EVENT-RELATED POTENTIAL (ERP) SIGNALS ANALYSIS

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ABSTRACT

The Event-Related Potential (ERP) components, P100 and P300 are thought to reflect visual and cognitive information processing, respectively. Recent studies suggested that these ERPs are influenced by natural or environmentally induced variables. The present study investigated the efficiency of visual and cognitive information processing over time and also the effect of caffeine intake on both visual and cognitive functions. The sample consisting of ten healthy individuals (five female and five male), was submitted to a visual discrimination task (oddball paradigm) at three different times (i.e., morning, afternoon and evening) within the same day. Also, subjects were required to undergo the same visual test after the administration of caffeine (65 mg). The main components of interest; P100 and P300 are studied and analyzed based on two variables used to quantify the components: latency and amplitude. Results suggest that visual and cognitive efficiency degrade over time (as observed by an increase in latency and a decrease in amplitude in most subjects). After the caffeine administration, the visual and cognitive efficiency for most subjects seemed to improve (a decrease in latency while an increase in amplitude). However, the caffeine effect was more prominent in a fatigue condition compared to well-rest condition. Though partially confirmed the hypotheses, this study provides evidences for the time-of-day and caffeine effects on both visual and cognitive processing efficiency as proposed in the current literature.

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LIST OF ABBREVIATIONS

Ca+	Calcium
Cl-	Chloride
CNS	Central Nervous System
CNV	Contigent Negative Variation
C_Z	Centroparietal mid-line lobe / Vertex
DAC	Data Acquisition Computer
DC	Direct current
DIN	Digital Input
ECI	Experimental Control Interface
EEG	Electroencephalography/ Electroencephalogram
EGI	ElectroGeodesic International
EP	Evoked Potential
ERP	Event - Related Potential
F_Z	Frontal Midline Lobe
HCGSN	HydroCel Geodesic Sensor Net
Hz	Herz (sample/ second)
K+	Potassium
KCl	Potassium Chloride
LCD	Liquid Crystal Display
LN	Late - negative
LP	Late - positive
LRP	Lateralized Readiness Potential

μV	micro-volt
ms	mili second
Na+	Sodium
O_Z	Occipital mid-line lobe
PVA	Polyvinyl Alcohol
P_Z	Parietal mid-line lobe
TCP/IP	Transmission Control Protocol/ Internet Protocol
T_Z	Temporal mid-line lobe
UTP	University of Teknologi PETRONAS
VTD	Visual Task Discrimination

CHAPTER 1
INTRODUCTION

1.1 Background of Study

In our daily lives, most of our activities involve sensory and cognitive information processing. The ability to process information from countless resources can be regarded as "cognitive efficiency". One of the event-related potential (ERP) components that is known to be as a general measurement of cognitive efficiency is the P300. Previous research [1] mentioned that the P300 reflects the Central Nervous System (CNS) activity related to cognitive operations and helps to differentiate the effects of CNS stimulant on brain functions.

Recent studies show that the P300 latency reveals the temporal aspect in cognitively processing a stimulus (task difficulty) while the amplitude reflects the degree of cognitively processing of the stimulus (attentional level) [2]. However, there are variations exist in the latency and amplitude of P300 which are due to many factors. According to [3], some of the inconsistencies for the ERP studies may stem from activity preference, previous food intake, and time-of-day. The differences of each factor can be observed if the other effects are properly controlled.

In this study, visual and cognitive processing efficiency of an individual are measured through the assessment of the cognitive ERP components (i.e P100 and P300). There are two factors of interest that will be mainly studied: the effects of i) time-of-day and ii) caffeine intake on the ERPs. To investigate the first factor, an individual will be assessed at three different times (i.e morning, afternoon and evening) within the same day to indicate the time-of-day process throughout a day.

Hypothetically, the ERPs of individual should demonstrate shorter latency (faster response) in the morning than if measured in the evening, while larger amplitude (higher attentional level) in the morning than if measured in the evening due to subjective mentally and physically fatigue state of an individual. Assuming that individuals tend to be under mentally or physically fatigue towards the end of the day, then their temporal and attentional aspect of the cognitive information processing is expected to degrade with respect to time. In other words, the P300 latency is prolonged while its amplitude is smaller.

As for the second factor, the effect of caffeine intake on the ERP components will be also tested on the same individual at the end of the day where caffeine will be induced during the evening session. On the contrary, after the caffeine administration, a decrease in latency and increase in amplitude are expected. This phenomenon is due to the fact that caffeine can improve cognitive efficiency by increasing attentiveness as fatigue is reduced and it has also been regarded as a neuromotor modulator as implied by previous studies [1,4].

1.2 Research Hypotheses

As explained in one of the ERP studies, the cognitive efficiency described by P300, where the two aspects of cognitive information processing: temporal (latency) and attentional (amplitude) may degrade with the accrual of fatigue over time [2]. Apart from that, with caffeine administration, the cognitive performance is said to be improved as the P300 latency is decreased while the amplitude is increased.

This project intends to verify the changes in the ERP components variables (amplitude and latency) based on the following reasons:

i. Cognitive efficiency of an individual may not be necessarily degraded over time due to the variation of the P300 components of different individuals which still can be modulated by other factors.

ii. Caffeine though is claimed to be a CNS stimulant, may not be effective to some individuals in rest conditions compared to those who are under physically and mentally fatigue state.

1.3 Significance / Relevancy of Project

Although various researches had been carried out to investigate the influencing factors on the ERPs, only few researches had been done to evaluate an individual's visual and cognitive performance over time. Also, provided that the underlying mechanism of caffeine effect on the central nervous systems are still under study, the effect of this substance on ERPs is not yet conclusive. Hence, this project is purposely designed to measure and evaluate the two factors combined (i.e., time-of-day and caffeine intake) on university students to validate the proposed hypotheses as claimed by the previous studies.

In addition to that, the outcome of the study may be useful in understanding the cognitive behaviour of students based on their assessment on visual and cognitive performance over time. Also, the study of effect of caffeine intake in improving the cognitive performance may suggest that one should drink coffee to keep him or herself stay alert especially when one is under mentally and physically fatigue state.

1.4 Objectives and Scope of Study

The primary objectives of this project are to validate the changes in latency and amplitude of ERP components in order to investigate the cognitive and visual information processing efficiency over time and also to assess the effect of caffeine intake on both visual and cognitive functions.

In addition to that, the project also aims to achieve the following objectives:

i. To comprehend the anatomy of brain and also the fundamental of neurophysiology that is relevant to the origin and phenomenology of biopotential inside the human brain.

ii. To understand the underlying theory of electroencephalography, and also the generation and measurement of event-related potential signals.

iii. To practically learn the operations of an electroencephalogram and how to measure and record the brain signals using the EEG technology.

iv. To be able to design a visual discrimination task (VTD) to elicit the ERP waves of interest under optimal condition.

v. To be able to identify the influencing factors of an ERP (i.e., time-of-day, food intake and etc.) and also the parameters used in recording and processing the ERP signals.

vi. To apply high-level signal processing concepts and techniques with the use of waveform tools available in NetStation in processing the acquired EEG signals.

vii. To correlate the effect of each influencing factor with individuals' cognitive performance through technical and statistical analysis.

CHAPTER 2
LITERATURE REVIEW

2.1 Fundamentals of Neurophysiology

2.1.1 The Human Brain Structure

In determining the electrode positions for EEG recordings, the human brain structure with its various functions and their associated locations are studied. Basically, there are four main structures of the human brain [5]:

i. Cerebrum
ii. Cerebellum
iii. Pons
iv. Medulla Oblongata

The largest area of the brain is the cerebrum. It consists of two hemispheres, which are the right and left cerebral hemispheres. Theoretically, the right hemisphere controls the left side of the body and vice versa. The cerebral cortex (outer layer of the cerebrum) is composed of nerve cells that control the brain activity is made up of grey matter. The inner portion of the cerebrum is white matter, composed of nerve cell axons that carry information between nerve cells in the brain and spinal cord. Each hemisphere of the cerebrum can be divided into four lobes as indicated in respective colours as shown in Figure 1:

i. Frontal Lobe: associated with planning, reasoning, planning, parts of speech, emotions, and problem solving
ii. Parietal Lobe: associated with movement, recognition, perception of stimuli and orientation

iii. Temporal Lobe: associated with auditory processing, speech and memory

iv. Occipital Lobe: associated with visual information processing

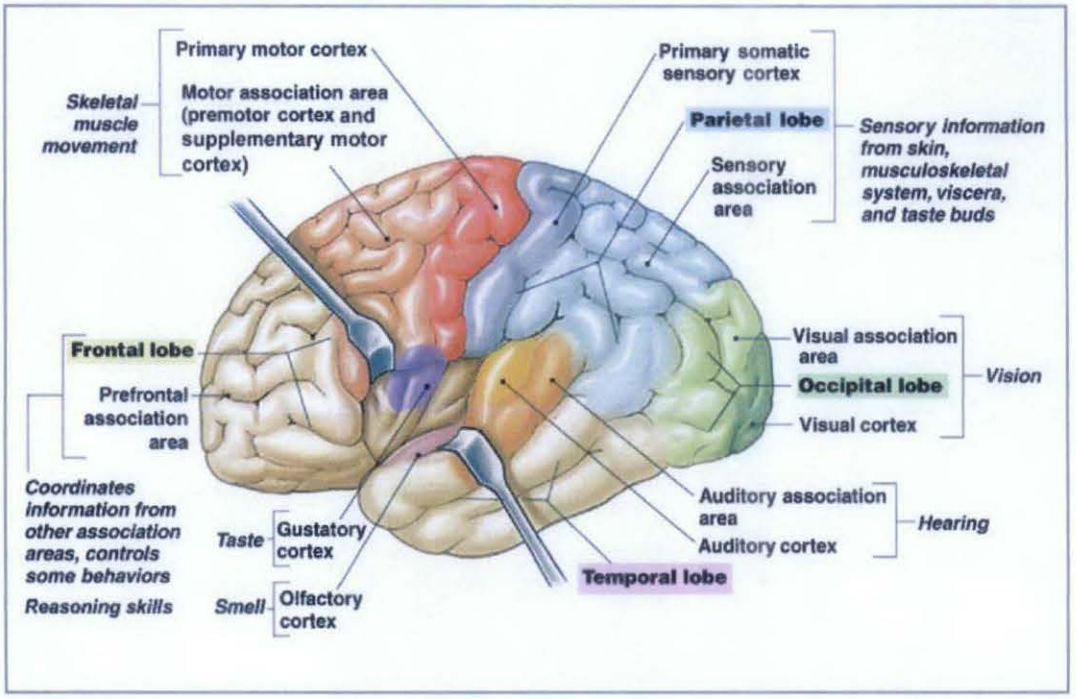

Figure 1: Four main lobes of the brain cortex (Figure from Pearson Education Inc., 2007)

2.1.2 Membrane Potentials

There are approximately 100 billion neurons or nerve cells inside the human brain. Each nerve cell body containing the nucleus and branches (processes) follow in all directions. Axon is the longest branch, which carries outgoing signals and may extend all the way from the CNS to toe, connecting with the muscle on which it acts upon [5].

2.1.2.1 The Resting Membrane Potential

According to [5], neurons send messages via an electrochemical process. The ions in the CNS are sodium (Na+) and potassium (K+), calcium (Ca+), and

chloride (Cl-) while there are also other negatively charged protein molecules. Nerve cells are enclosed by a semi permeable membrane that alternates by allowing certain ions to pass through while blocking the passage of other ions. When a neuron is inactive (i.e., not sending a signal), it is said to be at rest. As it is at rest, the inside of the neuron is more negative with respect to the outer side. The difference in the concentrations of various ions is an attempt to balance out on both sides of the membrane. However, it never reaches an equilibrium state of density due to the fact that only specific ions are allowed to pass through channels.

At rest condition, only the potassium ions (K+) can pass through the membrane while other ions such as, chloride ions (Cl-), sodium ions (Na+) and protein molecules are suspended. However, for every two potassium ions, three sodium ions can be transferred out of the neuron with the use of a biological pump. Ultimately, when the membrane is at equilibrium state, the potential difference between both sides of the neuron is said to be approximately -70 mV. Hence, is the resting membrane potential of a neuron [5].

This basic insight into the physical mechanics of the brain may assist in understanding how action potential differences are developed giving rises to various EEG techniques.

2.1.2.2 Action Potentials

The transmission of information from one neuron to another can be described by the mechanism of an action potential. The action potential is an electrical response that is created by a depolarising current. As a stimulus is presented, it causes the resting potential to move toward 0 mV (see Figure 2). The moment the depolarisation reaches the threshold value, -55 mV an action potential is generated and will constantly fire. Also, the size of the action potential is always the same for each neuron [5].

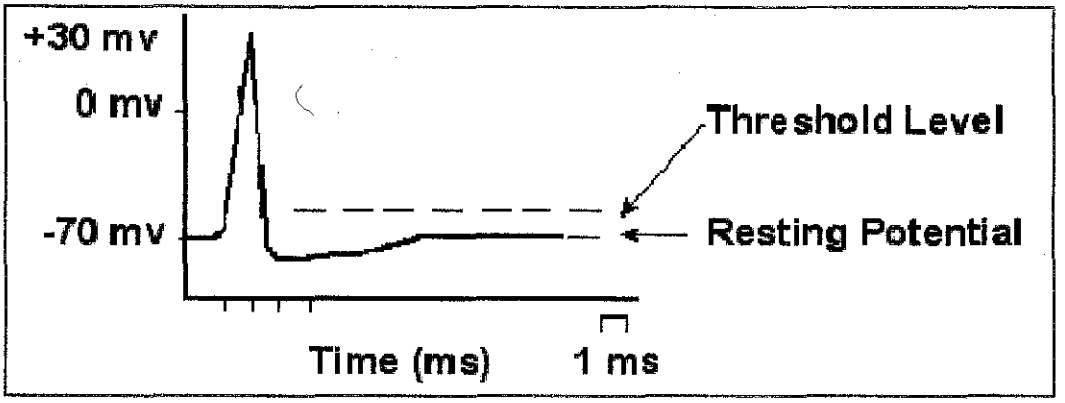

Figure 2: An action potential. (Figure from D. Novák *et. al*, 2004)

As mentioned previously, an exchange of ions across the neuron membrane triggers an action potential. A stimulus presentation will result in the opening of sodium channels. As the number of sodium ions on the outside is larger than the inside, sodium ions can penetrate into the neuron across the membrane. This will result in the neuron becoming a more positive element and subsequently it depolarises. However, there exists a small latency prior to the opening of potassium channels that allows the potassium, K+ ions to leave the cell, and reverse the depolarisation. As this happens, the sodium channels begin to close and cause the action potential to return to -70 mV (a repolarisation). In reality, the action potential may exceed -70 mV (a hyperpolarisation) since the potassium channels may remain open longer. The ion concentration however will gradually return to its equilibrium state (-70 mV) (see Figure 3) [5].

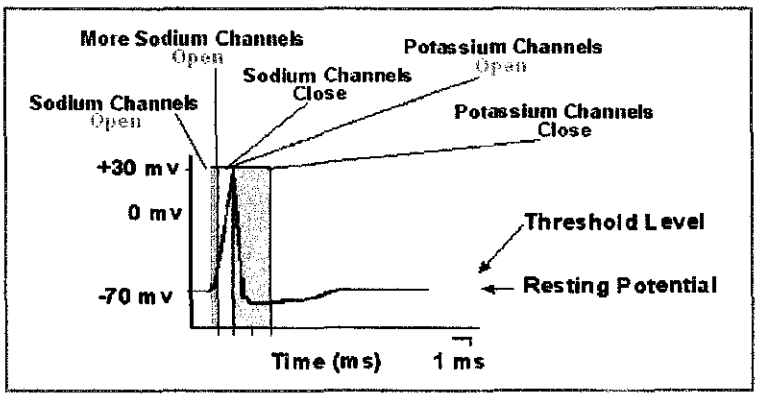

Figure 3: Chemical attributes of an action potential. (Figure from D. Novák *et. al*, 2004)

2.2 Electroencephalography (EEG)

2.2.1 Electroencephalography Overview

EEG is a representative of neuronal potentials, which are the electrical potential generated by neurons in the cortex of the brain. The dipoles which are the underlying electrical generator are produced by a single cell. Inside the human brain, there are approximately 100 billion of cells and when these cells act in synchrony or unison, then the synchrony reinforces the strength of the signal. So when the biological potential is measured from the scalp and is attributed to the brain, it is due to the synchronous activity of population of brain cells. This synchronous activity reflects the neuronal dynamics and the organization and processing the information that is going on inside the brain.

As one should be aware that while recording signals using EEG technique, other electrical sources could also be picked up. Hence it is important to understand the sources of artifacts (i.e., muscles tension) as well as the structure of the cortex and its behaviour in terms of generating biological rhythms. Also, we need to understand the origin of the different frequencies, amplitudes and connectivity measures (coherence, phase and etc.) as these components reflect the brain anatomy and physiology that indicate us what is happening underneath.

It is possible to measure the electrical activity from the brain as they are transferred to the scalp through volume conduction. Volume conduction is a mechanism where the scalp receives the electrical activity from the underlying tissues. The cerebral fluid, the brain tissues and even the skull are electrical conductors where the electrical energy can pass through them passively like a current flow in a resistive medium.

EEG waveforms are categorized according to their amplitudes, frequencies, and also the sites on the scalp at which they are recorded. The most common classification is based on frequency (eg., alpha, beta, and theta waves). The

information gained from the waveform frequencies and shapes are combined with the subject's age, state of alertness, and scalp distribution site to determine its significance [5].

2.2.2 Electrodes

Electrodes are used to make connections between the conducting fluid of the tissue (in which the electrical activity is generated or conducted) and the input circuit of the amplifier. In recording ERPs, standard gold disc or silver-silver chloride surface electrodes are recommended. The electrode impedances should be below than 5k ohm or equal [6].

The International 10-20 System of Electrode Placement (see Figure 5) is the most commonly used method to describe the location of the electrodes. The 10-20 system is based on the relationship between the location of an electrode and the underlying area of cerebral cortex. Each electrode site labelled using a letter (to identify the four lobes) and a number to identify the hemisphere location:

i. F- frontal
ii. C- central (Vertex)
iii. T- temporal
iv. P- parietal
v. O- occipital

The odd numbers are on the left hemisphere, while the even numbers are on the right side. The larger the number is, the further its location from the central part. The Z-notation associated with each lobe is to indicate the mid-line of the brain (i.e., F_Z, C_Z, T_Z, P_Z and O_Z).

