LOW RESOLUTION ELECTROMAGNETIC TOMOGRAPHY (LORETA) ANALYSIS OF THE BRAINS ELECTROPHYSIOLOGICAL RESPONSE TO EMOTIONAL VISUAL STIMULI UNDER DIFFERING CONDITIONS

by

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Current methods of diagnosing and monitoring stress include: observing changes in the severity of existing symptoms, the development of new symptoms, hormone level tests, and stress self-assessment surveys. Self-assessment surveys are subject to bias and false reporting. This project focuses on analyzing electroencephalogram (EEG) using Low Resolution Electromagnetic Tomography (LORETA) to identify differences within current source location of emotionally elicited event related potentials (ERPs), in order to aid physicians in stress diagnostics and management. For this study twenty-one participants took the Penn State Worry Questionnaire which classifies the participants into high-stress and low-stress groups. The individuals had their EEG recorded while viewing pleasant, neutral, and unpleasant stimuli. CURRY, the current reconstruction program, was used to filter, epoch, and average the data to obtain event related potentials (ERPs) for each participant. Using group-averaged ERPs as the data input, LORETA was used to calculate the current distribution within the brain. One and

two-tailed t-tests were performed to examine for current source distribution differences between

high-stress/low-stress conditions and pleasant, unpleasant and neutral stimuli. The results of the

experiment indicate that there is a difference in current source location between high-stress and

low-stress individuals. The current source distribution differences are within regions of the

frontal lobe and the parietal lobe associated with emotional processing.

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KEY TERMS

Event related potential (ERP): The measured brain response that is the direct result of a specific sensory, cognitive, or motor event. More formally, it is any stereotyped electrophysiological response to a stimulus.

Electroencephalography: the measurement of electrical activity in different parts of the brain and the recording of such activity as a visual trace (on paper or on an oscilloscope screen).

Low Resolution Electromagnetic Tomography (LORETA): A method which uses electroencephalography or magnetoencephalography to solve the inverse solution. The inverse solution takes measurements from electrodes and calculates the most likely current source, within the cortex, which would result in the measures scalp distribution.

Epoch: part of the procedure for analyzing event-related potentials from EEG is to later "chop" the signal into segments time-locked to an event such as a Stimulus.

Brodmann Area: a region of the cerebral cortex, in the human or other primate brain, defined by its cytoarchitecture, or histological structure and organization of cells.

Penn State Worry Questionnaire (PSWQ): a self-assessment survey designed to quantify the amount of worry/stress an individual in experiencing.

International Affective Picture Survey (IAPS): a database of pictures designed to provide a standardized set of pictures for studying emotion and attention.

Cortisol: a stress hormone produced by the adrenal gland responsible for long term activation of the sympathetic nervous system.

CHAPTER 1: INTRODUCTION

Stress is the body's physical or mental reaction to difficult or adverse situations. Stress is often called 'the fight or flight response'. When the body's sympathetic nervous system triggers a release of the stress hormones adrenaline and cortisol in order to resolve an immediate threat. There are positive and negative forms of stress. Positive stress gives a burst of adrenaline to help accomplish a task, improve bodily efficiency, and improve mental alertness. Positive stress is short-term in nature and the body naturally returns to its relaxed state shortly after the resolution of the stressor. Negative stress occurs when the body does not return to the relaxed state after the resolution of the stressor.

When a stressor is present for an extended period of time, the body's sympathetic nervous system remains in an elevated state, this is negative stress. A common occurrence of negative stress would be stresses related to work or family, for example, a sudden undesirable change such as job loss, divorce, or illness [1]. Individuals with negative stress have persistent elevated sympathetic nervous system activity. Constant activation of the sympathetic nervous system leads to elevated levels of stress hormones for extended periods of time, which can then lead to detrimental effects within the body.

