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Original Research Article

Electroretinogram and Visual Evoked Potentials recorded during Visual Imagery with closed eyes in humans - Temporal association between electrical activation of retina and visual cortex

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Abstract: ***Background:*** Visual Imagery is one of the extensively studied properties of human mind. Few of the previous studies showed centrifugal modulation of retinal ERG during visual imagery and few others showed activation of different brain areas. In the present study simultaneous electrical activation of retina and visual cortex during visual imagery was recorded in the absence of light and the characteristics of the ERG and VEP waveforms were studied. ***Methods:*** Ten healthy right-handed volunteers in the age group 25-30 years were involved in a free imagination task. Flat EEG electrodes connected to skin surface near eyes and the scalp were used to acquire ERG and EEG. Using the LabChart pro v8.9.1 software the amplitudes and times of ERG, VEP, and b waves were calculated and presented as median values. Temporal association between the activation of left and right eyes and left eye and visual cortex was studied. ***Results:*** Pattern Reversal type VEP with amplitude of 21.79µV [IQR 15.44-28.06] from O_2 and 19.99µV [IQR 15.68-29.97] from O_z were recorded from the scalp EEG. ERG, with amplitude 69.66µV [IQR 44.34-144.68] was predominantly recorded from left eye and b waves from right eye. ERG in left eye was followed by b waves in right eye by a time gap of 279.44±8.68ms mean value. VEP from O_2 were observed to follow ERG from left eye with variable times. ***Conclusions:*** Analysis of retinal signals suggest possible photoreceptor and postsynaptic cell activation, probably Bipolar cells, during visual imagery. VEP recoding suggests that, after centrifugal retinal activation, free visual imagery follows the visual pathway to reach the cortex. Retina hence is considered as a two-way transducer.

Keywords: Descriptors: Visual Imagery, ERG, VEP, Temporal association.

INTERDUCTION

Visual Imagery is one of the intriguing functions of human mind and thus is a good subject of study to many scientists. Whether done at a more basic physiological level or as an application in clinical neuroscience or psychology, the study promises fascinating results every time it is explored. Visual Imagery which was considered as non-veridical form of phenomenal vision continued to pose challenges to the researchers in finding out neural substrate for imagination. Various studies being done for 30 years have been attempting to answer one major question, are the visual imagery and visual perception, seemingly two independent processes, share a common substrate and if so for what extent (Bertolo, 2005). Most of them focused their study on various brain areas modulated during imagery and visual perception. Starting from a basic EEG recording to the sophisticated f-MRI and PET scans, studies demonstrated activation of cortical and subcortical areas similarly or differently during imagery and visual perception. Special attention was given to activation of the visual cortex, as the results were challenging being inconsistent. For instance, a PET scan study showed activation of area 17 during visual imagery (Kosslyn, Ganis, & Thompson, 2001) and another fMRI study showed activation of lateral geniculate nucleus along with the primary visual cortex (Chen *et al*., 1998). However, some other PET study revealed no activity in primary visual area during visual imagery (Mellet *et al*.,

1996). This disparity could probably be due to the ability of the individual participants involved in the study to imagine the given condition. As the visual imagery experience is difficult to assess and objectivise, various questionnaires and indices have been developed to substantiate the experimental findings. While a major group of scientists were involved in exploring the brain areas and hence the neural substrate for visual imagery, few other scientists started to study the role of centrifugal activation of the retina during visual imagery, wanting to address the receptor function in imagery. The power of imagination with variable strengths is found in all animals. It appears that evolution has made this character highly selected and enhanced in humans. Evolution of imaging and dreaming in warm-blooded animals and the importance of corticofugal innervation of mentally imaged sensations has been studied and documented (Kunzendorf, 2015). Centrifugal connections to retina have been found in fishes, reptiles, birds, and other mammals serving some important neuromodulatory and behavioural functions (Viktoria, 2007). They were also found in human optic nerve (Wolter & Knoblich, 1965). Centrifugal pathways to retina arise from few sub cortical areas which may be involved in sub-conscious perception of vision, emotions, and sleep states (Mazard *et al*., 2004). To describe the role of these centrifugal fibers, hence, has been an intriguing study to many. Studies done in these lines have shown that the retinal electrical activity (ERG characteristics) was modulated by the centrifugal fibers in human subjects upon presenting different experimental light flashes along with conditional stimuli. That centrifugal pathways play an essential role in learning in humans has also been proposed (Kunzendorf, Justice, & Capone, 2007). Recently, retina was found to be electrically activated by imagery producing ERG recorded through skin electrodes even in the absence of light stimulus probably occurring through the centrifugal fibers (Aswin & Rajkumari, 2017). Hence, the studies being carried on two different streams, one side exploring the brain areas involved in imagery and on the other side the receptor modulation need to be unified to a common point. The external world being transduced and internalized by the retinas is fed to the hierarchy of brain areas for parallel and interdependent processing ultimately helping in visual perception (DeYoe & Van Essen, 1988). In contrast, whether the visual imagery, which begins with either creation or recall of an internal world and ends in perception of this internal world is using the retina as a screen to project the imagery to same cortical areas or is using an alternate pathway must be addressed. The former idea seems logical because, the retina, which evolved as the transducer to internalize the world into the brain, may also be used by the brain to experience the internal world, which, we are calling visual imagery. Is visual imagery really a non-veridical form of phenomenal vision where there is no retinal involvement or is retina and thence visual pathway are active during visual imagery is the question addressed by the present study. If the activation of visual cortex follows the retinal activity, that means, the imagery is using the same neural substrate, the visual pathway, for its perception. Hence, our study attempts to record ERG and VEP waveforms from the retinas and primary visual cortex respectively without a stimulus and to further find out a temporal association between the two events.