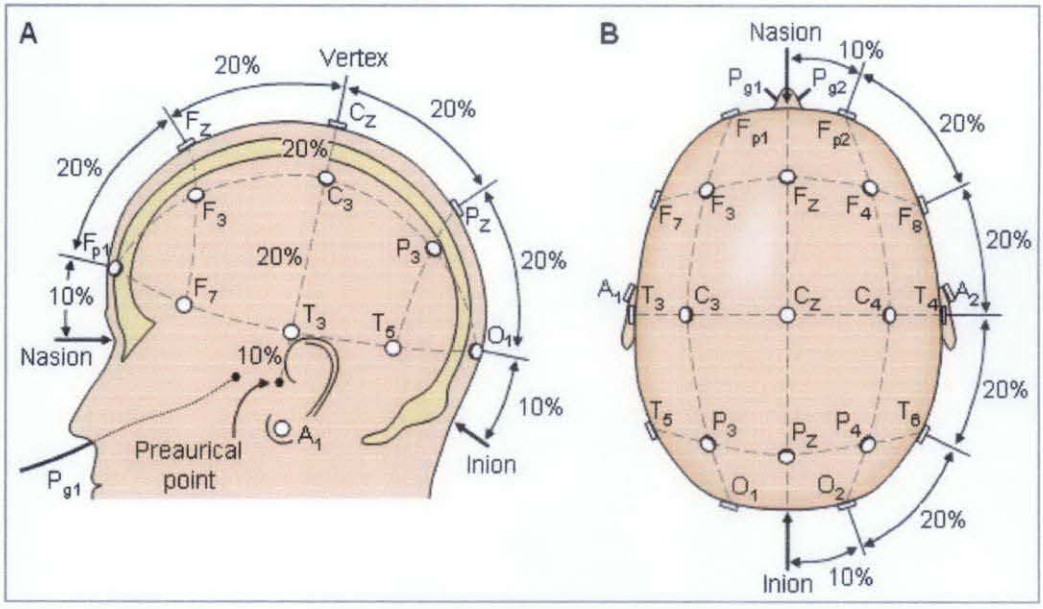

Figure 4: The International 10-20 System electrode placement. (Figure from BCI200, 2008)

According to this system, the electrodes are placed with respect to bony landmarks, in proportion to the size of the head. The measurements of the anterior/ posterior midline are based on the distance between the nasion and the inion over the vertex (centre of the head). The widely used ground electrode positions include the frontal, vertex, earlobes or linked earlobes, and mastoid.

One of the types of skin interface sensors is the electrocap, which can record the EEG signals from multi channels simultaneously. It is also a standard way to locate and measure at these spots as it is very important to get into the right place as we do the assessment. Traditionally, the electrode placements could be few centimetres off the spots and resulting in different readings from what is expected. However, it is safe to say that the electrocap is almost accurate as it could be adjusted accordingly and thus minimize the possibility of getting the wrong readings at the wrong spots.

2.3 Event – Related Potential (ERP)

2.3.1 What is ERP?

Event – Related Potential (ERP) signals are those EEGs that measure the electrical response of the cortex to sensory, affective, or cognitive events. They are voltage fluctuations in the EEG induced within the brain, as a sum of a large number of action potentials that are time-locked to sensory, motor and cognitive events which are typically generated in response to peripheral or external stimulations [7].

Since ERPs are relatively small (1-30 μV) to the EEG background, they often need the use of a signal-averaging procedure to be elicited.

2.3.2 Amplitude, Latency and Scalp Distribution

The waveform of an ERP can be quantitatively characterized across three main parameters [7]:

i. Latency

   It is the time point at which peak amplitude occurs that can reveal the timing of ERP activation.

ii. Amplitude

   The amplitude is an index of the extent of neural activity and how it responds functionally to a stimulus.

iii. Scalp distribution

   The distribution of the scalp provides the pattern of the voltage gradient of a component over the scalp instantly.

2.3.3 Types of ERP

The classification of ERP can be done in two ways, according to [8]:

i. the nature of the stimulus (i.e., visual, auditory or somato-sensory) or
ii. the latency at which the potentials occur after the stimulus presentation (i.e., short latency (less than 100ms) or long latency (more than 100ms) potentials)

To further the discussion, the generation of short latency potential is during the sensory stimulus processing stage thus it is known as exogenous (whose characteristics are controlled by the physical attributes of the stimulus such as presentation rate, intensity, and modality). On the contrary, the long latency potential is produced during the cognitive processing stage and is recognized as endogenous (whose characteristics depend on the nature of interaction between individual and the stimulus) [9]. This also means that long latency potentials are affected by the level of attention or task relevance although they are non-obligatory responses to stimuli.

In general, ERP consists of a series of positive and negative waves that can be named numerically (i.e. P3) or according to their latency (i.e. P300). For short latency potential or early components include N50, P100, N100, P200 and N200 while the long latency or late components mainly consist the P300, N400 and late positive (LP). These positive and negative peaks can be graphically described as in Figure 5 taken from one of the recent ERP studies [10] with the use of three different visual stimuli (i.e., Relevant, Irrelevant and Standard). The graph shows the grand average of ERPs generated at the occipital site (Oz).

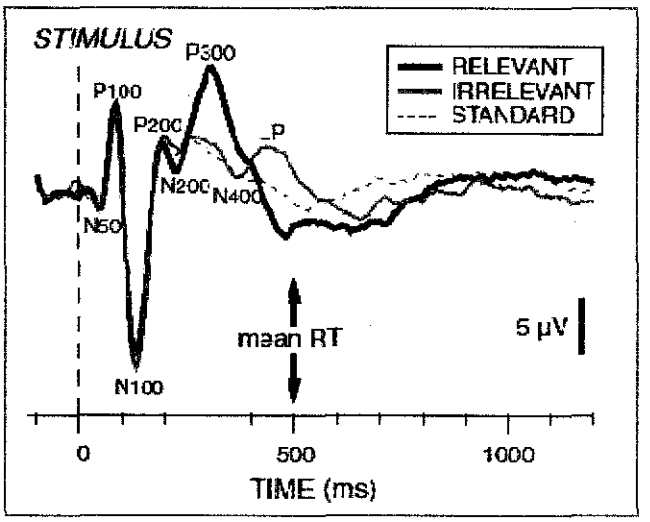

Figure 5: Early and late ERP components (Figure from S. C. Steffensen *et. al*, 2008)

These ERP waves' characteristics can be categorized into six families [11]:

Table 1: Classification of ERP waves

No.	Classification (latency range in ms)	Processes
1.	Sensory/ Perceptual (2-200 ms) - Auditory P50, N100 - Visual P100, N180, N200 - Mismatch Negativity	Early attentional selection, sensory memory, plasticity of sensory systems and specific perceptual modules.
2.	Discrimination/ Recognition (150-500 ms) - N200 (180 – 325 ms) - P300 (250 – 900ms)	Late attentional selection, feature discrimination, pattern recognition, classification & decision
3.	Memory-related (200-600 ms) - Late-positivities (LPs) - Late-negativities (LNs)	Storage and retrieval mechanism, implicit versus explicit memory

No.	Classification (latency range in ms)	Processes
4.	Language-related (200- 600 ms) - N400	Grammatical and semantic processing
5.	Readiness/ Preparatory potential - Motor potentials - Contigent Negative Variation (CNV) - Lateralized Readiness Potential (LRP)	Motor preparation, mental chronometry, continuous/discrete models
6.	Error-related Potential (150-400 ms)	Error detection/correction

2.3.3.1 Characterization of P300

Although there are many others ERP waves exist, the classical P300 as mentioned by Sutton *et. al* [12] is perhaps the most – studied ERP cognitive components as its amplitude is relatively large and facile elicitation in experimental context. The P300 is identified as the most positive component occurring approximately 300 ms after a stimulus presentation and appears after the N1-P2-N2 complex [13]. Even though the latency window of this component occurrence may vary; the range between 250 to 500 ms has been widely used in most research work. According to a study [14], the amplitude of P300 for visual and auditory event-related potentials varied from 5 μV to 20 μV, though amplitude of 40 μV has also been reported.

i. P300 latency

Depending on the nature of the experiment, the latency (ms) can be distinguished as active or passive. The elicitation of active latency occurs

when subjects are instructed earlier to only respond to the infrequent stimuli (or target) with button-presses. On the other hand, the passive latency may be observed in oddball tasks with no prior instructions given to the subjects.

ii. P300 amplitude

The P300 amplitude (μV) is the potential difference between pre-stimulus baseline. It is also the largest positive-going peak of the ERP waveform within latency window defined (eg., 250-500 ms, range may vary depending on subject, stimulus modality and etc.).

iii. P300 scalp distribution

The scalp distribution is the change in component latency or amplitude across the midline recording sites (F_Z, C_Z, and P_Z). The amplitude is typically increases from the frontal to the parietal site. This effect is considered important since the variation in amplitude for a given task has been used to deduce information about the P300 neural generators [3].

2.4 Human Vision

Visual information-processing begins in the retina, where cells that contain photo pigments capture light and initiate neural activity. Since visual cortex is activated mainly by the central visual field, visual ERPs depend on functional integrity of central vision of the visual pathway including the eyes, retina, the optic nerve, and occipital cortex [9] (refer Figure 6).

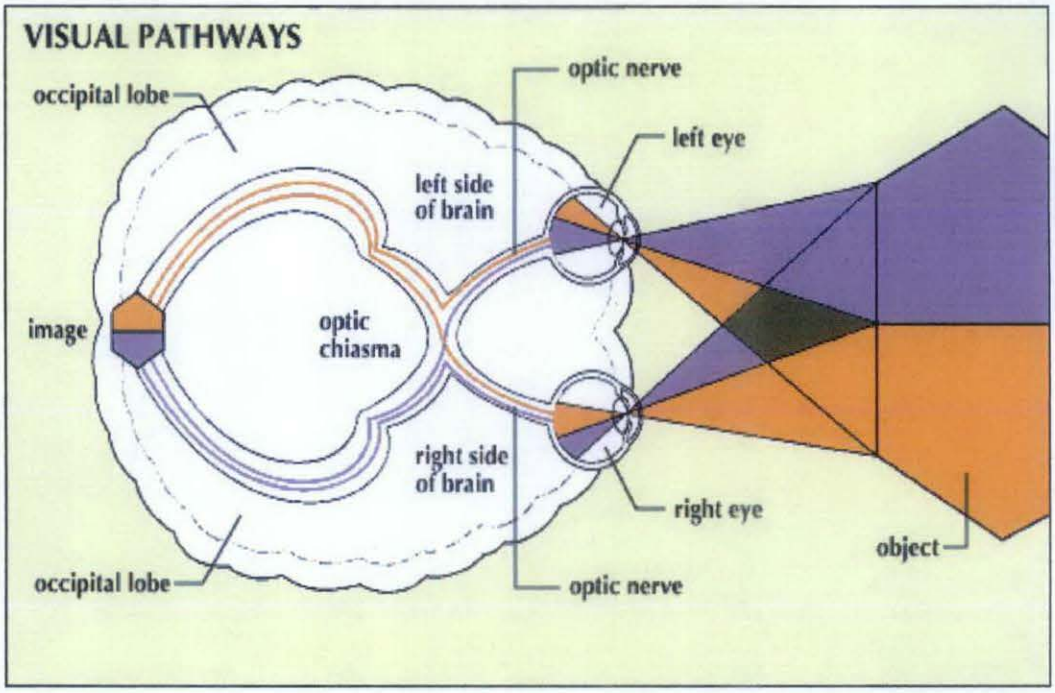

Figure 6: Human visual pathways (Figure from Encyclopaedia Britannica, 2011)

With the proper equipment and training, visual ERP can be reliably measured using EEG technology. As the EEG reflects thousands of simultaneously ongoing brain processes, the brain response to the event of interest presented by a stimulus, is not usually visible in the EEG recording of a single trial. So to observe the brain response, many trials (100 or more) are required. By averaging the recorded ERPs together, the random brain activity will be averaged out (signal averaging) while the relevant ERPs remain.

CHAPTER 3
METHODOLOGY

3.1 Procedure Identification and Process Flow

The overall process throughout this project is illustrated in the following flow diagram below:

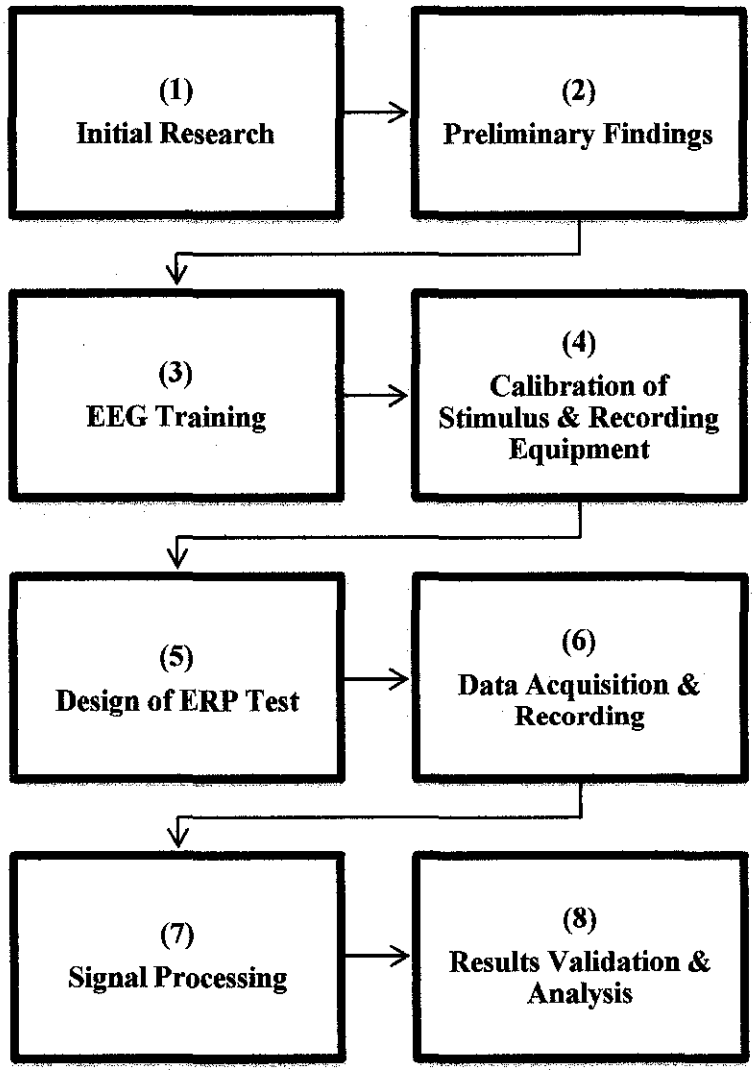

Figure 7: Project flow diagram

3.2 Initial Research

Initially, the project began with an extensive literature research on electroencephalography, event- related potentials and all its related subjects. The research was done by various means such as books, journals and websites and through these resources, I had learned the fundamental concepts in the generation of EEG signals and consequently ERP signals.

3.3 Preliminary Findings

Based on the knowledge that I gained which were mainly through various research publications, I had carried out some preliminary findings which were necessary for the formative stages of the experiment that I would need to design later. At this stage, I had performed an analysis on subjects, test environment, stimuli and task, where all of these were the core components in designing an optimal ERP test. Also, by identifying the factors that could influence the ERP parameters (i.e. amplitude and latency), I could minimize the risk of data contamination and have a better control for my experiment when acquiring data from the subjects. This was done merely by conducting a survey while selecting subjects as well as when preparing the test protocols.

3.4 EEG Training

In this project, the main equipments that will be extensively used are the electrodes and the recording equipments. For the electrodes, an array of sensors called HydroCel Geodesic Sensor Net (HCGSN) will be used to acquire the EEG signals from subjects. For the recording equipments which involve the use of Net Station acquisition software that was installed in a data acquisition computer (DAC) where the acquired EEG signals will be first amplified and filtered by an amplifier (Net Amps 300 Amplifier) that is connected to the Sensor Net.

To be able to use these equipments properly, I had attended a five-day EEG training which was held by the developer, ElectroGeodesic International (EGI) school in Johor Bahru on February 21 to 25, 2011. Other than Malaysia, the training was also attended by several other researchers from other countries such as Australia and Singapore. From the training, besides learning on how to handle the recording equipments (both software and hardware), I also learned about the waveform tools which are also available in the Net Station that can be very useful in analysing the acquired EEG signals. Apart from that, I also had the chance to discuss with other researchers who were also had been working on ERP projects which I found very helpful for me especially when I start planning and designing my experiment for my project later.

3.5 Calibration of Stimulus and Recording Equipment

Before going to experiment designing stage, first, I had to setup the stimulus and recording equipments. To do this, I need to perform a calibration to all equipments involved in order to avoid inaccuracy and data contaminant. Several tests and configurations were performed such as Audio Video (AV) Timing Test, Acquisition Setup Workbench, and E-Prime Timing Test. The tests were to ensure that the communication between the stimuli (presented by the experimental control computer) with DAC existed and also the recorded time for the acquired data was in synch with the time when the stimuli were presented.

3.6 Design of ERP Test

Once all equipments were successfully calibrated, I then proceeded with the design of ERP test. The experiment was designed to elicit the ERP components (i.e. P300) by employing the "oddball" paradigm test and this test was carried out on all subjects at three different times and also with or without the caffeine intake within the same day of experiment.

3.6.1 Subjects

Ten university students (five male and five female) of age ranging from 21 to 24 years old with normal or corrected-to-normal vision and no known neurophysiological impairments participated as volunteers in this study. Subjects were selected among undergraduate and post graduate students from University of Teknologi PETRONAS (UTP). Each subject was informed earlier about the experimental conditions through 'Participant Information' sheet (see Appendix B) and was given a written 'ERP Test Consent Form' (see Appendix C) to read and sign before participating in the study. All subjects were required to answer a simple questionnaire (see Appendix D) regarding their daily activities which will be used as future reference in the study. The questionnaire also helped to identify possible P300 biological determinants, such as food intake, sleeping hours, drugs, and among others. Laterality was used as an inclusion criterion; hence subject with either right or left handedness could participate in the study.

