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Intrinsically photosensitive retinal ganglion cells and its role on Human Alertness

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Abstract

INTRINSICALLY PHOTOSENSITIVE RETINAL GANGLION CELLS AND ITS ROLE HUMAN ALERTNESS

LALITHA SAHI NANDINI WUPPUKONDUR

MASTER OF SCIENCE IN VISION SCIENCE

PACIFIC UNIVERSITY, 2019

ABSTRACT

PURPOSE: To investigate if exposure to cool (blue) light with a peak wavelength at 450nm can affect alertness, performance and EEG brain waves.

METHODS: Twenty healthy individuals were studied in a balanced cross-over design with exposure to two different light settings of equal illumination (1000 lux): cool blue light (7000K, peaked at 450nm) vs. warm orange light (1800K, peaked at 612 nm). Morning or afternoon sessions had 30-minute dark adaptation and 30-minute light exposure. Alertness was quantified by accuracy and reaction time in a two-back memory task, and performance was quantified by accuracy in a memory recall task (word pair task). Amplitudes of EEG brain waves (delta, theta, alpha, beta, and gamma) were measured during the one hour using a MUSE headband with two temporal (TP9, TP10) and two frontal electrodes (AF7, AF8). Paired t-tests compared the differences between the dark adaptation and experimental light. Mixed model Analysis of covariances was used to compare color temperatures. Factor analysis was used to reduce the dimensionality of the 16 different brain waves and locations.

RESULTS: Exposure to cool light showed a faster reaction time on the two-back memory task (Mean $_{\text{Blue light}}$ =1226.3, Mean $_{\text{Orange Light}}$ =1317.9,Effect size= 0.5) and a Word-Pair task had higher performance (Mean $_{\text{Blue light}}$ =81.8 %, Mean $_{\text{Orange Light}}$ =78.4 %, Effect size= 0.9) when compared to warm light at $p<0.05$. Accuracy on the two-back memory task did not differ significantly between light conditions. Increase in amplitudes from dark adaptation to light was seen in TP gamma and beta factor (Mean $_{\text{Blue light}}$ =0.28, Mean $_{\text{No light}}$= -0.40, Effect size=0.74)in cool light ($p=0.002$) but not in warm light ($p=0.5$). Theta, delta and alpha factor showed increase in amplitudes in both AF and TP with warm light exposure (Mean $_{\text{Orange light}}$=0.01, Mean $_{\text{No Light}}$= -0.45, Effect size=0.56, $p<0.05$) but not in cool light. EEG during learning word pairs, factor AF 8 theta, delta, and alpha showed an increase in amplitudes in both cool light and warm light conditions ($p<0.05$). During the two-back task, increase in amplitudes of AF8 beta and gamma was seen under warm light.

CONCLUSION: Our findings from the performance in the two-back memory task, the word pair task as well as the EEG data recorded during the tasks suggest that exposure to cool light may enhance the alertness and performance relative to warm light. Results were consistent with the hypothesis that these effects may be mediated by Intrinsically photosensitive Retinal Ganglion Cells which are sensitive to 450 - 480 nm light.

KEYWORDS: Non-image functions of light, IpRGC, EEG Brain waves, Alertness.

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We, the undersigned, approve that the thesis completed by the student listed above, in partial fulfillment of the degree requirements for Master of Science in Vision Science, for acceptance by the Vision Science Graduate Program.

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Program Approval

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Director of the Vision Science Graduate Program: Yu-Chi Tai, PhD

INTRINSICALLY PHOTOSENSITIVE RETINAL GANGLION CELLS AND ITS ROLE ON HUMAN ALERTNESS

By

LALITHA SAHI NANDINI WUPPPUKONDUR

THESIS

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

.

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CHAPTER 1: INTRODUCTION

Light exerts a strong non-visual influence on human physiological parameters such as behavior and cognition. Additionally, light indirectly impacts phasing of the circadian rhythm, which directly promotes alertness in humans.[1] With the recent discovery of Intrinsically photosensitive Retinal Ganglion Cells (IpRGC) expressing the photopigment[2] melanopsin, which is sensitive to blue spectrum of visible light, more focus is being placed on better understanding its impact on and implications for ocular health as well as systemic health and physiology. Literature reveals that exposing retinal tissue to blue light at 430 nm can lead to cumulative damage and poses a risk for developing age related macular degeneration.[3] However, blue light at 450 nm - 480 nm appears to positively affect human alertness and cognitive performance via the retinohypothalamic tract, this mechanism is triggered by IpRGC projecting input to the Suprachiasmatic Nucleus (SCN) which mediates circadian rhythm by sending input to the Pineal gland to regulate production of melatonin secretion .[4]

The retina is the inner-most, light-sensitive layer in the human eye used to detect light for image formation and object tracking.[5] The primary light sensing cells (the photoreceptors) in the retina are the rods and cones, yielding visual perception through absorption of light in the 400 to 780 nm portion of the electromagnetic spectrum. However, light is also essential for regulation of several biological and physiological functions that are independent of image formation which are collectively known as non-image forming visual functions (NIF). These NIF's include neuroendocrine and neurobehavioral functions such as regulating the circadian rhythm and direct enhancement of sleep, mood, alertness, and cognition.[6]

Recently, a novel human opsin in the retina has been identified as a subpopulation of retinal ganglion cells acting differently from rods and cones. These cells express melanopsin (hence known as intrinsically photosensitive retinal ganglion cells, or ipRGCs) with a peak sensitivity to blue spectrum at 480 nm.[2] While these cells account for less than 1 % of the ganglion cells, they demonstrate widespread dendritic coverage around the entire retina exception of the fovea.

IpRGC's convey light information to different regions in the brain such as suprachiasmatic nucleus (SCN) to mediate the circadian rhythm, the olivary pretectal nucleus to dark adaptation the pupillary light reflex, and the lateral geniculate nucleus to regulate other NIF visual processing.[7] For example, Lucas et al., 2002 demonstrated that IpRGCs project to the pineal gland which is the main center for melatonin production and sleep regulation while also modulating cognitive functions like alertness and attention.[8] Although not the primary function of the IpRGC, Dacey et al., in 2005 demonstrated that IpRGCs also project to the lateral geniculate nucleus (LGN), which provides a small contribution to the image formation. [9] Acute or longer NIF responses are most sensitive to monochromatic light wavelengths between 450 – 480 nm. Neuroimaging studies demonstrated that light exposure can directly enhance alertness and performance with sensitivity to short wavelength light.[6,10] Current knowledge on melanopsin-expressing IpRGCs confirms that they can detect light on their own through retinohypothalamic tract which influence physiological functions of brain regions such as sleep-wake cycle, mood, attention and alertness.

Considering the evidence that ipRGC mediates both the direct effect of light on circadian and other NIFs, the Chellappa et al., in 2011 hypothesized that the short-wavelength (cool, blue-shifted) light serves as the main trigger of the acute effect of light. Once activated, this shorter wavelength induces greater melatonin suppression while enhancing subject alertness as was observed in the sustained Psychomotor Vigilance Task (PVT, typically used to quantify the level of alertness) as well as in reduced concentrations of melatonin in salivary secretions under light of 6500K.[4] However, not all studies agree that cool light better enhances alertness and cognitive performance than warm light.[11]

EEG is an electrophysiological monitoring technology used to record electrical activity of brain waves. Diagnostic applications involve studying event-related potentials which are based on the stimulus onset and response time or observing neural oscillations commonly referred as brain waves according to frequency domain.[12] Conventionally raw EEG data is obtained through several electrodes (up to 32) attached in strategic areas of the scalp which are connected to semi portable machine.

Five types of rhythms of brain waves are categorized and reported in the literature alpha, beta, gamma, delta and theta based on the frequency. Following is brief review adapted from a report by Tania Kotsos published in journal “Mind your reality:

- *Gamma* (30- 100Hz) fastest frequency, higher level cognitive processing.
- *Beta* (12-30 Hz) Beta brain waves are associated with wakefulness and state of alertness, logic and critical reasoning. It is reported that higher beta levels may suggest stress, anxiety.

- *Alpha* (8- 12 Hz) are seen in deep relaxation while the eyes are closed while dreaming. Often seen during meditation, alpha brain waves are also associated with imagination, and visualization.

- *Theta* (4-8 Hz) waves are presented in deep sleep, including REM (stage of sleep).

- *Delta* (0.5- 4Hz) is slowest and is present during deep sleep and when unconscious. Delta is associated with deep healing and regeneration.

Sahin & Figueiro in 2013 did a study on "*Alerting effects of short-wavelength (blue) and long-wavelength (red) lights in the afternoon."* During a 48-minute, post-lunch-hour period, subjects were exposed to either blue light at 470 nm or red light at 630 nm while their EEG brain waves were recorded every 10 minutes. The results indicate that, power in the alpha, alpha-theta, and theta was significantly lower ($p<0.001$) after exposure to red light than when the subjects remained in the darkness. Exposure to blue light reduced alpha and alpha-theta power compared to darkness, but these differences did not reach statistical significance ($p>0.05$). Their results demonstrated that subjects were more wakeful during light exposure in the daytime.

Overall, the literature suggests that blue enriched light may enhance alertness, but the results are not consistent among studies, and a direct link between objective and behavioral measurements of the alertness and their relationship with light of different spectrum is yet to be established. In the present study we aimed to investigate if exposure to cool (blue) light with a peak wavelength at 450nm will affect alertness, performance and the light effect on EEG brain waves.

Oscillatory activity of brain waves such as alpha, beta, theta, delta and gamma has been studied to demonstrate the behavioral change under light exposure. This study will promote understanding of blue light exposure at 450 - 480nm to human alertness and neural feedback can promote our understanding on the trends in brain waves as a function of light.

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CHAPTER 2: METHODOLOGY

2.1 Overview of Experimental design

This is a two-visit randomized cross over study design with a sample size of 20. A convenience sampling method was employed to recruit, and subjects were randomly assigned to the first experimental condition. 1800K and 7000K LED color temperatures were used as the experimental light conditions in a closed room atmosphere void of any external light source. Luminance was balanced at 1000 Lux. Real time recording of the power and average amplitudes of EEG brain waves (delta, beta, gamma, alpha, and theta) at left and right Temporal areas (TP 9 and TP10) and left and right Frontal areas (AF7 and AF8) were obtained using the Muse headband (InteraXon Ontario, CAN) during the two experimental light exposures with the data streaming directly to and being recorded on an Android mobile device. Alertness was evaluated and correlated with the performance of cognitive based tasks, two-back memory task and word pair task.