While it is true that few visual experiences like phosphenes and after images do not need the involvement of retina, it may also be true that a visual experience involving complex spatio-temporal content will need retina for processing and the visual cortex for the subsequent perception of the imagery. As the imagery is highly subjective, we cannot consistently record the ERG or VEP with one standard instruction to the subject like in asking them to imagine an object, character etc. They must be let free to imagine for a reasonable time to make them flow with thoughts and continuity in the imagery. Also, a VEP is recordable in a usual clinical setting only after signal averaging as it is a weak signal. Therefore, our study encourages free visual imagery by the subject for a reasonable time to acquire the ERG and VEP waveforms. Presence of Visual Evoked Potentials (VEP) is supposed to be the test to demonstrate integrity of visual pathway. Flashes of light thrown into eyes during EEG recording shows VEP. Therefore, by showing that during visual imagery with closed eyes, measurement of ERG from retinas and VEP from cortex is possible, it shall be proved that the retina is the screen for the visual imagery and visual pathway is the route followed by the imagery to reach cortex. Retina could therefore be treated as the two-way transducer. Once proved that retinas are activated during imagery, the analysis of waveforms from both eyes may be used to understand inter-eye differences in processing of the imagery and to ascribe the nature of the waves to the cellular substrate responsible for their production. Further, the temporal association between the events at retina and the cortex may be established in terms of the time difference between the onset of an ERG signal in eye and the VEP signal in cortex. The present study hence tries to record ERG and VEP during visual imagery and to study their characteristics.

Thus, this simple electro-physiological study acquires the ERG and VEP from the retina and visual cortex respectively during visual imagery with no stimulus. It also approximately measures the characteristics of the waveforms and few temporal associations between the retinal and cortical events.

METHODOLOGY:

Ten healthy right-handed volunteers (6 male and 4 female) in the age group of 25-30 years were involved in the study. An informed consent was taken from each as per the Institutional Ethical Committee guidelines. A brief questionnaire was administered to know about the habits and addictions, to rule out subjects with known addiction for smoking, alcohol, and drugs. Any significance in medical and surgical history was

considered for exclusion. After receiving the signed questionnaire, the study was initiated in the adequately dark laboratory. The subject was seated in a comfortable chair and asked to close the eyes for a dark adaptation period of 20 minutes (D'Anguilli, 2002). The skin was prepared with 7-% w/v isopropyl alcohol swab and with abrasive gel. Ten20 conductive EEG paste supplied by D.O. Weaver and Co., Aurora, USA was used to firmly place all electrodes in position. MLAWBT9 EEG gold plated flat electrodes were used and connected as four channels to the 8/35 Powerlab hardware provided by AD Instruments. Two channels were used to record the electrical activity from both retinas, surface electrodes placed near the lower lid and forehead (Leguire & Rogers, 1985; Esakowitz, Kriss, & Shawkat, 1993). As the subject is required to remain with eyes closed throughout the study, surface electrodes were chosen to reduce the discomfort. O_z and O_2 were marked on the scalp with a marker as per 10-20 International system. Two more channels recorded the Electroencephalogram (EEG) from the scalp points O_z and the O_2, referenced to the vertex (Odom *et al*., 2016). A Velcro strap was used to keep all electrodes in place and support the eye cover. After ensuring twenty minutes of dark adaptation the subject was asked to begin the free imagination. He/she was advised to imagine picture or scene of their choice keeping the eyes closed and trying to avoid the eye movements. Deliberate efforts to fix the eyes were discouraged. The LabChart Pro v8.1.9 software was used to record the data from all four channels under appropriate filter and frequency ranges (Odom *et al*., 2016). EEG from the two channels O_z, O_2 and electrical retinal activity in the form of complete Electroretinogram (ERG), isolated positive (b) waves and various components of ERG from other two channels were acquired simultaneously (Figure 1). Each subject was advised to carry on the imagery for about 25 minutes, after which, they were hinted to stop the process. After few minutes of relaxation, the subject was asked to narrate their imagery and their experience through a second questionnaire. As there was no stimulus presentation to evoke the VEP or ERG, the signal could not be time locked and averaged. Hence, manual identification and analysis of the waveforms was carried out (Figures 2-5). The overall data from all channels was carefully evaluated for the presence of Visual Evoked Potentials (VEP), ERG, and b waves of ERG.