3.6.2 Test Environment

The experiment took place at Centre for Intelligent Signals and Image Research laboratory (22-02-14), UTP. Subjects were seated in a chair with arm-rest to minimize discomfort that could possibly cause muscular artifacts. Also, during the visual test, the room was dimly lit to enable subjects to focus exclusively on the monitor screen. The distance between the subject and the visual stimuli (a 15.4" LCD display) was set to 0.1 meter away. Subjects were asked to remove any metal accessories (i.e. earrings, bracelets, or metal watch) and also to turn off their hand phones so as not to create impedances on the Sensor Net as well as to avoid disturbance while performing the test.

3.6.3 Test Time

Subjects were required to attend a series of recording sessions where there are four (4) sessions involved. All sessions must be conducted on the same subject within the same day of experiment. The sessions were classified as follow (see Table 2).

Table 2: ERP Test recording sessions time slots

No caffeine intake	
Session 1: Morning	(8.00 AM – 11.00 AM)
Session 2: Afternoon	(12.00 PM – 3.00 PM)
Session 3: Evening	(4.00 PM – 7.00 PM)
With caffeine intake	
Session 4: Evening	(4.00 PM – 7.00 PM)

3.6.4 Stimuli & Task

To elicit the P300, all subjects were asked to sit for an oddball test through visual presentation. In the visual oddball paradigm, the letter Xs, which served as target stimuli, were presented randomly with a low probability of occurrence of 20 % while the letter Os, represented the non-target or standard stimuli with 80% of occurrence. Subject's task was to respond only to the letter 'X' (target) each time it appeared on the screen by pressing any button on the Serial Response (SR) Box provided. However, they needed not to respond to the letter 'O' by simply doing nothing (i.e. not pressing any button). Although the reaction time (measured from the Response Box) does not depend on the ERP measures, it was used to verify the subjects' attentiveness and alertness while performing the task. The test was conducted in four blocks of 10 trials each and there was a short interval between each block. In other words, the letter X was presented 10 times in each block which gave a total number of 40, while the remaining 160 represented the frequency of the non-target occurrence. The test could be completed approximately within 10 minutes, and the subjects' overall reaction time was recorded for each session.

3.7 Data Acquisition and Recording

The next stage was the data acquisition and recording of the EEG signals from subjects. The tools required for data acquisition, recording and stimuli presentation are as follow:

3.7.1 Hardware

3.7.1 .1 HydroCel Geodesic Sensor Net (HCGSN)

Figure 8: HC Geodesic Sensor Net

During EEG recordings, subjects wear a Sensor Net. It consists of an array of sensors that tessellates the surface of the head, where small sponges wetted with electrolyte rest against the head. The Sensor Net is composed of an elastomer geodesic structure that equalizes tension. The sponges are made of Polyvinyl Alcohol (PVA) and are medical grade while the electrodes are silver and silver chloride plated carbon-embedded plastic. The sensor wires are coaxial wires with alloy-core, Teflon coated with shield. A subject wearing a 128-channel adult-sized HCGSN is shown in Figure 8.

3.7.1.2 Net Amps 300 Amplifier

Figure 9: Net Amps 300 Amplifier

A 128-channel Net was connected to an amplifier (Net Amps 300 Amplifier) to filter and measure the EEG signals recorded by the Net and sample them at milliseconds intervals. The digitized samples were then transferred to the data acquisition computer (DAC) in real time through a fire-wire cable. The amplifier is differential-referenced to the vertex electrode and also has DC coupling feature which allows the amplifier to detect slow waves. Besides that, it also has 200 Mega Ohm input impedance. This means that it allows higher scalp input impedance by decreasing the signal-to-noise ratio. The sampling rate for this amplifier is up to 20 Kilo Herz from 256 channels amplifier, but the maximum handling rate in Net Station is 1000 Herz (sample/ second).

3.7.1.3 Data Acquisition Computer (DAC)

Packets of data containing digitized EEG samples were sent from the amplifier to the data-acquisition computer (DAC). As the Net Station software resided on the DAC, the DAC was capable of continuously collecting dense- array EEG data for display and storage to disk. The DAC used was a 15 inch Mac book Pro with 2 dual core Intel processors, a 160 Giga Byte Hard Disk, and 2 Giga Byte of memory. In addition to the fact that Net Station required Mac Operating

System to run, the Mac was known best for its superior graphics abilities and user interface.

3.7.1.4 ERP Stimuli Computer

Another 15.4 inch computer (Dell) with 2.4 Giga Herz Intel processor was also being used in the experiment as an experimenter control computer (or ERP Stimulus). This computer was responsible to deliver the corresponding visual or audio stimulation to the subjects. Hence, it is important to ensure that the stimulation timing from this device is in sync with the recording timing. The computer uses a TCP/IP connection to the DAC and was connected to Serial Response Box connected to the EGI response pad.

3.7.2 Software

3.7.2.1 Net Station v4.4.2

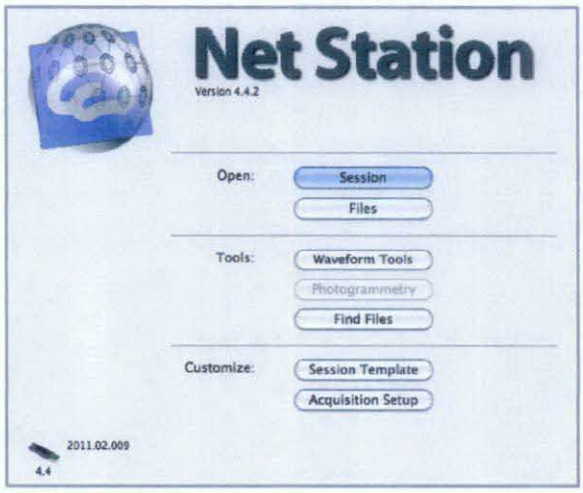

Figure 10: Net Station v4.4.2

Net Station acquisition software is designed for the acquisition of dense array EEG data. This project made use of the software to perform EEG data

acquisition, monitor and control the amplifier, and store subject information and technician markup events in data files (Session or Recording). This software resides on the hard drive of the data-acquisition computer (DAC) and communicates with the amplifier via the USB or Fire-wire cable that connects the amplifier and the DAC. Optionally, via Net Station, the Acquisition system can register and record external digital input (DIN) events and experimental control interface (ECI) events simultaneously with the EEG.

Net Station uses a workbench metaphor. Devices are assembled on the workbench and cabled together as needed (see Figure 11). It also uses a millisecond time base.

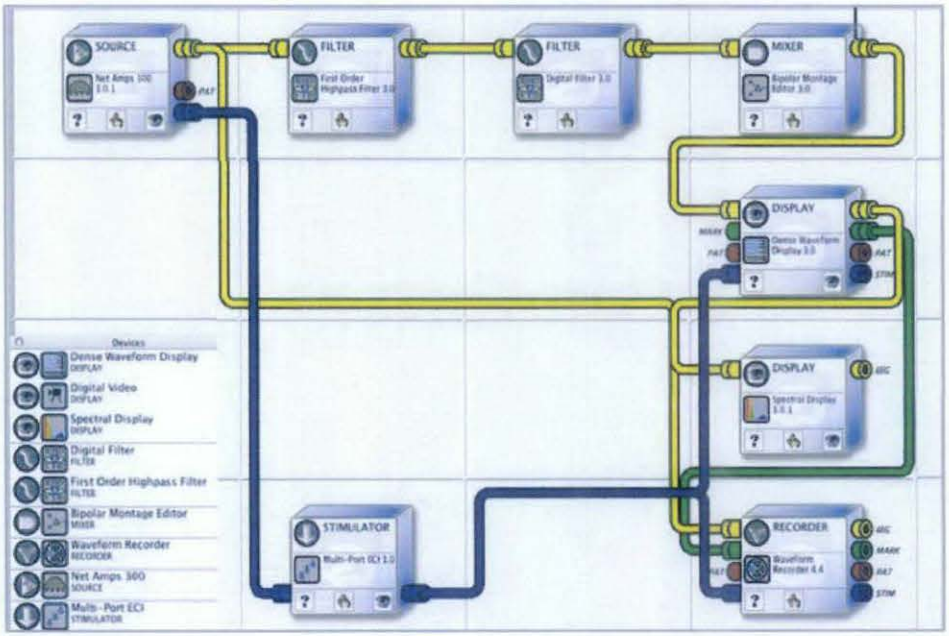

Figure 11: An ERP acquisition setup for in Net Station

The functionality is divided into devices:

1. Net Amp 300: provides software control of the Net Amps
2. Digital Video: control FireWire digital video which synchronized with the EEG waveform. The HandyCam records subjects' behaviors for later reference if needed.
3. Digital Filter device: controls the online lowpass, highpass and notch filtering.

4. First Order High Pass Filter: controls the online highpass filtering.
5. Bipolar Montage Editor: groups, mixes, and re-references selected channels. It also rearranges how channels are displayed.
6. The Multi-port ECI: interfaces with an external experimental control system via a TCP/IP connection.
7. The Dense Waveform Display: is responsible for displaying the data (EEG and events)
8. Waveform Recorder: records the EEG data from the Sensor Net connected to the amplifier.

A functional diagram between the core components is shown in Figure 12 below:

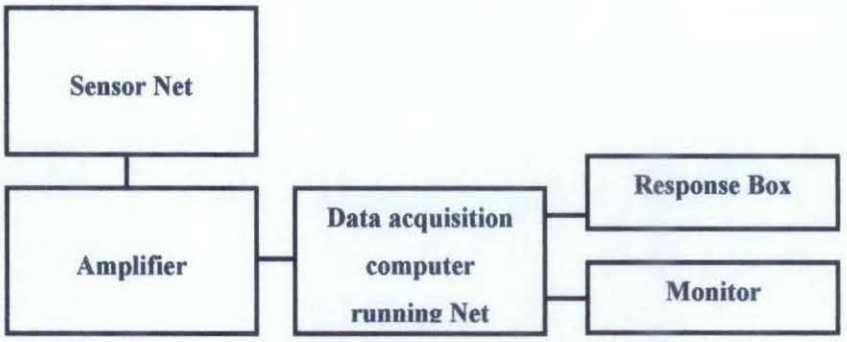

Figure 12: Physical connection diagram between core components

3.7.2.2 E-Prime v2.0

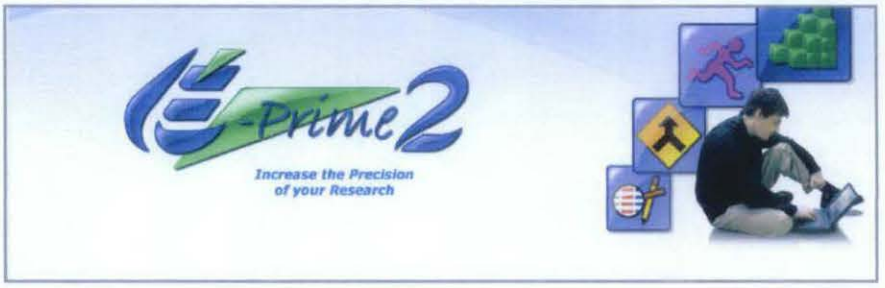

Figure 13: E-Prime v2.0

E-Prime v2.0 application software is used to design any event-related potential experiment that gives the user control over every aspect inherent to data collection in a research study. This project made use of this application software for the paradigm creation (oddball visual test).

3.7.3 Data Acquisition Workflow

3.7.3.1 Before recording session

Before subjects arrived, basic operations were checked (i.e. all the equipments are available and properly connected) and the electrolyte should be prepared earlier. Also, the session for the coming subject must be ready before the recording session begins because it took some time to setup the workbench. Upon subjects' arrival, they needed to wear a 128-channel Sensor Net where the correct size would be chosen after their head measurements had been taken. The Net was loaded with electrolyte (distilled water and Potassium Chloride, KCl) before applying the Net onto the head. While putting on the Net, subjects will be asked to close their eyes until they were told otherwise. Once the Net was applied and connected to the amplifier, the sensor impedances would be measured and the corresponding waveforms were also inspected. The workflow before the recording begins is summarized as follows:

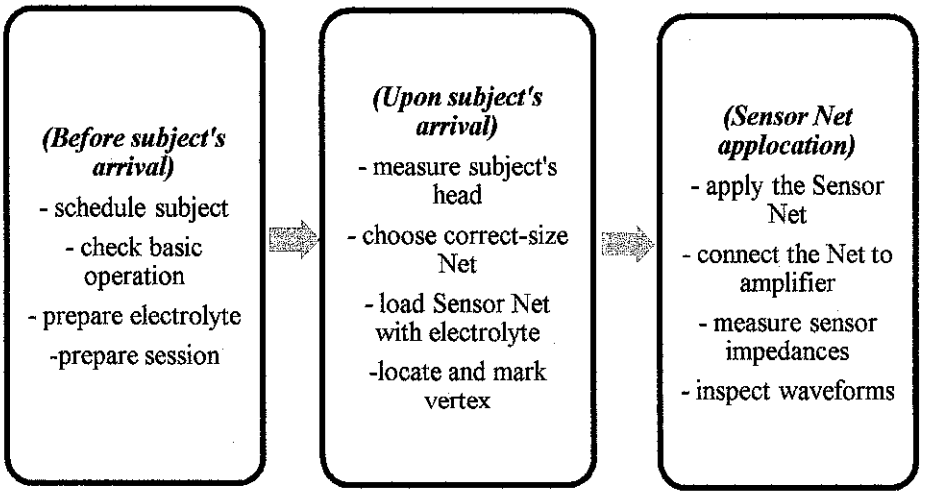

Figure 14: Pre- acquisition workflow

3.7.3.2 During recording session

Soon after the impedances were measured and there was no error could be found, subject would proceed with the visual ERP test. The distance between the

subject and the ERP Stimuli computer should remain 0.1 meter away. Also, subjects were reminded not to blink or move their eyes as much as possible when taking the test as to minimize the artifacts in the acquired EEG signals. During the test, subjects may take a break between each block of trials and they can resume doing the test whenever they are ready.

3.7.3.3 After recording session

When subjects had completed the test for that particular session, the Net will then be taken off and will be rinsed and disinfected for the cleaning purpose. This was to ensure there are no contaminant among subjects as the Sensor Net would be used again by another different subjects. For the first session, before subjects left, they were asked to answer a simple questionnaire regarding their daily activities. The questionnaire would be used as a mean of reference when analysing the EEG data.

The same test would be carried out for all sessions within the same day, however, for the last part of recording during the evening session (with caffeine intake), once the subjects had finished with the first evening session (while still wearing the Sensor Net), they were asked to drink a coffee and had to wait for about 15 minutes for the caffeine to start taking effect. Then after 15 minutes passed, they would resume the test and finally, the EEG data for all sessions were successfully recorded.

3.8 Signal Processing

Initially, the line noise was first removed using a 50 Hz notch filter which was set prior to recording session. The acquired EEG signals were then processed in two stages:
i) pre-processing and ii) post-processing.

3.8.1 Signal Processing Workflow

With the use of waveform tools in Net Station, the acquired signals were processed. The signal processing workflow is as shown below:

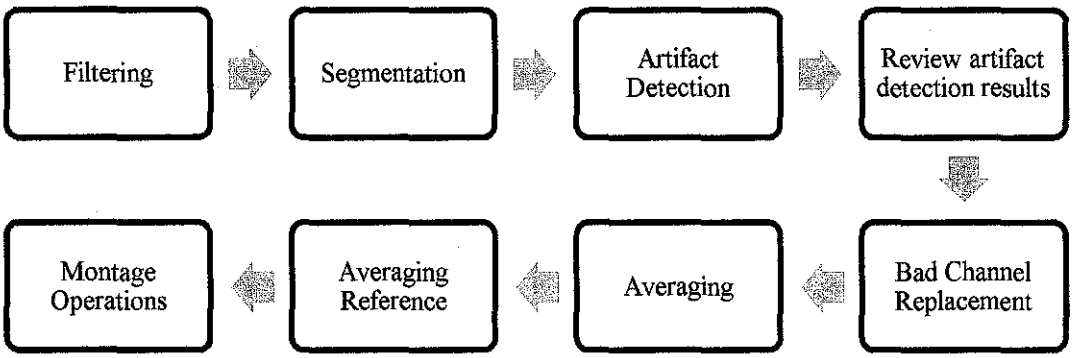

Figure 15: Signal processing workflow

3.8.2 Signals Pre-processing

Pre-processing involves filtering, segmentation and artifact detection. The tools specifications used are described as follows:

3.8.2.1 Filtering

The first step in processing the EEG signals is filtering. Filters were used to reproduce the range of frequencies contained in the EEG data and to minimize the amplitude of unwanted frequencies. According to [15], the approximate amplifier bandwidth required for clinical EEG signals are between 0.5 Hz to 70 Hz but opinions differ concerning the lowest frequency range. Instead of using a

bandpass filter, two filters were used in the study: i) 0.1 Hz high-pass filter (Figure 16) ii) 30 Hz low-pass filter (Figure 17). With the use of these filters, the EEG data can still be displayed adequately.

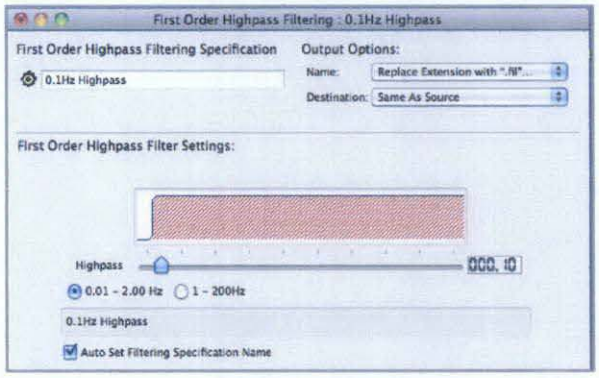

Figure 16: 0.1 Hz high-pass filter tool

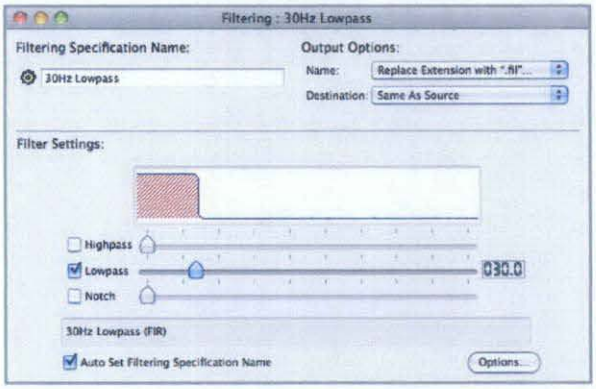

Figure 16: 30 Hz low-pass filter tool

3.8.2.2 Segmentation

Next, the EEG signals are segmented. In segmentation, the continuous EEG recording was broken into event-locked epochs (called segments) based on a set of selection rules and user-defined criteria. The purpose of segmentation is to organize the data into categories (standard or target) so that they can be averaged or other operations can be performed on them. This entails knowing which conditions belong to which events and understanding the concept of temporal relations.