Stress is difficult to diagnose and treat because multiple illnesses have symptoms that mirror the effects of negative stress. This overlapping in symptoms makes identifying stress as the primary cause of illness difficult. Current diagnostic methods for stress include: examining changes in existing symptoms, development of new symptoms, blood tests to measure hormone levels, and self-assessment surveys [2]. Monitoring symptoms requires individuals to make note of any changes in their symptom presentation and relay the information to their physician. Visits

to the doctor's office can range between weeks and months making it difficult for patients to remember all the changes in their symptoms. Blood tests to measure stress hormones are invasive, expensive, and can be skewed if the patient is nervous or afraid of getting his/her blood drawn which would result increased hormone levels during the test. Assessment surveys are subject to bias; these inconsistencies are due to false reporting of patients seeking attention or not reporting their symptoms correctly. This research project aims to offer a possible diagnostic tool that uses electroencephalogram to examine the brain's preconscious response to emotional visual stimuli.

Common symptoms of stress manifest as a mixture of physical, emotional and cognitive pathologies. Physical symptoms of stress can be directly related to the stress hormones. Adrenaline increases heart rate, elevates blood pressure and increases energy. Cortisol inhibits non-essential functions, suppressing the immune system and the gastro intestinal tract, and alters brain chemistry [3]. Individuals with negative stress have elevated levels of stress hormones in their system. A prolonged, elevated, adrenaline concentration in the body causes symptoms such as mild tachycardia high blood pressure leading to an increased risk of heart attack and stroke. Increased energy levels are necessary for the fight or flight response to address an immediate threat. However, over extended periods of time the increased energy expenditure leads to fatigue, muscle tension, and exhaustion [4]. The elevated cortisol level curbs appetite and suppresses the immune system, which can lead to weight fluctuation and malnourishment if not properly addressed [5, 6].

Stress affects an individual's cognitive well-being which can lead to impaired emotions and thought processes. Common mental symptoms of stress include increased frustration and aggression, difficulty relaxing, low self-esteem and an overwhelmed feeling. Cortisol alters the

brain chemistry predominately in the limbic system and parietal lobe the regions of the brain associated with emotions, mood, fear and motivation. Cognitive impairment is also associated with high-stress level. Elevated cortisol levels are believed to be the primary factor in cognitive impairment but physical symptoms such as increased blood pressure, and fluctuations in glucose levels can also affect cognition [6].

The objectives of this study is to analyze EEG data using the technique of low resolution electromagnetic tomography. The first objective is to filter the EEG data and segment it into the 1000ms post stimulus to obtain ERPs. The second objective is to average the ERPs together for each stimulus to obtain the average ERP to improve the signal to noise ratio. Objective three is to convert the averaged ERPs to LORETA files. The final objective is to statistically compare the conditions and stimuli to observe for differences in ERP source generation location.

CHAPTER 2: LITERATURE REVIEW

2.1 Literature Introduction

Stress is a complicated bodily process involving multiple organ systems throughout the body. Over the years hundreds of studies have been published each observing different aspects of stress. Its effects on different organ systems, different methods of quantifying and measuring stress and many others. In order to focus on stress literature to this study the literature included has been limited to EEG-based stress studies.

2.2 Frequency Domain Analysis

Frequency analysis of EEG signals examines the changes in fundamental frequencies, such as alpha, theta, beta, and gamma waves, over a period of time. Fundamental frequencies, can be compared against baseline brain activity to observe changes in contributing frequency bands [7]. In emotion studies, shifts in frequency band activity indicate a change in emotion or the lack of emotional stimulation. Shifts in frequency band activity can be mapped on the surface of the scalp and estimations can then made regarding the regions of the brain in which the changes occurred [2]. Frequency analysis is preferably performed on data that is longer than one second because of the Fourier Transform. The Fourier transform converts a time domain signal into the frequency domain so one may observe the frequencies which comprise the signal. More data in the signal yields a better estimation of the frequencies within the signal, therefore longer signals are preferred when analyzing frequencies. Frequency analysis of short signals is poor at estimating low frequencies within the signal because the recording may not be long enough to yield an accurate frequency estimate. Frequency analysis would not be preferred for observing the preconscious response of the brain to a stimuli that occurs within the first second of stimulus presentation.
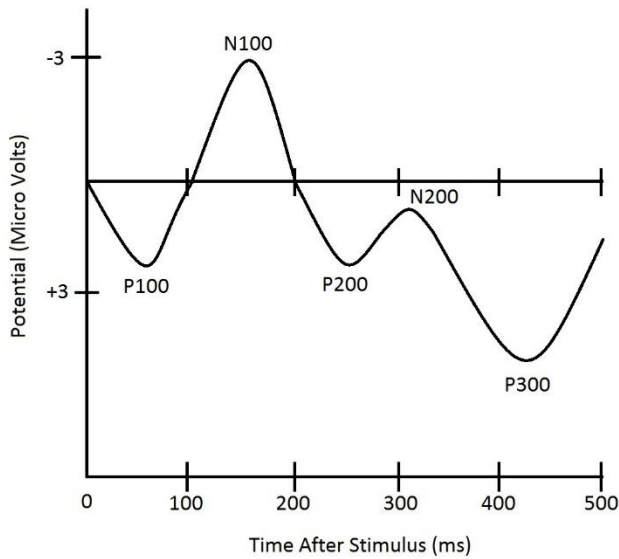
Figure 1. Event Related Potential (ERP)