2.2 Population and Sampling

2.2.1 Recruitment process

Upon Institutional Research Board (IRB) approval, we used convenience sampling method for identifying target population and sent study invitation to first, second- and third-year optometry students at Pacific university, Oregon.

2.2.2 Demographics

A Sample Size of 20 between 26 – 30-year-old, allows us to detect a 0.65 SD difference with power of 0.8 at an alpha 0.5 assuming a correlation between the baseline covariate and the outcome variable at r=0.7

2.2.3 Exclusion criteria

Subjects were excluded who had a history of neurological disorders, status-post head trauma, or surgical intervention with metal plates in head.

Inclusion Criteria

Subjects who did not meet Exclusion criteria were included in the study based on a screening,

2.2.4 Study Location

Room Jefferson 323, College of optometry, Pacific University, Forest Grove, Oregon

2.3 Materials

2.3.1 Pittsburgh Sleep Quality Index (PQSI)

Sleep plays an important role in health and well-being. Pittsburgh Sleep Quality Index is a self-rated questionnaire that is used for assessing an individual's sleep quality and disturbances over one week and one-month intervals. It was originally developed by Buysse et al.,1989.[13] In the present study we used the one-month interval questionnaire which has nineteen items with seven component scores such as subjective sleep quality, sleep latency, sleep duration, habitual sleep efficiency, sleep disturbances, use of sleeping medication, and daytime dysfunction.

Backhaus J et al., 2002 did a study to determine the test-retest reliability of PQSI questionnaire and reported correlation coefficient for test-retest reliability as 0.87. The PQSI has a good test retest reliability and has high validity for patients who report sleep quality complaints and insomnia. [14]

A PSQI score greater than 5 yields a diagnostic sensitivity of 89.6% and specificity of 86.5% (kappa = 0.75, p less than 0.001) in distinguishing good and poor sleepers. See Appendix A for the PQSI questionnaire for one-month interval for more information on seven components.

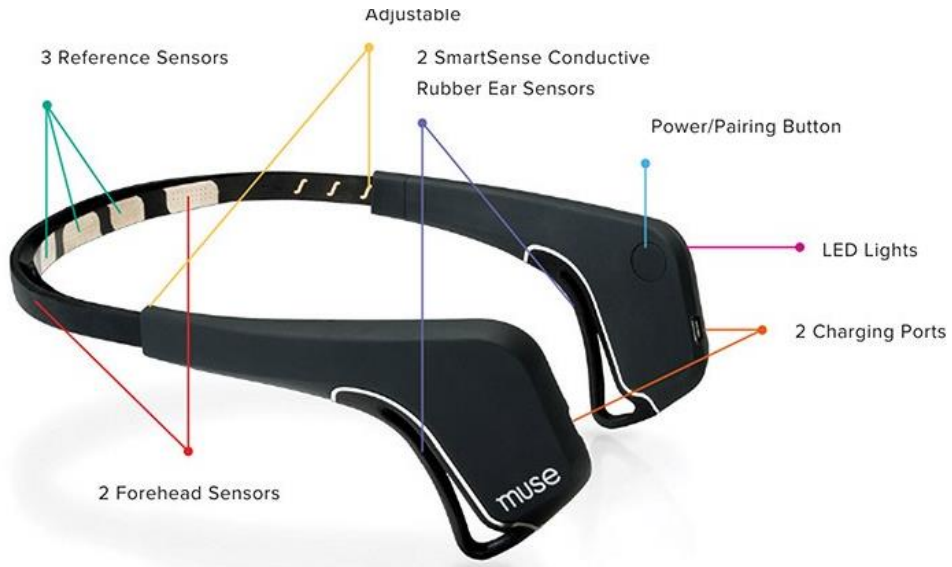
2.3.2. Muse Headband and EEG *Variables*

Originally designed as a meditation instrument, the Muse Headband is a device that provides an Electroencephalogram (EEG) using seven calibrated sensors (two on the forehead AF7,AF8 , two behind the ears TP 9,TP 10, and three reference sensors), it is capable of measuring each of the brain waves: delta (0.5-4 Hz), theta (4-8Hz), alpha (8-13 Hz), beta (13-32 Hz), and gamma (32-100 Hz) (Fig. 8)(Fig.9)

While it is principally marketed by the founders as a meditation training device, the Muse has enjoyed increasing success as a neurofeedback device. Neurofeedback enables to document cognitive state of mind.[15]Pouya et al., 2016 demonstrated that the Muse headband is well suited for differentiating cognitive states of mind by documenting subtle differences.[16] EEG data from 16 individuals was collected while watching a instructional video, recreational videos in two sessions.

Amplitudes of brain waves during EEG $_{\text{No activity,}}$ EEG $_{\text{Two-back memory task,}}$ EEG $_{\text{Learning Activity}}$ with average spectral power density values were compiled in Excel for delta, alpha, beta, theta, and gamma brain waves in Tp9, AF7, AF8, TP10 locations using Muse Monitor software.[17]

(a)



(b)

Figure 1: **(a)** Muse Headband. **(b)** Muse recording streaming through Muse Monitor app. Top left corner shows brain waves of delta, theta, alpha, beta, and gamma. Lower left corner with the horseshoe represents signal strength at 5 electrodes.

2.3.3 Light emitting diodes (LEDS) Lightbulbs

a) 1800 K LED

Five lights sources were used to (Two Corn Lamp LEDs manufactured by Sunlite at 1800 K, W 4.25/D 4.25/H 12 .5 inches, Two amber CFL Lights and one full spectrum light with red filter mounted a wooden plank with a peak wavelength at 612 nm and the 1000 Lux luminance balance. (Fig 2)

Figure 2 : Warm light (orange at 612 nm) set up on wooden plank and with a subject chair facing away from the light

b) 7000 K LED

A Corn lamp LED manufactured by AINIYO at 7000 K, W 5.8/ D 5.9/H 12.8 inches with peak wavelength at 450 nm was mounted on a same wooden plank and was used as second light condition with 1000 Lux luminance balance. (Fig 3)**.** Irradiance was documented for both the LED's with Spectrascan 670 Photometer (Photo Research, Syracuse, NY, USA).

Figure 3 : Cool light (Blue at 450 nm) set up on wooden plank and with a subject chair facing away from the light

2.3.4 Word pair Performance task

Word Pair Performance was the task employed in this study to measure each subject's recall and working memory. This task tests the system for learning numbers, words, or letters. The stimulus pair can be presented both verbally, both visually, or a combination of verbal-visual mode. Subjects respond based on the recall cues available at the moment.

In this study stimuli were presented verbally with a custom-designed program generated by Experiment Builder version 2.5.1 (SR Research, Ontario, CAN) to build auditory Word Pair performance tasks. Words were adopted from the Open American National Corpus with word frequencies ranging from 500 – 900 out of 22,164,985 tokens selected from texts of all genres and transcripts of spoken data since 1990. These words were converted to sound files and randomly paired based on categories; adjectives and nouns.

Fifty words pairs were presented during a 30-minute dark adaptation stage (See Appendix B - Word pair database). The subject learned these word pairs during dark adaptation. During light condition, word pairs were presented in random order and subjects were asked if the presented word pair was the same as it was presented during the learning phase. For instance, the word pair is "beautiful – campus" would be presented during the dark adaptation phase. Then, during the light condition, when this word pair is presented as "beautiful- campus" or "campus – beautiful", it would be considered the right word pair and subjects should respond "yes". If, on the other hand, a random word pair is presented such as "beautiful -day", it is a wrong pair and subjects should respond "no". Performance accuracy was recorded.

2.3.5 Two-Back Memory Task

Two-back memory task is a continuous performance task that is prominently used in psychology and cognitive research studies to test working memory. N-back was first introduced by Wayne Kirchner in 1958. N-back tests are those that require subjects to indicate when a current stimulus matches a stimulus presented in a previous sequence "N" steps earlier in the test. In the present

study, auditory two-back memory tasks were generated by Experiment Builder. Numbers 0 to 9 were used as the stimuli. The following variables were computed from the program:

- *Accuracy*. Accuracy is the ratio of total count of correct responses trials to total count of trials presented.
- *Reaction time.* Average reaction time of all correct responses and average of incorrect responses were calculated.

2.4 Procedure

This is a two-visit randomized cross over study. Each visit had two testing conditions (30 minute each). During the first 30 minutes, subjects received NO light exposure (Dark Adaptation). During this test condition subjects learned the word pairs and performed the two back memory tasks. During the second 30 minutes, subjects performed word pair association task and two-back memory task under light condition (blue, orange light). Real time measurement of EEG responses was recorded.

Subjects attended the study at the same time of day on two separate days. The light condition (peak 450nm or peak 612 nm) was assigned randomly during the first visit and the other light was used in the second visit. During both visits, the subjects spent the first 30 minutes with NO light exposure, 10 minutes of which included EEG collections with no activity while the last 20 minutes were devoted to obtaining EEG measurements while the subjects learned word pairs of 50-word pairs for 5 minutes for the Word pair performance task , 5 minutes of two back, and continuing to learn word pairs for word pair performance task for 5 minutes. After a 5 minute break, the testing light was turned on (blue or red) and the process was repeated in the same fashion. On a separate day for second visit the process was repeated using the as-of-yet to be used test light. In total, the testing sequence required one hour and twenty minutes to complete.