The segment length used was 1 second (1000 ms). The stimulus onset was at 100 ms and the remaining length was set to 99 ms. The offset value (16 ms) was predetermined during the calibration of the devices (see Figure 18 and Figure 19).

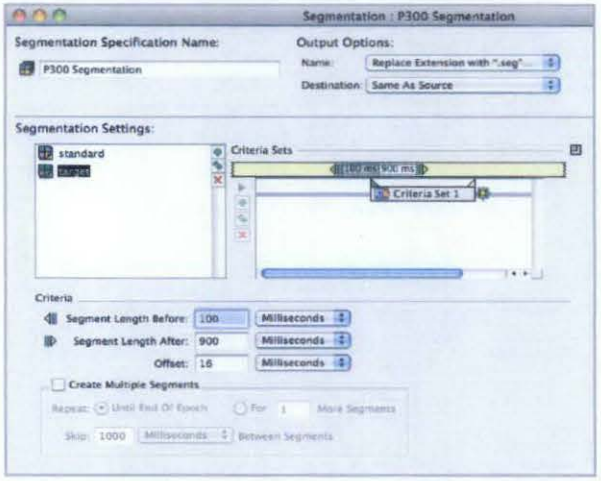

Figure 18: Segmentation length settings

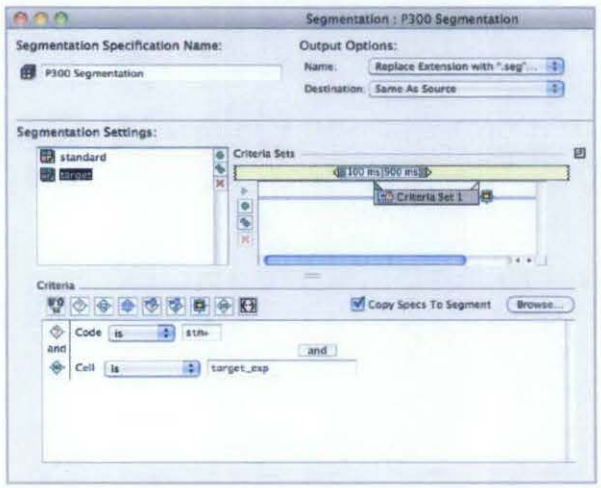

Figure 19: Segmentation criteria settings

Since there were two categories (i.e., Standard and Target), with Standard occurrence's probability is 0.8 while Target is only 0.2, hence with 200 of total simulations, there are 200 segments in total. In other words, the number of segments for Standard categories is 160 while Target is 40 segments.

3.8.2.3Artifact Detection

According to Woodman [16], eye blinks and eye movements are among can cause a massive electrical transient (i.e., over 100 μV) as compared to normal P100 amplitude (i.e., less than 10 μV). Hence, these eye blinks and eye movements are considered as artifacts in EEG recordings. However, according to a study, these artifacts can be detected with electrooculargram (EOG) as it is important to reject trials with artifacts to avoid data contamination. In the study, an artifact detection tool is created with the following specifications as shown in Figure 20.

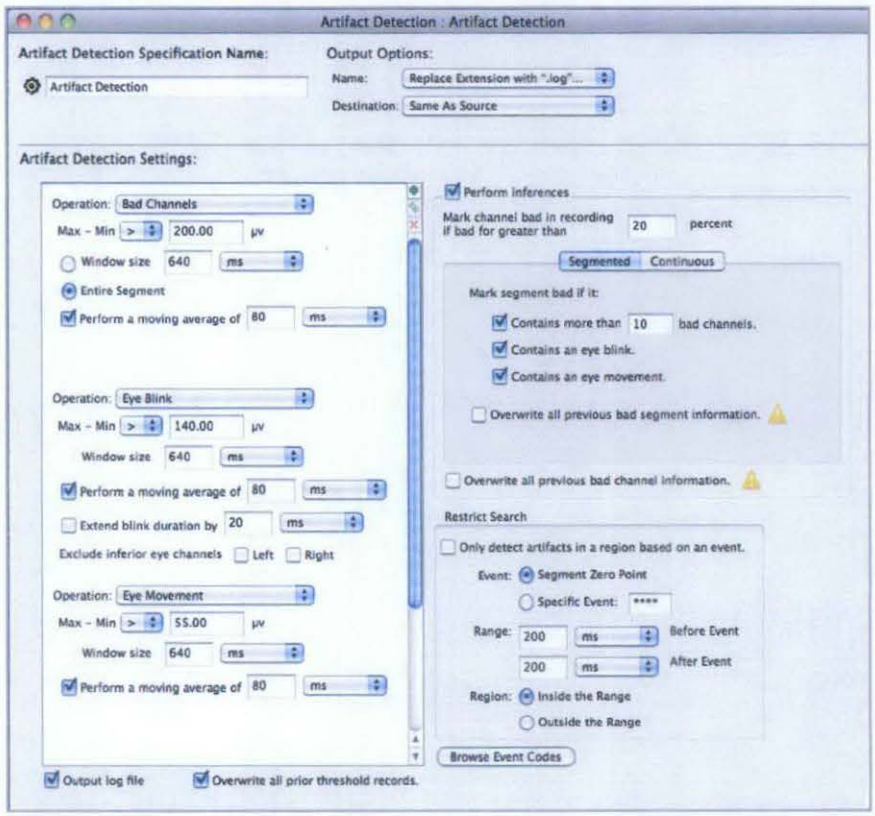

Figure 20: Artifact detection tool

3.8.2.4 Pre-processing Script

With all the pre-processing tools have been specified, the tools operations then can be chained together using the script tool. The tool is used to automate the

data processing once the data-analysis path has been satisfied. All the input files for each operation tools will accumulate and then generate the output files (see Figure 21) using this script tool.

Figure 21: Pre-processing script outputs

After the recorded data had been segmented and the artifacts were detected, the segmented file (.seg) was reviewed. The following figure (Figure 22) shows the total number of bad channels for every segment. In this figure, there are 132 good channels for the Standard stimuli while 36 of good channels for the Target. With reference to Segment 1 (Target), there are four bad channels in the segment. To verify this condition, the output file can be reviewed to see if there were any other missing bad channels that exist but could not detected by the artifact detection tool.

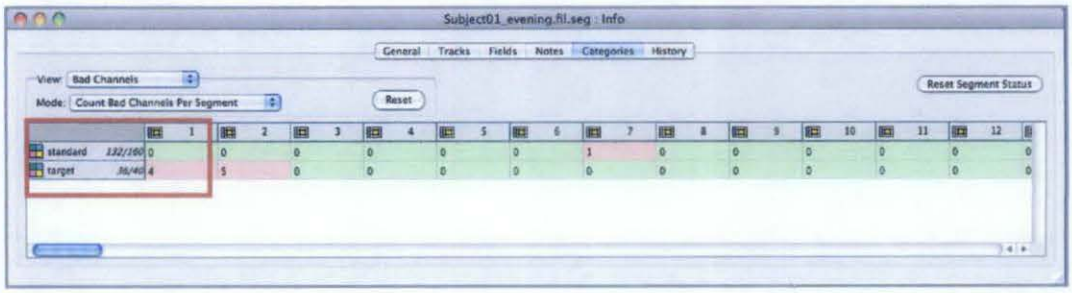

Figure 22: Summary of bad channels per segment

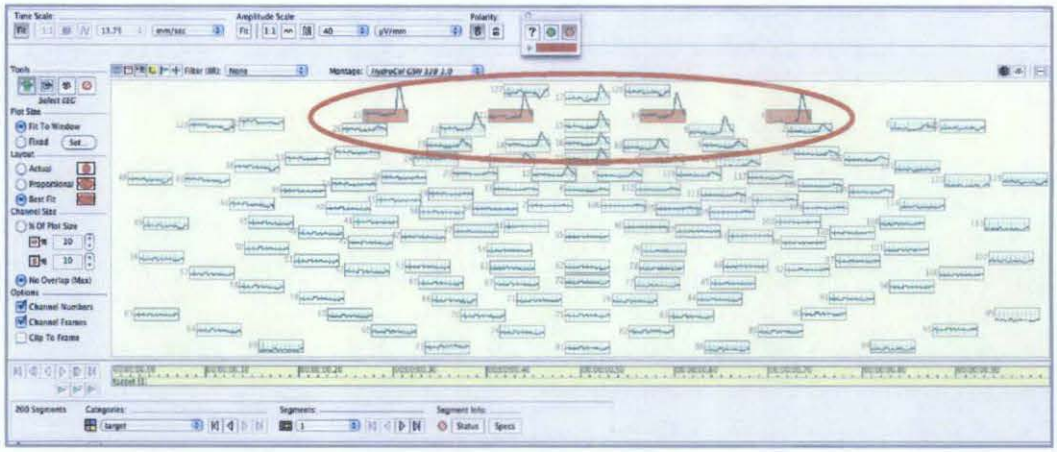

Figure 23: The segmented output file with bad channels are marked

As shown in Figure 23, the bad channels (in target segment 1) were marked in red because the subject might have blinked or moved her eyes while performing the task. However, note that there were several other channels which also had unusual waveforms (i.e., the amplitudes exceeded 200 μV) that also need to be marked as bad channels. Hence, the output files need to be reviewed by inspecting manually each segment (for target and standard categories) for any unusual waveforms which might not be detected by the artifact detection tool. The reviewed segmented file summary is shown in Figure 24. Note that the number of bad channels increased to ten (i.e., previously was six) as there were six more channels were marked as bad.

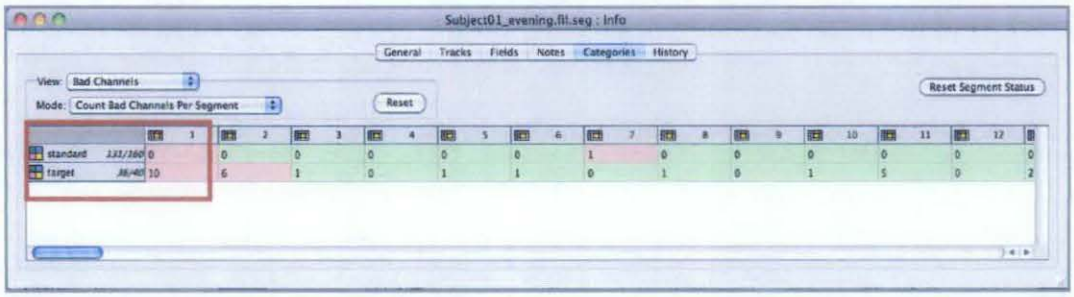

Figure 24: Summary of bad channels per segment after revision

3.8.3 Signals Post-processing

After the segmented output files had been reviewed, the next step is to further process the EEG signals. Pre-processing involves replacing bad channels, averaging, averaging reference, and finally, baseline correction. The tools specifications used at this stage are described as follows:

3.8.3.1 Bad Channel Replacement

This tool is designed to operate on the reviewed segmented file (after artifact detection process). It replaces the bad channels detected with the interpolated from the remaining channels (using spherical splines) and inactivates the "bad channel" status. Theoretically, due to volume conduction, scalp locations in proximity to each other have similar voltage values. Hence, the greater the number of channels, the better the approximation of the missing channels.

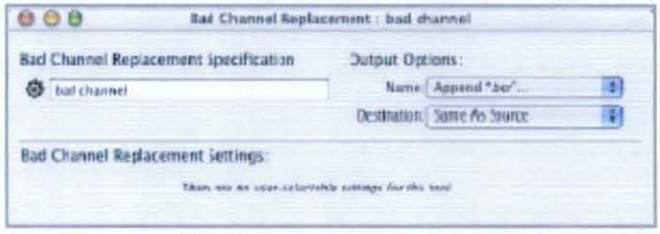

Figure 25: Bad channel specification tool

3.8.3.2 Averaging

In averaging, all the good segments are averaged for both Target and Standard categories. For each category, all the segments are collapsed to a single, averaged segment.

3.8.3.3 Average Referencing

In ERP derivation, the Montage Operation is used to rereference the data. The data can be rereferenced to any channel or to the average of any set of

channels. EEG is a measure of voltage, and voltage is a measurement of the difference between potentials. The purpose of rereferencing EEG data is to estimate a true, non-arbitrary zero value to which to reference the measurements of the voltage.

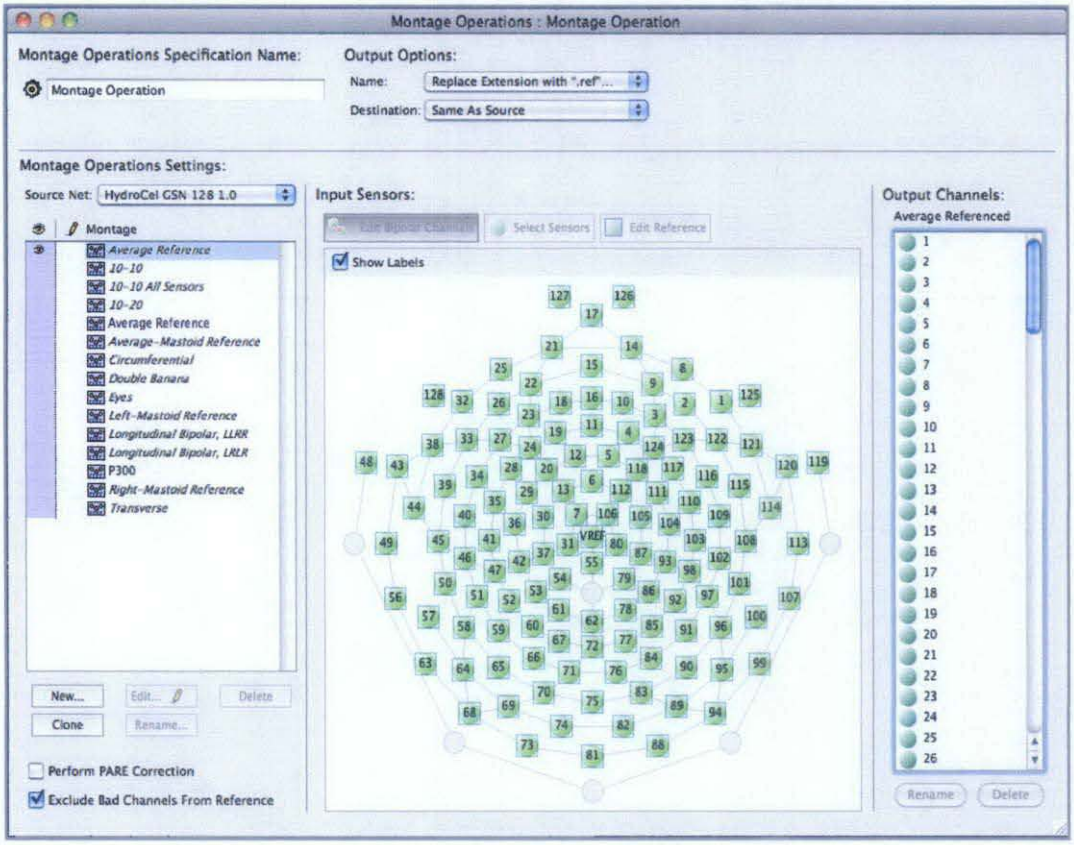

Figure 26: 128-channel montage operations

Also, if the specified set of channels is less than the set of channels in the input file, the data are downsampled. With the use of montage, the data can be displayed either with 128-channels (see Figure 26) or 10-20 system (see Appendix E-1).

3.8.3.4 Baseline Correction

In baseline correction, a new zero-voltage value is defined based on a baseline interval that already selected within the segment. The baseline interval could be either a portion of the segment or the whole segment. For stimulus

events, the baseline interval precedes the stimulus, where in this case 100 ms (see Figure 27). For every channel, the average of all samples within the baseline interval is subtracted from each sample in the segment.

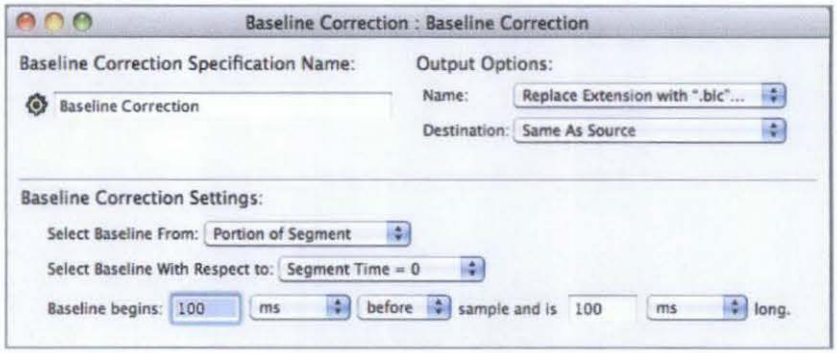

Figure 27: Baseline correction tool

3.8.3.5 Post-processing Script

Finally, as all the post-processing tools have been specified, all the input files for each operation tools will accumulate and then generate the output files using the post-processing script tool (see Figure 28).

Figure 28: Post-processing script outputs

3.9 Result Validation and Analysis

3.9.1 Visualising ERPs

The final stage of this project is the validation and analysis of the ERP results. In the following section, the critical steps taken to analyse the ERP data will be discussed. For this purpose of discussion, the data for P300 at C_Z for Subject 07, will be taken as an example.

Using the baseline correction output file (.blc), all the channels can be viewed using topo plot as shown in Figure 27 below. The data can also be viewed as 10-20 system (see Appendix E-2) .

