulkopuolisella valolla välittömiä vaikutuksia ihmisen aivojen sähköiseen toimintaan, teimme 18 terveellä koehenkilöllä sokkoutetun tutkimuksen, jossa korvakäytäviin vuoroin kohdistettiin silmän ulkopuolista valoa tai ei. Samalla mitattiin aivojen sähköistä toimintaa (EEG). Mittauksen aikana koehenkilöt suorittivat tarkkaavaisuustehtävää, joka sisälsi tunneärsykkeitä. Tilastollisessa analyysissä (toistomittaus ANOVA) saimme merkittävän interaktion tunneärsykkeen (neutraali vs. uhkaärsyke) ja silmän ulkopuolisen valon (päällä, pois päältä) välille. Tunnepitoinen ärsyke madalsi tarkkaavaisuuteen liittyvän herätevasteen, P300, amplitudia aivojen sentroparietaalisilla alueilla verrattuna neutraaliin ärsykkeeseen silloin kun silmän ulkopuolista valoa ei kohdennettu korvakäytävään. Kun koehenkilöiden korvakäytäviin kohdistettiin silmän ulkopuolista valoa, tunnepitoisella ärsykkeellä ei ollut vaikutusta P300 amplitudiin. Lisäksi osoitimme, että korvakäytävään kohdennettu valo läpäisee vainajan kallonpohjan.

Tutkimuksessamme havaitsimme, että korvakäytävään kohdennetulla valolla oli vaikutus ihmisen aivojen sähköiseen toimintaan ja erityisesti aivojen tarkkaavaisuusvasteiden tunnemodulointiin. Tämä silmän ulkopuolisen valon vaikutusmekanismi on toistaiseksi tuntematon ja lisätutkimuksia tarvitaan sekä tulosten vahvistamiseen että mahdollisten mekanismien selvittämiseen. Mahdolliset vaikutusmekanismit voisivat osin liittyä valoherkkiin proteiineihin, opsiineihin, joiden muita kuin näkemiseen liittyviä rooleja esitellään kirjallisuuskatsauksessa.

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1 Introduction

Light has an incredibly important role in many areas of physiology. It is arguably the most important type of stimulus, with which humans and animals alike sense their surroundings. The eye is the main organ responsible for perceiving light stimuli. Vision is the most prominent function of the eye. It is used to perceive and navigate the outside world, its dimensions and qualities. In addition to vision, light stimuli are utilized in multiple physiological systems, such as the vestibular system and the circadian rhythm. Most of these functions utilize the eye as their perceptive organ, but there are multiple light sensitive systems, which reside outside of the eye. These systems have been found to be present both in vertebrates and non-vertebrates.

Evidence of extraocular light sensitive systems in humans is sparse. This is mainly due to the difficulty and ethical problems of conducting research on human extraocular photoreception. There has, however, been multiple findings of novel light sensitive systems in other vertebrates in recent years. Surprising findings have also been made in human photoreception in the past years. These findings include but are not limited to the discovery of melanopsin-expressing intrinsically photoreceptive retinal ganglion cells (ipRGCs), which contribute to adjustment of the circadian rhythm (1).

We conducted a study using electrophysiological measurements and behavioural paradigms to determine if there is any immediate effect of extraretinal light stimulation to the brain physiology. Similar methods have also been used to detect the effect of the electrical neuromodulation, such as deep brain stimulation (2,3) and vagus nerve stimulation (4), on the human central nervous system. Using these techniques, we studied the immediate effect of administering extraocular light to the human brain. The subjects acted as their own controls in the experiment in a single-blinded study. As there is no conclusive evidence for photoreception in the human brain, the mechanism of the effect of the light is still speculative. There is, however, a multitude of findings of extraocular photoreception in other vertebrates and non-vertebrates, making the presence of extraocular photoreception in humans plausible.

In this literary review I go through some literary findings regarding photoreception outside of the eye.

2 Opsins

2.1 Opsins In General

All photoreceptors known to date are based on opsins, which are G-protein coupled receptor proteins. Their sensitivity to light is mediated by a vitamin A –based chromophore also known as retinal. (1) This photopigment can be converted from 11-*cis*-retinal to all-*trans*-retinal by a photon, which induces a phototransduction cascade (5). There are several different known types of opsins. They are found both in the eye and extraocularly. Human vision is traditionally thought to function based on rods and cones on the retina, but there are also other ocular opsins, which react to light stimulus. Intrinsically photosensitive retinal gangion cells (ipRGC) located in the retina affect the circadian rhythm instead of vision processing (6). All non-mammalian vertebrate brains have photosensitive functions in the pineal complex (1).

In a 2017 review by Leung & Montell, opsins were classified in three distinct types, ciliary opsins (c-opsins), rhabdomeric opsins (r-opsins) and RGR/G_o-opsins. This classification is based on the type of photoreceptor cells, which contains the photopigment. C-opsins and r-opsins are distantly related.

Traditionally c-opsins were associated with vertebrates and r-opsins with invertebrates, but there are some opsins, which contradict this association. Mammalian melanopsin (OPN4) for example is closely related to r-opsins, whereas c-opsins have been found in the mosquito (*Anopheles gambiae*) and the marine ragworm (*Platynereis dumerilii*).(5)

C-opsins are the more common type of opsins in vertebrates and among them are the human rod and cone opsins. They have stacked membranous discs whereas the r-opsins contain densely packed membranous microvilli. The effect of a light stimulus causes c-opsin to hyperpolarize and r-opsin to depolarize. In c-opsins, the activation of the chromophore dissociates it from the opsin protein and causes it to isomerize from 11-*cis*-retinal to all-*trans*-retinal, which in turn regenerates enzymatically to 11-*cis*-retinal in the retinal pigment epithelium. In r-opsins, the chromophore stays connected to the opsin protein and requires another photon to be absorbed for the all-*trans*-retinal to be converted back to 11-*cis*-retinal. (5,7)

In this section I will cover diverse findings and functions of individual types of opsins.