EEG from O_z and O_2 showed predominantly Pattern Reversal type of VEP (PR-VEP) followed by Pattern Onset-Offset type. The present discussion considers characteristics of PR-VEP only for analysis as it has relatively little variation between subjects, and the inter-ocular difference is also minimal with a given subject (Odom *et al*., 2016). Amplitude of the PR-VEP (amp-VEP) was measured peak-to-peak from the first negative deflection (corresponding to N75) to the immediate positive deflection (corresponding to P100) (Figure 3). Time-to-peak (ttp-VEP) was calculated from the first negative deflection to the first positive deflection. The ISCEV standards to measure time were not followed as the stimulus onset time could not be found out in this condition of free imagery. Record from left eye showed predominantly complete ERG waveforms (LE-ERG) and from that of right eye showed isolated b waves of ERG (RE-b). LE-ERG was analysed further to calculate the amplitude (amp-ERG) and time-to-peak (ttp-ERG) taken from the negative wave to the peak of positive wave as the stimulus onset could not be marked in this case (Figure 4). The isolated b waves in right eye (RE-b) were also calculated for the amplitude (amp-b) taken from baseline to the peak. Hence, from each subject, four different waveforms were collected, calculated, and analysed. The PR-VEP from O_z (O_zVEP) and O_2 (O_2VEP); the LE-ERG and RE-b. Each again measured for amplitudes and times as amp-O_zVEP, ttp-O_zVEP, amp-O_2VEP, ttp-O_2VEP, amp-ERG, ttp-ERG, amp-b (Table 1). All observations were presented as median values with inter quartile range Q1 and Q3. Mean values were represented with standard error.

RESULTS:

The total number of PR-VEP recorded from all the subjects from the visual cortex were 170 (87 from O_z and 83 from O_2). Amp-VEP compared between O_z and O_2 are 19.99 µV [IQR 15.68-29.97] and 21.79 µV [IQR 15.44-28.06] respectively, showing greater activation (about 9% more) of right visual cortex during imagery (Figure 6). Similarly, ttp-VEP values were 40 ms [IQR 35-50] and 45 ms [IQR 35-52.5] from O_z and O_2 respectively. The total number of LE-ERG recorded from all the subjects were 155 and the total number of RE-b were 116. The values of amp-ERG were 69.66 µV [IQR 44.34-144.68] and the ttp-ERG were 200 ms [IQR 160-260]. Amp-b values were 121.18 µV [IQR 69.68-215.28], showing 42.5% higher amplitudes than ERG (Figure 7). Interestingly, 93% of the total RE-b (108 out of 116) were observed to follow the LE-ERG in time, after a gap of 280ms [IQR 220-340], mean value 279.44±8.68ms. The above results suggest that during visual imagery, even with the eyes closed, the retinas and the visual cortices are activated electrically producing a measurable ERG and VEP respectively. ERG in left eye were followed by b waves in right eye with a greater amplitude and the time gap between them when analysed for the 116 observations, showed a near normal distribution (Figure 8). These findings suggest that the retinas are activated during imagery, probably by the functional centrifugal fibers reaching the retinas from the imagery areas. Subsequently, the activated retinas send impulses through the visual pathway to excite the visual cortex. As the left eye predominantly showed ERG waveforms, the impulses from the left eye must have reached the right visual cortex to produce O_2VEP. Hence, it is logical to calculate the time interval between the appearance of LE-ERG and O_2VEP. This time interval (t-diff) was measured from the start of the LE-ERG to the start of O_2VEP. It was found that 32 out of 83 total O_2VEP were observed to follow the LE-ERG with variable time intervals. The distribution of these t-

diff values was not normal with median value of 14.81s [IQR 6.91-28.16]. High internal variance in the observations of ttp-ERG, or ttp-O_2VEP as well as t-diff made it difficult to arrive at a correlation between these parameters. To find a temporal association between the retinal and cortical events, ttp-ERG, t-diff, and the ttp-O_2VEP for all the 32 observations of O_2VEP following a given LE-ERG were added up to arrive at a value called t-tot. The idea was to find out the relation between individual times to the t-tot. Through many trials it was found that the values of $\log_{(ttp-ERG)} t-tot$ and $\log_{(ttp-ERG)} t-diff$ showed a near normal distribution with n=32 (Figure 9,10). For the values of $\log_{(ttp-ERG)} t-tot$, the mean value was 1.823±0.04 and median, 1.81 [1.76-1.94] with variance of 0.05. Similarly, for the values of $\log_{(ttp-ERG)} t-diff$, the mean value was 1.816±0.06 and median value was 1.80 [IQR 1.75-1.94] with the variance of 0.05. This logarithmic relation suggests that the t-tot and t-diff are a function of the ttp-ERG which needs to be evaluated using mathematical modelling with more number of observations in the future studies.