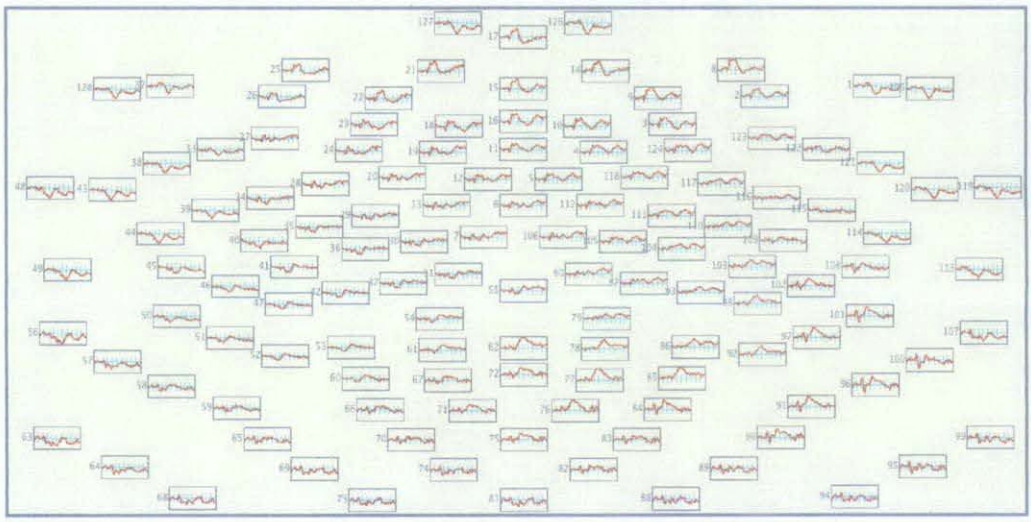

Figure 29: Data in 128-channel in topo plot view

3.9.2 Quantifying ERPs

The ERP components can be viewed in any channel of interest. Figure 28 shows the P300 at CZ. Since the data has been averaged, previous studies [16, 17] suggested that the peak (the highest point in amplitude) may not be the actual peak of the P300 as the averaged ERP components are filtered. In the study, in defining the P300, a temporal window of 250 to 500 ms was used. The most

positive going component within this latency window is selected as P300. This method had also been practised by many ERP researchers where the width of this window brackets the entire ERP component of interest across all subjects.

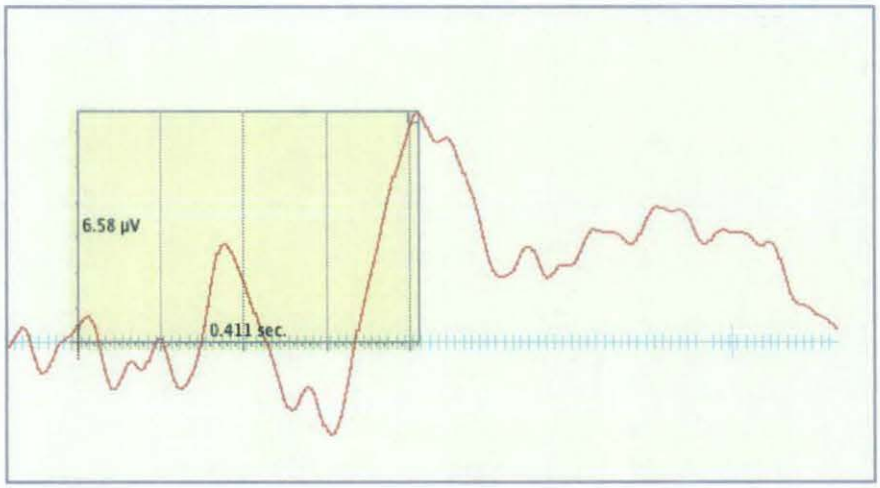

Figure 30: P300 at C_Z

For overall results for P100 and P300 components, see Appendix F and Appendix G, respectively.

3.10 Reporting

Upon project completion, all the research findings and the analysis results and discussions will be documented in the final technical report and thesis.

CHAPTER 4
RESULTS AND DISCUSSION

4.1 Electrophysiological Results

4.1.1 Time-of-day

The results of P100 and P300 components (i.e., amplitude and latency) at time-of-day at different electrode sites (i.e., O_Z, O_1, O_2, Fz, Cz, and Pz,) for all subjects are shown in the following graphs:

4.1.1.1 Latency

i) P100 latency across O_Z, O_1 and O_2 over time

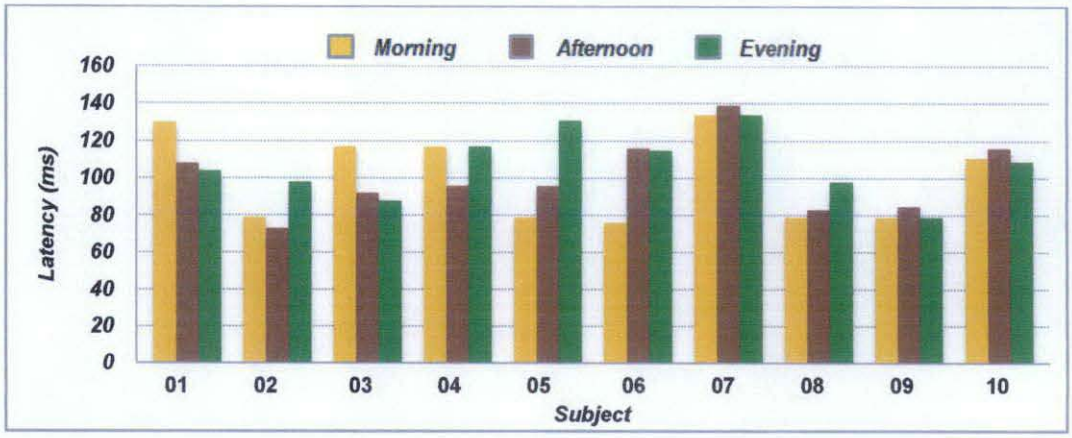

Figure 31: P100 latency at Oz over time across subjects

Figures illustrate P100 latency variations across subjects at the O_Z, O_1 and O_2 electrode sites over time. From Figure 31, it can be seen that the P100 latency in most subjects is relatively shorter in the morning than if compared in the afternoon and in the evening. Also, out of the ten subjects, six subjects showed

slight increase in latency during the afternoon in comparison with the morning session. However, later in the evening, only four subjects showed a significant increase in latency while the rest remained the same or had a slight decrease in latency.

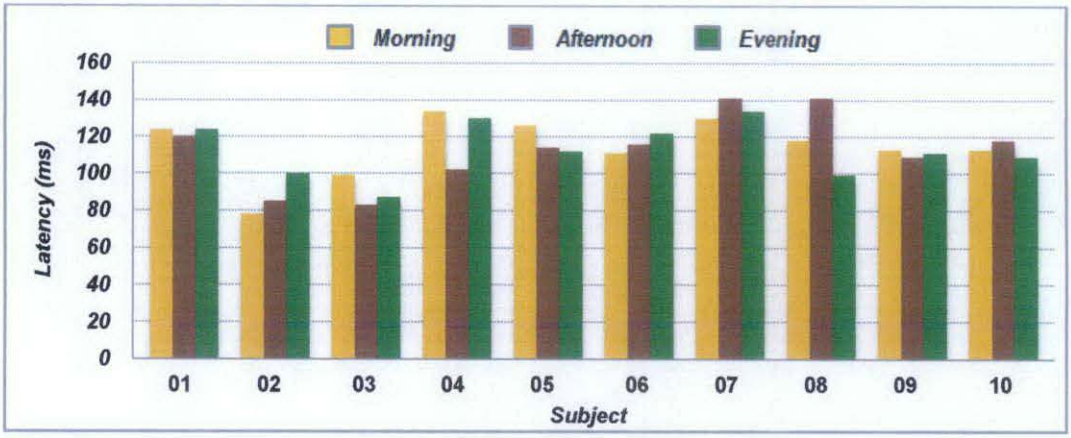

Figure 32: P100 latency at O_1 over time across subjects

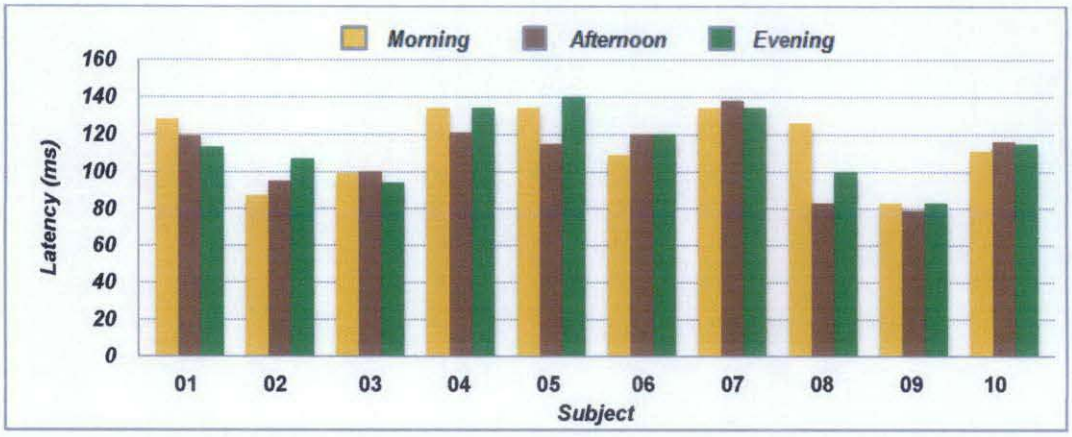

Figure 33: P100 latency at O_2 over time across subjects

On the other hand, in Figure 32 and Figure 33 shown above, the P100 latency measured at O1 and O2 sites, in most subjects is relatively longer in the morning than if compared in the afternoon. The latency varied among subjects in the afternoon, but six of them showed slight increase in the P100 latency towards the end of the day.

ii) P300 latency across F_Z, C_Z and P_Z over time

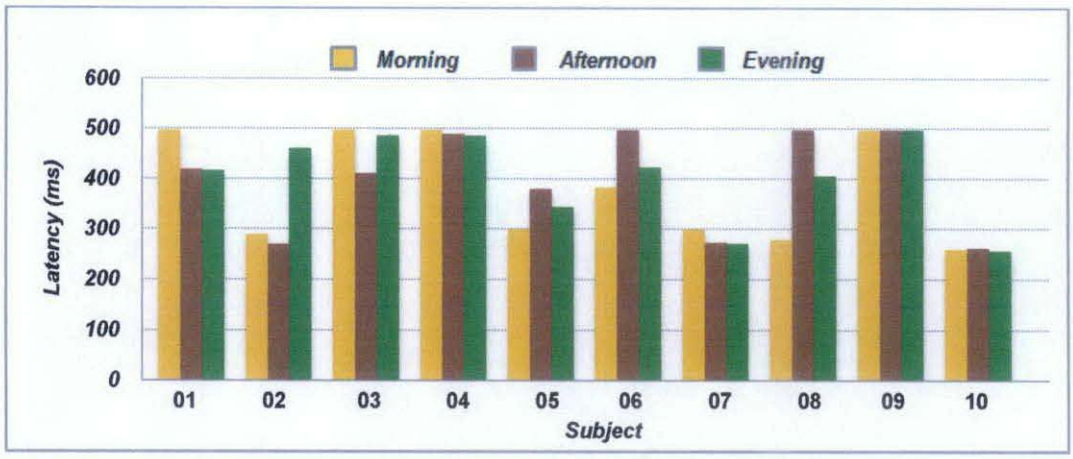

Figure 34: P300 latency at F_Z over time across subjects

From Figure 34, though there was no common trend found in P300 latency across subjects at F_Z, most of them showed shorter latency in the morning compared to the other two sessions. Besides that, the P300 latency in half of the sample remained the same for the afternoon and evening sessions.

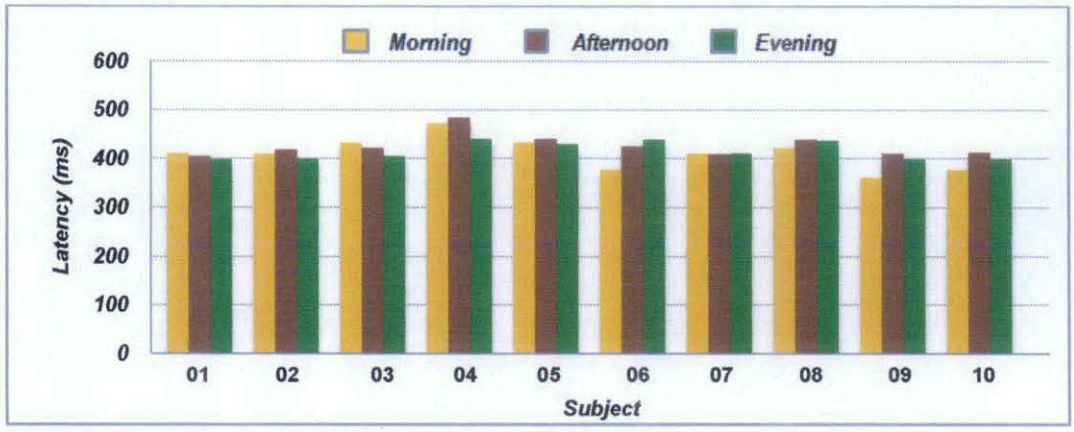

Figure 35: P300 latency at C_Z over time across subjects

In Figure 35, the P300 latency at C_Z for each subject showed small variance over time though most subjects showed a decreasing pattern (from afternoon to evening). The same results could also be seen at P_Z site (see Figure 36) where only three subjects showed significant changes. However at P_Z, most subjects showed an increasing pattern despite the small changes in the latency.

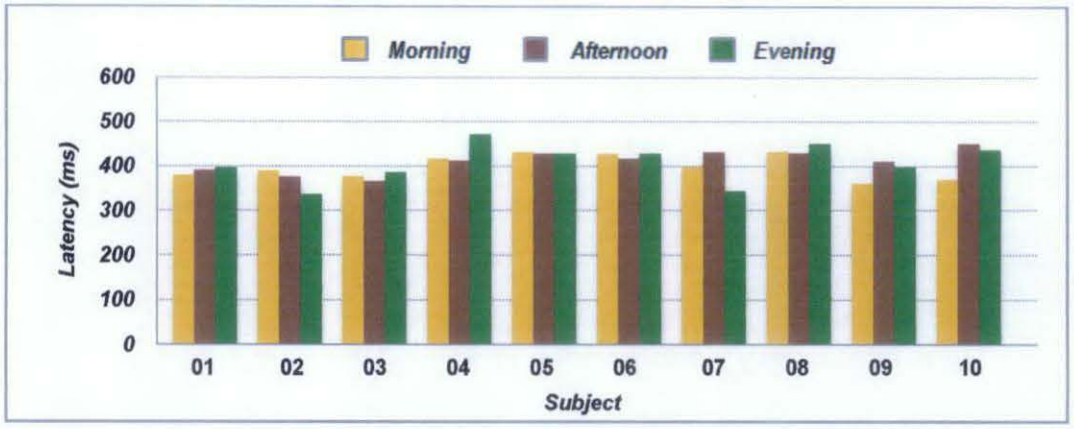

Figure 36: P300 latency at P_Z over time across subjects

4.1.1.2 Amplitude

i) P100 amplitude across O_Z, O_1 and O_2 over time

The amplitudes of P100 at the occipital sites were relatively small (i.e., below 6.0 μV). Nevertheless, the highest amplitude mostly occurred during the morning session as found in five subjects where as the remaining half showed the highest amplitude either in the afternoon or in the evening (see Figure 37 through Figure 39).

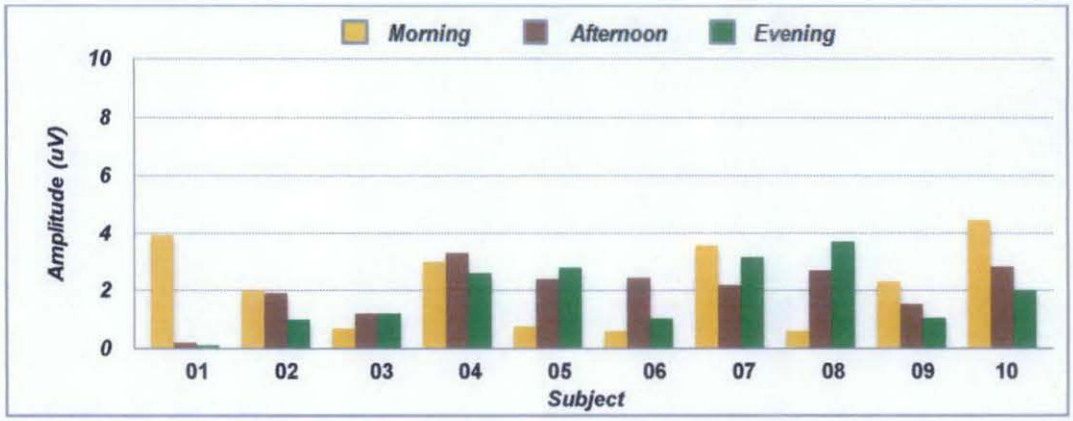

Figure 37: P100 amplitude at Oz over time across subjects

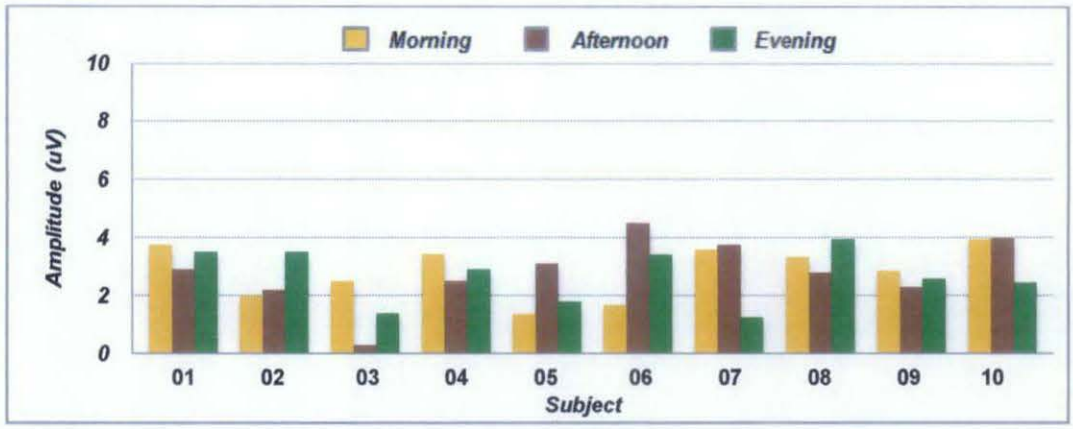

Figure 38: P100 amplitude at O_1 over time across subjects

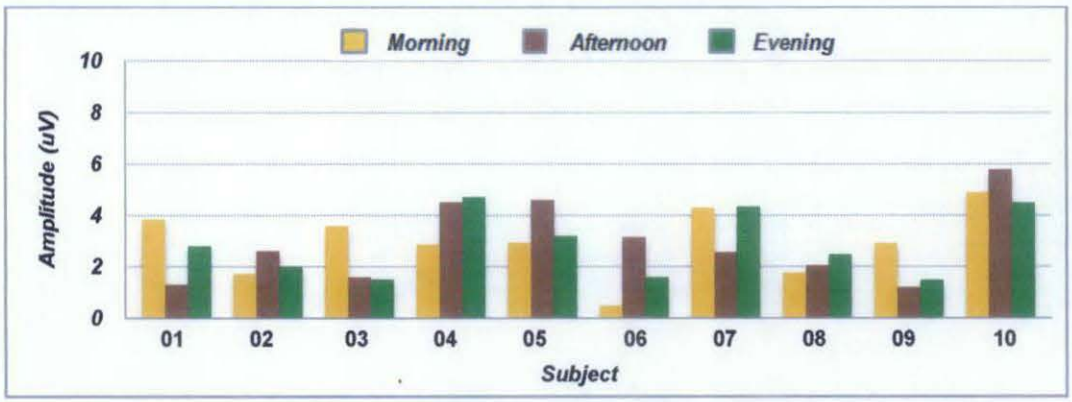

Figure 39: P100 amplitude at O_2 over time across subjects

ii) P300 amplitude across F_Z, C_Z and P_Z over time

Figure 40 shows that the morning session had the highest P300 amplitude as showed by five subjects. On the other hand, the lowest amplitude was found in most subjects during the evening in comparison with the afternoon session. This similar pattern can also be found at C_Z site (see Figure 41). However, at the P300 amplitude measured at P_Z in the afternoon (Figure 42) was found to be the highest for all subjects and again, most subjects had the lowest amplitude in the evening session compared to the morning session.