2.3 Time-Domain Research

Data for time-domain research is commonly recorded using an EEG. One of the more common signal patterns used to analyze EEG in the time-domain is the event related potential (ERP) which is the brains preconscious response to a stimulus. ERPs cannot be influenced by an individual as they are the reflexive response of the brain reacting to a stimulus. This reflex occurs before conscious thought begins, making the ERP an ideal gauge of the brains health, connectivity, and functionality [8]. The most common ERP waves that are observed in research are the P100, N100, P200, N200, and P300 as can be seen in Figure 1. The 'P' refers to positive potential, 'N' refers to negative potential, and the number following the letter refers to the time in milliseconds that the waveform presents. One of the most commonly studied ERPs is the P300, which is a positive wave which occurs 300ms after the onset of a stimulus [6,8]. P300 has been shown to be affected by different variables such as stress, anxiety, depression, and chemical changes within the brain or blood. Amplitude and latency (presentation post-stimulus) are the two primary methods of analyzing ERPs in the time domain. Variation in ERP amplitude and latency, in normal and clinical populations, reflects individual differences in cognitive activity. The amplitude of the ERP is a measure of the size of the neural population firing in synchrony; it is a reliable measurement to quantify the health and activity of the brain [6]. Individuals who show variation in P300 response times might have an underlying condition which is altering ERP