Table 1: Distribution of various waveforms and their characteristics

No.	Waveform identified	Total no. of waveforms identified for analysis	Calculated Parameter	Median value with Inter Quartile Range	Mean with SE
1	O_zVEP	87	amp-O_zVEP	19.99µV [15.68-29.97]	23.92±1.26
			ttp-O_zVEP	40ms [35-50]	44.55±1.47
2	O_2VEP	83	amp-O_2VEP	21.79µV [15.44-28.06]	24.26±1.42
			ttp-O_2VEP	45ms [35-52.5]	44.37±1.44
3	LE-ERG	155	amp-ERG	69.66µV [44.34-144.68]	103.61±6.42
			ttp-ERG	200ms [160-260]	222.19±7.91
4	RE-b	116	amp-b	121.18µV [69.68-215.28]	168.55±12.35

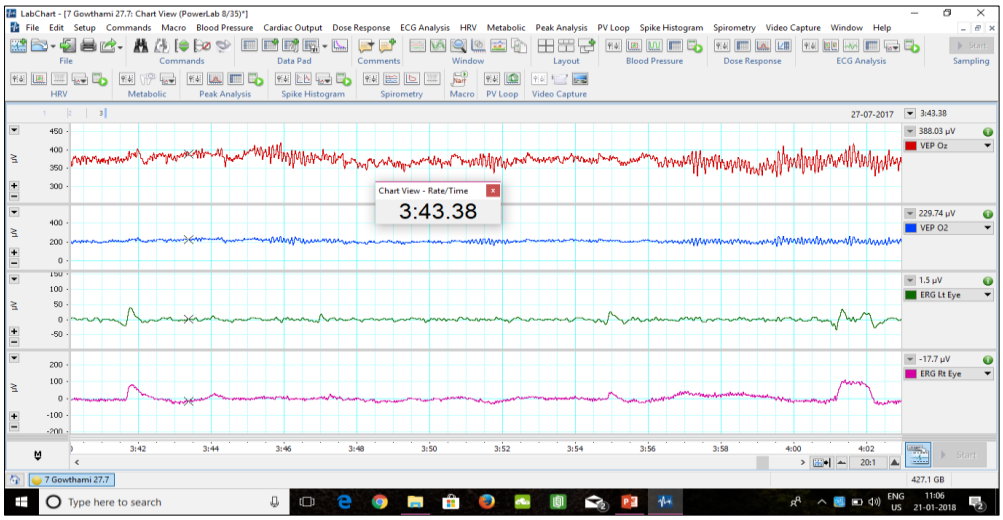

Figure 1: Screenshot of an overview of all channels LabChart pro v8.9.1 software, AD Instruments