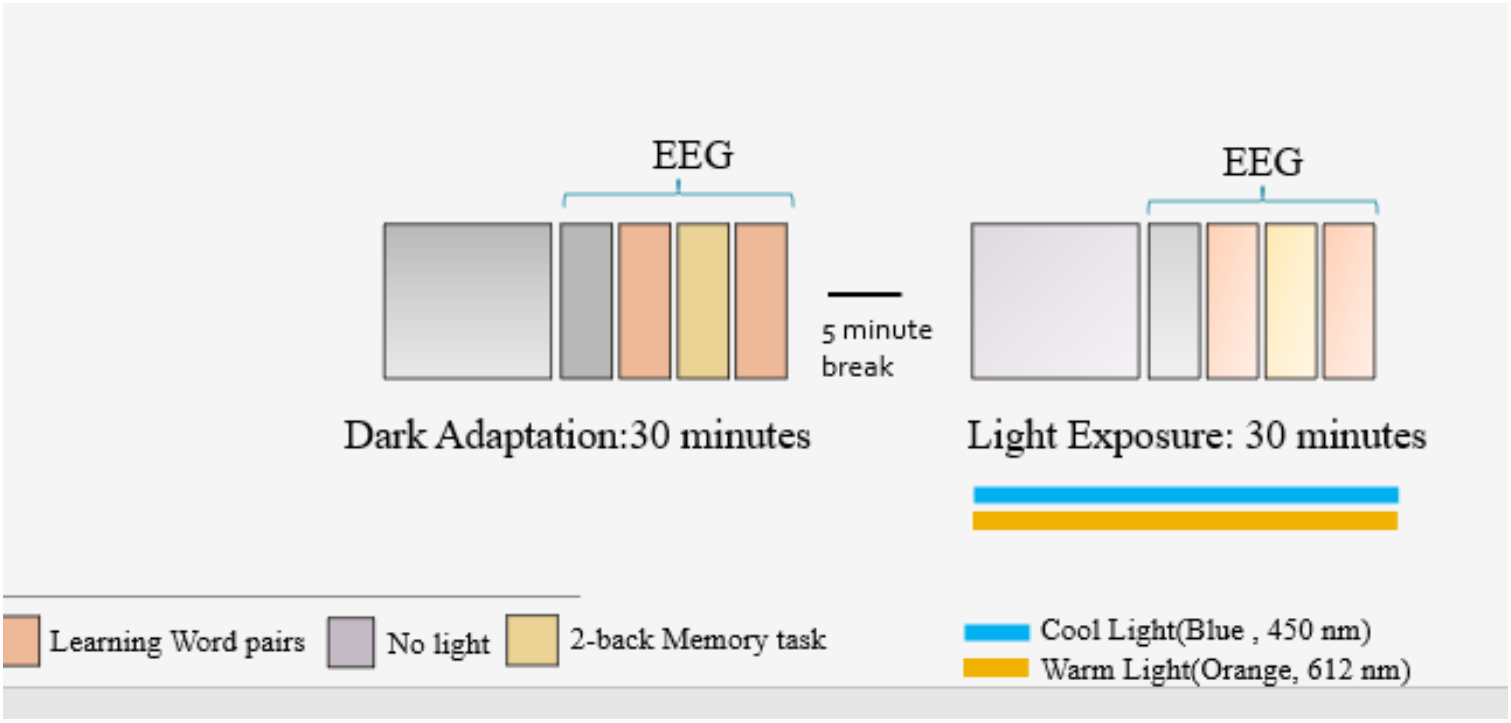


Figure 4: Representation of Experimental Procedure

2.5. Statistical Analysis

Two back memory task. Descriptive analyses were run to understand the population. Measures of means, medians, standard deviation and ranges were computed using SPSS software (IBM New York, USA). Mixed model analysis variance was used for statistical analysis and to test the hypothesis of equal light conditions.

Word pair performance Measures of means, standard deviation and ranges were computed using SPSS Software. Mixed model analysis of variance was used for statistical analysis and to test the hypothesis of equal light conditions.

EEG data acquisition Data was sorted based on subjectID, test, task and light color that was presented. Average amplitudes were computed for delta, beta, alpha, theta and gamma in AF 7, AF8, TP9 and TP10 locations. Principal axis factor analysis was applied to the 5 EEG waves by 4 locations (20) to reduce the dimensionality of the data. Mixed model analysis of variance was used to account for repeated measures and compute the estimates of parameters, means and standard deviations.

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CHAPTER 3: RESULTS

3.1 Two-back memory task

Two-back task was tested during dark adaptation and then again under the color light. Accuracy and response time were compared between the dark adaptation and the color light condition, and then again between the two color light conditions with performance during dark adaptation as covariate.

Accuracy

When compared to dark adaptation, there was no significant benefit of Blue light exposure on Accuracy ($Mean_{\_ Blue\ light}$ =0.51, $Mean_{\_ dark}$ = 0.49, Df. SD= 0.14, effect size= 0.10, p>0.05); No significant difference was found but a nontrivial increase in accuracy was observed between orange light and dark adaptation ($Mean_{\_ Orange\ light}$ =0.53, $Mean_{\_ No\ light}$ = 0.50, Df. SD= 0.11, Effect size= 0.23, p>0.05). (Fig 5)

Accuracy of two back memory task did not yield any significant differences between blue light and orange light. Mixed model analysis ANOVA was run to elicit means of accuracy and main effects of color of the light on accuracy ($Mean_{Blue\ light}$ = 0.50, $Mean_{orange\ light}$ = 0.53, F =0.62, p>0.05) (Fig 6).

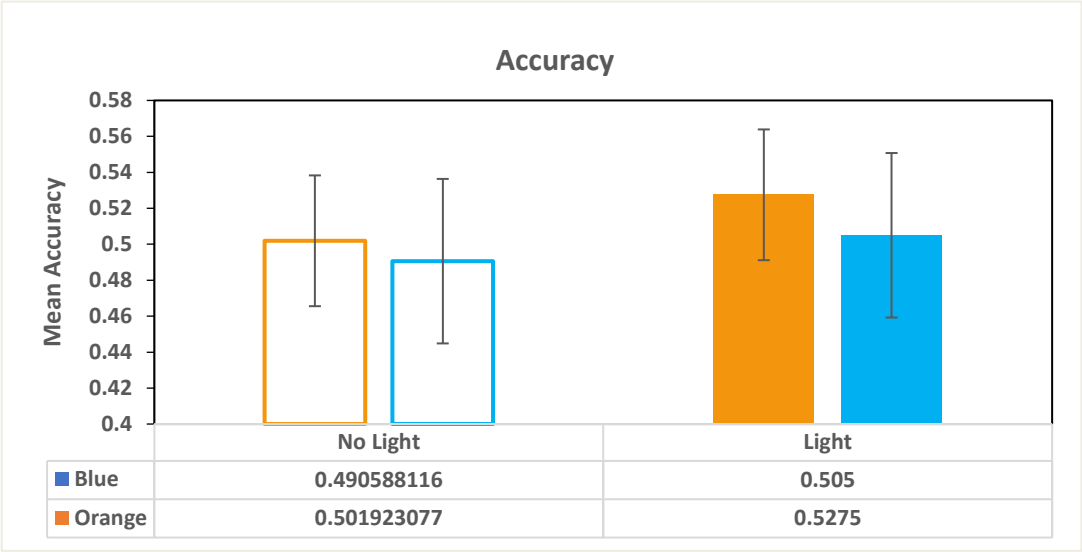


Figure 5: Means of Accuracy were plotted by Dark adaptation condition (No Light) to Color Light (Blue or Orange) condition.

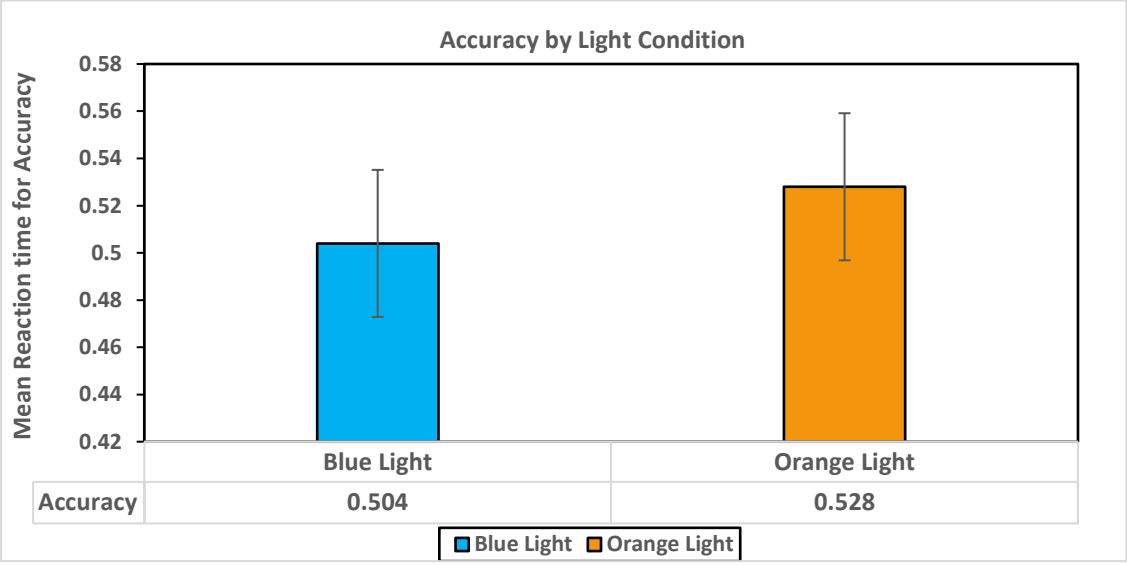


Figure 6: Estimated means of Accuracy comparison to Color Light (blue and Orange)

Reaction Time-Correct Responses for the two-back test

The small decrease in reaction time from dark adaption to blue light (effect size=-0.29) was not statistically significant ($Mean_{Blue\ light}$ =1200.81, $Mean_{no\ light}$ = 1272.1, Df. SD= 241.67, with Sig (2 tailed) p>0.05). (Fig 7)

The small increase in reaction time from dark adaption to orange light was not significant ($Mean_{Orange\ light}$ =1343.38, $Mean_{No\ light}$ =1357.05, Df. SD= 0.11, Effect size= 0.23 with Sig (2 tailed) p>0.05).