2.2 C-opsins

2.2.1 Visual rod/cone opsins (OPN1, OPN2)

The traditional photoreceptor opsins, which have been given the genetical identifiers OPN1 and OPN2, are expressed in the retina. Cone opsin (OPN1) are used in colour vision and rod opsins (OPN2) in night vision and peripheral vision. Different types of cone opsins are responsible for specific areas in the visible light spectrum, with wavelengths ranging from a wavelength maximum of 500-620 nm to a wavelength maximum of 355-435 nm. (1)

2.2.2 Encephalopsin (OPN3)

Blackshaw and Snyder (1999) were the first to identify a mammalian opsin that is primarily expressed in the brain, encephalopsin or OPN3. Transcranial illumination affects *OPN3* gene expression in mammalian brain. When light was administered transcranially on mice, there was a significant increase in the expression of *OPN3* in the brain compared to the control group (8).

OPN3 has a function in melanocytes in human skin. Blue light stimulation of human skin causes melanocytes to induce a long-lasting hyperpigmentation in skin types III-IV. OPN3 acts as the sensor for this process. (9)

OPN3 has also been found to be expressed in areas of the mouse brain associated with encephalic photoreception, the medial preoptic nucleus and the paraventricular nucleus of the hypothalamus. It is also expressed in areas, which are not associated with circardian photoreception, such as other hypothalamic areas, Purkinje cells and various neurons in the cerebral cortex. (10) In the rat brain, the pineal gland has also been shown to be photoreceptive during the neonatal period, but this effect disappears in adult rats (11).

2.2.3 VA opsin (VAL-opsin)

Vertebrate ancient long –opsins are expressed in the eyes and brains of birds, reptiles and fishes, where they have a role in the seasonal reproduction and gonadal development (12-25). VA-opsins have been demonstrated to have a role in chorion formation and embryonic hatching of zebrafish (14).

2.3 R-opsins

2.3.1 Melanopsin (OPN4)

Melanopsin is the main photopigment utilized in the ipRGCs for circadian rhythm photoentrainment in mice (6). The peak sensitivity of melanopsin is ca 480 nm, which is in the blue light spectrum. In gene knockout studies on mice, it has been found that blue light stimulation (470 nm) causes elevated corticosterone levels, arousal and prolongs sleep onset time (26). Melanopsin protein has been observed in the human brain outside of the retinohypothalamic tract (27), but there has been no link to physiological effects.

Melanopsin has also been found outside the central nervous system, for example in blood vessels. Sikka *et al.* have discovered that light stimulation on mice aorta causes vasorelaxation in the blue light spectrum at a wavelength of 430-460 nm. Intracellular membrane potential measurements have been used to determine that the effect is due to vascular hyperpolarization. In mice with *OPN4*$^{-/-}$ double knockout, this effect is absent. (28)

2.4 RGR/G_O-opsins

2.4.1 Neuropsin (OPN5)

In mammals, there is evidence of other functional photoreceptors in circadian regulation beside melanopsin, rods, and cones. The main circadian pacemaker, which synchronizes the physiological functions, is located in the suprachiasmatic nucleus (SCN), but tissues have circadian oscillators which can function independently but are synchronized by the SCN (29). In mice, only melanopsin, rods, and cones send information to the SCN (30), but the retina also has an independent circadian oscillator (31). This oscillator is paced by another photoreceptor, as it could function without rods, cones, and melanopsin both in vitro and in vivo (32). Interestingly, in a further study this necessary retinal photopigment was discovered to be neuropsin (OPN5), without which the mouse retina is not able to photoentrain either in vivo or ex vivo (33). In the same study, they showed that the absence of short wavelength cone opsin (OPN1SW) or encephalopsin (OPN3) has no effect on the retina's ability to photoentrain. When in turn OPN5 is absent; rods, cones, and melanopsin are not sufficient for photoentrainment with the missing OPN5 causing deteriorated vision. In addition to the retina, OPN5-mediated circadian photoentrainment has been also discovered in the mouse cornea (33).

2.5 The effect of opsins on physiology

Opsins have been found to have many different effects on physiology. The classical role of opsins is in the physiology of vision, but they are used in non-image-forming tasks in the eye and they have both light-dependent and light-independent extraocular roles. In addition to the previously mentioned ipRGCs, mouse and avian sphincter muscle cells of the iris (34,35) and mice retinal pigment epithelium cells (36) have been suggested to be intrinsically photoreceptive. The intrinsic light sensitivity of the mouse iris has since been debated to also be affected by ipRGCs (37). Light-dependent functions outside of the central nervous system include the OPN3 and OPN4-mediated photorelaxation of blood vessels of mice (28,38) and the modulation of skin color and pigmentation in fish (39-42).

Opsins also have light-independent functions. In *Drosophila* larvae, Rh1 opsin, a type of r-opsin, has a role in temperature discrimination. When given the choice, wild-type larvae would choose their ideal ambient temperature of 18°C from a range of comfortable temperatures, but when the gene encoding Rh1 opsin was mutated, the larvae were not able to distinguish between the comfortable temperatures. Interestingly, other *Drosophila* opsins (with the exception of Rh3) and mammalian melanopsin (OPN4) were able to function as a replacement for Rh1 opsin when expressed in its place. This thermotaxis can take place in the dark as light has no effect on it, making it light-independent. (43) Additionally, the *Drosophila* opsins Rh5 and Rh6 have been shown to have a significant role in mechanotransduction in the *Drosophila* auditory organ also independent from light (44).

