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TITLE

Neurobehavioural Effects of Monochromatic Light Exposure

Candidate

Dr. Antonio Costanzo

Supervisor

Prof. Giuseppe Barbato

Coordinator

Prof.ssa Maura Striano

Che io possa avere la forza

di cambiare le cose che posso cambiare,

che io possa avere la pazienza

di accettare le cose che non posso cambiare,

che io possa avere soprattutto l'intelligenza

di saperle distinguere.

A Gennaro

Michela

Giuseppe

Giovanna

La mia Famiglia.

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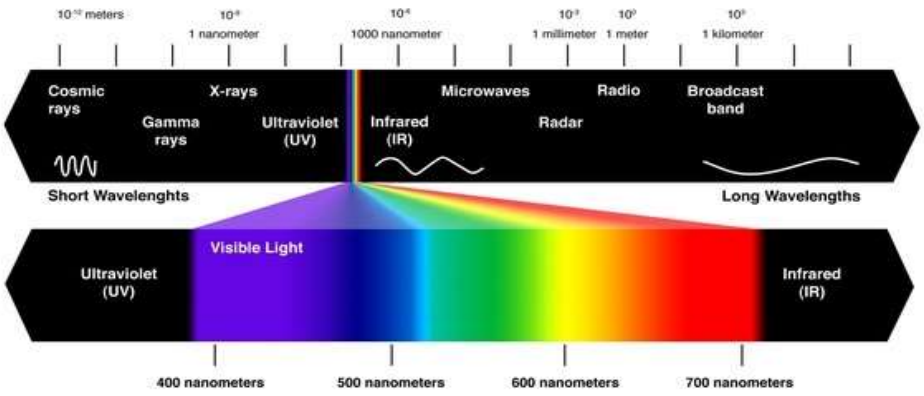
Chapter 1: The light, The Eye, The Brain

1.1 The Light

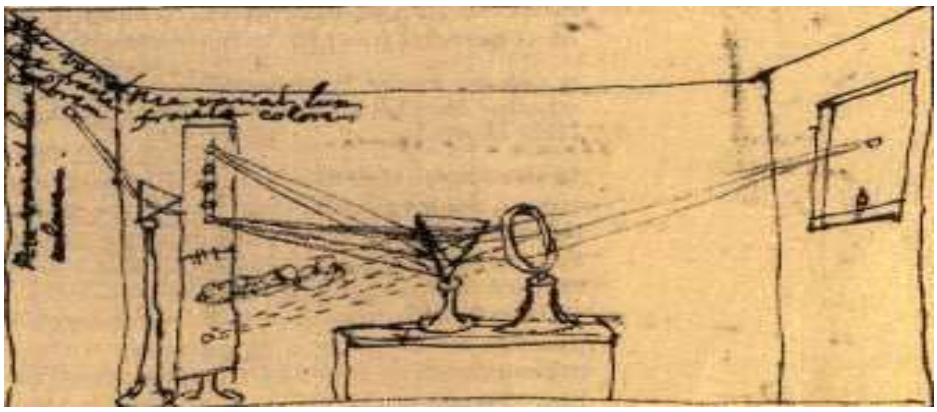
The electromagnetic radiation is oscillatory in its nature and, from a quantum mechanics perspective, a corpuscular nature can be also ascribable (Phillips, 1980). Such radiation has different properties and uses, depending on the emitting body (source), the distance covered by two crests of a wave (wavelength), the number of complete oscillation per time unit (frequency), their propagation media (vacuum, gasses etc.) and the surface hit by the radiation (receiver - Bagatti et al., 2010). The known entire frequency range of electromagnetic radiation (Spectrum) is comprised between few oscillations up to near three million of cycles per second. Adopted metering for oscillation is the Hertz (Hz), named after the German physicist (Heinrich Rudolph Hertz – 1857 -1894), that measured and modulated electromagnetic waves (Hertz, 1888) starting from James Clerk Maxwell (1831-1879) work published in 1873 The length of the wave is determined by the ratio between frequency of the wave and the constant propagation speed (Jenkins and White, 1957; Born and Wolf, 1999). In this way, every frequency can define its relative wavelength (Lambda – λ). Longer waves of slow frequency, (Radio

Waves of and Micro Waves) are widely used in communication technology such as transmission over radio and mobile phones. It is also well known that they have extremely dangerous effects on a living being if exposed to high frequencies and power of the radiation (Palladino, 2002). Wavelengths shorter than 100 nanometres (nm – 1 nm = 1e-9 meters) are defined as ionizing waves and includes ultraviolet rays, x-rays, gamma rays, and cosmic rays. Although these phenomena can be only measured by dedicated equipment, there is a narrow band of the spectrum presenting peculiar characteristics**.** Indeed, electromagnetic waves having a wavelength comprised between 760 nm and 380 nm are not far different from shorter and longer electromagnetic oscillation (Fig. 1.1). Within this narrow band, different wavelengths correspond to a colour. That is, if a radiation from an emitting body has a wavelength of 380 nm we observe a so called pure spectral colour, that is Violet in this case.



1.1: Graphical representation of the electromagnetic spectrum.

What is now easy to describe as Light, adopting basic physics definitions, has been indeed a complex topic that challenged scientists toward having great intuitions and observations across centuries. In one of his first *experimentum crucis* reported to the Royal Society (1666) Isaac Newton described a systematic observation of light behaviour (de Andrade Martins et al., 2001). A small ray of sun light, passing through a tiny hole in his window blind, hit a triangular glass prism and the result was that light was projected on a wall completely decomposed, showing different colours of the spectrum (Fig. 1.2).



1.2: Newton's Experiment on light decomposition in one of his drafts.

Bright light was then obtained converging decomposed rays into a single beam, where all of the single waves were evenly balanced. Despite his numerous experiments to discover nature of light, he proposed a corpuscular theorization of light that left most of the light phenomena unexplained. In 1690 a definitive scientific basis for modern light theory

was proposed by Christiaan Huygens in his work *Traité de la lumière* that paralleled Newton intuitions and experiments (Principles of Optics). Scientific revolution of late XVII century unveiled most of the characteristics of light. But light is also an important element of the history of men as a meaningful religious symbol in many cultures as well. Powerful Greek God, Apollo, was linked to sun and light, protecting arts and brightening intellect (Carassiti, 1996). Many other ancient cultures conferred to light the characteristic of bearing and sustain life of men and all the creatures. In this dualistic conception, light is contrasting darkness that instead belongs to death and obscure forces (Hornung, 1999).

1.2 The Eye

As mentioned above, just a small part of the electromagnetic spectrum is visible. This means that there is a body that receives the radiation can react with a specific pattern of events leading to superior organization of the light stimulation in a perceptive experience. Humans and many mammals possess a complex sensory organ that serve on this purpose (Purves et al., 2009). Eyeballs are natural optic devices. They are situated in bone sockets in the frontal part of the cranium. The most external layer of each eyeball (Sclera) is attached to 6 Extraocular muscles fibres that have the role of keeping the eyeball in place and to provide the eye with motion (Carlson, 2014). In the anterior region of the eye, the sclera becomes transparent and optically free thus allowing Light to penetrate the internal structure of the eyeball. In the anterior middle layer, light encounters the Uveal tract that consists of the Choroid, Ciliary body and the Iris. This latter structure has the important function of determining the amount of light that can penetrate the eye, regulating the size of the actual aperture (Pupil) of the Iris by means of muscle fibres (iris dilator and sphincter muscle). Once penetrated in the eyeball throughout the Lens, light travels toward the Vitreous Chamber, reaching the proper sensory part of the eye sitting on the inner layer that is housed in the eyeball (Carlson, 2014). Despite of its peripheral location, the Retina is

part of the Central Nervous System (CNS). This nervous structure is provided with blood vessels entering the eye throughout the Optic Disc. In this region of the Retina all the axons carrying the visual information are bundled together outside the eye in the Optic Nerve (Carlson, 2014). The electromagnetic radiation hitting the retina primes the photo-transduction process that involves five major classes of neurons. Classical photoreceptors lie in the back of the eye while other retinal cells lie closer to the optic elements of the eyeball. Therefore, light must travel through layers of other retinal unmyelinated interneurons before striking the photoreceptors. In a small region of the Retina (Fovea) neurons are shifted on the side, exposing receptors and allowing them to receive light with minimum amount of distortion (Kandel et al., 2014). In the outer nuclear layer, there are two types of photoreceptors, Rods and Cones, that are connected to Horizontal cells and Bipolar cells in the adjacent plexiform layer. In the inner layer, Bipolar cells and Amacrine are connected to Retinal Ganglion Cells (RGCs) that are layered together and from witch the information is transmitted to the optical nerve. Cellular body of Rods and Cones contains the light-transducing apparatus. This consists of membrane stakes in which photopigments are concentrated. Each receptor has different sensitivity to the electromagnetic radiation. Rods are more sensitive than Cones, thus in dim light condition, vision is substantially mediated by the larger number of Rods (Scotopic vision). Rhodopsin is the visual pigment contained in

Rods. The pigment has a protein portion (opsin) and a derivative of vitamin A (retinal) that is responsive to light by assuming different isomeric conformations (Imamoto and Shichida, 2014). When activated by light, the chromophore 11-cis-retinal changes his structure and in its transient states can activate a G protein (Transducine). This protein provides signalling for cyclic Guanosine Monophosphate (cGMP) which is therefore reduced in the cell inducing changes in the ionic membrane fluxes thus resulting in changes of membrane potential. Rods sensitivity to light is also the result of multiple synapses on the same target interneurons of the outer layer (Pinel, 2007). This connectivity allows the signal from a single receptor to be strongly detected by the Ganglion cells structure with a communication that is direct (longitudinally mediated by Bipolar cells) or indirect when Amacrine and Horizontal cells are involved. On the other hand, Cones are less in number if compared to Rods but they share the same biochemical functioning (Kuffler, 1953). In sufficient light conditions, the Rods contribution to vision is minimum and the Cones bring their major contribution with colour vision providing with high special resolution (Photopic Vision). There are three types of Cone, each containing photopigment that are selectively sensitive to the action of different part of the light spectrum. Short ones (S-Cones) have their absorption peak when reached by Electromagnetic radiation of ~437 nm, displaying maximum sensitivity for Blue-Violet colours. S-Cones are the lesser represented in the retina

(around 10% of cones) and almost not present in the Fovea. M-Cones react to a radiation peaking around ~533 nm thus being primarily sensitive to Green colours. L-Cones express a characteristic sensitivity to higher region of the spectrum of visible light, peaking at ~564 nm that corresponds to the "Red" portion of the electromagnetic radiation (O'Brien, 1982; Pinel, 2007). Before the discovery of three different wavelength-dependent receptors, a Theoretical approach that explain human colour perception had been made in XIX Century by Young (1801). The scientist proposed colour vision as mediated by three different receptors responding to Colours defined as Primary and the final colour perception is a situation in which different wavelength combines according to their mixture and produce a given colour. The trichromatic theory of colour vision was subsequently completed by Hermann von Helmholtz in mid '800 following "primary" colour matching experiments (for an extensive review see Mollon, 1982). Photopic and Scotopic system relay information through multisynaptic pathways to RGCs which innervate different areas in the brain for complex visual processing (Wassle, 2004; Palczewski, 2012; Kandel, 2014).