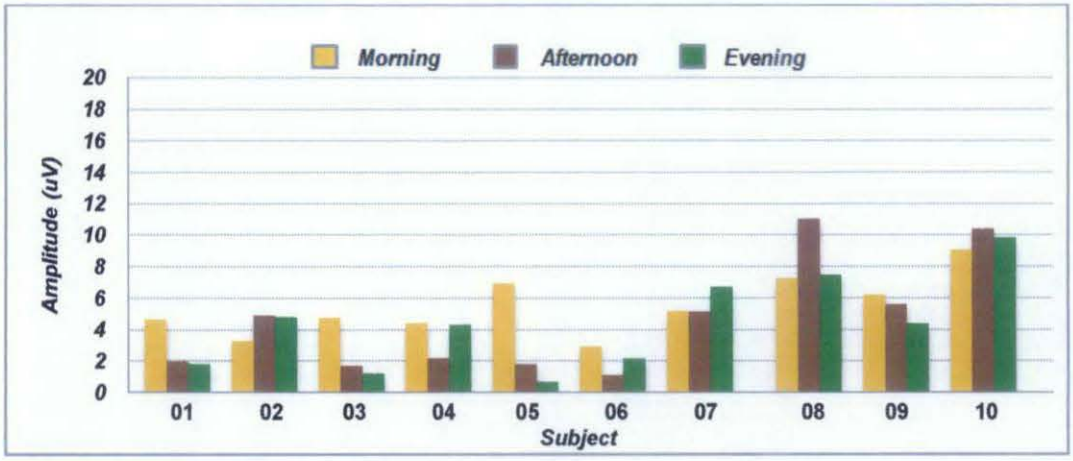

Figure 40: P300 amplitude at F_Z over time across subjects

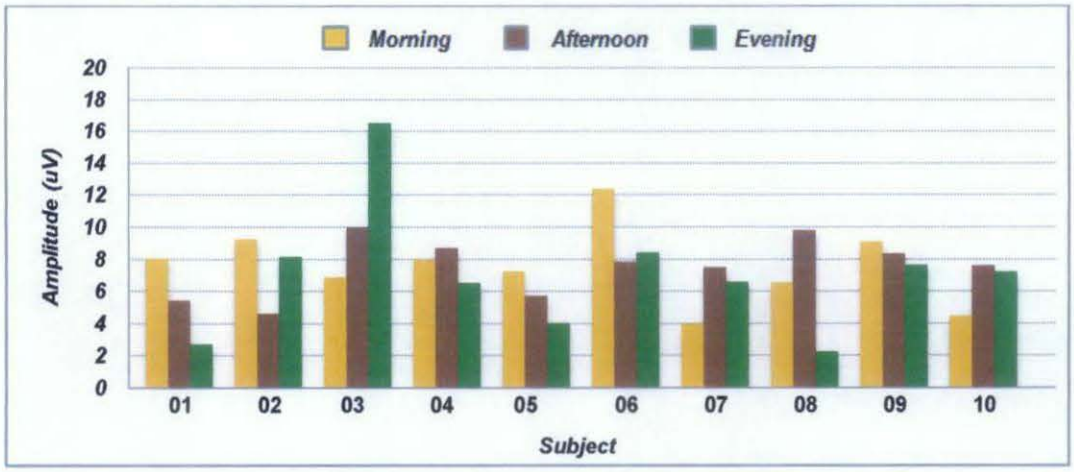

Figure 41: P300 amplitude at C_Z over time across subjects

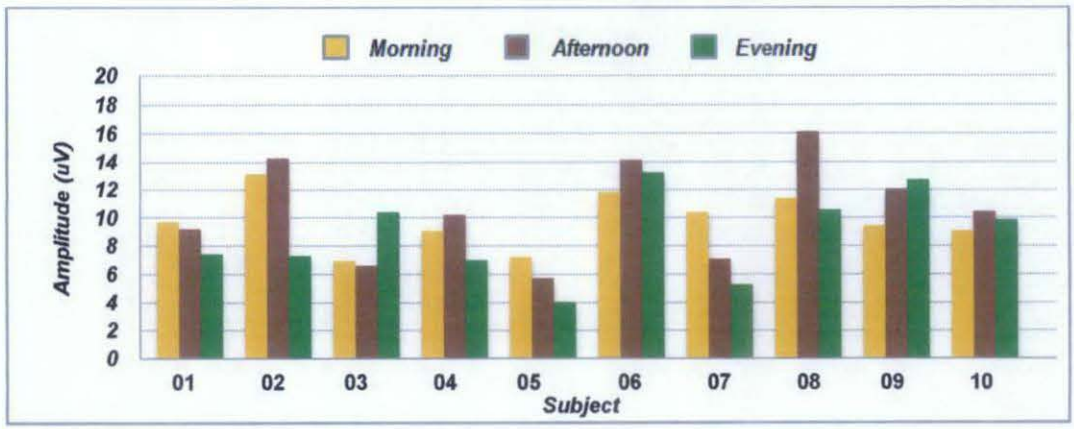

Figure 42: P300 amplitude at P_Z over time across subjects

4.1.1.3 Scalp Distribution

i) P300 latency across Fz, Cz and Pz at time-of-day

The following three graphs (Figure 43 through Figure 45) below in general shared similar trends. It can be seen that the P300 latency at FZ site was the shortest, as found in five subjects even at three different times. In addition, there was small variance between the P300 latency at CZ and PZ.

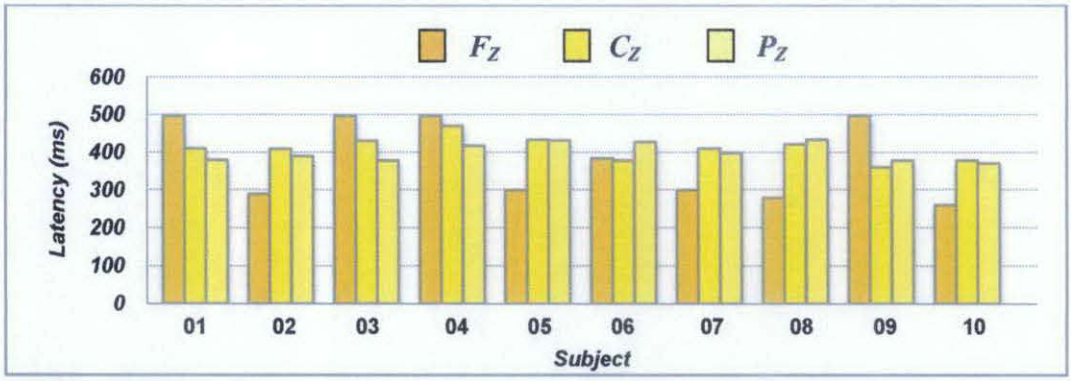

Figure 43: P300 latency at F_Z, C_Z and P_Z in the morning

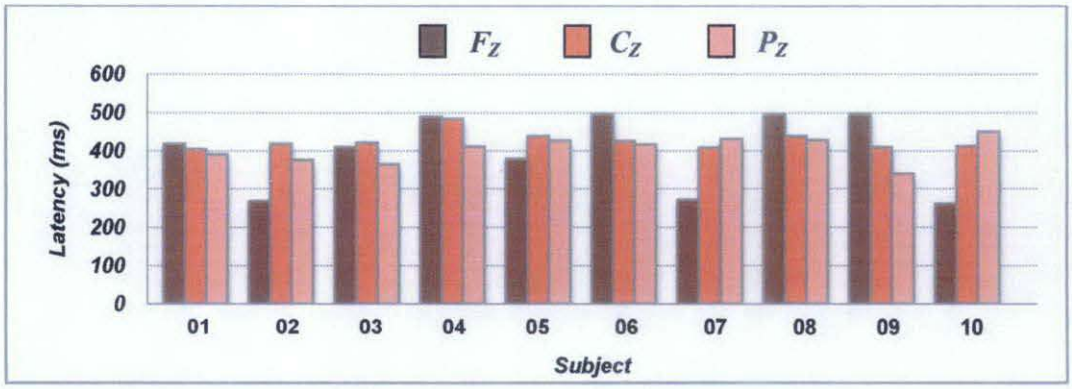

Figure 44: P300 latency at F_Z, C_Z and P_Z in the afternoon

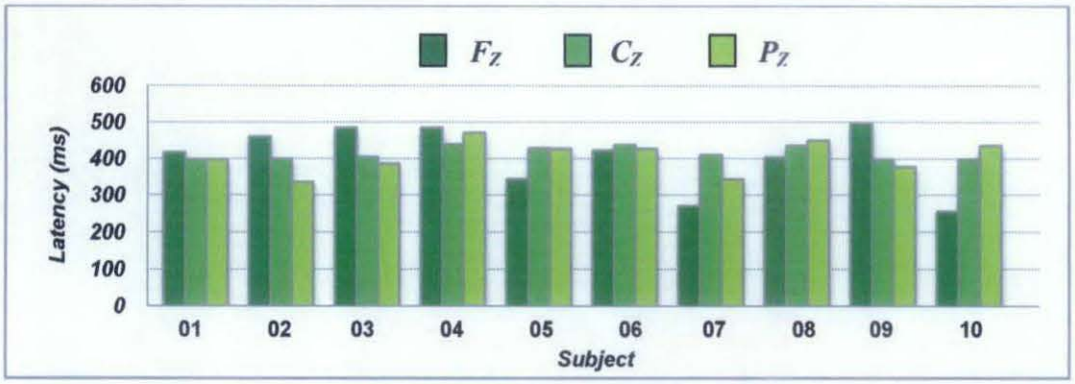

Figure 45: P300 latency at F_Z, C_Z and P_Z in the evening

ii) P300 amplitude across F_Z, C_Z and P_Z at time-of-day

For the amplitude, an increase trend was seen (i.e., $F_Z < C_Z < P_Z$) for most subjects with F_Z site had the lowest amplitude while P_Z site had the highest amplitude. This trend holds for all the three sessions as shown in the graphs (Figure 46 through Figure 48).

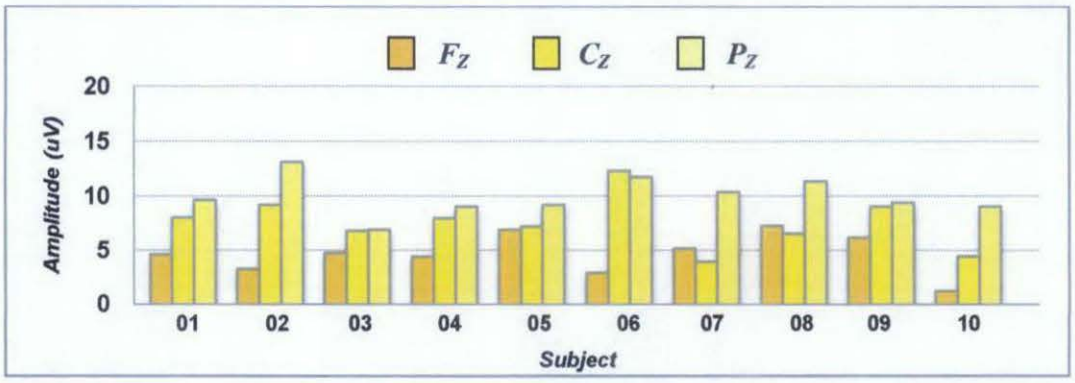

Figure 46: P300 amplitude at F_Z, C_Z and P_Z in the morning

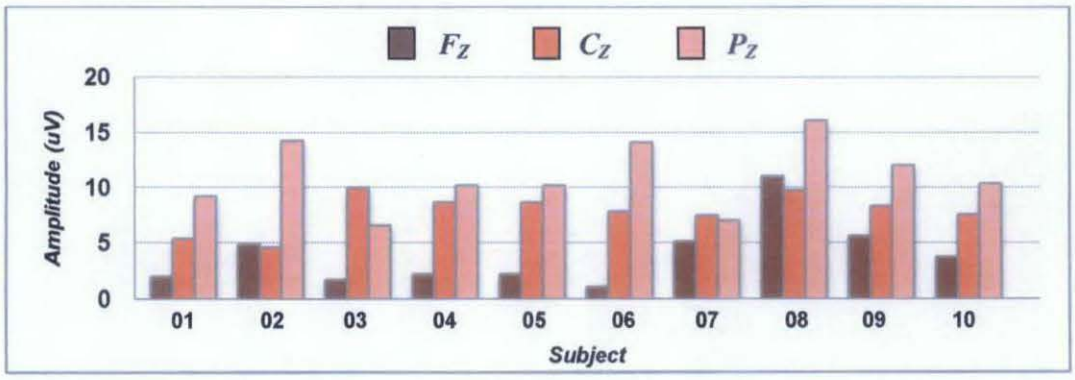

Figure 47: P300 amplitude at F_Z, C_Z and P_Z in the afternoon

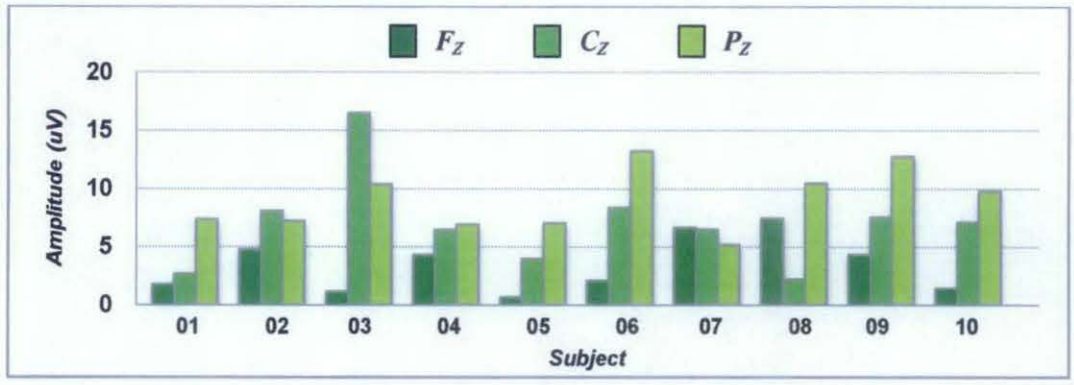

Figure 48: P300 amplitude at F_Z, C_Z and P_Z in the evening

4.1.2 Caffeine administration

The results of P100 and P300 components (i.e., amplitude and latency) before and after caffeine administration (in the evening) at different electrode sites (i.e., O_Z, O_1, O_2, F_Z, C_Z, and P_Z,) are shown in the following graphs:

4.1.2.1 Latency

i) P100 latency across O_Z before and after caffeine administration

Overall, the latency at O_Z for all subject were decreased after the caffeine intake except for few subjects whose latencies either remained the same or increased (see Figure 49).

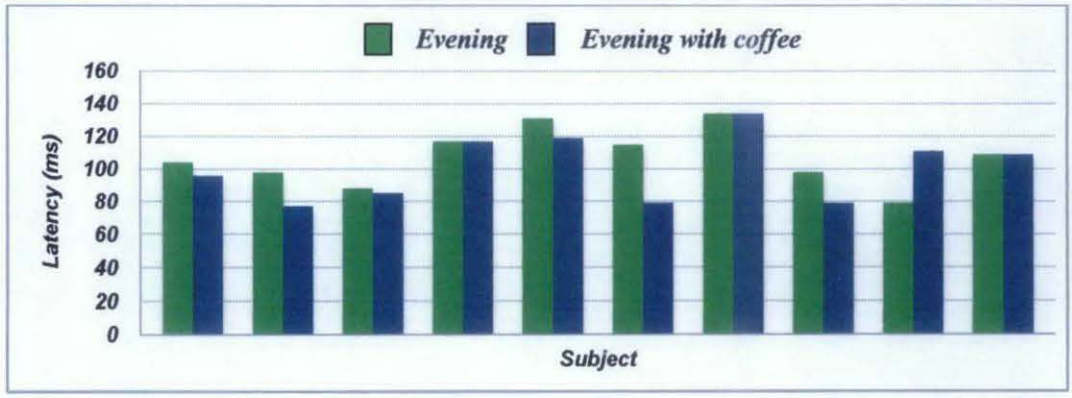

Figure 49: P100 latency at O_Z before and after caffeine administration

ii) P300 latency across F_Z, C_Z and P_Z before and after caffeine administration

However, as seen from Figure 50 through Figure 52, almost all subjects showed a shorter latency after drinking coffee.