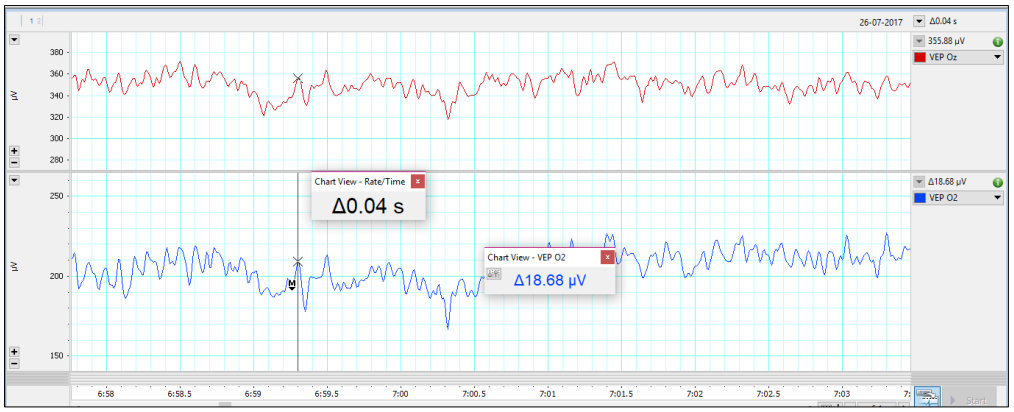

Figure 2a: Manual identification of VEP

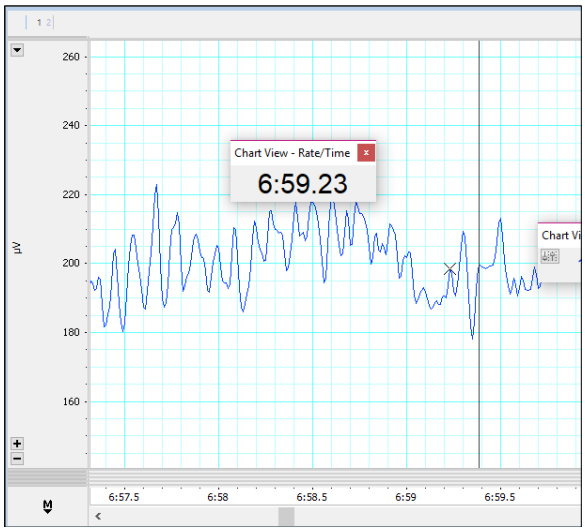

Figure 2b: Manual identification of VEP

Figure 3: Analysis of VEP – calculation of amplitude and ttp

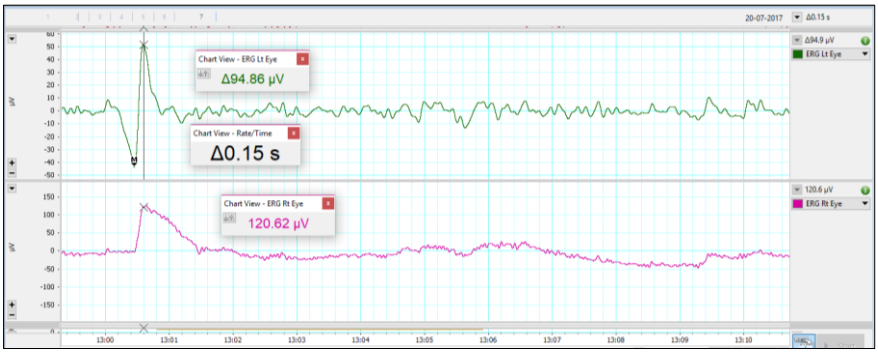

Figure 4: Manual identification of ERG from left eye and a positive wave from right eye simultaneously and calculation of amp-ERG