1.3 The Brain

The structural and functional organization of the Retina provides the Optic Nerve with the information to be carried to superior visual brain structures. From the Optic disc, the photic information is partially crossed over in the Optic Chiasm (Decussation). Two halves of the medial retina only transmit in a contralateral way the information, while the information coming from the distal portion of the retina is carried out ipsilateral. These nervous fibres are topographically organized and constitutes the first portion of the Optic Tract. Projection of these fibres reach three main subcortical structures in the brain, each serving to a specific visual function. The Superior Colliculus receive RGCs axons and Superior Cortical projection and controls eye movement (saccades). The visual information is integrated with other sensory information converging in this structure. The Pretectum of the Midbrain, that is situated behind the Thalamus and the anterior region of the Superior Colliculus, takes role mainly in controlling the pupillary reflex. The principal subcortical structure that receives almost all Optic nerve information is represented by the Lateral Geniculate Nucleus (LGN). As the information transported to these cells is topographically organized, the LGN contains an exact representation of each of the halves of the receptorial field, (with major representation of the information coming

from the Fovea) and that is therefore defined as retinotopic. This region consists of six distinct layers receiving different afferences from RGCs and divided in two main structures (Magno Cellular and Parvo Cellular – Kaas et al., 1972) each of them receiving specific signals from RGCs. First inner layer of this neural formation receives information from contralateral information from the retina and so layer number four and six (the nasal halves of the retina). Layer three two and five receive ipsilateral retinal information. This highly organized structure redirect via the Optic Radiation information about colour and luminance contrast to the Primary Visual Cortex (Hubel, 1963; Hubel, 1982). Situated in the occipital lobe of the brain, this portion corresponds to Broadman area 17. Mostly allocated in the Calcarine Fissure, it consists of five layers where visual information is received from the contralateral retina; therefore, the signal captured from the left eye is delivered to the right Visual Cortex and *viceversa*. This structure, together with other cortical brain region, is responsible for location of a stimuli in the space, to catch shapes and movement, and contributing massively to the entire sensory life of humans and many mammals. The highly-specialized structure and nature of receptors and interneurons are the starting point of the process that make vision possible. Their ability to detect and respond to the electromagnetic radiation has something else than “just” Vision to accomplish with.

Chapter 2: Light and biological Rhythms.

2.1 Day and Night

The Star of our solar system is accountable for most of the electromagnetic radiation that hits our Planet (Liu and Jordan, 1960). Earth rotation on its oblique axis is responsible for variability in the amount of light radiation on a given geographic spot, while the orbit around the Sun provides with variability on the duration of the light period in far from equator locations. This environmental variability marks the difference between two distinguishable periods on planet Earth. Living organisms mostly vision dependent had therefore to develop survival mechanisms that can account for this variability. In other words, the ability of keep track of the periodicity of Solar Day and Night (Yorukoglu and Celik, 2006). During the Light period, Humans and many mammals exhibit a degree of complex and active behaviour while resting states are allocated during the Night (Yorukoglu and Celik, 2006). Based on a 24hours (24-h) duration of the solar cycle of day and night, these rhythms are called circadian (circa= about; diem = one day). This periodicity is centred by the capacity of light detection in the environment, and the use of photic stimulation, as an input to a master biological rhythms pacemaker so for other than visual purposes. Today

we know that this is localized in the Suprachiasmatic Nuclei (SCN), a small structure in the anterior hypothalamus, above the optic chiasma, on either side of the third ventricle (Hastings, 1998; Klein et al., 1991; Jones, 2000; 2003; Saper et al., 2001; Siegel, 2000; Sutcliffe and de Lecea, 2002; for a review see Mistlberger, 2005). Output from SCN determinates the phases of entrainment of physiological events such as melatonin onset and duration of secretion, motor activity and core body temperature (CBT, Lack and Wright, 2007).

2.2. Light and Biological Rhythms of Sleep and Wake.

Sleep and Wake Rhythm is one good example of adapted behaviour to variable light conditions in the environment. Its regulation is strictly linked to the light tacking capacity of the SCN. In healthy humans, sleep and wake occurs at stable time point of the solar day and night. These time points are characteristics phase markers of biological rhythms that depends on the SCN regulation of Melatonin secretion. (Lewy et al., 1999). In dark light cycle, Melatonin shows a rhythm that is entrained to external light condition. The SCN receives the photic retinal information via glutamatergic projections in Retino Hypotalamic Tract (RHT; Knauer et al., 1980; Hendrickson, 1972; Lewy et al., 1995). Melatonin production is controlled by SCN projections to the autonomic subdivision of the para-ventricular nucleus of the Hypothalamus (Moore, 1996). The para-ventricular nucleus projects directly to the upper thoracic inter-mediolateral cell column. Preganglionic sympathetic neurons project to the superior cervical ganglion that provides a direct sympathetic noradrenergic projection to the pineal gland which controls melatonin production through ß-adrenergic receptors. The onset of melatonin production occurs with the decrease in SCN neurons firing rate in late day, when light decreases, while is suppressed by light. Analysis of the onset and the offset of melatonin signal is a reliable marker of

endogenous entrainment to external light. In environmental controlled situations, such as constant darkness, researchers have found that Melatonin rhythm is preserved under direct control of the SCN (Czeisler et al., 1980). Knauer and his collaborators in 1980 provided evidence on the effect of light of a certain intensity in suppressing melatonin levels in humans. In the context of an interrupted sleep episode, participants were exposed to two different light intensity. Melatonin suppression occurred only when participants were exposed to the equivalent light intensity of a bright solar day (c.a. 2500 lux) within 30 minutes, reaching daytime levels and returning to night levels within 40 minutes as soon as they continued the sleep episode. These results matched other observation on animals, with the difference that Humans required high intensity light and that Melatonin levels in animals were found not to subsequently increase (Cardinali et al., 1972; Minneman et al., 1974; Deguchi and Axelrod, 1972). Sleep and wake cycles are also regulated on the basis of another oscillatory activity under control of the SCN. In an experiment of free-scheduled sleep in an environment with no temporal cue, sleep onset, sleep structure and sleep duration resulted linked to the internal phase of body temperature. Sleep/Wake patterns observed were consistent with a propensity to place the sleep episode at the lowest level of CBT displaying a free-running pattern. The *ad libitum* sleep was more frequently interrupted during the rising limb of CBT, regardless of prior sleep duration (Czeisler et al., 1980). Effect of light in resetting the time

phase of these two endogenous rhythms come from evidence retrievable in Shanahan and Czeisler of 1991. Authors observed by means of constant routine protocol (CR; Mills et al., 1978) that Melatonin maximum secretion anticipated CBT minimum with a 2h offset. This established phase between Melatonin and CBT was shifted when participants underwent Bright light administration schedules without influencing the oscillatory nature in both variables (Shanahan and Czeisler, 1991). This result led to the conclusion that both CBT and Melatonin were driven by a common clock mechanism entrained by light as a Zeitgeber (time giver, German). Taking into account CBT minimum, is possible to shift the Melatonin rhythm in either an advance or delay of phase (Czeisler et al., 1989; Jewett et al., 1997; Lack and Wright, 2007; Kräuchi, 2002). Timing of light administration before temperature minimum lead to a phase delay, whereas a light stimulation presented after the temperature minimum can induce a phase advance. Phase shifting also depends on the number of repeated stimulation presented, with greater shifting effects after repeated light stimulation (Minors et al., 1991). Four to seven hours of phase shifts were induced following three repeated light exposure while only two hours shifting was obtained with a single light presentation. A comprehensive model for sleep regulation also considers time-dependent mechanism, that regulates internal features of sleep, in constant interaction with physiological clock-dependent events (Borbely, 1982; Daaan et al., 1984; Achermann et al., 1993;

Borbely et al., 2016). The “two process” model for sleep regulation as proposed by Borbely (1982) is mostly based on the restorative function of sleep and that circadian timing can maintain sleep entrained into a functional organization. Electroencefalographic (EEG) studies revealed that during episode of prolonged wakefulness, a range of brain slow waves (delta e theta - SWA) increases as a function of time spent awake (Steriade et al., 1993). When participant to these sleep studies were allowed to recover from sleep deprivation, a longer sleep episode occurred together with higher representation of SWA that is the main characteristic of non-REM (NREM) sleep with a time course decrease of SWA. Studies on sleep deprivation and naps also showed that the amount of NREM of recovery sleep was related with the time spent awake and prior NREM sleep history (Dijk et al., 1987; Daan et al., 1988). In the model, SWA is then conceptualized as a marker (S) of the sleep that is needed in the first occasion for initiating sleep, reaching an upper threshold that initiate sleep and decreasing during sleep until a lower threshold is reached. Recent investigations reported that S could also be a marker of behavioural and learning processes sustained during wake. Tononi and Cirelli (2006) proposed a model for sleep debt as expression of the magnitude of synaptic potentiation during the wake and synaptic downscaling action of the SWA during the subsequent sleep in a periodic Energy/Space saving model for brain connectivity. In this view, sleep and wake is regulated on the tendency of sleep debt in being maintained

within a certain range; in a constant interaction with circadian (C) timepoints of sleep propensity or opposition entrained by external Zeitgebers fitting the proper alternation of behavioural state response to environmental and endogenous demands. (Dijk and Beersma, 1986).

2.3 Light, Alertness and wavelength

Exposure to light is not only a stimulation paradigm used for the study of biological rhythm. Light has been found to produce alerting effect on arousal and neurobehavioral measures. In a study by Cajochen and colleagues (2000) an alerting effect of bright light was found even at common indoor illuminance level (intensity) establishing a dose response curve for alerting effect of light. Night Exposure before the CBT minimum to a range of bright light pulses (3 lux -9100 lux) produced a decrease in reliable measures of alertness. In particular, they found a reduction of the presence of Slow Eye Movements (SEMs), in EEG slow waves and self-reported levels of fatigue. The same light intensity required to suppress Melatonin was also correlated to alertness measures. The dose response curve assessed by Cajochen et al. (2000) was best fitted by a logistic function, giving the fact that half of the maximum alerting effect of the entire range was obtained at 100 lux. Phase shifting response of Melatonin and CBT were fitted in the same function shape in an almost contemporary experiment (Zeitzer et al., 2000). The possibility that alerting effect of bright light administration was related to shifting of the circadian phase was tested by a daytime bright light administration paradigm (Rüger et al., 2005). The authors compared the effects of 4h of daytime bright light with 4h of the same light administered at night. They found that bright light administration provided physiological alerting

effect just in the night time condition, showing a reduced drop in CBT and an increase in Heart Rate (HR) in agreement with results from previous studies (Badia et al., 1992; Scheer et al., 1999). An interesting result from the same authors (Rüger et al., 2005) was that they found some alerting effect on self-report measures of fatigue, energy and sleepiness that were not attributable to CBT drops. The authors suggested that light must have provided an alerting effect via other neural pathways that are linked with sleep and wake promotion, mechanisms previously found in animal studies (Aston-Jones and Cohen, 2005). The evidence that this response is mediated by a functioning eye came also from studies on people whose sight is compromised to different levels of light perception (Skene and Arendt, 2006). Studies that investigated the role of pigments in retinal receptors using an Action spectrum paradigm (Thapan et al., 2001) found that there was no specific contribution of classical photoreceptors in the light induced response of melatonin suppression. Final report of the study anticipated the possibility of a novel photopigment as a candidate element to explain the peak of melatonin sensitivity to the exposure at a specific short wavelength of λ=459nm (corresponding to the light spectrum region of "Blue" colours). Animal studies confirmed the presence of a sub population of RGCs that can mediate the non-visual response to light stimulation. The entire cellular body of these RGCs contain novel photopigment (melanopsine) that has been found to be the best candidate (Provencio et al., 1994; Berson et al.,