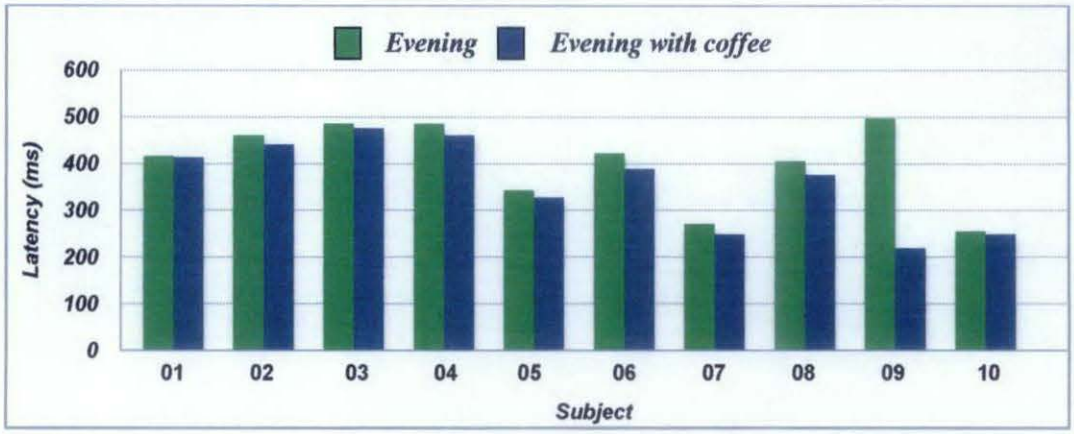

Figure 50: P300 latency at F_Z before and after caffeine administration

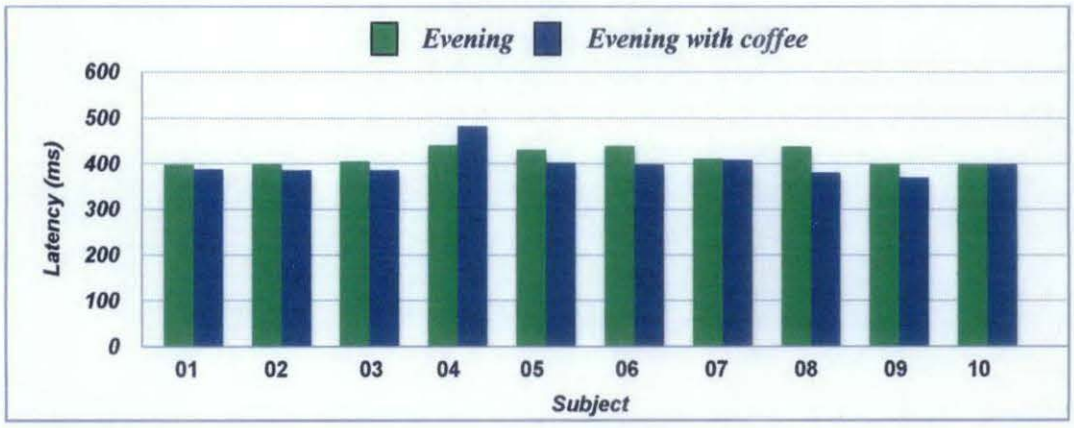

Figure 51: P300 latency at C_Z before and after caffeine administration

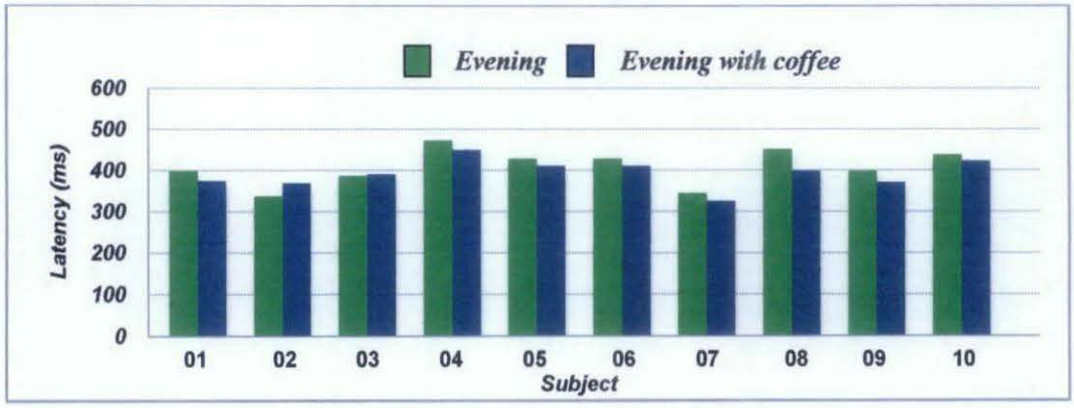

Figure 52: P300 latency at P_Z before and after caffeine administration

4.1.2.2 Amplitude

i) P100 amplitude across O_Z, before and after caffeine administration

Though most subjects showed positive effects in the latency (shorter latency), the case is not the same for the amplitude. We expected to see an increase in the amplitude after the caffeine ingestion. As shown in Figure 53 through Figure 56 however, there were subjects whose amplitudes are decreased or remained even after the caffeine intake.

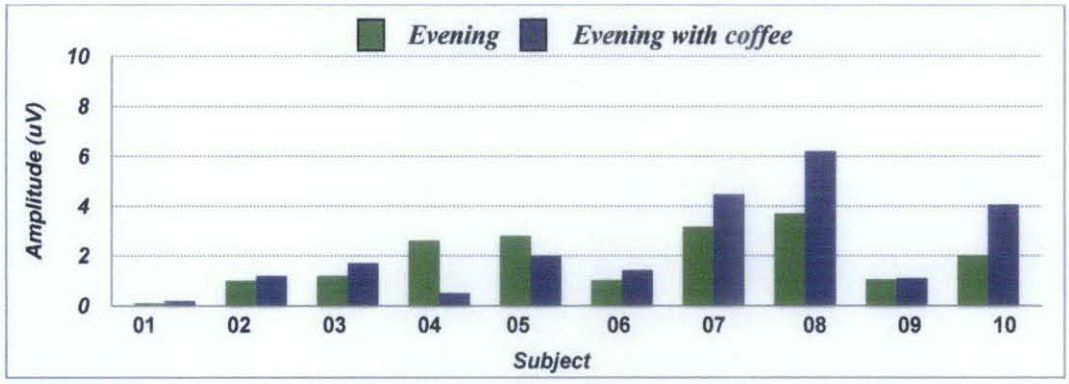

Figure 53: P100 amplitude at O_Z before and after caffeine administration

ii) P300 amplitude across F_Z, C_Z and P_Z before and after caffeine administration

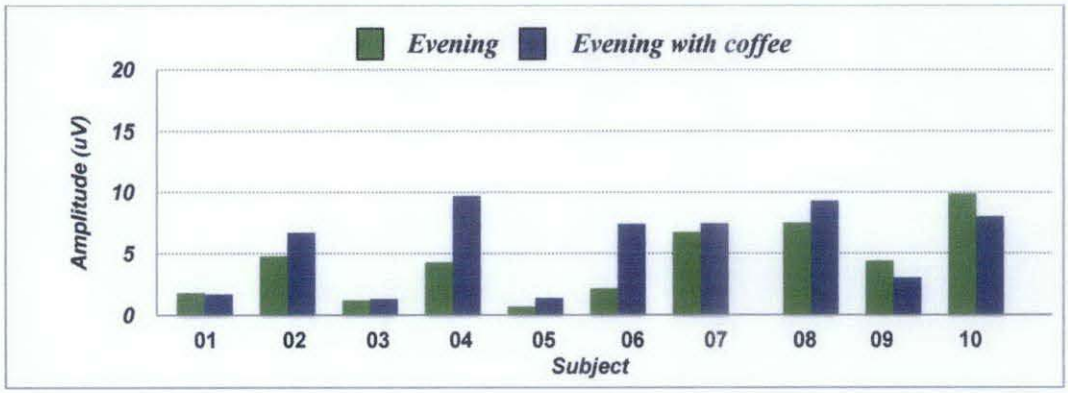

Figure 54: P300 amplitude at F_Z before and after caffeine administration

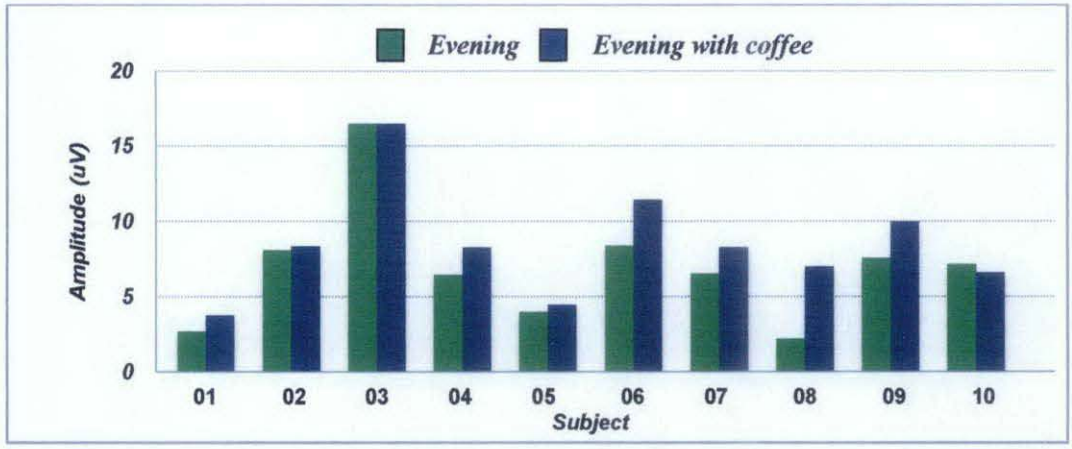

Figure 55: P300 amplitude at C_Z before and after caffeine administration

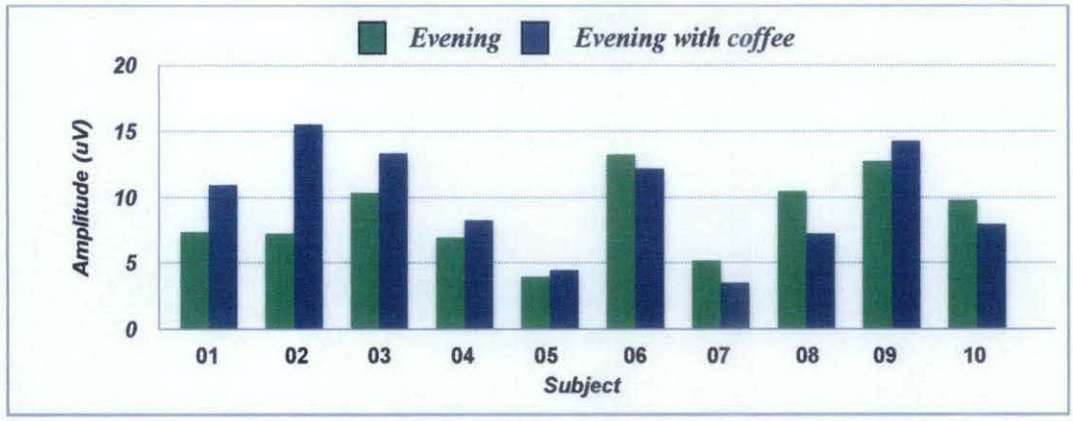

Figure 56: P300 amplitude at P_Z before and after caffeine administration

4.2 Discussion

This study aims at investigating the effect of time-of-day and caffeine administration on visual and cognitive processing efficiency, through the inspection of the ERP components (i.e., P100 and P300) variables: latency and amplitude. The results analysis for P100 and P300 components will be discussed in the following discussions.

i) P100

As mentioned earlier, the P100 component is associated with the early stage of visual information processing as it is distributed at the occipital region and thus reflects the visual inputs nerve conduction velocity from the retina to the primary visual cortex. Moreover, the early stage of information processing which involves the detection of stimuli and also perceptual classification of different stimulus modalities might compromise P300 component as speculated by Puga *et. al* [18]. Another study suggested that the integrity of information processing and the reliability of early visual inputs seem to be dependent. In other words, any impairment of the visual inputs may be the cause of failure of an individual's high-order cognitive processes such as memory, attention or sensorimotor performance.

To test the effect of time-of-day on P100, the visual discrimination task had been carried out at three different times within the same day. We expected to see longer latencies and lower amplitudes towards the end of the day and the obtained results partially confirmed this hypothesis. This is because few subjects had shown longer latencies and higher amplitudes in the morning which could indicate that individuals with slower visual processing may not be necessarily have low attentional level or alertness, respectively. However, later in the evening, most subjects tend to have longer latencies but with only half of them showed lower amplitudes within the same session. This may be because the arousal or fatigue level for every individual varied due to many factors (i.e., workloads).

As for the second factor, though many researches had proven that caffeine proved to improve cognitive performance, very few studies focused on its effect on the early stage of visual processing component, namely P100, hence the results of this experiment are controversial and not yet conclusive. However, the results showed that the latencies in most subjects tend to decrease while the amplitudes were increased after the caffeine intake. This may imply that caffeine has positive effect in vision as shorter latencies as well as higher amplitudes was observed in most subjects.

ii) P300

The P300, which is an index of cognitive efficiency as it is often regarded as one of the main cognitive components involved in decision making processes. As claimed by Deslandes *et. al* [19], it reflects the neuroelectric activity related to cognitive processing such as memory updating and attentional allocation. Individuals may vary to one another due to several factors among which, the time-of-day and caffeine intake are investigated in this study.

To validate the changes in latency and amplitude of the P300 over time, subjects were submitted to a visual oddball paradigm task in the morning, in the afternoon and finally, in the evening. Prior to the visual test, they were instructed to respond only to the infrequent stimuli (target) which in this case was the letter 'X', by pressing the source response box. Though no significant differences for latency values were shown at C_Z and P_Z, a trend of decrease and increase were found at the respective electrode sites. In general, shorter latency in P300 indicates superior cognitive performance, where in this sense, subjects with shorter P300 latencies are capable to recognize and discriminate stimuli faster than those who had longer latencies. Also, the action of responding to only the targeted stimuli reveals the alertness of the subject which can be assessed through the amplitude values. Higher amplitudes indicate higher attentional level and vice versa.

Interestingly, looking at the pattern of the latency distribution of P300 across F_Z, C_Z and P_Z, with F_Z having the shortest latency, followed by P_Z and finally C_Z. This tells us that as subjects saw the stimulus, first, there was a generation of P300 at F_Z, then the visual information reached the cognitive part located at the parietal site, Pz. This is actually where the integration of visual and cognitive information processing occurs, thus subjects could discriminate and decide provided that both visual (letter 'X') and cognitive (respond only to 'X') information are available. Finally, if the stimuli made any sense to the subjects, they would respond to it by pressing a button on source box and this motory action was controlled by the motor areas located at the centralparietal near to the vertex (C_Z). Also, taking note on the small variations which were consistent between the P300 latency across C_Z and P_Z over time signifies that the time difference for one to decide and act upon the decisions made is relatively small.

Lastly, the effect of caffeine on the P300 components from this study are still contradictory. Even though most subjects showed positive effects of caffeine, there were also negative effects or no dramatic effects on certain subjects. Previous studies shown that caffeine effect is more prominent to subjects that were under physically or mentally fatigue state compared to those who were under well rested condition. Lorist *et. al* [20] stated that caffeine affects information processing through an effect on the perceptual system and on output related processes. Also, the consequences of caffeine also varied with the dosage. In the study, the amount of caffeine in a 240 ml black roast coffee is approximately 65 mg [21] which considered as lower dose. According to [19], low doses of caffeine affect an individual's cognitive function related to alertness, mood, and energy perception and also has been associated with the improvement in motor performance.

CHAPTER 5
CONCLUSION AND RECOMMENDATIONS

In conclusion, though the results partially confirmed the hypotheses, this study provides evidences for the time-of-day and caffeine effects on both visual and cognitive processing efficiency as proposed in the current literature. However, the effects for each factor studied were not consistent and also varied between subjects due to individual differences stemming from other factors that could also contribute to the ERP measures. The observed P100 and P300 latencies in most subjects in the morning were shorter and gradually increased over time. Furthermore, despite the higher amplitudes in the morning, a trend of decrease-and-increase was found over time for both P100 and P300. After the caffeine administration however, the amplitudes for most subjects were increased with the exception on the one or two subjects. The findings further indicate that the effects of caffeine are most evident in fatigued subjects compared to well rested subjects who showed negative or no significant after the administration of caffeine.

Finally, there are few areas of improvements that could be made in future ERP studies. The following recommendations are made based on challenges faced during the present study. They include:

i) More exposure on ERP experimental designs – This will help researcher to design the experiment where the factor that is being investigated can be well studied exclusively through practises and familiarization with ERP experiment designs.

ii) Training subjects – Subjects play important role in data acquisition phase since it is critical to have good data for the analysis purpose. If the study is looking for a trend/ common behaviour for a particular factor, it is recommended to make use

of a large sample size or number of subjects participating in the study. However, the most important part in data acquisition is to train the subjects. A well-trained subject after going several trainings, can provide more consistent data which can be very useful in many ERP studies.

iii) Control group – experiments that aim to study the effects of caffeine on visual and cognitive performance on certain conditions should be made in comparison to control groups. The control groups may include those subjects who are healthy, non-smokers, and have no known cognitive impairments.

iv) Optimal test environment – In getting more accurate results, the ERP recording should be performed in a sound attenuated room where the subject is isolated from other environment.

Finally, future studies are needed to further investigate the specific mechanisms responsible for these experimental results as these new findings can contribute to enhance understanding of the underlying neurophysiological process involving fatigue and caffeine ingestion.

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APPENDICES

APPENDIX A

Summary of Project Activities

No.	Activities	Week of completion
	Final Year Project (I)	
1-1	Selection and confirmation of project title	1
1-2	Literature review on EEG and ERP	4
1-3	Submission of Preliminary Report	4
1-4	Identification of ERP components and test parameters	7
1-5	Literature review on visual stimulus and EEG recording	8
1-6	Submission of Progress Report	9
	Seminar	12
1-7	Submission of Interim Report	14
1-8	Oral Presentation	15
	Final Year Project (II)	
2-1	EEG Training	1-4
2-2	Calibration of stimulus and recording equipments	5
2-3	Design of ERP experiments	6-7
2-4	Data acquisition and EEG recording - Subjects preparation (10 subjects) - Literature review on ERP recording	8-13
2-5	Signal Processing	8-13
2-6	- Pre-processing & Post-processing signals	
2-7	Results Validation and Analysis	10-13
2-8	Submission of Final Report	14
2-9	Oral Presentation	15

APPENDIX B

PARTICIPANT INFORMATION

Project title:

Event- Related Potential (ERP) Signal Analysis

Introduction:

You are kindly invited to participate in a research study to assess individuals' cognitive efficiency over time and also the effect of caffeine intake on the ERP signals.