Mixed model ANOVA on reaction time for correct responses showed significant decrease in reaction for blue light relative to orange light Fig 8: $Mean_{Blue\ light}$ = 1226.28 , $Mean_{orange\ light}$= 1317.91 , F= 17.96 , p<0.001, effect size = -0.45)

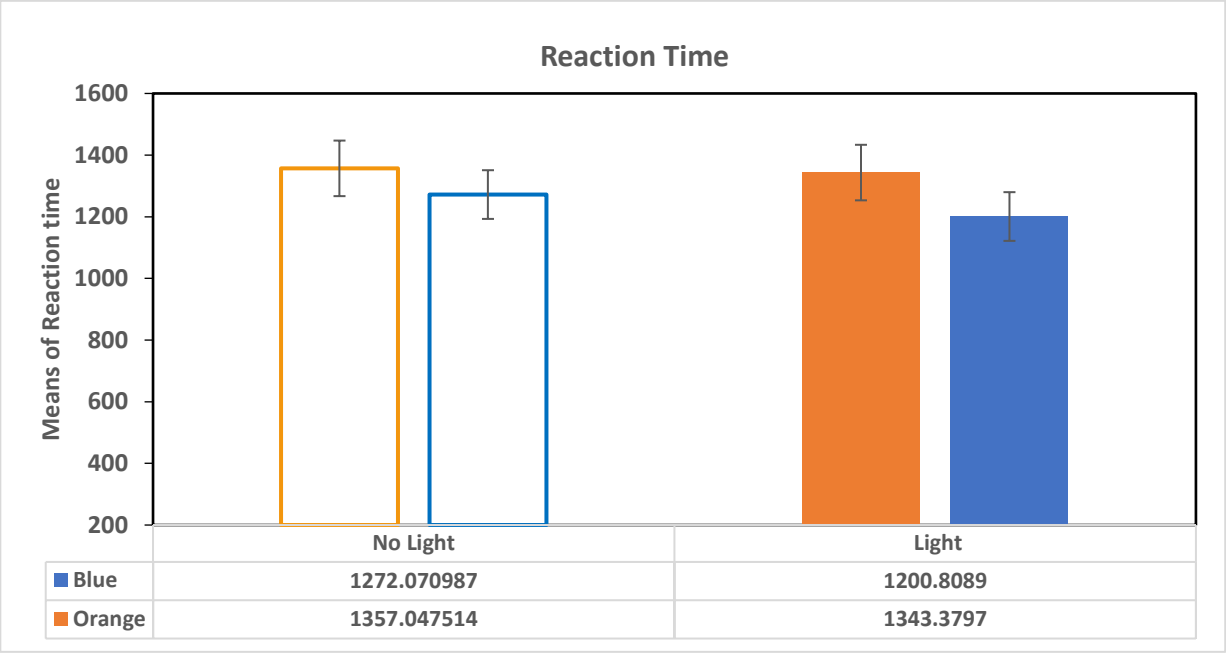


Figure 7: Means of Reaction time _correct responses were plotted by dark adaptation(No Light) to Blue Light condition. Means of Reaction time for Correct responses were plotted by dark adaptation(No Light) to Orange Light condition.

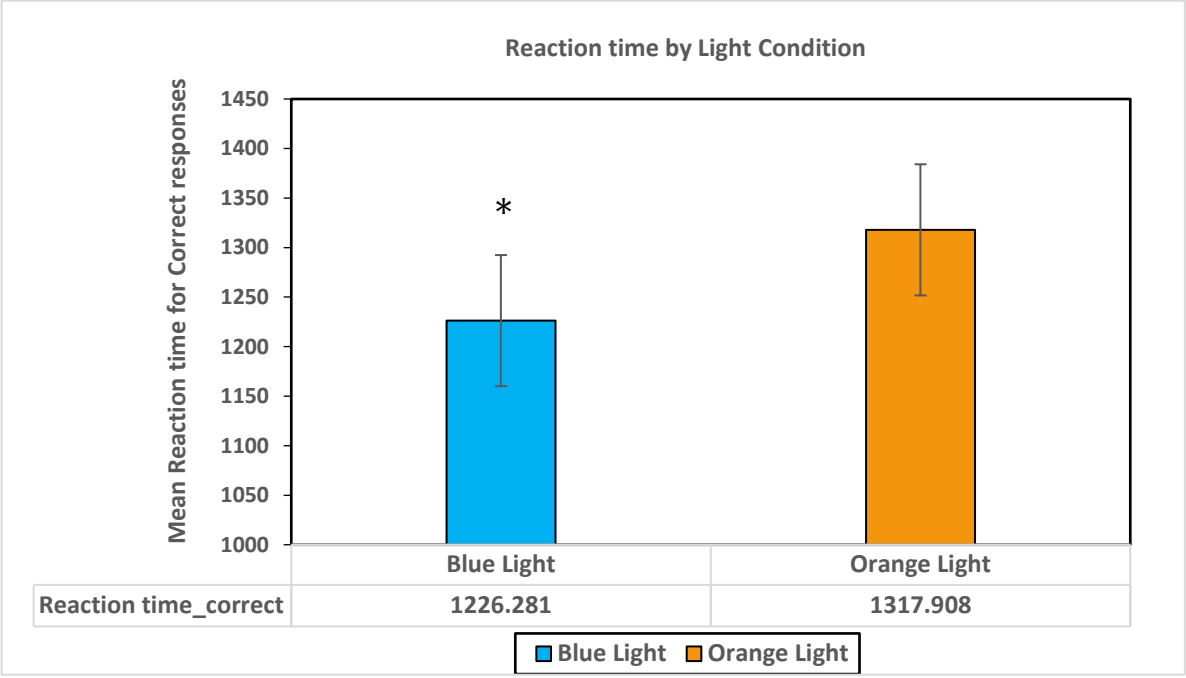


*Figure 8 : Estimated means of Reaction time for correct responses in comparison to blue light at 450 nm with an effect size of -0.45, *p<0.001 after adjusting for dark adaptation reaction time.*

3.2 Word Pair Performance task

Word pairs were learned for 10 minutes and then tested for 10 minutes during the dark adaptation and light conditions. Accuracy was compared between the dark adaptation and light conditions, and between the two color light conditions.

Performance in blue light was greater better than orange with a significance difference between Mean performance % $_{\text{Blue Light}}$ = 81.8% and Mean performance % $_{\text{Orange Light}}$=78.4 %, SD= 3.87with a large clinically relevant effect size= 0.88, *p<0.05.

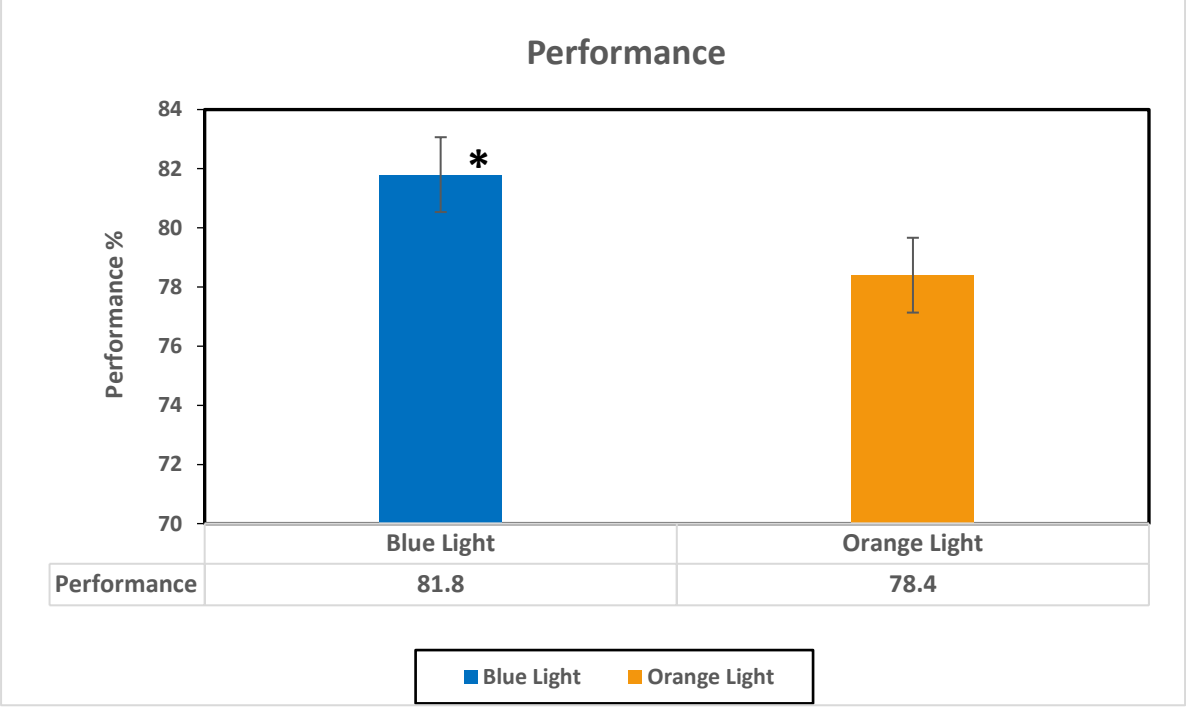


Figure 9: Graph showing performance % by light condition

3.3 EEG

Factor analysis was used to reduce the dimensionality of the 16 different brain waves and locations. AF 7 Alpha, theta, delta, beta, gamma, TP alpha theta delta, TP gamma beta, AF8 Theta delta alpha, AF8 beta gamma were identified as factors. Factor 1- AF 7 Alpha theta delta beta gamma is factor which involves left frontal lobe activation of all the brain waves, factor 2 – TP Alpha, theta, delta is activation of brain waves alpha, theta, delta in right and left temporal lobe, factor 3 – TP gamma, beta is activation of brain waves gamma and beta in right and left temporal lobe, factor 4 – AF 8 theta delta alpha is activation of brain waves theta delta, alpha in right frontal lobe and factor 5 – AF 8 beta, gamma is activation of brain waves beta, gamma in right frontal lobe.

3.3.1 EEG with No Activity

When compared to dark adaptation, there was significant increase in amplitude by Blue light exposure on factor TP Gamma beta (Fig 9: $Mean_{\_ Blue\ light}$ =0.28 $Mean_{\_ No\ Light}$ = -0.40, effect size= 0.74 with Sig (2 tailed) $p<0.05$) and no significant difference on other factors. Factor AF8 Theta delta alpha showed a significant increase in amplitude between orange light exposure and dark adaptation (Fig 10: $Mean_{\_ Orange\ light}$ =0.02, $Mean_{\_ No\ light}$ = -0.45, Effect size= 0.56 with Sig (2 tailed) $p>0.05$).