The physiological effects opsins have been proven to be difficult to research, resulting in a difficulty to determine their meaningfulness on a macro-scale. There is little to no evidence of extraocular light affecting the human nervous system, even though there are commercially available products, which are marketed by such effects. The finding by Regazzetti *et al.* (2017) that encephalopsin (OPN3) is not only found in the human skin, but also has a direct effect on melanocytes is promising for future findings of new physiological effects of opsins.

3 Effect of extraocular light

The research of the effect of light for non-visual pathways has long roots. The first evidence of an extraocular effect of visual light was in 1911, when Karl von Frisch discovered that European minnow fish (*Phoxinus Phoxinus)* change their skin colour according to changes in light even after being blinded and pinealectomized. Since then there has been many discoveries in non-visual photoreception, some of which have been already discussed above.

Visible light has been shown to affect the brains of mammals. In a rat brain, applying light on the removed cortical tissue in amounts that could penetrate the rat skull increased release of γ-aminobutyric acid (GABA) (45). The mechanism for this effect remained unclear.

Extraocular light has also been found to have an effect on human sleeping patterns when administered in the extremities. When light of the blue-green spectrum was administered in the popliteal area of the legs of human test subjects for 3-hour periods, it was found to reduce the length of REM/non-REM sleep cycle. The overall duration of REM sleep remained unaltered. It was suggested that this effect was created by the smooth muscle tissue, vascular system or peripheral nerves being directly stimulated by light, but there is no conclusive evidence for this. (46)

The direct effect of extraocular light administered to the ear canal on the salival melatonin levels and performance on a psychomotor vigilance task has also been studied. Human test subjects have been subjected to ocular light and extraocular light has been administered through the ear canal in the evening. Salival melatonin levels and reaction times were lowered after ocular light exposure. With light administered through the ear canals, these measurements were similar to the control group. (47)

In our study we found, that delivering extraocular light to the ear canal diminishes the difference between centro-parietal P300 amplitudes measured during emotional and neutral distractors. The mechanism of this effect is yet unknown, as there is no previous evidence of extraocular light affecting emotion-attention interaction, calling for future studies to confirm these results. Our research paradigm did not control for possible heat transmitted by the light stimulating device to the ear canal, which introduces a potential confound that needs to be controlled for in future studies. It is theoretically also plausible that the effects observed in our study would be caused by some light reflected to the back of the eye from the base of the skull via cerebrospinal fluid transmitted by the visual pathways, possibly activating retinal cells sensitive to even low levels of light. While these are only theoretical speculations, they could, for example, be addressed with the measurement of retinal potentials and the impact on them by extraocular light delivered via ear canals.

4 Conclusion

As this literary review shows, light has many different effects on the physiology of vertebrates and non-vertebrates alike. For this reason the notion, that light may have yet unknown physiological effects is plausible.

Opsins have a significant role outside of traditional vision-related processing. Surprisingly, even though opsins are strongly associated with light processing they have completely light-independent functions as well. This could be explained by opsins being a more primitive sensory receptor which have since developed to be used mainly in vision-related processing (5). Because of this, it is credible, that opsins could be part of many yet unknown sensory systems or remnants of such systems.

Many of the findings in this literary review are quite recent, suggesting that there will be more significant findings of the physiological effects of light in the near future. Future studies are needed to confirm the results of the current study and if confirmed, to assess potential direct and indirect ways transcranial light might influence brain physiology.

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APPENDIX 1. Original Research Article

Human brain reacts to transcranial extraocular light

Short title: Extraocular photosensitivity of human brain

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Abstract

Transcranial extraocular light affects the brains of birds and modulates their seasonal changes in physiology and behavior. However, whether the human brain is sensitive to extraocular light is unknown. To test whether extraocular light has any effect on human brain functioning, we measured brain electrophysiology of 18 young healthy subjects using event-related potentials while they performed a visual attention task embedded with emotional distractors. Extraocular light delivered via ear canals abolished normal emotional modulation of attention related brain responses. With no extraocular light delivered, emotional distractors reduced centro-parietal P300 amplitude compared to neutral distractors. This phenomenon disappeared with extraocular light delivery. Extraocular light delivered through the ear canals was shown to penetrate at the base of the scull of a cadaver. Thus, we have shown that extraocular light impacts human brain functioning calling for further research on the mechanisms of action of light on the human brain.

Introduction

Ambient light guides behavior not only through the traditional visual pathway, but also by regulating numerous nonvisual physiological functions, including circadian, neuroendocrine, neurobehavioral responses and mood [1, 2]. While the main route for light to impact the brain is via photoreceptors on the retina, some birds are known to have deep brain photoreceptors in the hypothalamic and septal regions that react to transcranial extraocular light. These deep brain photoreceptors modulate seasonal changes in physiology and behavior of birds, such as seasonal breading [3, 4]. The effect of extraocular light on reproduction is most commonly studied on birds with eyes and pineal gland surgically removed to avoid ocular light interference. Long-term exposure to light is also needed for measurable changes in physiology [3-5]. Study of extraocular photosensitive proteins led to the discovery of deep brain photoreceptors in birds, e.g. Opsin 5, which detects blue and ultraviolet light and modulates seasonal breeding [3]. However, whether the human brain is sensitive to extraocular light is unknown and a similar approach of studying extraocular photosensitivity of birds is not applicable for humans.

In animals it has been shown that the skull is not completely impenetrable to light [4, 6] and that the measurable density of skull-penetrated light was found to affect neural metabolism. For example, transcranial bright light has been reported to enhance potassium-induced release of γ-aminobutyric acid (GABA) in cortical neurons of rats [7]. Penetration of light via the skull is also supported in a study showing that covering the head of blind ducks diminished photoperiodicity, where normal testicular growth in response to long daylight stimuli was abolished due to preventing the cranium from exposure to light [8]. Thus, although effect of the extraocular light is subtle it might be important in regulating physiological responses.