Project Background:

Most of our daily activities involve information processing where this useful information will be processed in our brain to help us in making decisions, recognizing objects and etc. However, towards the end of the day, we tend to get physically or mentally tired, hence our cognitive efficiency may be affected. This project aims to study how individual differences stemming from time-of-day and caffeine intake would affect the behavior of ERP components (P100 and P300). So in the study, an ERP experiment will be performed to test the ability of a person in classifying and discriminating stimuli throughout the day. To test this condition, the ERP test will be carried out at three different times (i.e. in the morning, in the afternoon and in the evening). Apart from that, the effect of caffeine will also be investigated on the same individual within the same day, where the latencies and amplitude in both conditions (with and without caffeine intake) will be analysed.

When and where will the test be held?

If you choose to participate, you will be invited to attend a series of recording sessions of a ERP test which will be conducted on the following days: **April 23 & 24, 2011(Sunday and Saturday)** at **Block 22 (22-02-14).** You may only need to

choose one day to come for the test. Within the same day, there will be three (3) sessions that you need to attend:

(No caffeine intake)

Part I: Morning session (8AM -11AM)

Part II: Afternoon session (12PM- 3PM)

Part III: Evening session (5PM – 8PM)

(With caffeine intake):

Part IV: *Evening session (5PM – 8PM)

*participant will be given a coffee drink after Part III has finished (within the same session)

NOTE: ALL three (3) sessions must be conducted on the same participant within the same day. Participants may not choose different sessions on two different days.

What should I do to get ready for the test?

1) Participants are advised to have dry, freshly washed hair on the day of experiment. You are recommended to use only basic shampoo and make sure your hair does not have any conditioners, rinses, mousses, oils, hair sprays or any styling products applied on it.
2) Do not wear any makeup.
3) Make sure your body is free from any oils, lotions, and moisturizers.
4) Take your medications. Be certain you have told your physician what medications you take.
5) Eat as normal, but **avoid caffeine products**, such as coffee, tea, soft drinks and chocolate.
6) If you are wearing glasses, you can wear them as usual. However, if you are wearing contact lenses, you must bring your own case to the lab. You might need to remove the lenses before doing the test.
7) Do not wear any earrings, hair ties or clips.

What will I be asked to do during the test?

i) Before recording session: (approximately 10 minutes)

You will need to wear a 128-channel Sensor Net where the correct size will be chosen after your head measurement has been taken. The Net will be loaded with

electrolyte (distilled water and Potassium Chloride, KCl) before applying the Net onto your head. While putting on the Net, you will be asked to close your eyes until you are told otherwise. Once the Net is applied and connected to the amplifier, the sensor impedances will be measured and the waveforms will be inspected.

(NOTE: to ensure that the sensors are in good contact with the scalp, each sensor will be gently scrubbed after the Net is placed onto your head and the electrolyte may need to be reapplied on any sensor that gives high impedance. Please inform the experimenter if you are experiencing any discomfort while the Net is being applied. The Net will be adjusted until you are feeling comfortable).

Possible Risk associated with Potassium Chloride

Skin irritation – may cause temporary skin irritation on sensitive skin.

(NOTE: The potassium chloride (11g) used for the electrolyte is diluted with 1 Liter of distilled water. However, please inform beforehand if you have previously experienced an allergic response or skin irritation due to Potassium Chloride.

ii) During recording session: (approximately 10 minutes)

In this session, you will be asked to sit for a visual test where you will be presented with a series of letter 'O's and 'X's on a screen display for a short duration. The letter 'O' is known as a standard, while the 'X' is a target. Your task is **to respond only to the letter 'X'** (target) each time it appears on the screen by pressing any button on the Source Box. However, you need not to respond to the letter 'O' by simply doing nothing (i.e. not pressing any button). The test will be conducted in four blocks and there will be a short interval between each block. Once the test is completed, your overall reaction time and accuracy will be recorded.

The same test will be carried out for all the four sessions, however, for the last session (Part IV), participant will be asked to drink a coffee and will wait about 15 minutes before sitting for the test again.

(NOTE: The type of coffee (250ml) will be used for every participant is black roast with no milk added. Please inform in advance if you are allergic with any caffeine intake).

iii) After recording session: (approximately 2 minutes)

As you have completed the test, you will be asked to stay put in your position. The Net will then be taken off from your head while your eyes are kept closed. The Net will be rinsed and disinfected for the cleaning purpose.

(NOTE: You may use the towels given to you to dry your hair if you feel your hair is wet).

How long will it take?

For this experiment, we need people who can participate in ALL FOUR sessions of testing and recording. **Each session may take 1 hour to complete** and must be completed within the same day. The time for each session is as suggested and you may choose which day you would like to come. Participants will be scheduled and notified about their appointments through email.

Will I be reimbursed for my time?

No. You will not be reimbursed for attending the sessions as this is based on a voluntary participation.

Is my personal information protected?

Yes. All your personal details and your confidentiality of your test results will be protected to the fullest possible extent. All measurements and records taken as part of this study will remain confidential. Your name will not appear in any publications or reports arising from this study and any references to your personal information that might allow other to guess your identity will be removed.

Will I receive a feedback?

You may request for a written summary of the findings based on your test results after the study has been completed.

What if I do not want to be involved?

Your participation in this study is entirely voluntary. However, if you already chose to participate but you wish to withdraw your participation, you may do so.

Please contact the person in charge as soon as possible to make arrangements for the test schedule.

In what conditions I may not be able to participate?

There are several conditions where participants may not be able to participate in the study. Participant who has skin allergic or allergic to caffeine, cornrows/ dreadlocks hair or hair lice are recommended not to participate to avoid further complication as well as to avoid contamination among the participants.

How do I agree to participate?

If you choose to participate, please indicate that you have read and understood this information by reading and signing the accompanying consent form, and returning it to the person in charge.

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Department: Electrical & Electronics Engineering (UTP)

Experimenter: Raja Nur Hamizah bt Raja Khairuddin (also person in charge)

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APPENDIX C

CONSENT FORM FOR EVENT-RELATED POTENTIAL (ERP) TEST

Test description

You will take an event-related potential (ERP) test. The same test will be carried out in all four (4) sessions as stated in the Participant Information. During the test, you are required to wear a 128-channel Sensor Net and you will be presented with certain stimulations displayed on a screen to respond to. The stimulation test results for all sessions will be recorded with the use of electroencephalogram (EEG) system.

Risks and discomforts

There exists the possibility of risk and discomfort occurring during the test that could include skin irritation or allergy. To minimize these conditions, you will be frequently asked by the experimenter if you are experiencing any discomfort and your electroencephalogram will be closely monitored.

Inquiries

Any questions about the procedures used in the test are welcome. If you have any doubts or questions, please contact the person in charge for further clarification.

Freedom of consent

Your participation in this test is entirely voluntary. You are free to deny consent if you so desire.

I acknowledge that I have read and understand all the information and conditions stated in the Participant Information and I consent to participate in this study. I do hereby give my consent to the experimenter to perform the test referred to above and use the data for research and publications. If at any time during the test I have discomfort or pain, I will inform the person in charge.

Participant's Name:
( )

Experimenter's Signature

Date:

APPENDIX D

PERSONAL INFORMATION & QUESTIONNAIRE FORM

This personal information and questionnaire form is to be filled up by participant prior to the ERP test and will be used as a future reference for this study. Any personal information from this form will not be disclosed without the participant's first approval.

Participant Personal Information

Name:

Contact Number: **E-mail:**

Gender: ☐ Male ☐ Female **Race:**

Date of Birth: ___ (DD)/ ___ (MM)/ ___ (YY) **Age:**

Head measurements: *(to be filled up by the experimenter)*

Circumference: _____cm **Nasion-inion distance:** _____cm (midpoint: _____cm)

Net Size: S / M / L **Pre-auricular distance:** _____cm (midpoint: _____cm)

(Note: Small: 54-56 cm; Medium: 56-58 cm; Large: 58-61)

Test information: *(to be filled up by the experimenter)*

Subject: #___

Test Date:

Test time:

(Morning session: 8.00 – 11.00 AM)

Start time: _____ End time: _____

(Afternoon session: 12.00 – 3.00PM)

Start time: _____ End time: _____

(Evening session: 5.00 - 8PM)

Start time: _____ End time: _____

(NOTE: In the event where participant comes later than the indicated time for any session, please put a remark. Please ensure that participant attends all four sessions within the same day).

Which hand was being used by the participant to click the response button?

☐ Right ☐ Left

(NOTE: Please ensure that participant uses the same hand in all four sessions for test consistency).

Questionnaire

1. Have you ever experienced an EEG test before?

☐ Yes ☐ No

2. Are you taking any daily medications?

☐ Yes ☐ No

3. How many hours do you usually sleep? Please tick ONE box only.

☐ less than 6 hours ☐ 6 to 8 hours ☐ more than 10 hours

4. What time did you sleep last night? Please state your:

Sleep time : _____(previous day) Wake up time: _____ (today)

5. Do you smoke?

☐ Yes ☐ No

6. How many hours per day (approximately) do you spend in front of a computer? Please tick ONE box only.

☐ less than 3 hours ☐ 3 to 6 hours ☐ 7 to 10 hours
☐ more than 10 hours

7. Do you wear glasses or contact lenses?

☐ If yes, please state your power: _____ ☐ No

8. Please state if you experience any discomfort while taking the test. Or do you have any suggestions regarding the test convenience? (We will take into consideration on your suggestions/ comments for our future reference).

_____

_____

_____

THANK YOU VERY MUCH FOR YOUR PARTICIPATION! ☺

APPENDIX E

10-20 System

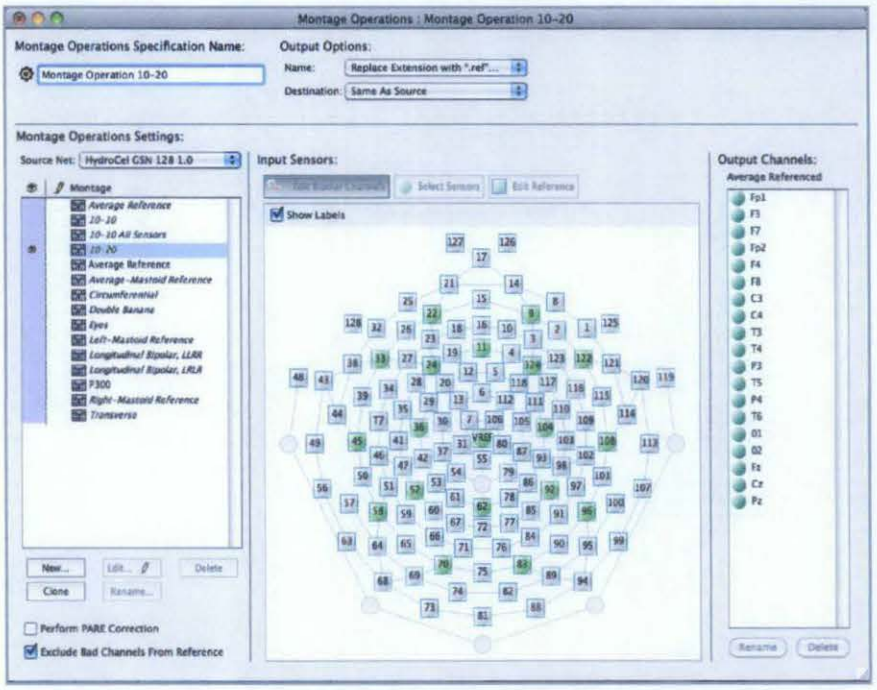

Figure E-1: Montage operation of 10-20 system

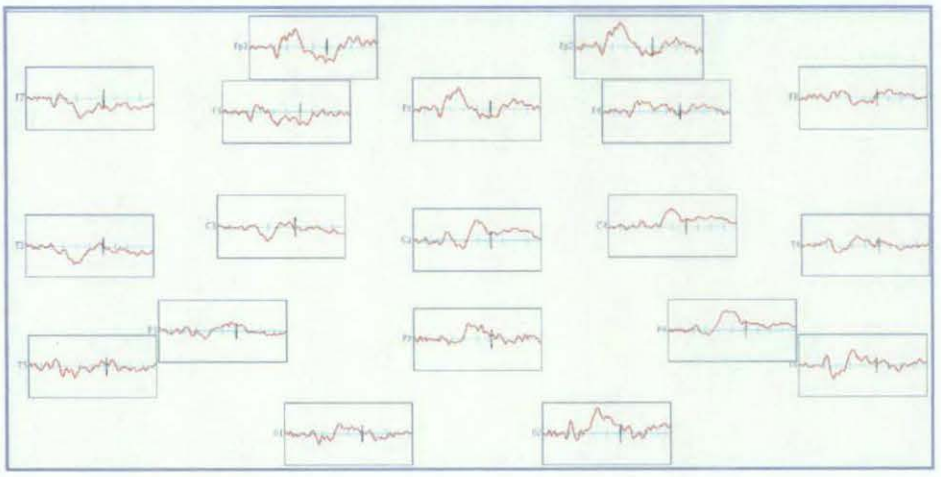

Figure E-2: Data in a10-20 system topo plot view

APPENDIX F

P100 latency and amplitude across O_Z, O_1 and O_2 over time

Subject	Time-of-day							
	Morning		Afternoon		Evening		Evening with coffee	
	Latency (ms)	*Amplitude (μV)*	*Latency (ms)*	*Amplitude (μV)*	*Latency (ms)*	*Amplitude (μV)*	*Latency (ms)*	*Amplitude (μV)*
P100 at O_Z								
1	130	3.94	108	0.2	104	0.1	96	0.2
2	79	2.01	73	1.9	98	1	77	1.2
3	117	0.7	92	1.2	88	1.2	85	1.7
4	117	3	96	3.3	117	2.6	117	0.5
5	79	0.77	96	2.4	131	2.8	119	2
6	76	0.6	116	2.43	115	1.03	79	1.42
7	134	3.58	139	2.19	134	3.16	134	4.48
8	79	0.6	83	2.7	98	3.7	79	6.2
9	79	2.33	85	1.52	79	1.05	111	1.1
10	111	4.49	116	2.83	109	2.01	109	4.06
P100 at O_1								
1	124	3.75	120	2.9	124	3.5	114	2.5
2	78	2.01	85	2.2	100	3.5	95	2.4
3	99	2.5	83	0.3	87	1.4	89	0.8
4	134	3.43	102	2.5	130	2.9	134	2.8
5	126	1.38	114	3.1	112	1.8	120	2.1
6	111	1.68	116	4.51	122	3.41	109	3.73
7	130	3.58	141	3.76	134	1.26	128	2.52
8	118	3.33	141	2.8	99	3.95	122	0.98
9	113	2.85	109	2.29	111	2.59	109	3.99
10	113	3.94	118	3.98	109	2.45	111	4.06
P100 at O_2								
1	128	3.83	119	1.3	113	2.8	107	2.1
2	87	1.73	95	2.6	107	2	89	1.5
3	99	3.59	100	1.6	94	1.5	87	2.3
4	134	2.87	121	4.5	134	4.7	134	0.2
5	134	2.94	115	4.6	140	3.2	130	4.5
6	109	0.48	120	3.15	120	1.59	112	1.66
7	134	4.3	138	2.56	134	4.34	134	4.48
8	126	1.77	83	2.05	100	2.47	79	2.77
9	83	2.91	79	1.2	83	1.49	99	1.14
10	111	4.89	116	5.79	115	4.48	111	5.04

APPENDIX G

P300 latency and amplitude across F_Z, C_Z, and P_Z over time

Subject	Time-of-day							
	Morning		Afternoon		Evening		Evening with coffee	
	Latency (ms)	*Amplitude (μV)*	*Latency (ms)*	*Amplitude (μV)*	*Latency (ms)*	*Amplitude (μV)*	*Latency (ms)*	*Amplitude (μV)*
P300 at F_Z								
1	498	4.65	419	2	417	1.8	415	1.7
2	290	3.3	270	4.9	461	4.8	442	6.7
3	498	4.76	411	1.7	486	1.2	477	1.3
4	498	4.43	490	2.2	486	4.3	461	9.7
5	300	6.95	380	1.8	344	0.7	329	1.4
6	384	2.94	498	1.1	423	2.15	390	7.4
7	300	5.2	273	5.17	271	6.72	250	7.45
8	279	7.28	498	11.04	406	7.5	376	9.3
9	498	6.23	498	5.63	498	4.39	220	3.02
10	260	1.28	262	3.74	256	1.45	250	3.75
P300 at C_Z								
1	411	8.04	405	5.4	398	2.7	388	3.8
2	409	9.24	419	4.6	399	8.13	386	8.4
3	432	6.85	422	10	405	16.5	386	16.5
4	472	7.98	484	8.7	440	6.5	482	8.3
5	434	7.23	440	5.7	430	4	402	4.5
6	378	12.37	426	7.82	439	8.43	399	11.47
7	411	3.97	409	7.47	411	6.58	409	8.31
8	422	6.56	439	9.77	437	2.24	380	7.05
9	361	9.08	411	8.34	399	7.61	370	10.02
10	378	4.48	413	7.59	399	7.2	399	6.66
P300 at P_Z								
1	380	9.68	392	9.2	398	7.4	375	11
2	390	13.15	377	14.3	337	7.3	369	15.6
3	378	6.94	366	6.6	387	10.4	391	13.4
4	418	9.07	412	10.2	472	7	449	8.3
5	432	9.24	428	9.7	428	7.1	411	12.4
6	428	11.81	418	14.14	428	13.3	411	12.24
7	399	10.41	432	7.05	344	5.23	325	3.53
8	434	11.39	430	16.12	451	10.53	399	7.3
9	378	9.43	340	12.04	378	12.8	384	14.3
10	370	9.06	451	10.42	437	9.86	422	8.01