2002; Foster et al., 2005) for transducing the light information. Although these cells have no membrane specialization for melanopsine, photoreception is substantially different from rods and cones (Bellingham et al., 2006), but an intrinsically photosensitive nature can be attributed to 5 different subtypes of RGCs (ipRGCs M1-M5; Tu et al., 2005; Baver et al., 2008). These cells are able to incorporate light signals over extended period of time resulting in an increased sensitivity during prolonged light stimulation. Rodless and Coneless animals and blind humans have been found to positively respond to light stimulation in abolishing melatonin signalling (Vigh et al., 2002; Lucas et al., 1999) Moreover, ipRGCs are most sensitive to wavelengths that are in the "Blue" region (λ max = 482–484 nm) of the light spectrum (Lupi et al., 2008; Zelinski et al., 2014; Melyan et al., 2005; Qiu et al., 2005) close to the light spectrum (λ max = 459 nm) responsible for melatonin suppression (Thapan et al., 2001). Consequently, effects of different wave-length lights have been investigated in monochromatic light exposure paradigms. Lockley and colleagues (2006) reported increased cognitive performance, and decreased signs of both physiological (EEG slow waves) and psychological sleepiness following 6.5h of monochromatic light exposure. Light at 460nm ("Blue") induced a greater alerting response and melatonin suppression magnitude compared to monochromatic light exposure at 555 nm ("Green") concluding that the light elicited variations in the increasing homeostatic need for sleep

Further evidences that even less exposure time and intensity can exert an activating response are reported by Cajochen (2005) and his group. Results showed that low levels of “Blue” light (5 lx at the cornea of narrowband radiation peaking at 460 nm) for a duration of about 40 minutes at night increased heart rate and self- reported alertness, as well as melatonin suppression and CBT increase compared to a dark condition and a 550 nm monochromatic light (Cajochen et al., 2005). In 2009, Phipps-Nelson found that low intensity (1 lx) “Blue” light (λ= 460nm) was able to suppress slow wave delta (1.0 – 4,5 Hz) and theta (4,5-8 Hz) as well as to increase performance on PVT and reduction of slow eye movement during night time testing if compared to bright light and “Red” light (λ= 640nm) at same levels. Surprisingly, these alerting effects were not associated to a significant reduction of salivary melatonin levels nor perceived sleepiness or performance to a more complex task (driving simulator). In parallel, Figueiro and colleagues (2009) found that both “Blue and “Red” light (respectively λ max = 470 nm, and λmax = 630) can induce a reduction of EEG Alpha power (8-12 Hz) and to increase EEG Beta (12-30Hz) during the night if compared to dark condition. In this within-subject study, participants were exposed to four experimental light conditions, administering the two monochromatic lights at different light intensities (40 lx and at 10 lx each). Melatonin suppression was found only in the High intensity “Blue” light condition (40 lx at the cornea). There results further suggested that alerting effects

of lights are not solely relative to circadian sensitivity to light spectrum. This possibility has been recently tested during the day, a natural condition in which Melatonin is virtually absent. Sahin and Figueiro, (2013), assessed their participants during two monochromatic lights exposure periods, timed around post prandial hours. The monochromatic "Red" light (λ max = 630) exposure at 40 lx during the day has been found to affect EEG measures of alertness with a statistical significant reduction of EEG activity in the Theta and Alpha rhythms if compared to the preceding dark adaptation, to "Blue" light and a darkness condition.

Chapter 3: Neurobehavioral Effects of Monochromatic Light Exposure.

3.1 Overview and Purposes of the Study

Our study is an attempt of partial replication of the study on the effects of monochromatic lights from Sahin and Figueiro (2013). Despite the considerable novelty and ecological consistency in terms of timing and duration of the exposure, we found that the research on alerting effects of monochromatic light exposure during the day should be further investigated. While timing was taken in consideration, there is no indication of prior alertness levels. Furthermore, while "Red" light has been found to be effective in reducing Theta and Alpha activity in the waking EEG, there is no evidence that such stimulation could have provided some effects on reducing sleepiness following exposure. On this purpose, we conducted a two-way crossover comparison of the effects of two Light Emitting Diodes (LED) Monochromatic Lights ("Red", "Blue") administered at 40lx. The Study was thus divided in two parts. In the first part (Part I) we explored the possibility of an alerting effect of light comparing basal morning alertness levels, post prandial alertness and post light exposure. According to previous results by the authors (Sahin and Figueiro, 2013), we tested the hypothesis that monochromatic lights can induce changes in the EEG Theta and Alpha

bands, lower self-reported sleepiness levels following exposure to “Red” light during post prandial hours.

In the second part (Part II) we were partially replicating the study by Sahin and Figueiro (2013). Adopting the same light technology and specifications, alerting effect of light were evaluated by means of repeated EEG measurements of alertness. According to previous report from the authors (Sahin & Figueiro, 2013), our hypothesis was that significant reduction of EEG activity in the Theta and Alpha bands were induced by “Red” Light Exposure.

3.2 Procedures and Materials.

3.2.1 Participants

Participants have been recruited among a population of university students using poster and university email advertising. Volunteers who reported disturbed sleep, ophthalmic or other relevant medical condition, history of psychiatric illness, use of recreational drugs and/or alcohol, night time working schedules, food or seasonal allergies were excluded from enrolment. An Italian version of the Morningness Eveningness Questionnaire (M.E.Q.- Natale et al., 2006.) was also administered to exclude extreme chronotype. Anxiety was also assessed by means of State- Trait Anxiety Inventory Form Y2 (STAI-Y2, Spielbergher et al., 1989). At the end of the recruitment campaign and screening we enrolled 17 participants (Males n=8; Females n=9) that were negative for exclusion criteria. Sample description is available in Table 1.1. Participants signed informed consent containing detailed procedures and were made aware that they were free to withdraw the experiment at any time. The study received favourable opinion by the Ethical Commission of the Department of Psychology.

Tab 1.1: Sample Description

	Tot=17		M=8		F=9	
	Mean	**S.D.**	**Mean**	**S.D.**	**Mean**	**S.D.**
AGE	23.29	3.44	24.13	4.26	22.56	2.55
M.E.Q.	50.18	4.3	50.13	5.25	50.22	3.60
STAI-Y2	40.71	10.28	39.5	9.43	41.78	11.44

M.E.Q., Morningness Eveningness Questionnaire; STAI-Y2; State- Trait Anxiety Inventory Form Y2; SD, Standard Deviation; M, Males; F, Females; Tot, Total.

3.2.2 Procedures

Enrolled subjects were instructed to adhere to regular sleep schedules during the week preceding each experimental session. Compliance was assessed with regularly held sleep logs. Participants arrived at the lab at 9.00 on each experimental session and left the lab at 17.00: explanation on procedures were repeated on arrival and participant verbally renewed their consent. Experiments took place at Psychophysiology of Sleep Laboratory of the Department of Psychology, University of Campania "Luigi Vanvitelli". Participant were allocated in a controlled temperature room (23°) with dark windows (room lights off = 0.01lx eye level), freed of time cues. Volunteers were constantly exposed to an environmental artificial bright light (c.a. 300lx) and asked to refrain from adopting photophobic behaviours (reading, eyes closing or covering), using light emitting devices (smartphones, tablets) other than the Desktop Computer Monitor allocated in the laboratory for cognitive assessment. In Part I of our study, differently from Sahin and Figueiro. (2013), measures of alertness were collected at 3 time point in indoor artificial bright light condition. Part I started with alertness levels assessed at Baseline (T0) and before Monochromatic light exposure (T1) respectively at 10.00, 13.30. A sandwich was served between 12.00 and 12.30 avoiding timing advice or awareness across each session. Part II started at 14.00, turning on the Light apparatus. We conducted four EEG trials (seven in the

original experiment) adopting a time span of fifteen minutes between each trial (seven minutes in the original experiment). Participants were exposed for 60 minutes to Monochromatic lights preceded by 5 minutes of dark adaptation. Subjects were asked to sit in front of the open panel of the Light Box, assuming a comfortable position and to stare at the inner panels of the box minimizing body movements. During exposure, room light was turned off and alertness measurements were limited to continuous Electrophysiological recording. At the end of the sixty minutes (as in the original experiment) the Light box was then turned off and alertness measures were taken following exposure at 15.15 (T3) under artificial room lightning condition thus completing both Part I and Part II. Participants were then discharged at 17.00 after a brief wellness assessment by the experimenter (A.C.). Each experimental session was separated by a week washout period. On the second experimental session either consent and exclusion criteria were assessed again. Order of condition presentation was randomized accounting for gender. Experiment diagram representation available in Table 1.2.

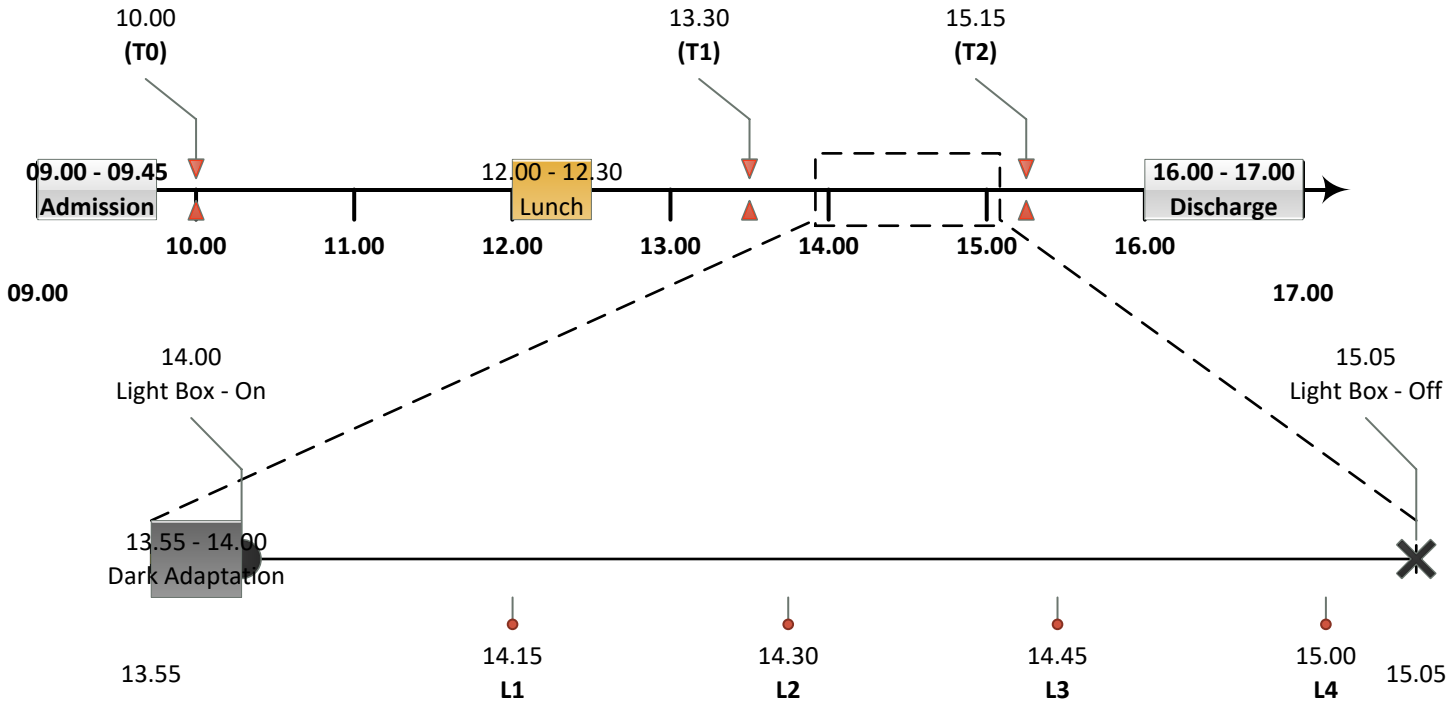


Table 1.2. Procedures Diagram. T0, Basal Assessment; T1, Post Prandial Assessment; T2, Post Exposure Assessment; L1-4 EEG assessment at 15 minutes intervals.