Human neuroimaging studies report that exposure to ocular blue light modulates brain activity, higher cognitive functions and emotional brain responses to auditory tasks in visually blind individuals [9-11]. This effect is believed to occur via a novel class of photosensitive retinal ganglion cells distinct from the rods and cones called intrinsically photosensitive retinal ganglion cells (ipRGCs) expressing the photopigment melanopsin that is maximally sensitive to short wavelength blue light (~480 nm) [12]. While amphibians, fish, reptiles and birds have been reported to possess deep brain photoreceptors that mediate behavior

[13, 14], it is thought mammals lack a similar photosensitive receptor and the evidence for a homologous receptor in mammals remains circumstantial [15-17]. Further, it is neither known whether the comparatively thick human skull bone is penetrable by visible light.

In order to study whether there is any effect of extraocular light on human brain function, we investigated the effect of extraocular light on brain's electrophysiology using event-related potentials (ERPs) and on behavior. ERPs are well suited for studying potential effects of extraocular light on brain physiology with vastly studied components such as the P300 sensitive to attentional and cognitive processes as well as biological and environmental factors [18]. With centro-parietal distribution of the classical P300 or P300b we chose P300 amplitude in the centro-parietal region of interest as a measure for the possible effect of extraocular light. In the present study, transcranial extraocular light was delivered via the ear canals and subject EEG was recorded while they performed a computer based Go/NoGo visual attention task embedded with emotional distractors, i.e. the Executive - Reaction Time (RT) test. Combined with EEG the Executive - RT test allows the study of subtle alterations in attention related brain responses and how they are modulated by emotional stimuli [19-21]. We investigated whether the transcranial extraocular light had any effect on centro-parietal P300 amplitude evoked in the Executive-RT test. Hypothesizing that extraocular light has an effect on brain physiology we expected this effect would be reflected in P300 amplitude and/or performance of the task. We further investigated whether the ear-canal-delivered light could penetrate the base of a skull in a human cadaver.

Methods

Subjects. Eighteen young healthy subjects (mean age = 25 y, sd = 6y, 3 male and 15 female) provided their written consent and voluntarily participated in the study according to the guidelines set forth in the Declaration of Helsinki governing the treatment of human subjects. The study was approved by the Regional Ethical Committee of Tampere University Hospital, Tampere, Finland and the permission number is R12237.

Extraocular light delivery. Extraocular light was delivered using a commercial Bright Light Ear Headset (NPT1100, Valkee Oy, Oulu, Finland; Fig 1a). UV-free and blue-enriched LED light with maximum of 3.5 Lumens was presented via both ear canals. The light has a photon density of 1.13×10^{16} photons $\cdot$ $cm^{-2} \cdot s^{-1}$ with a peak in the blue region around 448 nm. Detailed information of the LED light can be found in the previous study by Jurvelin et al. [22].

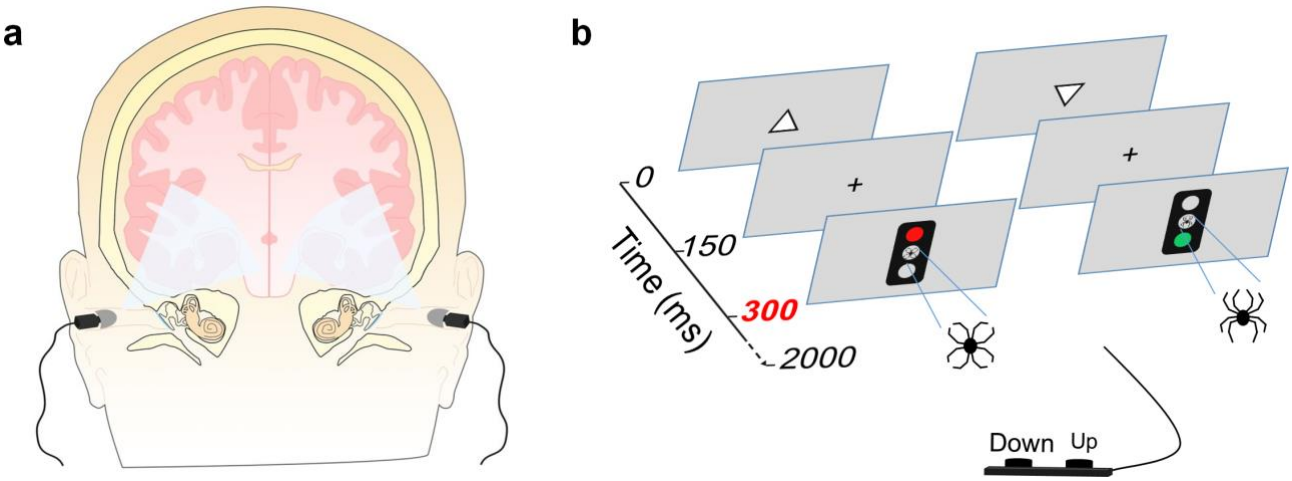

Fig 1. Experimental design.