Factors did not yield any significance between the blue light and orange light conditions; however, TP Gamma beta showed a minimal clinical significance with effect size = 0.44 (Fig 11)

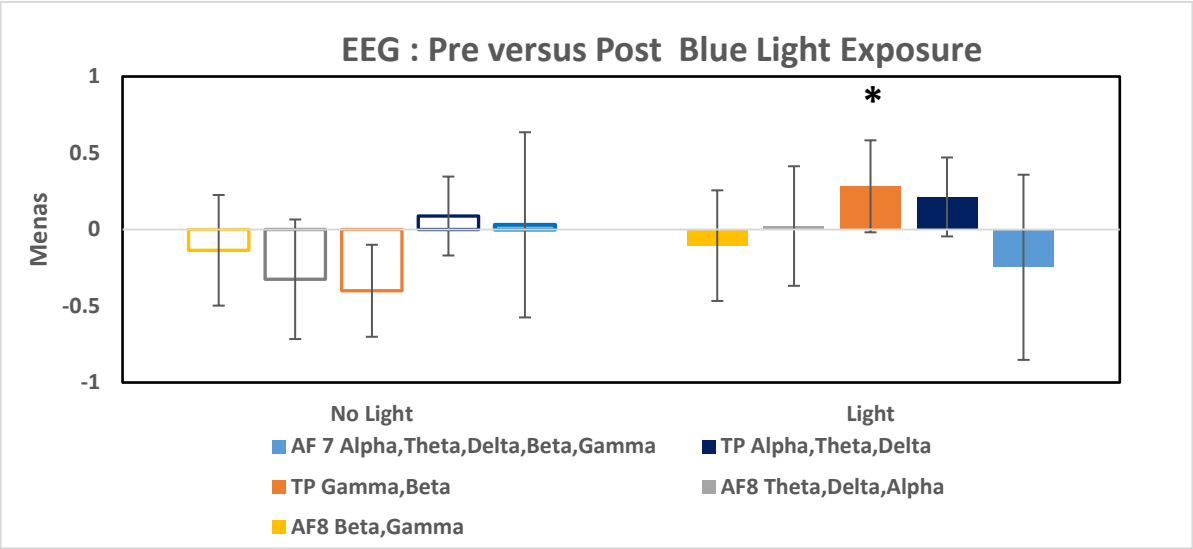


Figure 10: Graph showing the Mean amplitudes of EEG between the no activity portion of dark and light adaptation. The y-axis shows the deviation from a mean of zero in standard deviation units.

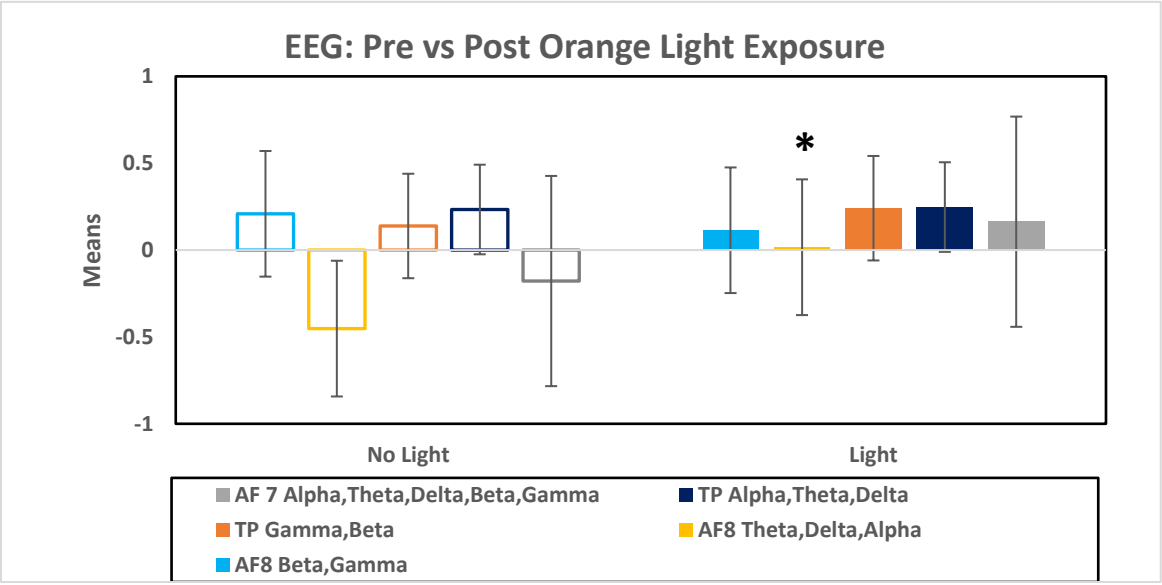


Figure 11: Graph showing the Mean amplitudes of EEG: No activity by Dark adaptation light to Orange light . The y-axis shows the deviation from a mean of zero in standard deviation units.

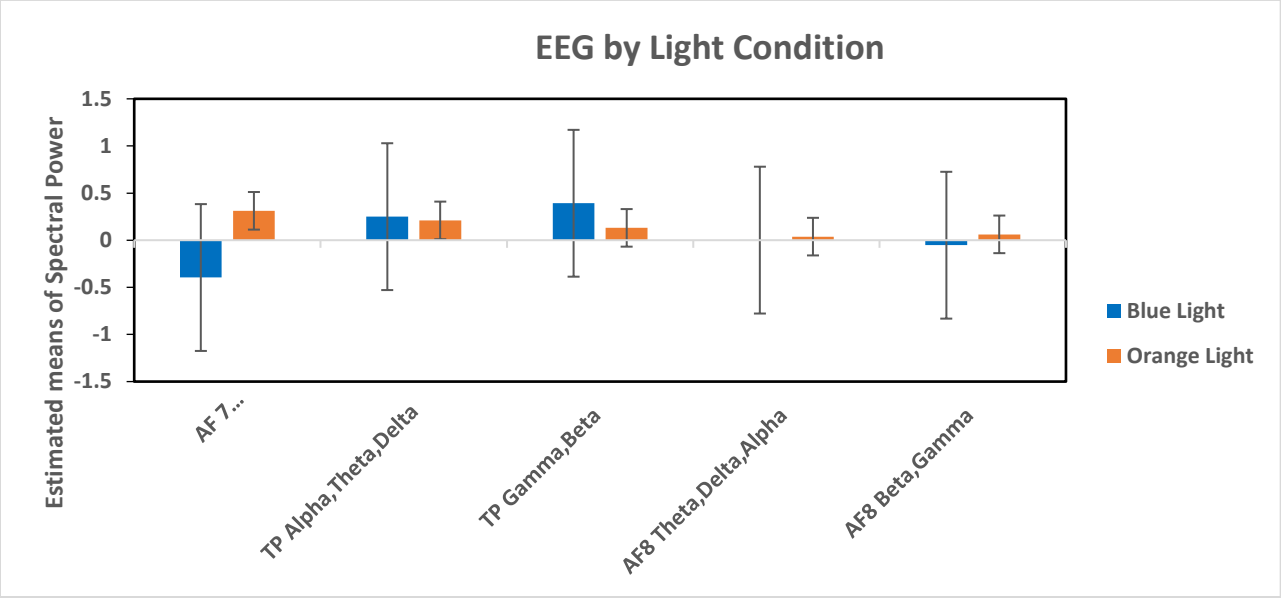


Figure 12: Graph showing the Mean amplitudes of EEG: No activity under Blue vs. Orange light .

Table 1 : Estimates of Mean Amplitudes of EEG No activity by light condition

	Blue Light	Orange Light	
EEG	Mean	Mean	Effect Size
AF 7 Alpha, Theta, Delta, Beta, Gamma	-0.39	0.31	-0.29
TP Alpha, Theta, Delta	0.25	0.21	0.06
TP Gamma, Beta	0.39	0.13	0.44
AF8 Theta, Delta, Alpha	0.001	0.03	-0.05
AF8 Beta, Gamma	-0.05	0.06	-0.23
Dependent variables did not reach statistical significance			

3.3.2 EEG: Learning Word pair task

When compared to dark adaptation (No light), there was significant increase of amplitudes by Orange light exposure on factor AF 7 Alpha, Theta, Delta, Beta, Gamma and a significant decrease in amplitude of AF 8 Beta gamma was seen and no significant difference on other factors (Fig 14). Factor AF8 Theta delta alpha showed a significant increase in amplitudes between orange light exposure with dark adaptation. Factor TP Alpha, Theta, Delta did yield significant increase in amplitudes with blue light at 450 nm to orange light at 630 nm with $p<0.05$ and clinical significance of 0.88 (Fig 13; Fig 15; Table 2 Estimates of Mean Amplitudes of EEG by light condition).