Participants repeated procedures after a week Washout period completing exposure to two light condition..

3.2.3 Subjective Measures

An Italian translation of the Karolinska Sleepiness Scale (KSS; Åkerstedt and Gillberg, 1990), a 9-point Likert-type scale with anchors 1: Extremely alert and 9: Extremely sleepy, fighting sleep, was administered at T0, T1 and T2. Subjective Vigilance (VIG) and Mood (MOOD), were assessed by Visual Analogue Scales (VAS - Monk and Embrey, 1989). In addition to these widely used self-report scales, Subjects were required to assess their Comfort/Discomfort (C/D) by means of a VAS created on purpose. The scales were 100-mm horizontal lines, anchored by word descriptors at each end. Participants were asked to mark the point on the line which best described his or her current state between the extremes of a continuum from 0 (Very low) to 10 (Very high). VAS scales were administered at T0, T1 and T2. At the end of Monochromatic Light Exposure, participants were requested to rate lights as Pleasant or Unpleasant (P/U) by means of a VAS.

3.2.4 Cognitive Measures

We administered two computer-based performance test. An adapted Psychomotor Vigilance Task (PVT – Basner and Dinges, 2011; Basner et al, 2011; Dorrian et al, 2005**)** and a Choice Reaction Time (CRT). In the 5 minutes PVT, subjects were asked to stare to a fixation point on the screen and react by pressing a keyboard key as soon as the randomly

timed targets stimuli (a Square) appeared. Similarly, the CRT consists of 20 trials that were presented at fixed timing and in a non-randomized order across measurements so that learning was predictable. Participants were instructed to react to two different target stimuli (a Cross and a Circle) selecting the assigned key on a computer keyboard. This task was adopted to verify and eventually contrast learning effects on PVT measures. PVT and CRT were administered at T0, T1 and T2.

3.2.5 EEG and EOG Measures

An adapted version of the Karolinska Drowsiness Test (KDT; Åkerstedt and Gillberg, 1990) was performed, recording participants eyes closed (EC; 3 minutes) and eyes open (EO; 3 minutes) EEG activity. During EO, subjects were requested to direct gaze at a neutral wall, minimizing body and head movements. Each timepoint for EEG measures were recorded on a Grass model 12 (Astro-Med, Inc.) applying gold plated cup electrodes on Oz derivation according to "10-20" International System (Jasper, 1958). Bipolar horizontal Electrooculogram (EOG) was recorded with gold plated cup electrodes placed above and below the left eye to measure spontaneous Eye Blink Rate (EBR; Barbato et al., 2000). A ground electrode was placed on the forehead while EEG derivations were referenced to mastoid derivations (M1+M2). Electrode impedance was

kept below 5 kohm and signal was digitized at a sampling rate of 100 Hz (Polyview version 2.1, Astro-Med, Inc). EEG and EOG tracks were exported in European Data Format (EDF) and processed under the supervision of Professor Dirk-Jan Dijk at Surrey Clinical Research Centre (University of Surrey, Guildford – UK). Signal were uploaded on Vitabase-Vitascore software, version 1.50B (Instruments B.V., Kerkrade, The Netherlands) to estimate EEG spectral power in the Theta band (4.5 – 7.75Hz), Alpha (8 – 12.75Hz), Beta 1(15 – 19.75Hz) Beta2 (20 – 25Hz) by means of Fast Fourier Transform (FFT) analysis. Four-seconds sub-epochs annotated as artefacts were excluded from the analysis of the power spectra. The data were weighted with a squared cosine window, implemented in the Vitascore software. Spectra computed according to sub-epochs length resulted in a 0.25Hz resolution. Frequency bins were then calculated relative to total power value of each trial. The obtained Relative EEG Power Spectrum for each band was thus calculated as the cumulative power (uV^2/Hz) expressed within each band ranges (Theta, Alpha, Beta1-2). EEG measures were performed at T0, T1 and T2. Same EEG specifications will be used for the 4 additional KDTs that were performed during light exposure (Part II) at regular intervals of 15 minutes each (L1, L2, L3, L4) for a total retina irradiation of 45 minutes.

3.2.6 Light Box and Exposure Procedures

A Light Box was used for Monochromatic Light Exposure. The Box (60x60x60 cm) was equipped with two different LED systems which were hidden from participant direct sight. Spectral irradiance of "RED" system (λ max = 630 nm - 2.31E-1W/m^2 – 7.37E+17 photons/s - 40lux) and the "BLUE" system (λ max = 470 nm - 5.41E-1W/m^2 – 1.27E+18 photons/s - 40lux) was assessed using Konica Minolta CS2000 Spectroradiometer. LED Systems of the Light Box were energized and turned off by a remote control. Before each exposure session, light intensity was measured at the level of the eye of the participant (HD 8366 luxometer, Deltahom, Padua, Italy). Participants were invited to assume a comfortable position sitting on a chair, to avoid closing their eyes if not requested by the experimenter and to minimize head and body movement. At the end of the exposure, Subjects were asked to complete a VAS rating the light condition as "Pleasant" or "Unpleasant" (P/U). Light Box apparatus has been provided by Majorano, Inc (http://www.majorano.it/azienda/)

3.3 Statistical analysis of Part I.

Subjective Measures

In order to verify variations in the time course of subjective alertness we conducted a set of 4 separate Repeated Measures Analysis of Variance (rANOVA) with a first repeated factor (CONDITION; 2 levels – RED/BLUE) and a second repeated factor (TIME, 3 Levels - T0/T1/T2) on average levels of VIG, MOOD, C/D and mean scores of KSS respectively. Furthermore, a One Way ANOVA was conducted on average levels of P/U with a single repeated factor (CONDITION; 2 levels; RED/BLUE).

Performance.

In order to verify variations in the time course of Performance Levels, a set of 4 separate rANOVAs were conducted with a first repeated factor (CONDITION; 2 levels - RED/BLUE) and a second repeated factor (TIME; 3 Levels - T0/T1/T2) on mean reaction time (RT) on PVT, mean of the 10% Fastest reactions, mean of the 10% Slowest reactions, and average RT on CRT respectively.

EEG and EOG Measures

In order to verify variations in the time course of EEG measures we conducted a set of 4 separate rANOVAs with a first repeated factor (CONDITION, 2 levels - RED/BLUE) and a second repeated factor (TIME, 3 Levels - T0/T1/T2) on average EEG Frequency Power in the selected bands (Theta, Alpha, Beta-1) in the EO period. Changes in the time course of EBR were identified by conducting rANOVA with a first repeated factor (CONDITION, 2 levels - RED/BLUE) and a second repeated factor (TIME, 3 Levels - T0/T1/T2) on rate of spontaneous eye blink defined as number Eye blinks/minutes.

3.4 Statistical Analysis of Part II

Effects of Light Exposure on EEG and EOG Measures

In order to verify variations in the time course of EEG measures we conducted a set of 4 separate rANOVAs with a first repeated factor (CONDITION; 2 levels - RED/BLUE) and a second repeated factor (TIME; 4 Levels – L1/L2/L3/L4) on average EEG Frequency power in the selected bands (Theta, Alpha, Beta) in the EO period. Changes in the time course of EBR were identified by conducting rANOVA with a first repeated factor (CONDITION, 2 levels - RED/BLUE) and a second repeated factor (TIME, 4 Levels – L1/L2/L3/L4) and a second repeated 7 levels factor (L1/L2/L3/L4) on rate of spontaneous eye blink defined as number Eye blinks/minutes.

For all rANOVAS, Huynh-Feldt degree of freedom (df) correction were adopted when sphericity assumption was violated but original df were reported. Statistical significant was set at $\alpha.= .05$. Post-Hoc comparisons were performed in case of statistical significant differences to describe directions of effects. All statistical analysis was conducted using Statistical Package for Social Science (SPSS), version 18.

3.5 Results

3.5.1 Subjective measures

Time course of subjective measures of alertness are detailed in tab 1.3

Karolinska Sleepiness Scale.

We found a statistically significant effect of TIME ($F_{(2,32)}$ = 14.333; p <0.001) with higher KSS average scores in T2 compared to T1 and T0 and higher average KSS scores in T1 if compared to those reported in T0. No statistically significant interaction effect of TIME*CONDITION has resulted ($F_{(2,32)}$ = 0.651 ; p = .529 - Fig 1.3)

VAS Vigilance

We found a statistically significant effect of TIME ($F_{(2,32)}$ = 11.757; p <0.001) with lower Vigilance average scores in T2 compared to T1 and T0 and lower Vigilance average scores in T2 compared to T1. No statistical significant interaction effect of TIME*CONDITION has resulted ($F_{(2,32)}$ = 0.225 ; p = .800) - Fig 1.4)

VAS Mood

We found a statistically significant effect of TIME ($F_{(2,32)}$ = 6.343 ; p < .05) with participants reporting lower mean Mood scores in T2 compared to T1 and T0 and lower mean Mood scores in T1 compared to T0. No

statistically significant interaction effect of TIME*CONDITION has resulted ($F_{(2,32)}$ = 0.897; p = .400 - Fig 1.5)

VAS Comfort/Discomfort

No statistically significant effect of TIME were found ($F_{(2,32)}$ = .423; p = .643) and no statistically significant interaction effect of TIME*CONDITION has resulted fig ($F_{(2,32)}$ = .897; p = .418 - Fig 1.6)

VAS Pleasant/Unpleasant

One Way ANOVA conducted (tab 1.4) reported no statistically significant differences between ratings ($F_{(1,32)}$ = 0.493; p = .487 - Fig 1.7)

Tab1.3: rANOVA for Subjective measures

				Condition		Time		Condition*Time	
	T0	T1	T2	$F_{(1,16)}$	p	$F_{(2,32)}$	P	$F_{(2,32)}$	P
KSS Scores	3.58±0.29*	4.41±0.3*°	5.5±0.315						
λ max = 630 nm (Red)	3.76±0.42	4.41±0.31	5.35±0.38	0.003	0.957	14.33	<0.001	0.651	0.529
λ max = 470 nm (Blue)	3.41±0.40	4.41±0.38	5.34±0.42						
VAS Vigilance (mm)	70.61±3.91*	60.96±4.59*°	46.64±3.72						
λ max = 630 nm (Red)	69.23±5.33	61.5±4.32	47.47±4.95	0.000	0.998	11.75	<0.001	0.220	0.800
λ max = 470 nm (Blue)	72±4.95	60.35±5.51	45.82±4.41						
VAS Mood (mm)	72.08±3.87	69.71±3.92*	61.26±3.76						
λ max = 630 nm (Red)	72.11±4.42	67.48±4.11	58.41±5.05	0.617	0.444	6.343	0.005	0.897	0.418
λ max = 470 nm (Blue)	72.05±4.47	71.94±4.97	64.11±4.48						
VAS C/D (mm)	71.6±4.12	68.32±5.15	69.35±5.20						
λ max = 630 nm (Red)	68.41±4.49	66±5.33	68.47±5.15	3.610	0.076	0.423	0.659	0.897	0.418
λ max = 470 nm (Blue)	74.80±3.65	70.64±5.33	70.23±5.78						

*, significantly different from T2; °, significantly different from T0; KSS, Karolinska Sleepiness Scale; VAS, Visual Analogue Scale; - C/D, Comfort, Discomfort, mm, millimetres; ±S.E.M. (Standard Error of Means)