topology. Imbalances in brain chemistry from depression, stress, or even low blood sugar can alter ERP presentation [9]. The current literature emphasizes the analysis of the ERP amplitude and latency, and provides insight into the precognitive reaction of the brain [10]. Examination of the ERP's amplitude and latency has been heavily researched, contributing hundreds of studies analyzing the changes in ERP topology to different stimuli and conditions. Stress and worry studies have primarily focused on the presentation of the ERP and if the amplitude of the wave has changed or shifted over time [11]. Other studies looked for lateralization, which is where one hemisphere of the brain is more active than the other. Lateralization is normal within the brain due to communication between the different structures within the cortex. Emotional processing, in a healthy brain, occurs within the right cerebral hemisphere, therefore an emotional ERP would tend to be most evident on the front right portion of the scalp [10]. Multiple emotion ERP studies look for lateralization in individuals who are classified as high stress/worry [10,6,12]. Research has indicated one of the most common effects of stress on ERP presentation is a delay of the P300 ERP from 300ms after stimulus onset to approximately 500ms. This delay in the ERP presentation is also accompanied by an increase in amplitude. This change in ERP amplitude is believed to be the brains attempt to compensate for the late ERP. A time domain signal gives little insight into the contributing current sources or their true locations within the cortex. Only inferences to the true activity of the brain may be made based on the location of the electrodes contributing the largest amplitude ERP. The electrodes with the highest contribution to the ERP are closest to the volume of the brain responsible for the generation of the ERP [12]. Given this, the location within the cortex that differs between conditions can be estimated. If, for example, the electrodes: FP2, F8, and F4 in Figure 2 contribute the most amplitude to the ERP then the structures within the brain responsible for the ERP are most likely in the right pre-
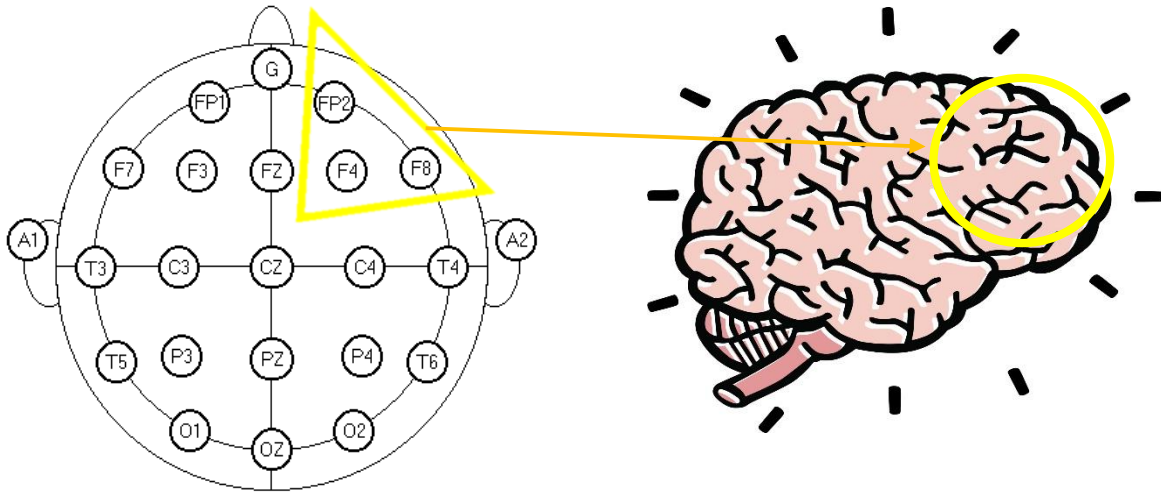
Figure 2. Modified 10/10 electrode array; The electrodes within the triangle record activity from the circled portion of the frontal lobe.

frontal cortex. This is why there is a limit to spatial conclusions which can be drawn from the amplitude of the ERP regarding anatomical source of the signal within the brain, even with electrode arrays with 100+ channels [10].

In the early 2000's the advent and advancement in dipole and current reconstruction techniques made it possible to draw better conclusions regarding the calculated current source locations within the cortex. The advancement in understanding allowed for greater accuracy in the estimation of the structures within the brain which are contributing to the ERP.

2.4 Dipole Source Analysis

Dipole source analysis (DSA) is a method of solving the inverse problem for estimating the sources of surface evoked potentials after generating a scalp potential distribution map (SPDM) then solving for a point within the cortex known as a dipole. A dipole is the flow of electrons or ions between a source and a sink. It can be represented as a directional vector with a magnitude representative of the current strength. In this case it is the flow of ions through the axons of neural tissue [13]. An SPDM is the estimated potential (voltage distribution) on the

scalp. Given the SPDM, DSA estimates a point or points within the cortex that would most likely produce the SPDM. DSA estimates five nonlinear parameters per dipole: the x, y, and z dipole position values, and the two angles necessary to define dipole orientations in 3D space [14]. Dipoles can be estimated as moving or rotating as seen in Figure 3.
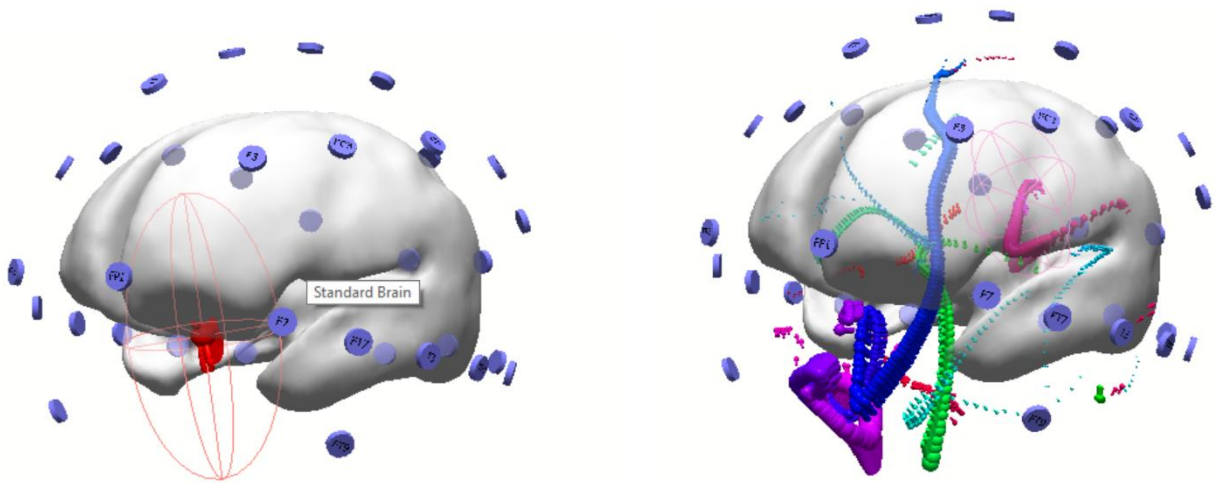
Figure 3. Dipole source analysis computation; The image on the left is an example of a fixed rotating dipole and the image on the right is an example of a moving dipole.