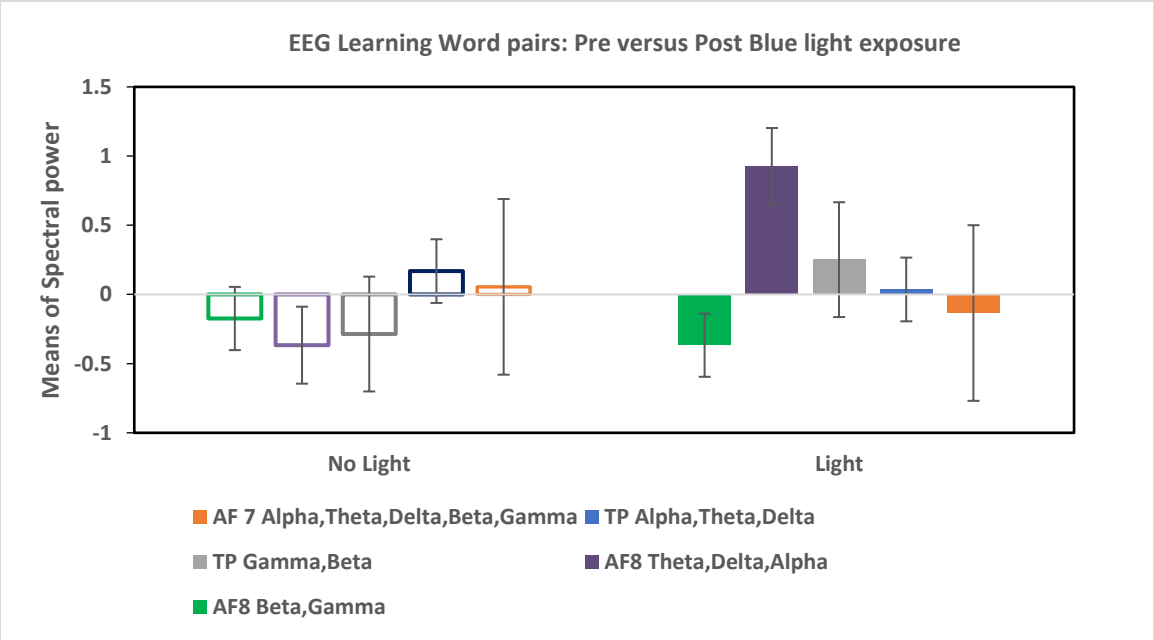


Figure 13: Graph showing the Mean EEG Amplitudes by Dark adaptation light to blue light

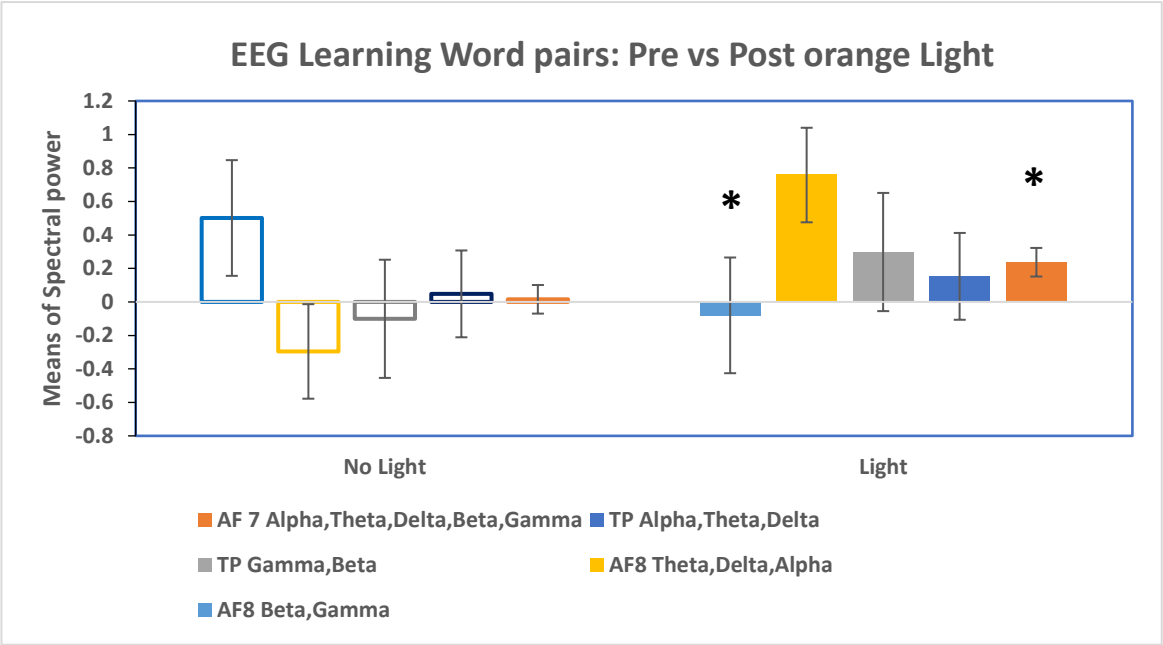


Figure 14: Graph showing the Mean EEG Amplitudes by Dark adaptation light to orange light

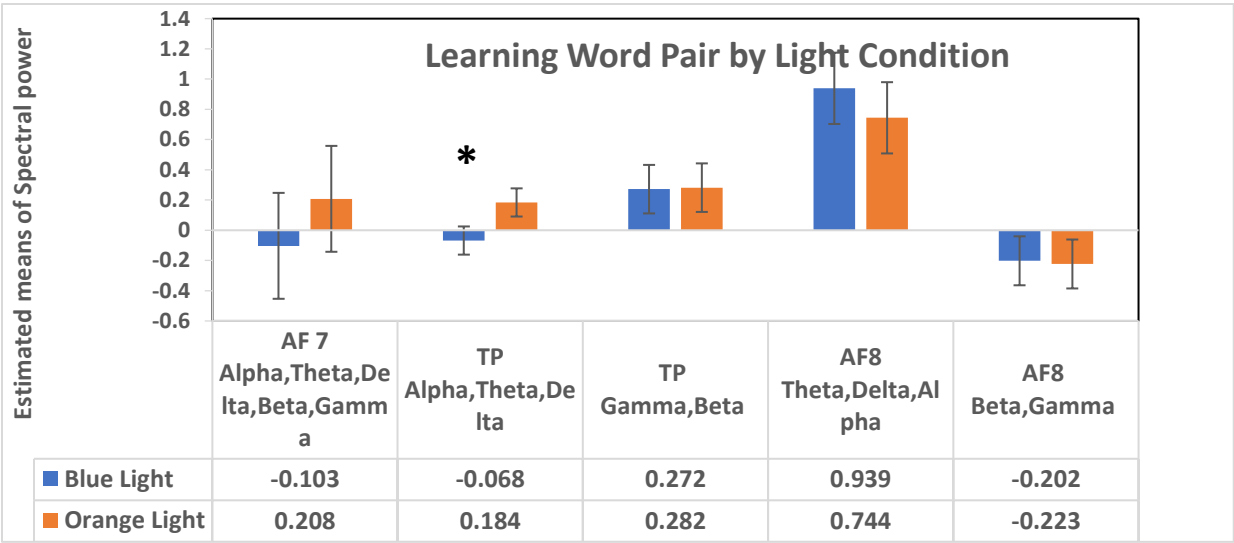


Figure 15: Graph showing the Mean amplitudes of EEG by light Condition

Table 2: Estimated means of main factors by light condition during EEG Learning Activity

EEG: Learning Word pair	Blue Light	Orange Light	Effect Size
	Mean	Mean	
AF 7 Alpha, Theta, Delta, Beta, Gamma	-0.10	0.21	-0.29
TP Alpha, Theta, Delta	-0.06	0.18	-0.88 *
TP Gamma, Beta	0.27	0.28	-0.02
AF8 Theta, Delta, Alpha	0.94	0.74	0.27
AF8 Beta, Gamma	-0.20	-0.22	0.04
* Dependent variables showed significance p<0.05			

3.3.3 EEG: Two back Memory Test

When compared to dark adaptation (No light), there was significant decrease in of amplitudes by Orange light exposure on factor AF 8 Beta gamma and no significant difference on other factors (Fig 16; Table 3 summarizes the mean amplitude differences between dark adaptation to Light condition). Factors did not yield any significant differences in amplitudes between the light condition- blue light at 450 nm and orange light at 630 nm with $p<0.05$ (Fig 17; Table 4 Estimates of Mean Amplitudes of EEG by light condition).