The Behavioral Test. The behavioral test was conducted in a sound-attenuated room under soft white ceiling light. During the behavioral test, i.e. the Executive-RT test (Fig 1b), subjects sat one meter in front of a 21" computer screen. Presentation software (Neurobehavioral System, Inc.) was used to present the visual stimuli and collect the behavioral data. The first visual stimulus is a triangle (pointing either up or down) lasting 150 ms in the center of the screen. Thereafter, there was a 150 ms delay with a fixation cross in the center of the screen before onset of the Go/NoGo signal, i.e. the traffic light. The traffic light was presented for 150 ms leaving approximately 1550 ms for response before the next trial. Each trial lasts for 2000 milliseconds, with each block consisting of 64 trials and a total of 16 blocks. The response rules was switched between each block, i.e. if a green traffic light was the Go signal in the first block, then a red traffic light was the Go signal for the following block. Presented in the middle of the traffic light was a distractor, a schematic drawing in the shape of either

a spider (emotional, threatening) or a flower (neutral, non-threatening) [23, 24]. The order of the Go/NoGo signals and the emotional/neutral distractors were randomized.

Prior to testing, the Bright Light headset ear plugs were placed into the ear canals of the subject. When the headset was ON, extraocular light was delivered and when it was OFF, no light was delivered. Light was either ON or OFF for approximately six minutes, thereby allowing subjects to finish two blocks of behavioral tests; thereafter the light status alternated. Eight blocks were completed with ON status and the remaining half of the test completed with OFF status. Subjects did not know whether the ear-canal light delivery was on or off.

EEG recordings and data processing. Continuous electroencephalography (EEG) was recorded using a 64-channel actiCAP Ag/AgCl electrodes (Gilching, Germany) and digitized at 500Hz. Impedance for all electrodes was kept below 5 kΩ. Common reference was used during recording and in offline EEG data analysis. The offline EEG signal was processed using BrianVision Analyzer 2 (Brain Products, Gilching, Germany) software for event-related potential (ERPs) study. The signal was down sampled to 250Hz. Ocular movement correction was performed using "ICA ocular correction" function of Brain Analyzer 2, where EEG was decomposed into independent components using extended Informax algorithm and components (typically one or two) corresponding to artifact due to ocular movement were rejected. Following this, a band-pass filter (0.01-30 Hz) was applied. After filtering the EEG was then segmented into 2200 ms segments with 200 ms baseline before onset of the trial. The segments were baseline-corrected and processed for further artifact rejection, where segments with EEG amplitude higher than ±70 μV were rejected. ERPs were yielded by averaging the remaining segments for each condition. There were eight condition combinations composed of two types of emotional distractors, two extraocular light statuses and two response types.

ERP amplitude of the centro-parietal brain region of interest (covering electrodes C1, Cz, C2, CP1, CPz, CP2, P1, Pz and P2) was analyzed. In the ERP time window analysis, average amplitude of each 100-ms ERP window was exported for analysis. In P300 peak analysis, P300 peak was detected as the biggest positive

peak between 300 ms and 550 ms after onset of the traffic light (i.e. the Go/NoGo signal), corresponding to 600-850 ms on the ERP real-time window. Detected P300 peaks were visually inspected. The P300 amplitude was an average amplitude of 20 ms around the detected peak.

Statistical methods. Repeated-measure-analysis of variance (ANOVA) was used for analyzing ERPs and for reaction times in the behavioral test. Analysis of ERPs, including both analysis of both P300 amplitude and ERP time window analysis, was done using Extraocular light (ON vs. OFF), Emotion (neutral vs. emotional) and Response type (Go vs. NoGo) as factors.

In the reaction time analysis, we only involved trials of correct button press with reaction time longer than 150 ms. Analysis of reaction times was done using Extraocular light and Emotion as factors.

Logistic regression analysis was used for analyzing behavioral error types. Two categories of trials (Go/NoGo) generated three types of errors: incorrect button press (i.e. incorrect report of triangle orientation in Go trial), miss (i.e. no button press in Go trial) and commission errors (i.e. any button press in NoGo trial). Separate binary logistic regression models were generated for each error type. For each error type, trials were dichotomized into either “error” (e.g. incorrect button press in Go trials) or “other” (other outcome of Go trails, i.e. miss or correct response). Subject, Extraocular light, Emotion and interaction between Emotion and Extraocular light were used as predictors.

To account for multiple comparisons, the significance criteria was Bonferroni-adjusted to 0.006. All statistical analysis was performed in using R (version 3.1.3) with ez-package (version 4.2-2) [25].

Skull penetrability to light. We included an autopsy case with the base of skull photographed in order to determine the penetrability of light. This case belongs to the Tampere Sudden Death Study (TSDS), where people died out-of-hospital and underwent medicolegal autopsy at the Department of Forensic Medicine, School of Medicine, University of Tampere. The study protocol was approved by the Regional Ethical Committee of Tampere University Hospital with permission number R09097. In Finland when a study has ethical committee approval and an autopsy is routinely done as is the case with sudden death, no next of kin consent and no previous permission from the diseased subject or their relatives is required, similarly as previous studies [26-29].

Results

Analysis of centro-parietal P300. Analysis of centro-parietal P300 amplitude revealed a significant interaction between Extraocular light and Emotion, F (1, 17) = 25.48, p = .0001. Post hoc analysis revealed the main effect of Emotion existed only in situations with no extraocular light delivered, F (1, 17) = 10.83, p = .004. In contrast, when extraocular light was delivered, no effect of Emotion on P300 amplitude was found, F (1, 17) = 2.48, p = .13 (Fig 2).