When a moving dipole is estimated, the 3-dimensional location changes for every time sample. This represents the path of the current source over time. A rotating dipole is fixed within the brain and it rotates as its angle and magnitude change. In general moving dipoles are used to estimate the current source of an ERP. This is because currents within the brain are constantly moving to different neural volumes changing direction and strength. DSA is performed using the average of multiple ERPs of a participant. Multiple ERPs are averaged together to improve the signal to noise ratio and thus the accuracy of the dipole calculation [15]. This method is useful but it is up to the researcher to determine how many dipoles should be estimated. Estimating the number of dipoles is often done by looking at past research and determining what structures within the brain are theorized to be active. In doing so, one can limit the number of dipoles to the

number of suspected active structures within the brain. Often the estimated dipoles are not within the brain and are outside the skull, which represents one of the flaws with dipole source analysis.

2.5 Low Resolution Electromagnetic Tomography (LORETA)

LORETA, like DSA, is another method used to solve for the inverse solution. However, the method by which LORETA calculates the solution to the inverse problem differs greatly from DSA. LORETA is a functional imaging technique which models the cortex as a collection of volume elements (voxels) in a digitized talairach atlas provided by the brain imaging center, Montreal Neurological Imaging center [16]. LORETA uses a standardized 3-Sphere head to model the properties of the scalp, skull, and brain to aid in the estimation of the current sources within the brain, accounting for the different conductive properties of the head [16]. LORETA's calculations restrict the solution to the cortical gray matter (CGM). Bounding the solution to the CGM insures that estimated current sources are located within cortex. The biggest advantage of

Figure 4. Result of LORETA computation; The image depicted is a rendering of the human cortex with the estimation of the current source locations colored in yellow.

LORETA is that the solution is a 3D volumetric representation of the neuronal activity within the cortex as opposed to a few dipole point solutions with DSA. For example, Figure 4 is a computed LORETA 3-dimensional distribution of active neuronal generators in the brain as a current density value (A/m²) at each voxel [17]. In a review performed by Pascual et. al, LORETA successfully identified the source location for an ERP when it was contaminated with

noise, showing the robustness of this analytical method, with as few as 16 electrode channels [18].

LORETA offers a high time resolution estimation of the current sources within the brain at the cost of spatial resolution. High time resolution makes it possible to examine the active regions of the brain on a millisecond scale allowing one to view how the brain makes connections to different structures throughout the cortex. Functional magnetic resonance imaging (fMRI) and positron emission tomography (PET) have been used to study the brains reaction to stimuli by monitoring metabolic activity, and both fMRI and PET scans offer high spatial resolution, with voxels as small as 1mm, estimates to the active regions within the brain [19]. While these modalities have a high spatial resolution they do not have a fast time resolution. fMRI and PET scans can only offer an average of the metabolic activity over a 5 to 10 second period. Multiple studies have used fMRI and PET scans to validate the results of LORETA analysis [16, 20]. With these validations LORETA has become one of the most accurate current reconstruction algorithms available [16]. The most recent versions of the LORETA algorithm are sLORETA (standardized) and eLORETA (exact). Both versions offer an improvement on the original LORETA spatial resolution by reducing the voxel size from 7mm to 5mm and allowing more voxels to occupy the same cortex volume. sLORETA and eLORETA also provide higher estimation accuracy of the current density and location while maintaining a low error rate for sLORETA and theoretically no error for eLORETA [18]. sLORETA will be referred to as LORETA throughout the remainder of the paper.