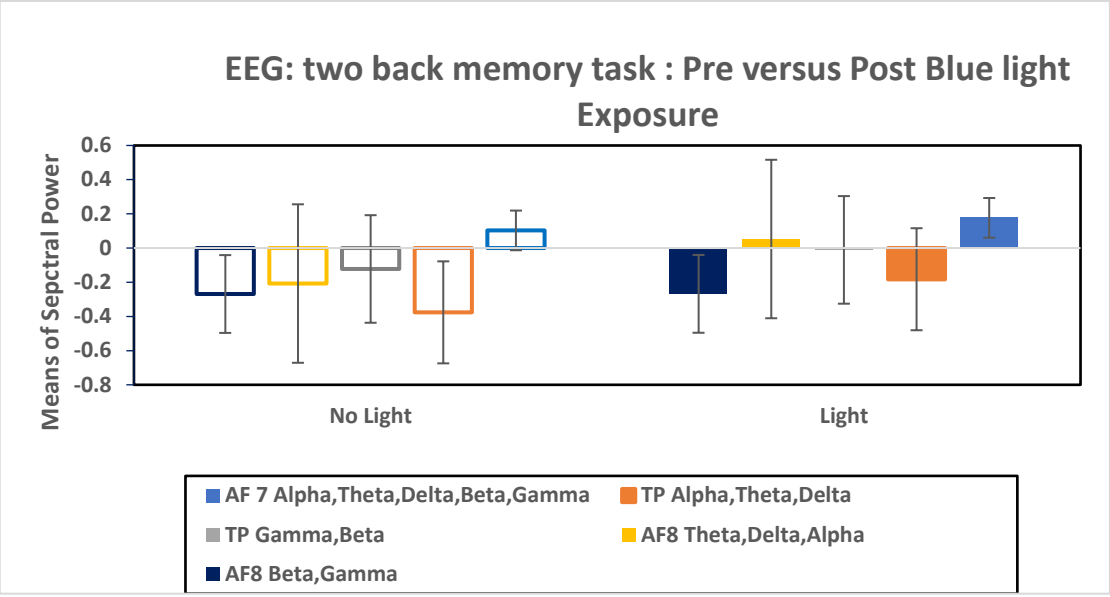


Figure 16 Graph showing the Mean EEG Amplitudes by dark adaptation to blue light

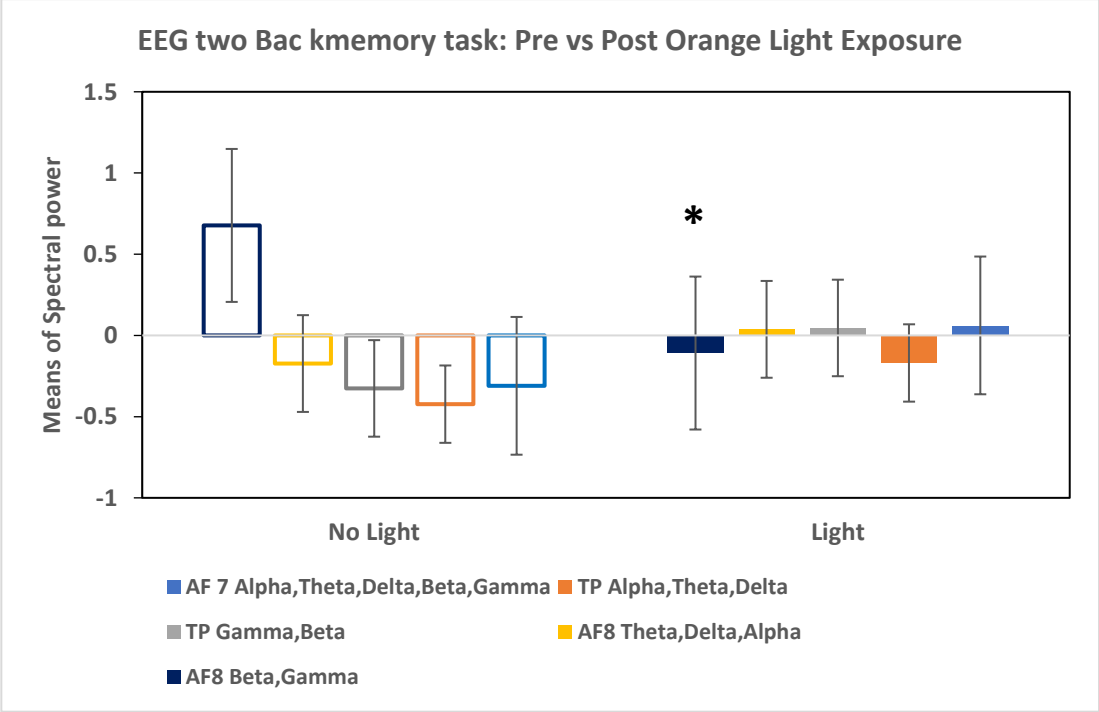
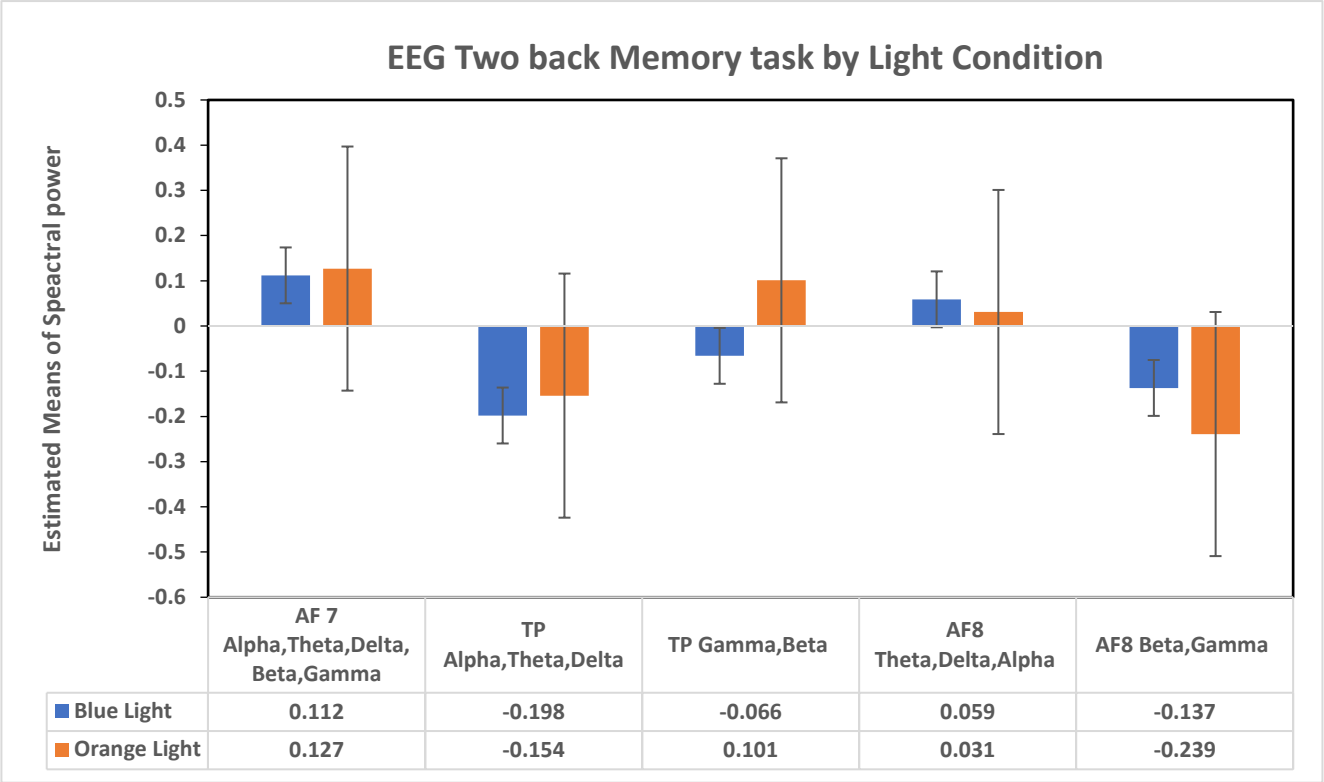


Figure 17: *Graph showing the Mean EEG Amplitudes by Dark adaptation light to Orange light*



	AF 7 Alpha,Theta,Delta, Beta,Gamma	TP Alpha,Theta,Delta	TP Gamma,Beta	AF8 Theta,Delta,Alpha	AF8 Beta,Gamma
Blue Light	0.112	-0.198	-0.066	0.059	-0.137
Orange Light	0.127	-0.154	0.101	0.031	-0.239

Figure 18: *Graph showing the Mean EEG Amplitudes by light Condition*

Table 3 Mean difference between dark adaptation to Light condition during Two -Back Memory Task

EEG: Two Back memory task	Dark adaptation to Blue Light			Dark adaptation to Orange Light		
	Df. Mean	Df.SD	Effect Size	Df. Mean	Df.SD	Effect Size
AF 7 Alpha, Theta, Delta, Beta, Gamma	0.073	0.35	0.20	0.37	1.29	0.28
TP Alpha, Theta, Delta	0.19	0.91	0.21	0.25	0.72	0.34
TP Gamma, Beta	0.11	0.96	0.11	0.37	0.90	0.40
AF8 Theta, Delta, Alpha	0.26	1.41	0.18	0.21	0.91	0.23
AF8 Beta, Gamma	0.0009	0.69	0.001	-0.78	1.43	-0.54 *
* Dependent variables showed significance p<0.05						

Table 4 Estimated Means of factors by Light condition

EEG: Two Back memory task	Blue Light	Orange Light	Effect Size
	Mean	Mean	
AF 7 Alpha, Theta, Delta, Beta, Gamma	0.11	0.13	-0.08
TP Alpha, Theta, Delta	-0.19	-0.15	-0.05
TP Gamma, Beta	-0.06	0.10	-0.23
AF8 Theta, Delta, Alpha	0.06	0.03	0.03
AF8 Beta, Gamma	-0.14	-0.24	0.16
Dependent variables did not reach statistical significance			

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CHAPTER 4 DISCUSSION

In the present study, we investigated how exposure to cool light and warm light affects objective measures of alertness using performance and EEG Brain wave powers at TP 9, TP10, AF7, AF8. Results are discussed by EEG brain waves and performance at the Two-back memory task and Word pair performance task.

EEG Brain waves

Our results showed that, in comparison to the dark adaptation (no light) condition, significant increase in the amplitudes of the TP gamma, beta Factor was seen under cool light and significant increase in amplitudes of AF 8 Theta, delta, alpha factor under warm (orange) light. Increased amplitude of Gamma, beta factor indicates increased alertness under cool light while increased amplitudes of theta, delta and alpha suggests more sleepiness under warm light suggests that less sleepiness (alertness) in cool light and more sleepiness in warm light. Accompanying to the increase in gamma beta amplitude, increased alertness is also manifested by faster reaction time in the two back memory task and better accuracy in the word pair task. While no literature was found to report gamma and beta activity together, our results are consistent with the animal study by Jan Kaminski et al., in 2012, which reported increase in beta oscillations activity with an increased alertness with faster responses of target stimulation.[18]

Our results report an increase in AF 7 Alpha, theta, delta, beta, gamma and AF 8 Beta, gamma in warm light ($p<0.05$) when compared to dark adaptation light with learning activity suggesting that with warm light there is more sleepiness. However, we could not establish any difference between cool light and warm light by brain waves. It might be because of the time of the day Brain waves during the two back memory tasks did not change significantly with cool light but AF 8 Beta, gamma showed a significant decrease with warm light ($p<0.05$). This suggests that less alertness is seen with warm light. However, in a study done by Levent et al.,[11] in 2013 showed a reduced alpha, theta and alpha, theta ranges with red light exposure during day time. This study suggests that exposure to orange light elicited higher alertness than blue light. These responses might be different with present study because they collected data with 470 nm cool light and 630 nm warm light during post lunch hours and our subjects were more during afternoon.

Results were encouraging in terms reaction time in two back memory tests which quantify alertness objectively. Reaction time is relatively faster with cool light (blue) condition at 450 nm when compared to orange light at 612 nm which is consistent with Chellappa et al.,[4] study who also showed faster reaction time with blue light. Their study did not demonstrate any exposure to blue light was effective in increasing cognitive performance or benefit to higher cognitive tasks. Our present study accuracy of the two back memory tasks showed a similar result. Accuracy did not reflect any possible understanding of effect between cool light and warm light condition. However, we could establish a better performance % with cool light.

Light illumination might have been a contributing factor to affect the present results. We controlled illumination at 1000 Lux for cool light and warm light. A study done by Min et al., 2013 reported that bright illumination might affect (reduce) the activity of alpha brain waves.