Tab 1.4, One Way Anova for Lights Rating

VAS P/U	Mean±S.EM	F(1,16)	P
λ max = 630 nm (Red)	52.7±6.65	1.460	0.244
λ max = 470 nm (Blue)	59.82±7.63		

VAS, Visual Analogue Scale, -P/U, Pleasant/Unpleasant nm Nanometres; ±S.E.M., Standard Error of Means

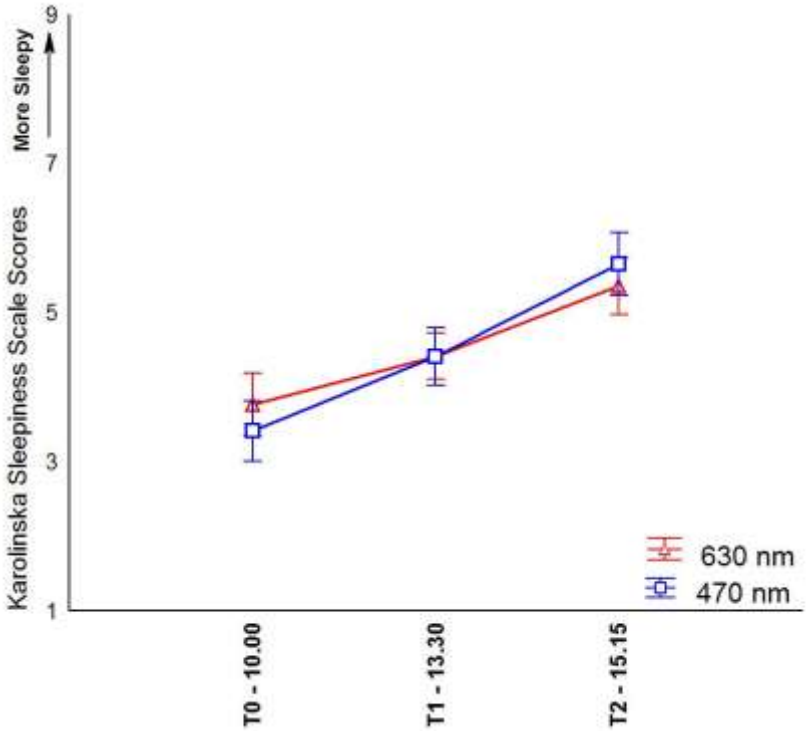


Fig 1.3. Average Karolinska Sleepines Scale scores

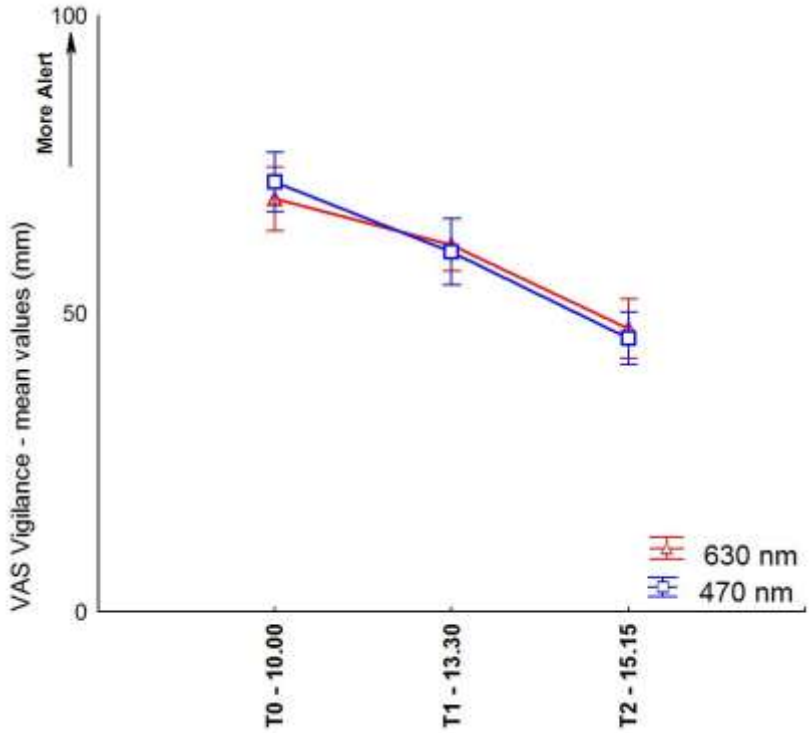


Fig 1.4. Average VAS Vigilance scores; VAS, Visual Analogue Scale; mm, Millimeters.

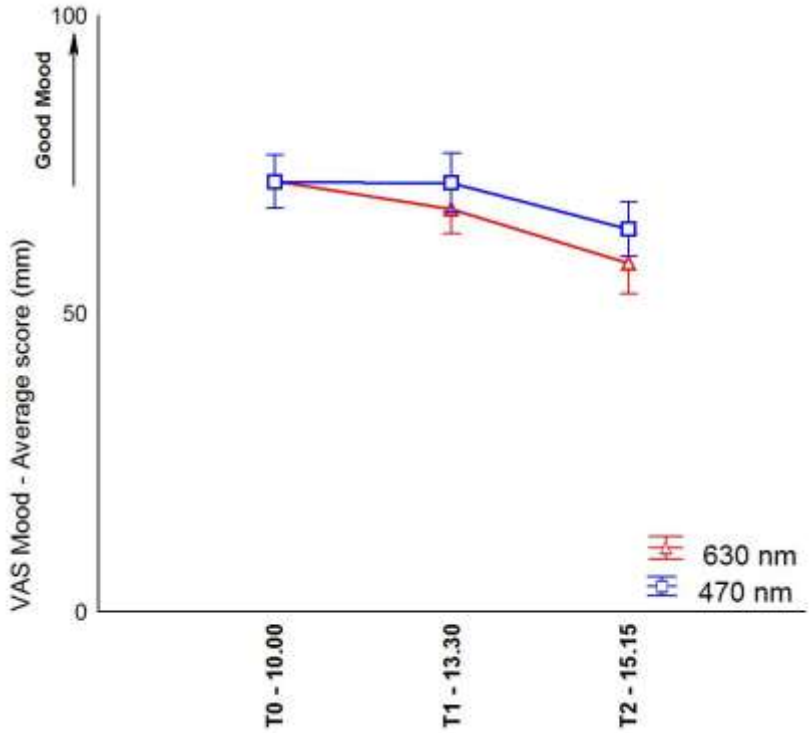


Fig 1.5. Average VAS Mood scores; VAS, Visual Analogue Scale; mm, Millimeters.

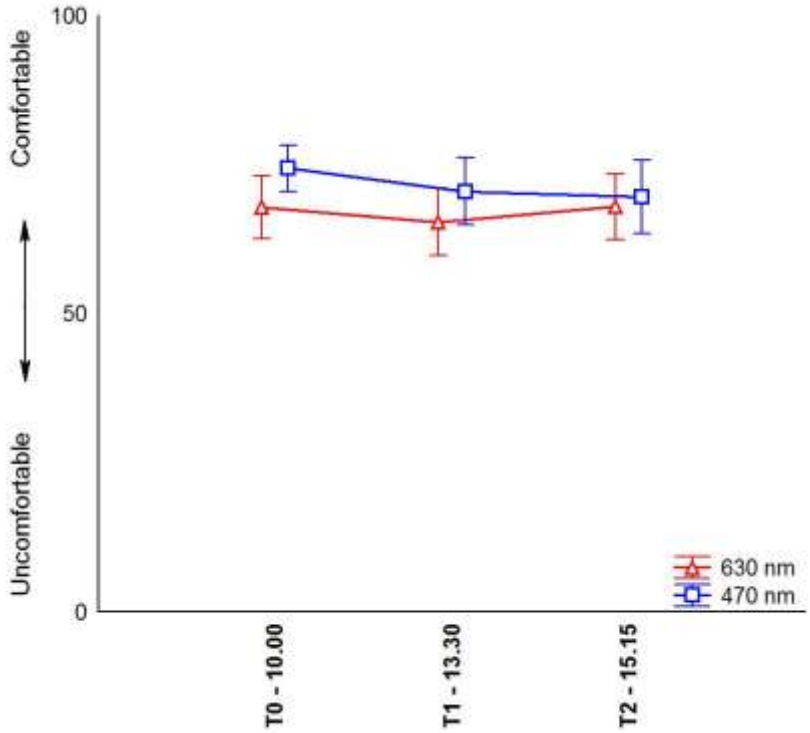


Fig 1.6. Average VAS Comfort/Discomfort scores; VAS, Visual Analogue Scale; mm, Millimeters.

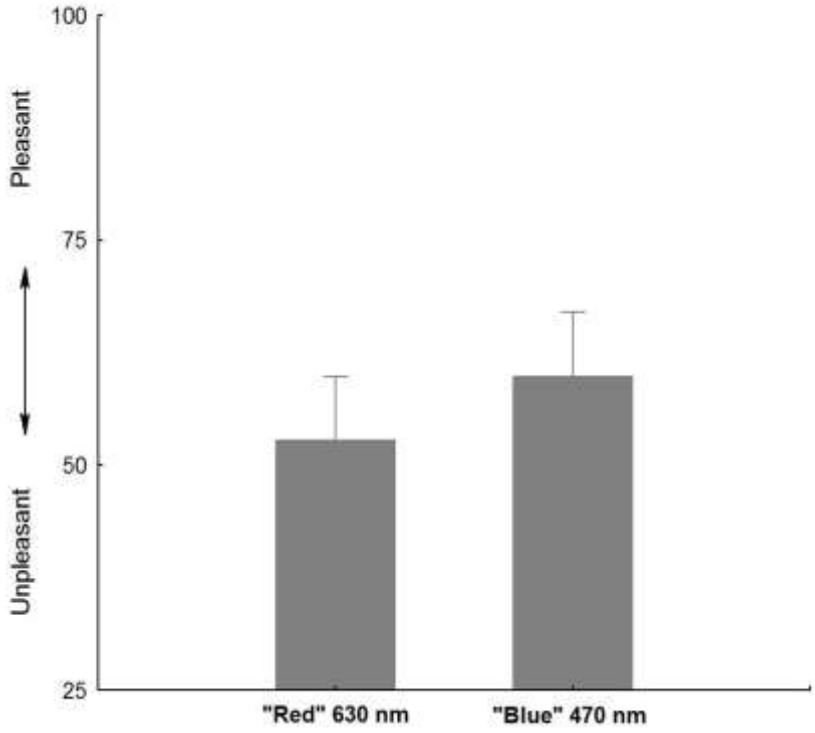


Fig 1.7. Average VAS Light Ratings (Pleasant/Unpleasant); VAS, Visual Analogue Scale; mm, Millimeters.

3.5.2 Part I: Time course of Performance

Time course of Performance measures are detailed in tab 1.5

PVT – Mean RT

We found a statistically significant effect of TIME ($F_{(2,32)}$ = 9.597; p = .001) with participant reporting slower average RT in T2 and in T1 if compared with those reported in T0. No statistically significant interaction effect of TIME*CONDITION ($F_{(2,32)}$ = 1.499; p = .240 - Fig 1.8)

PVT - 10% Fastest RT

We did not found a statistically significant effect of TIME ($F_{(2,32)}$ = 1.414; p = .258) and no statistically significant interaction effect of TIME*CONDITION were reported ($F_{(2,32)}$ =0.080; p = .923 - Fig 1.9)

PVT - 10% Slowest RT

We found a statistically significant effect of TIME ($F_{(2,32)}$ = 4.654; p = .017) with participant showing slower average RT in T2 if compared with those measured in T0. No statistically significant interaction effect of TIME*LIGHT resulted from the analyses ($F_{(2,32)}$ =1.465; p = .247 - Fig 1.10)

Choice Reaction Time.