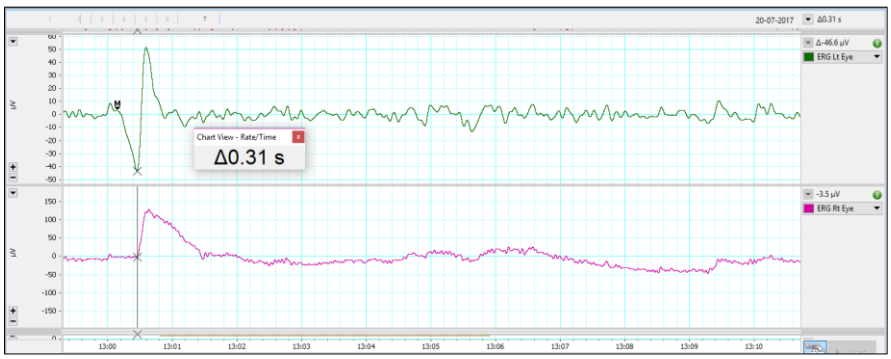

Figure 5: Calculation of time gap between LE-ERG and RE-b

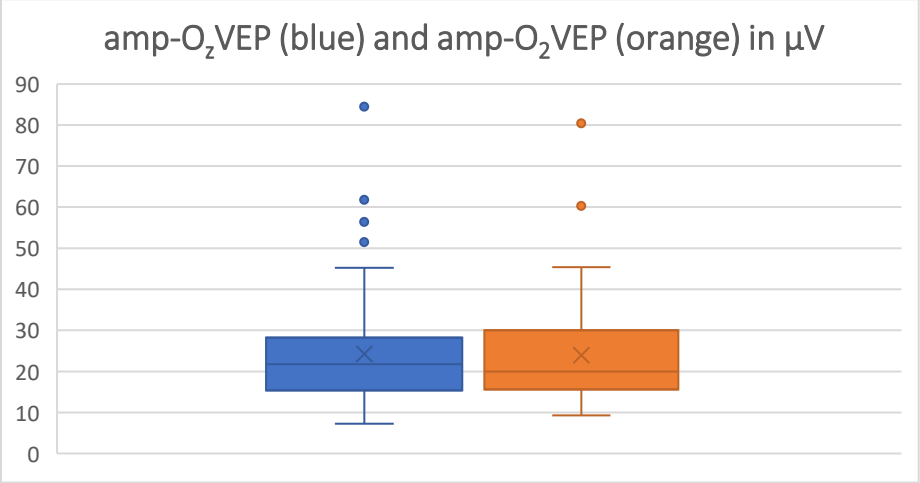

Figure 6: Comparison of amp-O_zVEP and amp-O_2VEP

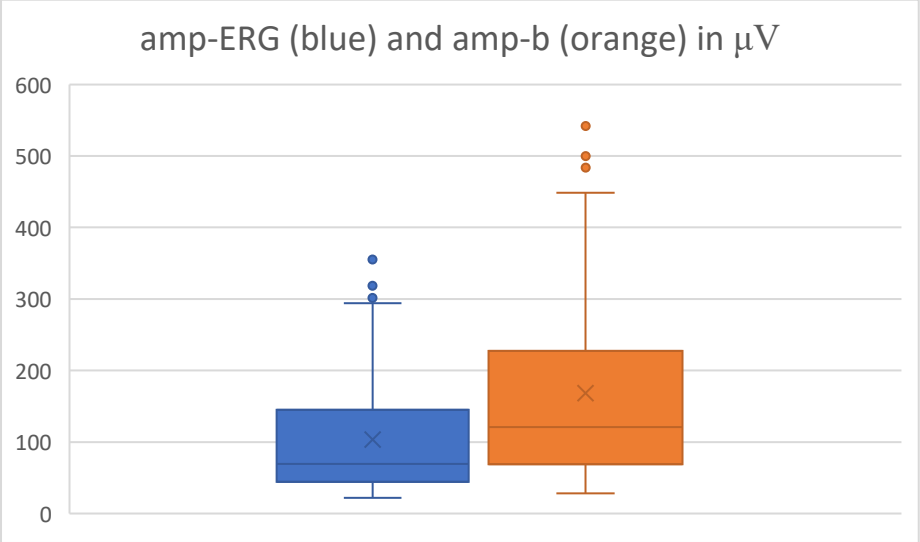

Figure 7: Comparison of amp-ERG and amp-b

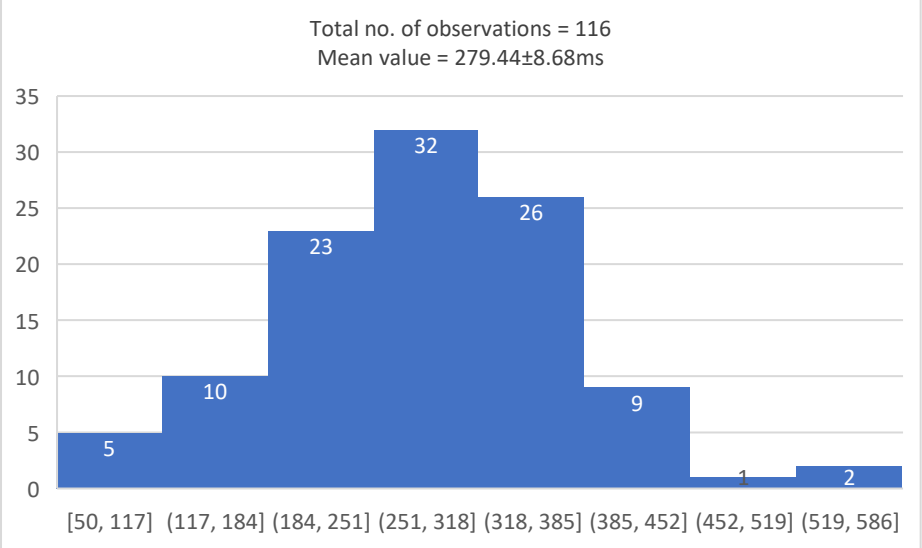

Figure 8: Distribution of the time gap between LE-ERG and RE-b

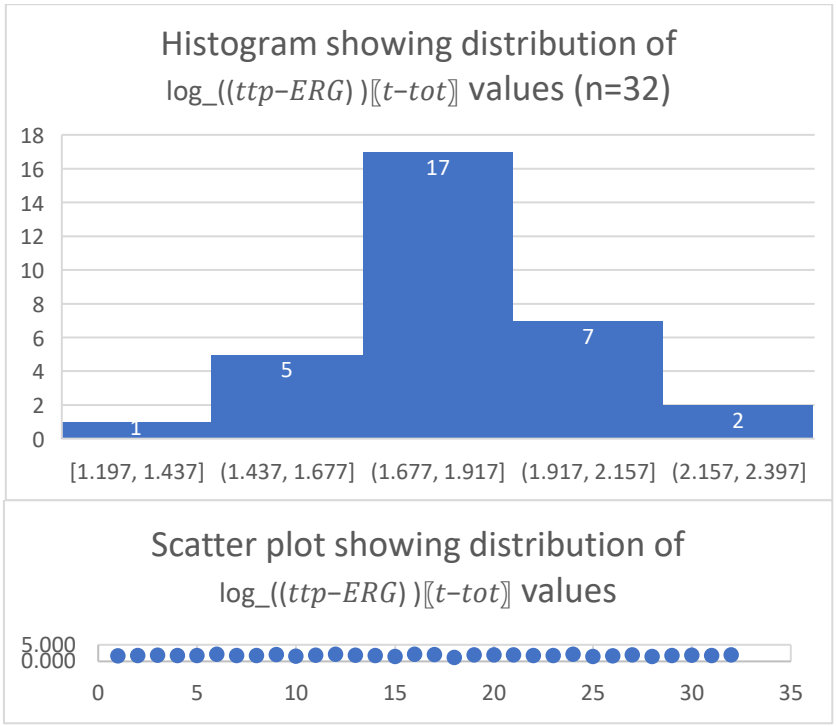

Figure 9: Distribution of $\log_{(ttp-ERG)}\, t - tot$ values

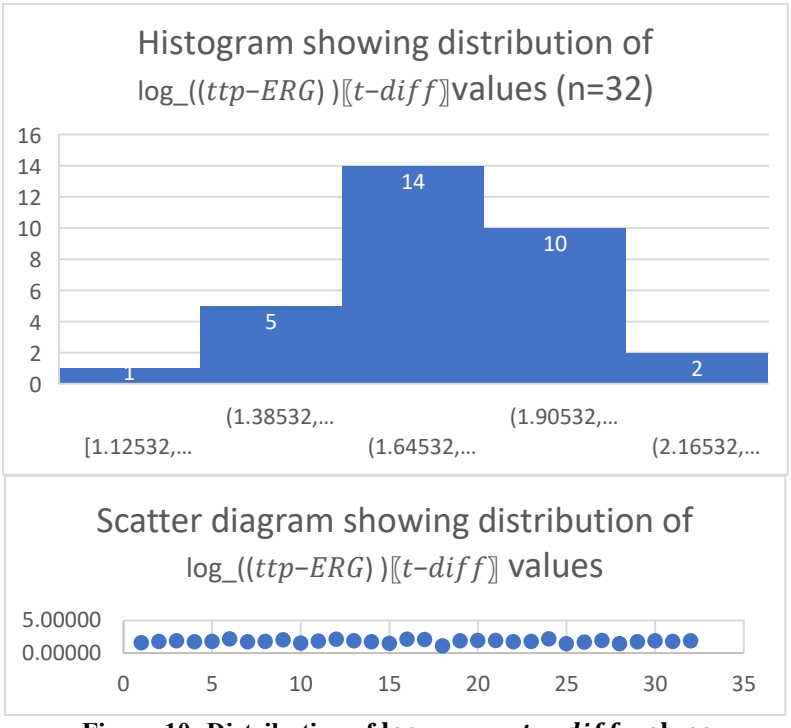

Figure 10: Distribution of $\log_{(ttp-ERG)}\, t - diff$ values

DISCUSSION AND CONCLUSION:

The present study employs electro-physiological measurement of simultaneous cortical and retinal activity during visual imagery to demonstrate the functionality of the centrifugal retinal fibers in humans and activation of visual cortex via visual pathway. It shows that retinas are activated during an imagery process even with eyes closed and upon activation, the visual pathway conducts the information to the visual cortex for perception of the imagery. By calculating the parameters of the ERG and VEP waveforms, the study attempts to establish a temporal association between them. For the total 155 LE-ERG waveforms recorded, there were 116 RE-b waves and 83 O_2VEP and 87 O_zVEP. Out of the total 116 RE-b waveforms, 93% (108) of them were observed to follow the LE-ERG after a near constant time gap. This suggests that activation of right eye in the form of b waves is almost always accompanied

by left eye even though an independent activation is possible (7%). Out of the total 83 O_2VEP waveforms, 38.5% (32) were observed to follow the LE-ERG after a variable time gap. The less number of observations may be due to absence of standard stimulus presentation to evoke a VEP, or the differential content of visual imagery, or due to differential attention by the subject to the imagery. However, besides clearly establishing that the ERG and VEP could be recorded during imagery, the study raises two important questions. One why the electrical activation of both retinas is predominantly dissimilar even though temporally correlated? Two why is the time gap between the LE-ERG and O_2VEP variable when both events were separated by a fixed distance, the visual pathway? The later part of discussion attempts to answer these questions appraising the novelty as well as limitations of the present study.