IpRGC can contribute to image formation function, these effects mediate through photoreceptors (rods and cones) which are sensitive to 480 nm.[6] In the present study we used 450 nm cool light and 612 nm warm light, results would have been more effective with 480 nm cool light and 630-680 nm warm light. Vandewalle et al. measured FMRI and showed a greater alerting effect after exposing to 480 nm than to long wavelength at 530 nm. Review report by E. Basar et al. in 2001, suggests that Brain wave oscillations are correlated with multiple aspects based on task, stimulus and structures involved with it. This review also reported that cognitive tasks also affect alpha activity and might result in increase in delta activity.[19] Moreover, present study is limited with 20 subjects. Color might be a confounding factor as more subjects were randomly assigned to Orange light condition as the first trial led which might also have a practice effect. Additionally, while handling EEG data acquisition, for single missing data point and bad signal we removed the entire data row for all the other locations. This might have potentially affected our results by reducing the number of seconds covered in each condition.

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CHAPTER 5 CONCLUSION

In the present study, we attempted to demonstrate how exposure to cool light (blue light at 450 nm,7000K) relative to orange light could enhance alertness and performance by employing EEG measuring instrument for brain wave activity, a two back memory task, and Word pair performance task. Increases in TP gamma, beta activity in cool light and less sleepiness compared to the dark accompanied with faster reaction time and higher percent correct suggests that alertness is cool light shifted. Activity of other brain waves factors AF 7 Alpha theta delta, beta, gamma, TP Alpha, theta, delta, AF8 Beta, gamma did not demonstrate any difference between the light conditions. Accuracy in two-back memory task did not report any differences between light conditions.

From the present study results, it should be noted that wide-ranging amplitudes of individual brain waves suggests that it is not possible to assign brain waves with a single functionality as these waves travel through different brain regions and hen can have multifacited functions. The present results extend to those studies who performed EEG and alertness in the daytime. Our results are encouraging and demonstrate some consistency with our hypothesis that exposure to cool light will enhance the alertness and performance relative to warm light and these effects may be mediated by Intrinsically photosensitive retinal ganglion cells which are sensitive to 450 -480 nm light. In future studies we plan to investigate Melatonin levels pre and post light exposure with 480 nm cool light and 680 nm warm light during morning and afternoon sessions with a higher sample size.

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APPENDIX A

Buysse et al 1989 developed Pittsburgh sleep quality index

Sleep Quality Assessment (PSQI)

What is PSQI, and what is it measuring?

The Pittsburgh Sleep Quality Index (PSQI) is an effective instrument used to measure the quality and patterns of sleep in adults. It differentiates "poor" from "good" sleep quality by measuring seven areas (components): subjective sleep quality, sleep latency, sleep duration, habitual sleep efficiency, sleep disturbances, use of sleeping medications, and daytime dysfunction over the last month.

INSTRUCTIONS:

The following questions relate to your usual sleep habits during the past month only. Your answers should indicate the most accurate reply for the majority of days and nights in the past month. Please answer all questions.

During the past month,

1. When have you usually gone to bed? _____
2. How long (in minutes) has it taken you to fall asleep each night? _____
3. What time have you usually gotten up in the morning? _____
4. A. How many hours of actual sleep did you get at night? _____
   B. How many hours were you in bed? _____

5. During the past month, how often have you had trouble sleeping because you	Not during the past month (0)	Less than once a week (1)	Once or twice a week (2)	Three or more times a week (3)
A. Cannot get to sleep within 30 minutes				
B. Wake up in the middle of the night or early morning				
C. Have to get up to use the bathroom				
D. Cannot breathe comfortably				
E. Cough or snore loudly				
F. Feel too cold				
G. Feel too hot				
H. Have bad dreams				
I. Have pain				
J. Other reason (s), please describe, including how often you have had trouble sleeping because of this reason (s):				
6. During the past month, how often have you taken medicine (prescribed or "over the counter") to help you sleep?				
7. During the past month, how often have you had trouble staying awake while driving, eating meals, or engaging in social activity?				
8. During the past month, how much of a problem has it been for you to keep up enthusiasm to get things done?				
9. During the past month, how would you rate your sleep quality overall?	Very good (0)	Fairly good (1)	Fairly bad (2)	Very bad (3)

Scoring

Component 1	#9 Score	C1 _____
Component 2	#2 Score (<15min (0), 16-30min (1), 31-60 min (2), >60min (3)) + #5a Score (if sum is equal 0=0; 1-2=1; 3-4=2; 5-6=3)	C2 _____
Component 3	#4 Score (>7(0), 6-7 (1), 5-6 (2), <5 (3)	C3 _____
Component 4	(total # of hours asleep) / (total # of hours in bed) x 100 >85%=0, 75%-84%=!, 65%-74%=2, <65%=3	C4 _____
Component 5	# sum of scores 5b to 5j (0=0; 1-9=1; 10-18=2; 19-27=3)	C5 _____
Component 6	#6 Score	C6 _____
Component 7	#7 Score + #8 score (0=0; 1-2=1; 3-4=2; 5-6=3)	C7 _____

Add the seven component scores together _____ *Global PSQI* _____

A total score of "5" or greater is indicative of poor sleep quality.
If you scored "5" or more it is suggested that you discuss your sleep habits with a healthcare provider

APPENDIX B

Word pair Performance task: Please follow the link for word pair database that is used for the auditory program.

https://1drv.ms/x/s!ApA8Gxdvyz4k7Rrx9eLbj7m8D1i0

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Curriculum Vitae

Summary

• Research Assistant at Vision Performance Institute, Pacific University, Oregon

• Teaching Assistant for Ophthalmic dispensary course in fall 2018

• Workplace responsibilities as Optometrist included recording patient history, vision assessment, Retinoscopy, slit lamp examination and dilated posterior segment evaluation

• Performed Visual Field Testing, Optical Coherence Tomography, and assessed Intra ocular pressure through Tono pen or applanation.

• Experienced user SPSS & MATLAB programming

• Experience in performing and develop Eye tracking & related experiments with SR Eyelink, it's an experiment builder to develop a computer-based eye tracking & related experiment

• Active student representative in Vision Science graduation committee at Pacific University , my role involves curriculum development, student affairs, contributing my decisions for Vision science program development.

Education

Master Of science in Vision Science – Pacific University, Oregon,2019

Professional Course Certification -Design & Interpretation of Clinical Trails – Johns Hopkins University,2018

Bachelor of Science in Optometry – Birla Institute of Technology & Science, Pilani Jul 2015

Technical Skills

• Statistics and programming: SPSS, MATLAB (beginner), SR Eyelink (Computer programming to develop eye tracking & related experiments)

• Ocular Diagnostics: Optical coherence Tomography-OCT (Anterior & Posterior), Corneal topography, Slit lamp photos, Fundus photos, Humphrey Visual fields.

• Electro diagnostics: Electroencephalography, Electrooculogram, Electroretinogram

• ELISA analysis – BIORET, Gen5

• Additional Software: MS Office- Word, Excel, Endnote, RefWorks

Certifications

• Collaborative Institutional Training Initiative CITI certification
https://www.citiprogram.org/verify/?w2414fcd0-bd6a-42f9-8dba-71acaa7d1d57-27824945

• Health Insurance Portability and Accountability Act of 1996 HIPPA Certification
https://www.healthstream.com/HSAPP/Transcript/Certificate?courseInstanceId=7c3d0787-0187-e811-bfce- 005056b15f08

• National Institute health certification

• CPR certification

Member of

• American Academy of Optometry

• Optometry council of India

• India Vision Institute

Working Experience

- *Research Assistant, Pacific university, Oregon August 2017 – May 2019*

The Vision Performance Institute (VPI) is a leading research organization in the vision sciences. Job responsibilities were to create a study protocol as per IRB guidelines and process the documents for approval from IRB, recruit subjects and collect the data, enter the data and perform statistical analysis. Responsible to execute the projects within the timeline, perform research tasks which involve data recruitment, performing procedures, data analysis. Skills: SPSS, MATLAB, SR EYE LINK, Powerref

- *Teaching Assistant Pacific University, Oregon Fall 2017 Semester*

Coursework: Ophthalmic dispensing, 2 hour per week Assist in teaching lab coursework, guiding optometry graduate students during lab proficiencies

- *Ophthalmic dispensing, Pacific University, Oregon September 2017 – January 2019*

Objective of this position is to make prescription eyeglass lenses by cutting, grinding, edging, and finishing the lenses according to instructions provided by dispensing opticians, optometrists or ophthalmologists.

- *Adjunct Optometrist- Glaucoma department, L.V. Prasad Eye Institute, Oct 2015 – Nov2015*

Detailed posterior segment evaluation (Retina and Glaucoma evaluation) , Disc evaluation, HVF interpretation and diagnostic tools usage. Administered visual field tests, eye drop administration for dilation, tonometry, fluorescein angiography Gdx, refract patients, and assisted Doctor in minor surgeries for pediatric refraction on table

- *Optometrist- Comprehensive, Orient Eye Hospital, since Feb 20,2016*

Comprehensive eye examination and patient counselling

- *Clinical Internship, L.V. Prasad Eye Institute, Hyderabad, Jun 2014 - Jul 2015*

  Comprehensive Eye Examination

  Refraction: Objective and subjective refractive assessment (case specific)

  Slit lamp examination (Anterior segment &Posterior segment evaluation)

  Contact lens dispensing and Low vision device dispensing

  Observation in Neuro/Genetic /Vision science research work and relevant knowledge gain.

  Hands experience with advanced research equipment in area of field

  Ocular Diagnostics –Optical coherence Tomography-OCT (Anterior & Posterior), Corneal topography, Slit lamp photos, Fundus photos, Humphrey Visual fields.

Research Work

1. Intrinsically Photosensitive retinal ganglion cells and its effect on human alertness master's Thesis, Pacific University, Oregon

The main aim of the study is to determine the effects of stimulated IpRGC's at 480 nm on human alertness and to differentiate the powers of Brain waves by using EEG

Internal funding Student faculty research fund, Haynes Foundation.

2. Objective measurement of cataract using Hartmann-Shack scanning Aberrometer - L.V. Prasad Eye Institute, Hyderabad.

The main aim of the project is to develop a new objective method based on objective scatter index to estimate the amount of scattering that effects severity of cataracts.

Academic Achievements

Vision Science Student fund 2018

Kikuchi Scholarship 2017

Zeiss scholarship for academic year 2011.

First prize for poster presentation in 2013 for world optometry day conducted in university of Hyderabad

Participated in screening camps conducted by ICARE (LVPEI)

Participated in Myopia-current research trends and treatment strategies workshops-India vision institute