We found a statistically significant effect of TIME ($F_{(2,32)}$ = 24.973; p < .001) with participant reporting fastest average RT in T2 and in T1 if compared with those reported in T0. No statistically significant interaction effect of TIME*CONDITION was found ($F_{(2,32)}$ =.740; p = .485 - Fig 1.11)

Tab 1.5, rANOVAS for Performance

				Condition		Time		Condition*Time	
	T0	T1	T2	$F_{(1,16)}$	*p*	$F_{(2,32)}$	*p*	$F_{(2,32)}$	*P*
PVT – Mean RT (ms)	2268.82±6.74	291.88±5.93°	289.37±9.5°						
λ max = 630 nm (Red)	273.20±8.97	283.14±6.54	290.77±10.12	0.089	0.769	9.597	0.001	1.499	0.239
λ max = 470 nm (Blue)	264.45±6.58	300.62±9.65	287.98±12.36						
PVT – 10% Fastest RT(ms)	213.67±3.29	222.6±3.34	217.44±5.01						
λ max = 630 nm (Red)	216.41±22.74	224.20±4.38	218.61±6.1	0.426	0.523	1.414	0.258	0.080	0.923
λ max = 470 nm (Blue)	210.94±3.41	221±5.82	216.26±6.43						
PVT – 10% Slowest RT (ms)	375.17±16.95*	410.38±16.99	444.11±27.04						
λ max = 630 nm (Red)	387.23±25.69	395.42±17.79	463.70±38.9	0.274	0.608	4.654	0.017	1.465	0.246
λ max = 470 nm (Blue)	363.11±15.94	425.34±20.97	424.52±31.37						
CRT – Mean RT (ms)	441.40±19.62	406.17±20.19*°	394.83±18.38*°						
λ max = 630 nm (Red)	450.38±23.68	406.42±25.57	405.62±25.38	0.624	0.441	24.973	<0.001	0.740	0.485
λ max = 470 nm (Blue)	432.41±21.75	405.93±18.336	384.04±15.06						

*, significantly different from T2; °, significantly different from T0; PVT, Psychomotor Vigilance Task; RT, Reaction Time; ms; Milliseconds; Choice Reaction Time Task; nm, nanometres; ±S.E.M. (Standard Error of Means)

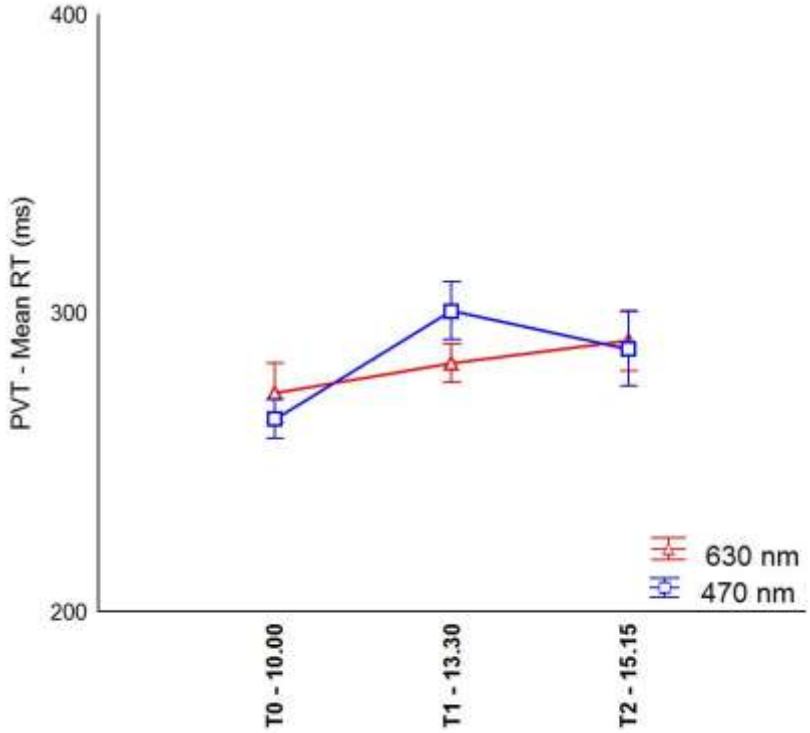


Fig 1.8. Mean Reaction Time; PVT, Psychomotor Vigilance Task; RT, reaction time; ms Milliseconds.

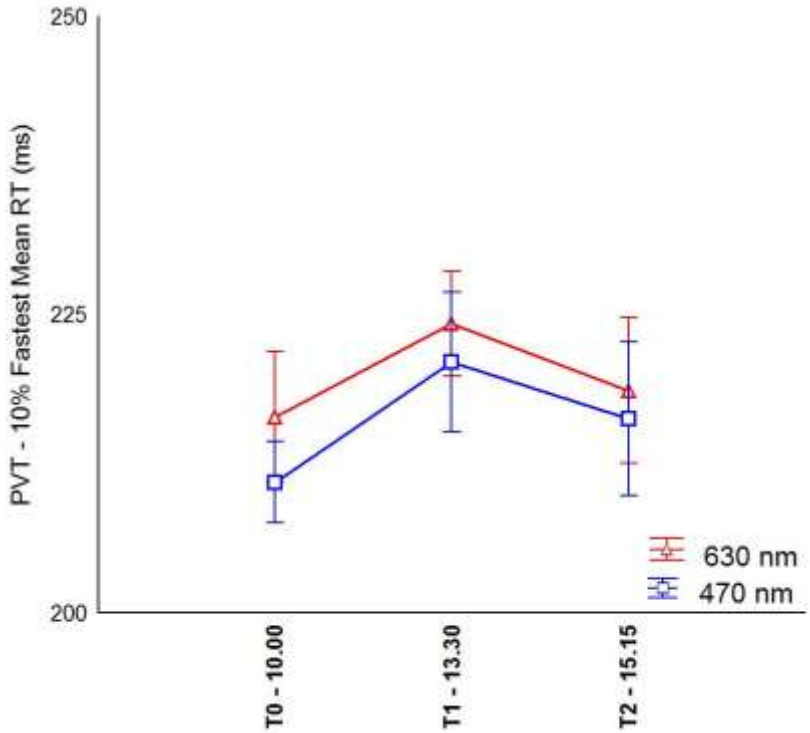


Fig 1.9. 10% Fastest Reaction Time; PVT, Psychomotor Vigilance Task; RT, reaction time; ms Milliseconds.

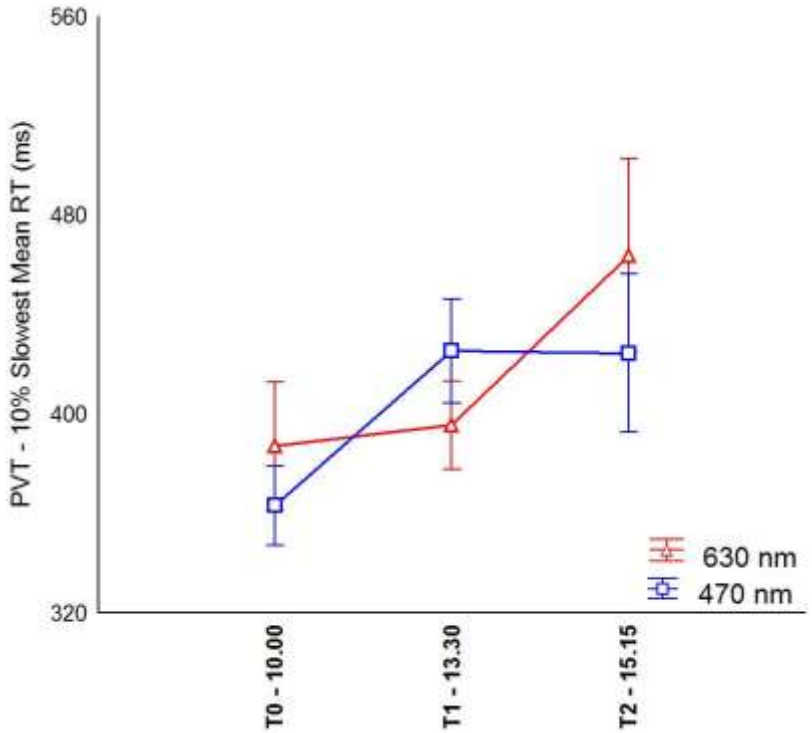


Fig 1.10. 10% Slowest Reaction Time; PVT, Psychomotor Vigilance Task; RT, reaction time; ms Milliseconds.

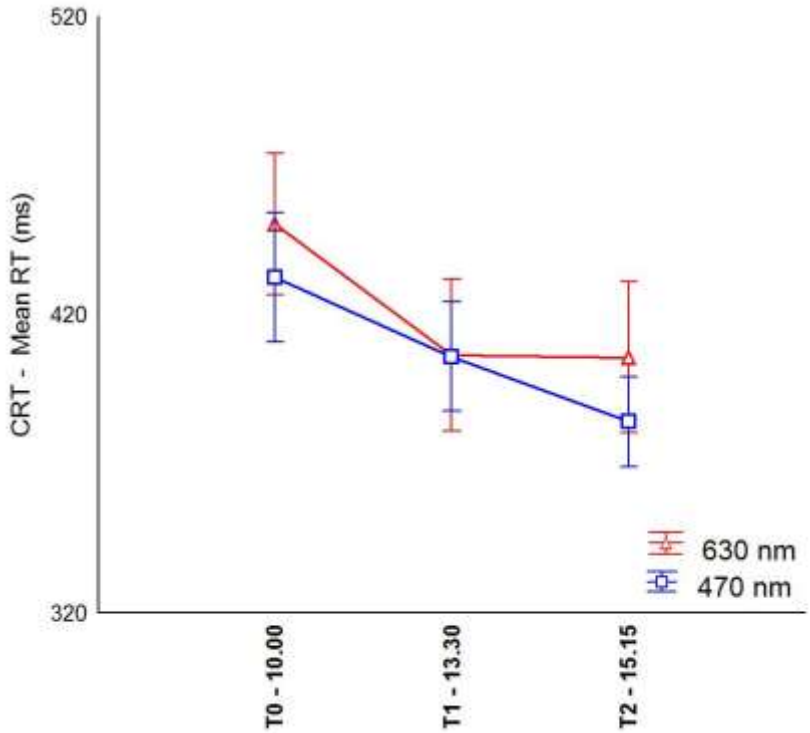


Fig 1.11. Choice Reaction Time (CRT); RT, reaction time; ms Milliseconds.

3.5.3 Part I: Time Course of EEG and EOG measures

Time course of EEG and EOG measures are detailed in tab 1.6

Theta (4.75 – 7.75Hz)

We did not found a statistically significant effect of TIME ($F_{(2,32)}$ = 1.005 p =.363) as no statistically significant interaction effect of TIME*CONDITION was reported from the analyses ($F_{(2,32)}$ =.2.355; p = .111)

Alpha (8 – 12Hz)

We found a statistically significant effect of TIME ($F_{(2,32)}$ = 3.802; p = .033) with higher Spectral Power measured in T2 if compared to T1 and T0. Moreover, we found statistically significant interaction effect of TIME*CONDIITION ($F_{(2,32)}$ = .4.755; p < .05) with higher Spectral Power at T2 in the BLUE condition if compared to values measured in T2 of the RED condition (Fig 1.12 - A).