In the ERG from a dark-adapted eye negative 'a' waves are due to photoreceptor activation and positive 'b' waves are due to the activation of cells postsynaptic to photoreceptors, usually Bipolar cells and maybe Muller cells (Ido Perlman 2003; Odom *et al*., 2016). Hence, during visual imagery, photoreceptor activation is predominantly seen in left eye while Bipolar cells are activated in both the retinas possibly. A comment may be made now on the amplitudes. A surface skin electrode in scotopic conditions will usually record 14% of the actual amplitude of the b wave relative to that recorded with a standard Burian Allen electrode (about 125µV). But the amplitude of b waves recorded in our study are about 121.18 µV which is 97% of the value which could have been recorded with standard electrode to a standard flash of light (Esakowitz, Kriss, & Shawkat, 1993). This means that the activation of retinas due to imagery is as good as presenting a flash of light (or even more) from within! The present study setting differs from a clinical ERG recording in the fact that there is no presentation of a standard light stimulus. The stimulus was from within, centrifugally reaching the retinas. In such scenario, the centrifugal fibers which were to carry the imagery to their receptors must have been closely associated with the photoreceptors and Bipolar cells by some unknown mechanism. Because in most of the right-handed individuals the right brain is said to be the seat of imagination the right brain must have been activated at the beginning of imagery. That the centrifugal fibers mostly end on the Amacrine and the Bipolar cells of the retina, they may be responsible for the production of b waves. Few studies show that retinopetal axons in mammals including human arise from hypothalamus and midbrain that contain histamine and serotonin respectively. While serotonin receptors are localized to photoreceptors, histamine receptors are found on ON-Bipolar cells, affecting the b wave (Gastinger, 2006). Few other studies demonstrate the role of Muller cells and retinal astrocytes in propagating calcium waves which have a role in glial-neuronal signalling through some internal messengers which may activate photoreceptor cells (Eric, 2001). Whatever the mechanism could have been, the activation of retina is indeed possible during imagery and that photoreceptor activity in left eye preceded the probable Bipolar cell activity in right eye. Hence, measurement of time gap between the beginning of LE-ERG and the beginning of 'b' wave in right eye will depict the approximate time for which the 'a' wave persisted in left eye. This time gap of about 280ms is probably the approximate time for which photoreceptors were active in the left eye for processing each of the imagery signal received from right brain. The present study is novel in calculating these approximate time intervals. However, if the study were supplemented by other neuro-imaging techniques, imagery areas in the brain and the centrifugal pathways connecting them to the retinas could have been studied in detail. The other limitation is with accuracy in the values because the signal could not be averaged and time-locked.