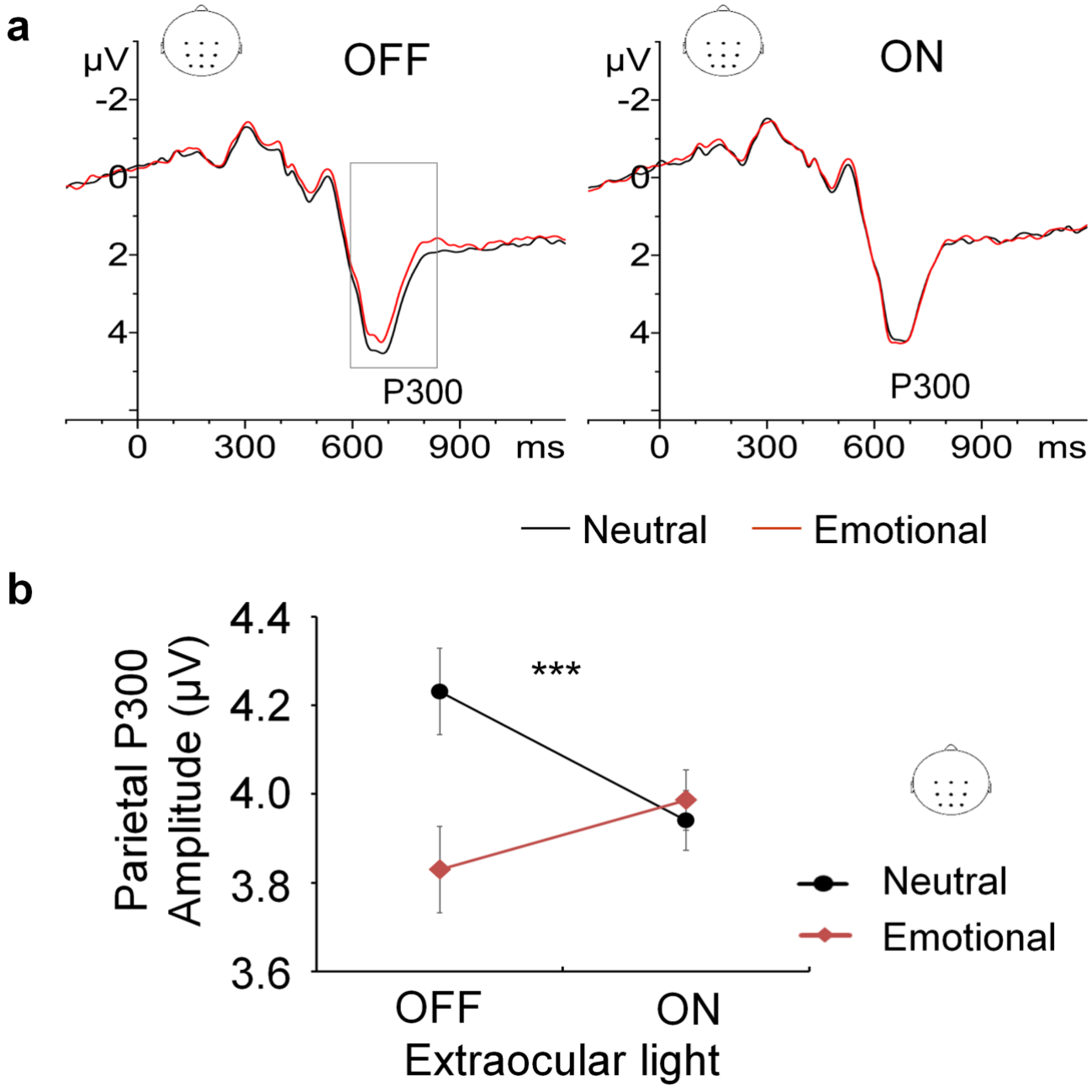

Fig 2. The effect of extraocular light on emotional modulation of P300 amplitude

ERP time window analysis. ERP time window analysis with 100-ms windows was also performed for the brain centro-parietal region, S1 Table. Analysis of ERP time windows revealed an interaction effect between Emotion and Extraocular light within two consecutive time windows 600-700 ms ($F(1, 17) = 13.22$, $p = .002$) and 700-800 ms ($F(1, 17) = 12.86$, $p = .002$). Post hoc analysis resulted in a main effect of Emotion when Extraocular light was OFF, but not when it was ON (Fig 3). These two consecutive time windows correspond to the latency of P300.