Beta (15 – 20Hz)

We did not found a statistically significant effect of TIME ($F_{(2,32)}$ = .3111; p = .735). rANOVA showed statistically significant interaction effect of TIME*CONDITION ($F_{(2,32)}$ = 4.632; p = .017) with higher Spectral Power at T2 in the BLUE condition if compared to values measured in T2 of the RED condition (Fig.1.12 - B)

Eye Blink Rate

We did not found a statistically significant effect of TIME ($F_{(2,32)}$ = .216; p = .736) as well as no statistically significant interaction effect of TIME*LIGHT was reported from the analyses ($F_{(2,32)}$ = 2.720; p = .08)

Tab 1.6 rANNOVAs for EEG and EOG measures

				Condition		Time		Condition*Time	
	T0	T1	T2	$F_{(1,16)}$	p	$F_{(2,32)}$	p	$F_{(2,32)}$	p
Theta (4.75 – 7.75Hz) – Relative Power (uV^2/Hz)	12±1.04	12.65±0.83	11.31±0.91						
λ max = 630 nm (Red)	12.27±1.73	14.63±1.47	11.65±1.32	1.48	0.241	1.005	0.377	2.355	0.111
λ max = 470 nm (Blue)	11.73±1.08	10.68±0.97	10.97±0.89						
Alpha (8 – 12Hz) – Relative Power (uV^2/Hz)	23.51±2.63+	26.53±2.8	27.881±2.58						
λ max = 630 nm (Red)	22.29±2.61	24.70±2.35	22.29±2.99	5.672	0.030	3.802	0.033	4.755	0.016
λ max = 470 nm (Blue)	24.73±3.1	28.37±3.66	33.47±3.572*						
Beta (15 – 20Hz) – Relative Power (uV^2/Hz)	5.84±0.41	6.02±0.58	5.85±0.47						
λ max = 630 nm (Red)	5.3±0.452	5.9±0.94	4.43±0.64	1.741	0.206	2.167	0.131	1.988	0.154
λ max = 470 nm (Blue)	6.38±0.62	6.14±0.42	6.93±0.62*						
EBR (Eye blinks/Min)	18.22±2.02	17.41±1.93	17.67±1.96						
λ max = 630 nm (Red)	14.97±1.94	15.64±1.77	16.8±1.59	3.622	0.075	0.216	0.087	2.72	0.081
λ max = 470 nm (Blue)	21.47±2.73	19.17±2.66	18.55±2.83						

+ significantly different from T2; * significantly different from T2 in Red Condition Hz, Hertz; uV, microvolts; Min, Minutes; nm, nanometers; ±S.E.M. (Standard Error of Means)

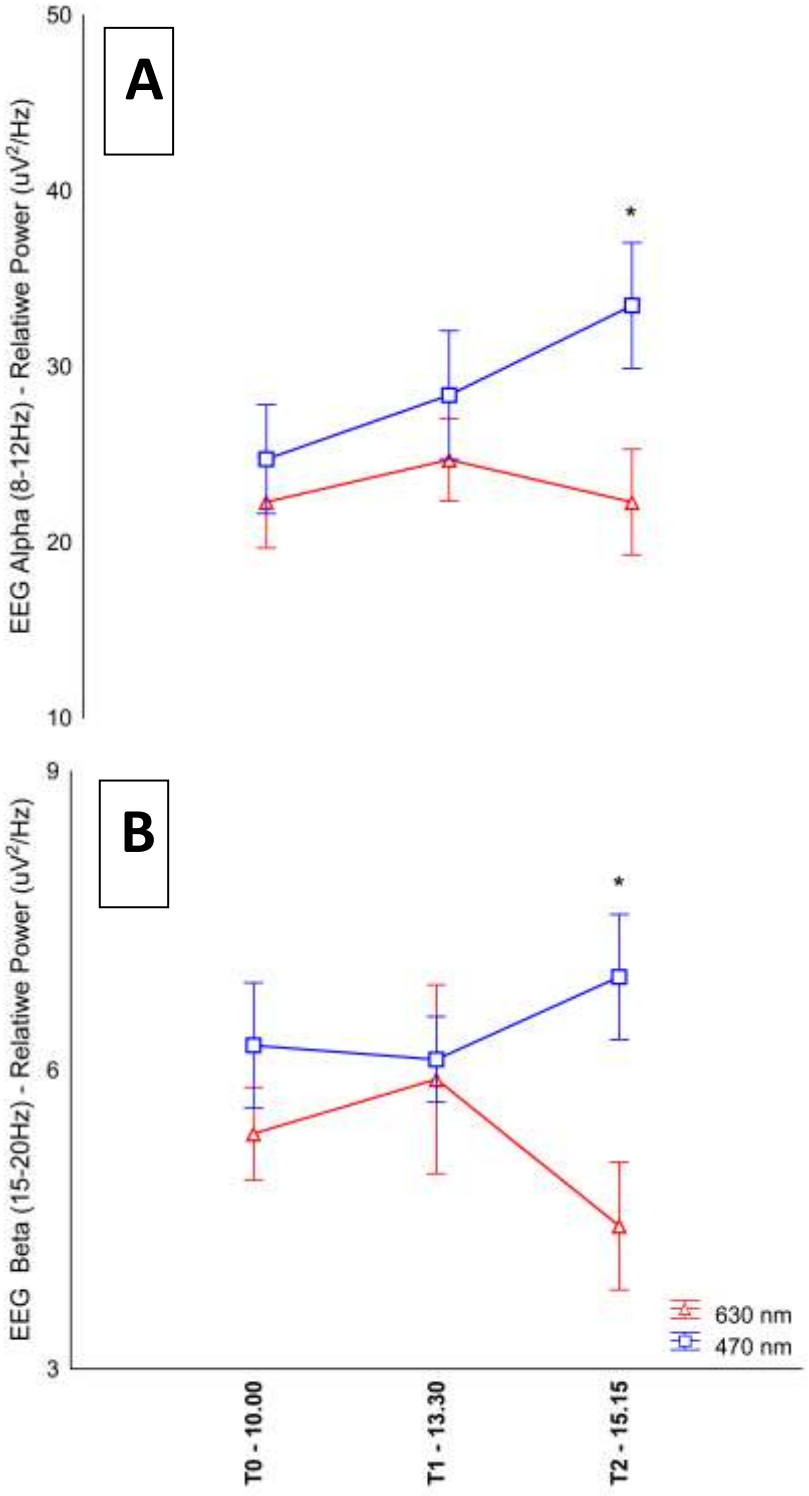


Fig 1.12. Average Power Spectral for Alpha (A) and Beta (B) * significantly different from T2 in Red Condition Hz, Hertz; uV, microvolts; Min, Minutes; Colour coded according to Wavelength

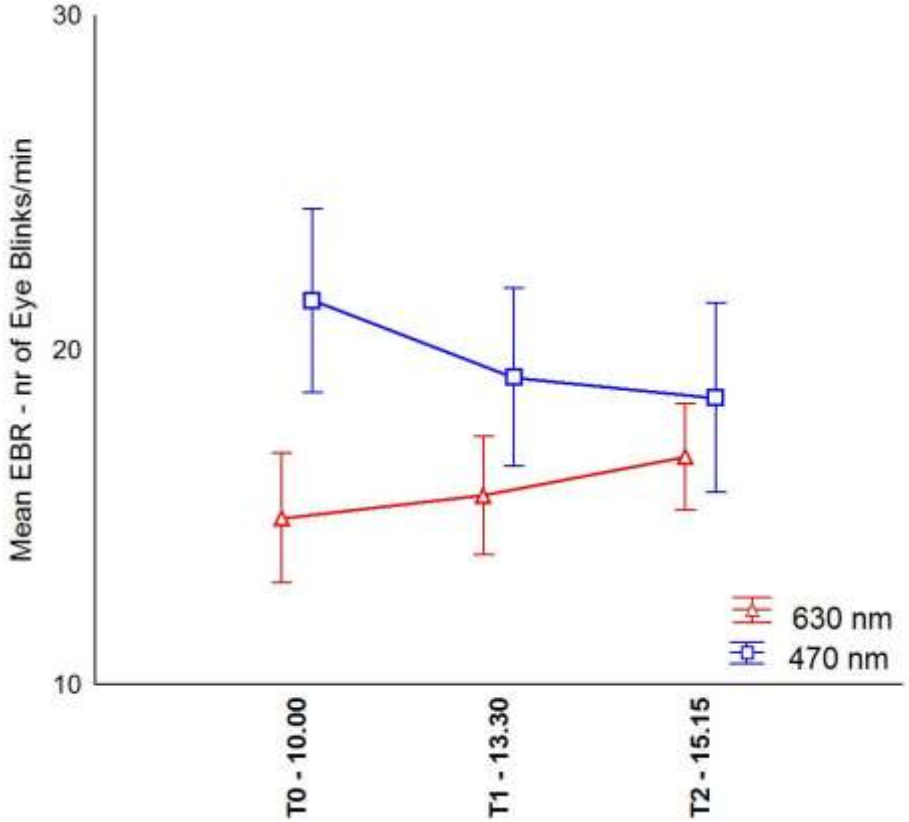


Fig 1.13. Mean Eye Blink Rate; nr, Number; min, Minutes

3.5.4 Part II: Effects of light Exposure on EEG and EGO Measures

Time course of subjective measures of alertness are detailed in tab 1.7

Theta (4.75 – 7.75Hz)

We did not found a statistically significant effect of TIME ($F_{(2,48)}$ = 1.050 p =.379) as well as no statistically significant interaction effect of TIME*LIGHT has resulted ($F_{(2,48)}$ = 1.581; p = .206)

Alpha (8 – 12Hz)

We found a statistically significant effect of TIME ($F_{(2,48)}$ = 7.455; p = .001) with higher Spectral Power measured in L2 if compared to Spectral Power measured in L1 and L3. No statistically significant interaction effect of TIME*LIGHT ($F_{(2,48)}$ = 1.159; p = .331 - Fig 1.14 -A)

Beta (15 – 20Hz)

We found a statistically significant effect of TIME ($F_{(2,48)}$ = 3.989; p = .022) with higher Spectral Power measured in L4 if compared to Spectral Power measured at L1, L2 and L3. rANOVA showed also statistically significant interaction effect of TIME*LIGHT ($F_{(2,48)}$ = 4.331; p = .015 fig 1.14 - B) higher Spectral Power measured at L4 in the BLUE condition if compared to Spectral Power measured at L1, L2 and L3 of the RED condition.