Following the centrifugal activation of the retinas by the imagery areas it is observed that the visual cortex processed the imagery. The events at the retina and the cortex seemed to be temporally independent but still, a significant number of observed O_2VEP (32) indeed followed LE-ERG with variable times (t-diff) which is of great value for us to begin with some analysis. Owing to the fixed distance between the receptor and the cortical cells, it is expected that the t-diff values may remain constant. The variation in the t-diff values could have arisen from the differential processing in the receptor, or, at the thalamus; the study explores the first possibility. As the LE-ERG were recorded in left eye predominantly, the t-diff were analysed between left eye and O_2VEP, signifying right visual cortical activity. Inter individual differences in the amount and time of the processing of the imagery may have produced variable amplitudes and times of LE-ERG. When either the amplitude or time of this LE-ERG waveform were correlated to t-diff values, no significant association was observed. Hence, authors have tried with various parameters and equations to find an association between observed LE-ERG and t-diff and t-tot values. Out of all such trials, the values of $\log_{(ttp-ERG)} t - tot$ and $\log_{(ttp-ERG)} t - diff$ estimated between them were found to be near normally distributed with minimal variance for the calculated values (n=32) studied in all the subjects. The ttp-ERG is thus exponentially related to the t-diff and the t-tot. Looking at the numbers, a possible approximate assumption could be made. The ttp-ERG time in the left eye, raised to a value in the range of 1.816-1.823 approximately, will decide the total time taken for the retinal output after the processing of imagery to reach and initiate cortical activation in the right brain. The mean ttp-ERG calculated was 222.19±7.91ms while the median value was 200ms [160-260] (Table 1). The ttp-ERG values calculated from the peak of a wave to the peak of the b wave indicate the time taken for most of the retinal post-synaptic cell activity including the later part of photoreceptor activity in left eye. The post-synaptic cell activity which is mostly affected by the centrifugal fibers during imagery is what

shown to be affecting the time delay of impulses to reach the cortex for processing of the imagery. Hence, after the photoreceptor and possible Bipolar cell activity, impulses must have been produced in the retinal ganglion cells and followed through the visual pathway to the right visual cortex. The degree of temporal separation of the retinal and cortical events is therefore significantly under the effect of retinal events. In fact, there may be many other factors which could have contributed to the variable times between LE-ERG and O_2VEP like-processing at thalamus, sensory attention, interval between the successive signals produced at the retina, inhibitory systems in the visual pathway. The present study, however projects the importance of the retinal events alone. Whether thalamic processing could also contribute to the variation in t-diff values, and the contribution of either the receptor level processing of imagery or the thalamic level is more significant, needs to be studied.

The present study could establish an approximate association between ttp-ERG and t-diff and t-tot values. The authors also propose to continue the study further attempting to identify the role of thalamic processing in the t-diff values. If the study were conducted on a large sample of observed t-diff values, a more deeper understanding into their variation must have been possible. This remains one of the limitations of our study. Even though it is proved that VEP could be recorded from the cortex during imagery which have a temporal association with the ERG in the eye, EEG signal was acquired only from two montages, O_z and O_2. This is the next limitation. The production of VEP akin to a standard pattern reversal VEP would however prove activation of striate and extra-striate cortex during imagery in our study. Analysis from other cortical areas would have thrown light on the activation of other cortical areas during imagery.

Besides providing some interesting observations such as photoreceptor activity time during imagery, the exponential relation between ttp-ERG and t-diff for the first time, the study will sure encourage many researchers in this line to explore further into the various components of ERG and patterns of VEP, the authors strongly believe. The knowledge on the characteristics of the VEP signal acquired from a large sample would help establish comparative data with a clinical VEP signal and would serve as an important tool for psychophysiological research.

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