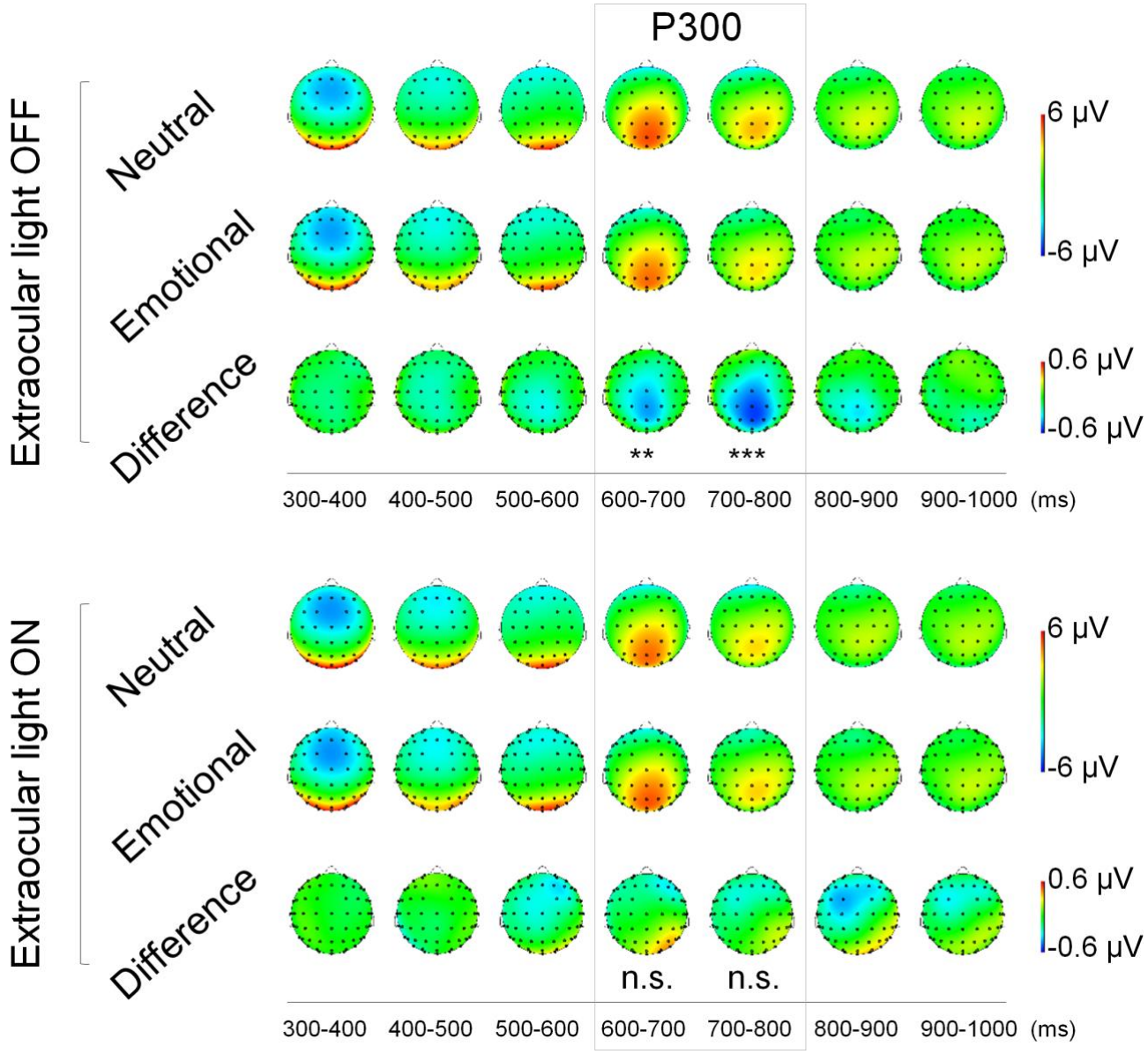

Fig 3. Extraocular light abolished the normal emotional modulation of attention related ERPs at 600-800 ms (corresponding to the 300-500 ms after Go/NoGo signal).

Behavioral analysis. In the behavioral data analysis, no effects were found due to delivery of extraocular light, S2 Table.

Transcranial light penetration via human ear canals. Possible penetration of light through the ear canals was investigated on a human cadaver after the brain was removed upon autopsy. Light penetration of the skull was visible when viewed both in lighted (Fig 4a) and dark (Fig 4b) conditions. Light was able to reach intracranial space through the ear canals and was visible at the base of the skull under the temporal lobes.

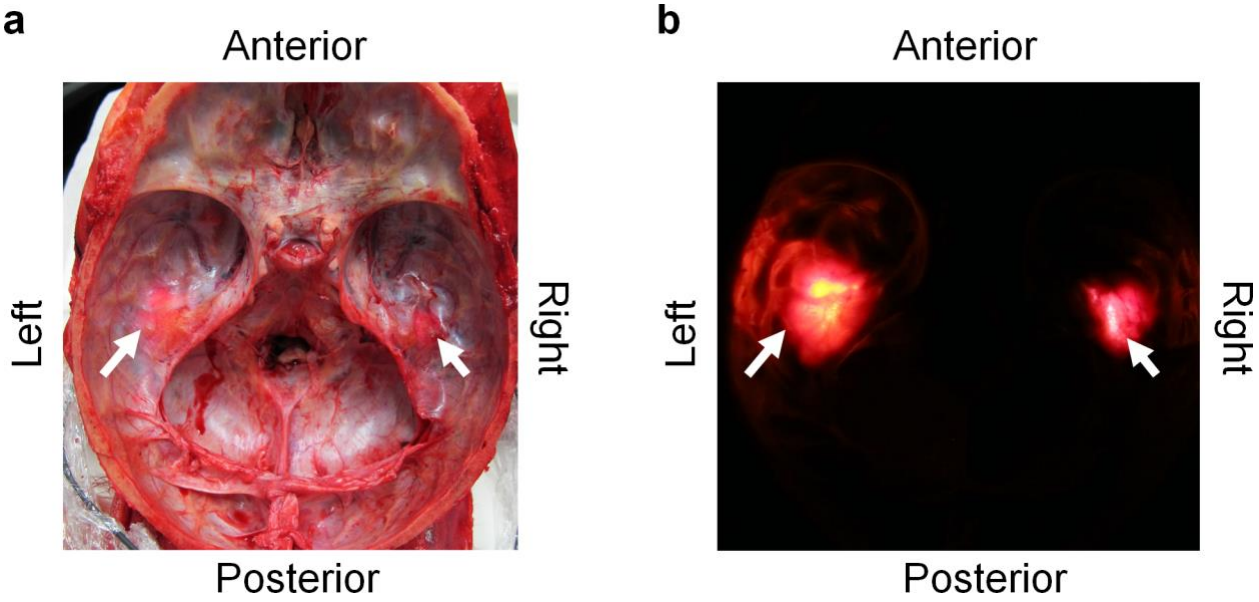

Fig 4. Light is able to penetrate human skull via ear canals.

Discussion

The present study demonstrates that extraocular light affects human brain functioning. Extraocular light modulated attention-related brain responses, specifically related to emotion-attention interaction. This light abolished emotional modulation of centro-parietal P300 brain response suggesting the extraocular photosensitivity of the brain. The light was able to penetrate the base of the skull and reach the brain's temporal lobes, as demonstrated in a human cadaver (Fig 4). Uncovering the subtle effect of extraocular light leads to new insight of human brain functioning.