Eye Blink Rate

We did not found a statistically significant effect of TIME ($F_{(2,48)}$ = 1.463; p = .247) as well as no statistically significant interaction effect of TIME*LIGHT has resulted ($F_{(2,48)}$ = 2.686; p = .057 – Fig. 1.15)

Tab 1.7, rANOVAs for EEG and EOG measures (PartII)

					Condition		**Time**		**Condition*Time**	
	L1	L2	L3	*L4*	$F_{(1,16)}$	*p*	$F_{(3,48)}$	*p*	$F_{(3,48)}$	*p*
Theta (4.75 – 7.75Hz) – Relative Power (uV^2/Hz)	9.31±0.44	8.26±0.73	9.55±0.4	8.77±0.33						
λ max = 630 nm (Red)	9.93±0.73	9.48±1.27	9.28±.066	9.21±0.67	0.781	0.390	1.050	0.379	1.581	0.379
λ max = 470 nm (Blue)	8.8±0.51	7.76±0.57	9.822±0.59	8.22±0.71						
Alpha (8 – 12Hz) – Relative Power (uV^2/Hz)	28.5±2.65$^{\#}$	35.18±2.88	29.02±2.67$^{\#}$	31.74±2.66						
λ max = 630 nm (Red)	23.14±2.48^{+}	31.43±3.49§	26.94±2.94	28.05±3.17	10.877	0.005	7.455	0.001	1.159	0.331
λ max = 470 nm (Blue)	33.86±3.17	38.93±3.09^{+}	31.09±3.35	35.43±3.33						
Beta (15 – 20Hz) – Relative Power (uV^2/Hz)	4.43±0.45$^{\%}$	4.62±0.46	4.64±0.34	5.44±0.48						
λ max = 630 nm (Red)	4.4±0.73*	4.5±0.81*	3.72±0.27*	4.42±0.58	2.199	0.158	3.989	0.022	4.331	0.015
λ max = 470 nm (Blue)	4.47±0.34	4.7±0.3	5.55±0.55	6.45±0.68						
EBR (Eye blinks/Min)	19.23±2.15	17.97±2.16	19.13±2.66	20.33±2.02						
λ max = 630 nm (Red)	15.94±1.81	14.79±1.88	17.29±2.38	19.47±2.16	6.088	0.025	1.463	0.247	2.686	0.057
λ max = 470 nm (Blue)	22.52±3.1	21.14±2.83	20.97±2.55	21.2±2.38						

#, Significantly different from L2; *, Significantly different from L4 in the Blue Condition; %, Significantly different from L4; +, Significantly different from L1 in Red condition; §, Significantly different from L2 in Red condition Hz, Hertz; uV, microvolts; Min, Minutes, nm, nanometres; ±S.E.M. Standard Error of Means.

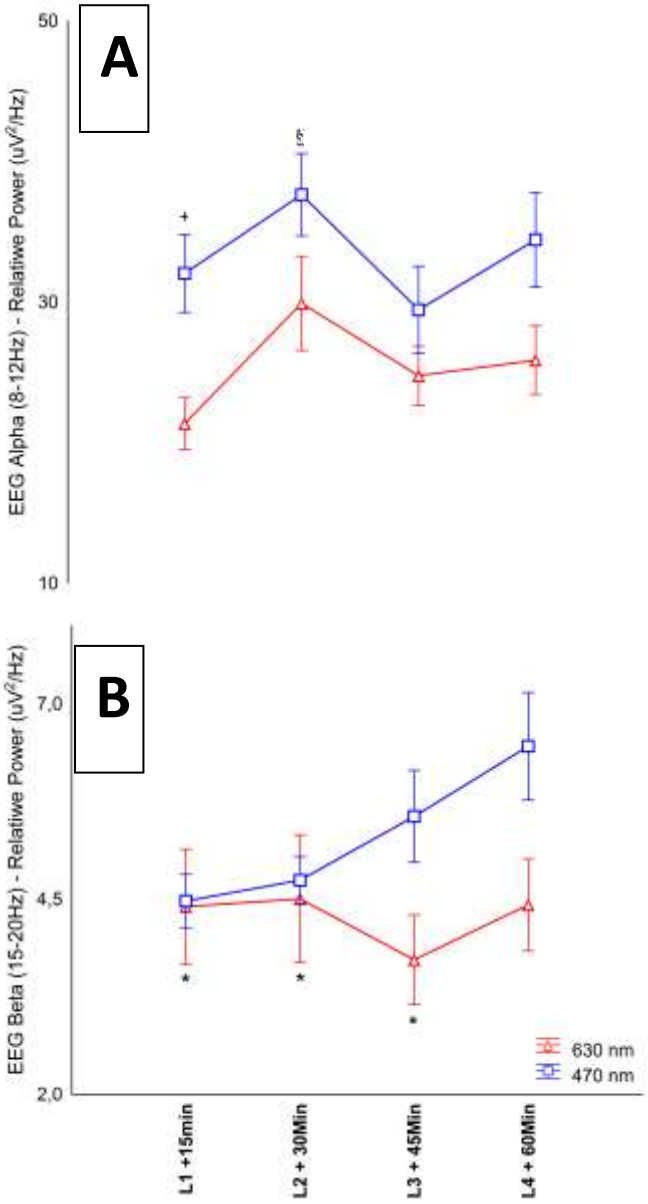


Fig 1.14. Average Power Spectral for Alpha (A) and Beta (B) *, Significantly different from L4 in the Blue Condition; +, Significantly different from L1 in Red condition; §, Significantly different from L2 in Red condition Hz, Hertz; uV, microvolts; Min, Minutes; Colour coded according to Wavelength

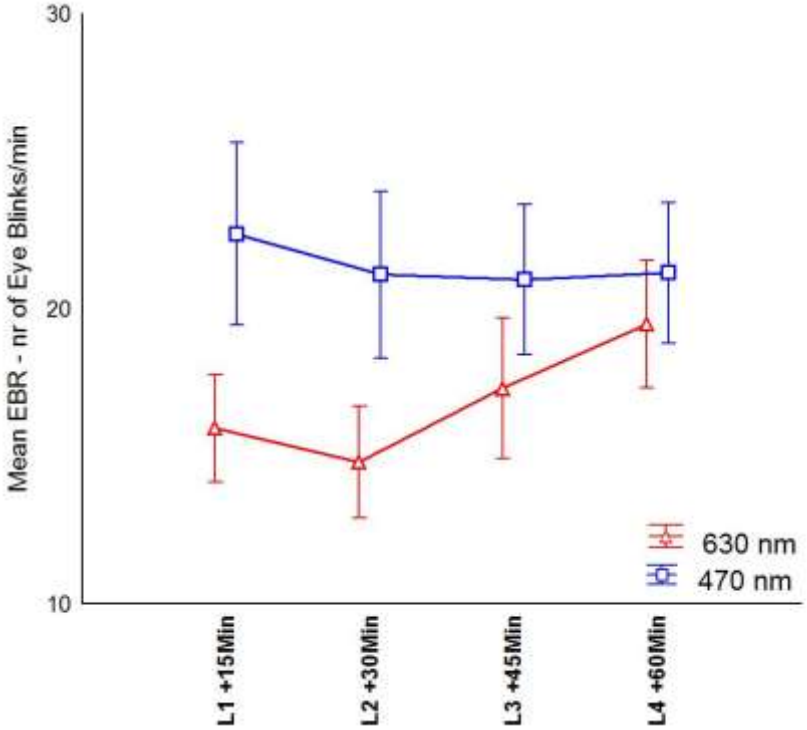


Fig 1.15. Mean Eye Blink Rate; nr, Number; min, Minutes

3.6 Discussion

Our Study aimed to explore the alerting effects of Monochromatic light exposure during the day. The observed effects on subjective measure of sleepiness (increasing levels of self-reported sleepiness and decreasing levels of vigilance and mood) provided evidence that accumulating need for sleep is a phenomenon that one person can easily recognize and report. In our data, this is supported also by contrast measures of general Comfort assessed in our participants, that contrarily to self-reported alertness measures, remained stable across the time of observation. As regards our observation on alerting effects of Monochromatic light on subjective alertness exposure, we observed no differences between two different wavelength stimulation, thus failing to confirm that Monochromatic lights tested can counteract the predictable increasing need for sleep as a function of time spent awake (Borbely, 1982; Daaan et al., 1984; Achermann et al., 1993; Borbely et al., 2016). Moreover, an increased reaction time to monotonous computer based tasks assessing objective alertness is frequently reported to be affected by a progressive need for sleep, leading our hypothesis acceptance in the same direction adopted for subjective measures. (Dorrian et al., 2005; Horne, 1993). However, in our study, we observed a decreasing reaction time across repeated trials of a slightly complex computer based task. This task was implemented in our paradigm to contrast learning effects on PVT. It is

reported that increasing need for sleep can exert different impact on tasks in which a top-down processing is requested (Harrison, Horne and Rothwell (2000). As predicted by the Two Process model for sleep and wake regulation, Slow waves in the waking EEG increasing is detectable on the basis of prior sleep history and time spent awake, increasing consistently after 16h spent awake (Cajochen et al, 1999). In addition, post prandial hours are classically reported as the circadian timepoint in which both physiological and behavioural sleepiness increases (Schmidt et al, 2007; Garbarino et al., 2001; Horne, 2010). At this particular time of day (between 14:00 and 16:00) sleep propensity is facilitated, and is higher than other hours of the day (Lack and Wright, 2007; Strogatz et al, 1987). In our study, the hypothesis that monochromatic light can elicit a lasting alerting response by suppression of EEG Theta and Alpha activity has been verified by comparing prior exposure levels and post exposure levels. We observed that, while the time course of Theta activity remained stable across trials, Alpha activity increased monotonically, with a greater increase following short-wavelength radiations (Blue). Moreover, an increase in Beta levels has been measured following Blue light administration. This partially corresponds to previous reported temporal dynamics in the EEG bands: Alpha levels seems to decrease as a function of sleep pressure, while higher levels of Theta and Delta (0.75-4Hz) are detected in the waking EEG (Åkerstedt and Gillberg, 1990). Adopting this explanation, we should assume that Red light failed in

exerting a lasting alerting response based on the observation of attenuated Alfa levels while a major increase in Theta band is still undetectable. These observations are also supported by increased levels in Beta waves, that could be attributable to higher levels of EEG Beta Power as a possible alerting response elicited by Blue light on a sleepiness-opposing Physiological response. This kind of response could have been modulated by other arousal related subcortical structures (locus coeruleus, hypothalamus and dorsal and posterior portions of the thalamus) that are connected to the SCN (Vandewalle et al., 2007; Perrin et al, 2004). These structures are in constant interaction with the Ventrolateral Preoptic Nucleus (VLPO). The sleep promoting signal coming from the activity of these latter cells results in constant interactions with brainstem structures that promotes wake. (Deurveilher and Semba, 2005; Saper et al, 2001). Photic information could have thus facilitated a wake promoting priming via caudal projection (Moore, 1996) of the hypothalamus; in waking individuals, the mechanism of action appears to be mediated by ipRGCs that are sensitive to short-wavelength lights as the one we have tested.

These interpretations could also fit other results observed.

First. In Part I, we found attenuated detrimental effect of time awake on PVT. Following Light stimulations, Mean RT assessed were very close to Mean RT assessed prior exposure. Is it possible that light esposure

elicited an alerting response with no particular modulation based on the wavelength.

Second. In Part II of the study, we observed a significant increase in EEG Beta waves starting from 30minute exposure to Blue light together with higher levels of Alpha across 4 trials. Theta waves resulted unaffected, failing to provide evidence that longer wavelength could suppress Theta EEG activity as previously reported (Sahin and Figueiro, 2013). Monochromatic lights tested in our study did not affected EBR profiles, that has found to not respond to this particular photic stimulation.

A final consideration is of course about limitations of our study. Our results interpretation could have been improved by assessing Melatonin levels and contrasting light exposure conditions with a Dark condition as the authors of the original experiment did. In addition to this, actigraphy measures could have provided robust control on sleep prior each experimental session. Moreover, CBT assessment in constant routine condition prior experiments could have provided information about the circadian phase and light could have been administered in its relation. However, we tested our Hypothesis in a more ecological way: the actual possibility that a purpose built light apparatus could counteract diurnal sleepiness. Our participants were tested in artificial light (Part I), that is a most common situation, considering that a person is not usually experiencing near to dark environments during the day. Dark conditions are commonly used in these type of studies, but on the other hand could

have facilitated sleep propensity as a means of comparison of the Alerting effect of Monochromatic light.

3.7 Conclusions

Our study showed that there is no effect of Monochromatic Light exposure on subjective and objective measure of alertness. Results observed in the analysis of EEG signal highlight a possible role of subcortical regions responsible for arousal regulation that are influenced by exposure to lights of short wavelength. These effects have been found during light exposure; but our results support the possibility that these alerting effects can influence EEG activity even after Monochromatic Light Exposure.

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