We confirmed in our investigation of a human cadaver skull that light is capable of penetrating the human skull via ear canals and reach the temporal lobe of the brain. Because the brain is floating in cerebrospinal fluid, transmitted light might be widely dispersed, thus illuminating the basal surface of temporal lobe. The mechanism by which extraocular light may affect human brain functioning is unclear.

There might be several mechanisms of action of light on brain function depending on species, cell type and the physical properties of light. Deep brain photosensitive molecules OPN3 and OPN5 have been shown in mice and birds acting as signal transmitters for light [3, 30, 31]. Visible light has been reported to enhance potassium induced release of the neurotransmitter GABA of cortical neurons of rats [7]. On the other hand, near-infrared (NIR) light, found to increase adenosine triphosphate production in mitochondria, modulate reactive oxygen species and induce cellular transcription factors [32, 33]. NIR also penetrates human skull [34] and is used to study human brain hemodynamics (NIR spectroscopy) [35]. Furthermore, transcranial NIR has been studied as a potential therapy to treat mild traumatic brain injuries [36, 37].

Event-related potentials provide online information of brain functioning even with no behavioral changes [38-40]. In our study, ERP analysis revealed that extraocular light abolished the modulatory effect of emotion typically found on P300 amplitude [41-44]. This finding is consistent with previous EEG and fMRI studies showing changes in brain activity with ocular blue light exposure of less than a minute in visually blind subjects performing an auditory task [9]. Meanwhile, exposure to extraocular light delivered via the auditory canal did not alter performance on an Executive-RT task engaging several executive functions with threat-related and emotionally neutral distractors. The lack of behavioral signs might be due to extraocular light effects being subtle and the insensitive nature of behavioral measures. This is also in line with a previous study by Bromundt et al using light delivered via the auditory canal that found no evidence of performance improvement on a 10-minute psychomotor vigilance task [16]. Nevertheless, brain neuroimaging findings of these subtle effects, both due to extraocular and ocular light exposure, are likely to be demonstrated only when subjects are actively engaged in a cognitive task as was the case in our study and the previous ones [9, 10].

Beyond the apparent consistency with previous findings [9, 10, 16], uncovering the extraocular pathway of light in the human brain is revolutionary. With extraocular bright light delivery via both ear canals, centro-parietal P300 responds differently toward emotional distractors, indicating that the human brain reacts to extraocular light. The centro-parietal P300 has been associated with attentional resource allocation [45], with emotional stimuli able to capture attentional resources [19, 23, 46] and modulate centro-parietal P300 amplitude [41-43]. The emotional modulation of centro-parietal P300 amplitude due to emotional distractors disappeared during extra-ocular light delivery. Thus, extraocular light modulated emotion-attention interaction.

The current study has demonstrated that extraocular light has immediate effects on brain potentials of healthy subjects. Uncovering that brain functions may be modulated by extraocular bright light has broad implications for future research on brain physiology. Furthermore, our findings might also promote investigation on potential clinical applications. Whether chronic bright light delivery via the ear canals bears clinically applicable benefits is beyond the scope of this study. Transcranial bright light treatment has been previously reported by Jurvelin et al to relieve depressive symptoms associated with seasonal affective disorder [47]. While the study by Jurvelin et al lacked an adequate placebo control group and there was no dosage effect, the results suggested that transcranial light might impact mood. The current findings showing altered emotion-attention interaction due to transcranial light is consistent with potential effect of bright light on mood.

In conclusion, we have found that extraocular light impacts human brain physiology. Whether similar photosensitive brain receptors exist in the human brain as in birds is still unclear. The results from this study call for future research on the mechanism of action of light on the human brain. Demonstrating how extraocular light influences emotional reactivity might provide additional insights regarding how light directly affects mood [2]. The subtle effect of extraocular light might be critical for healthy human brain functions and disease. Therefore, the results from this study have potential widespread impact on understanding the effect of light on the healthy brain as well as its potential involvement in brain disorders.

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Supporting Information

S1 Table. List of p values for the analysis of ERP time windows.

S2 Table. Analysis of error types did not reveal significant predictors.

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Figure legends

Fig 1. Experimental design. (A) Theoretical penetration of light via ear canals. (B) Schematic presentation of the Executive-RT test. In case of a Go trial subjects were required to report the orientation of a previously presented triangle pointing either up or down with a corresponding button press. In NoGo trials subjects were required to withhold from responding. The Go/NoGo signal was a green or a red traffic light embedded with an emotional (i.e. a line-drawing of a spider) or neutral distractor.

Fig 2. The effect of extraocular light on emotional modulation of P300 amplitude. (A) Grand-average ERP of the centro-parietal region. When extraocular light was OFF, emotional distractors diminished P300 amplitude compared to neutral distractors. When extraocular light was ON the valence of the distractor had no effect on the P300 amplitude. (B) Extraocular light abolished the normal emotional modulation of centro-parietal P300 amplitude. This effect was highly significant ($p = .0001$). Error bars: Fisher's least significant difference.

Fig 3. Extraocular light abolished the normal emotional modulation of attention related ERPs at 600-800 ms (corresponding to the 300-500 ms after Go/NoGo signal). When extraocular light was OFF (upper panel), the valence of the distractor had an effect on the ERP waveforms with Difference waveform (Emotional-Neutral) leading to centro-parietal negativity at 600-800ms. In contrast when extraocular light was ON (lower panel) the valence of the distractor did not have a significant effect on ERPs. The main effect of Emotion is marked; n.s. = no significance.

Fig 4. Light is able to penetrate human skull via ear canals. (A) Light penetration through the ear canals at the base of the scull on a cadaver after inserting the Bright Light Ear Headset into both ear canals under normal surgical lights in the autopsy room and (B) same skull base after turning the surgical lights off and darkening the room.