CHAPTER 3: METHODOLOGY

3.1 Analysis Method of Choice

LORETA provides the necessary time resolution to calculate on a millisecond scale the

current distribution of the ERP. While LORETA has a relatively low resolution when compared

to that of an fMRI or PET scan, it has a fine enough resolution to identify the volume of cortical

matter that is active at each point in time based on the recorded EEG data. Dipole source analysis

is a common method of determining source location but is has a few complications which

LORETA does not. DSP is useful to investigators who have prior knowledge of the location of

source generators and the number of dipoles. DSP does not bound solutions to the cortical grey

matter leading to solutions outside the brain. LORETA calculates estimated volumes of voxels,

not just one point in space per data sample as with DSP. All solutions in LORETA are bounded

to the cortical grey matter and no prior knowledge about the theoretical number of source

generators is needed. For these reasons outlined above and in Table 1, LORETA was selected as

the method of analysis for this study.

Table 1. Analysis Methodologies and Attributes.

Analysis Method	Time Resolution	Spatial Resolution	Cortex Visual	Solution Bounded to Cortex
Frequency Analysis	Low	N/A	No	N/A
Time Domain Analysis	Varies	N/A	No	N/A
ERP	High	N/A	No	N/A
Dipole Source Analysis	High	High	Yes	No
LORETA	High	Moderate	Yes	Yes

3.2 Experimental setup

Data acquisition was conducted by Dr. Ervin Davis of the East Carolina University neuropsychology department in 2008. All data was completely de-identified prior to Dr. Davis handing over the data. Participants were asked to complete a self-reported trait worry assessment using the Penn State Worry Questionnaire (PSWQ) which consisted of a 16-item self-report measure of trait worry in a 5-point Likert format, maximum score of the questionnaire was 80 points [2]. Participants who scored at or above 50 out of 80 were classified as high-stress the rest as low-stress. Forty electrodes were used to collect the EEG data. Due to the limitations of the LORETA programs electrode registration module and the NuAmp data collection equipment only 30 channels were used in the study. Electrodes on the front of the scalp were used to measure eye-blink and eye-movement artifacts. Each participant was sat in an isolated quiet room and shown, via testing monitor, three series of 20 pictures selected from the International Affective Picture System (IAPS), a database of images specifically designed to elicit emotional affect [20]. The series of IAPS pictures were categorized into pleasant, unpleasant and neutral conditions. Each image was shown for 10 seconds.

Pictures were presented continuously within and between categories with no interstimulus interval. Before recording, the order of the conditions was counterbalanced across participants to minimize the probability of biasing the results by a learned pattern. A NeuroScan 40 channel, PC-based EEG system with Scan 4.4 software was used to collect the data. The data provided for these experiments had been de-identified, meaning participants became anonymous with only the raw testing data and PSWQ score being accessible.

3.3 Data Preparation

The recorded data was collected as a continuous data file with data markers used to identify the different stimulus presentations, Pleasant (P), Unpleasant (U) and Neutral (N). All raw participant data was loaded into the Current Reconstruction Suite (CURRY) under a new experiment tab. With all of the participant data loaded into CURRY a parent configuration file was created to ensure that all participants' data underwent identical filtering. The band-pass filter with a range of 1-15Hz was applied in order to remove extraneous noise from the raw data as to focus on the lower frequencies that contribute most to the ERP.

3.4 Filtering and Signal Conditioning

The LORETA program used was version 20150415. This build version did not contain all of the channels in the modified 10/10 electrode setup that were recorded during the collection phase of the experiment. Macro was written in CURRY to speed up the removal of electrodes A1 and A2 from all participants' data, excluding them from further steps of analysis. A macro is a basic programming script that is written to perform repetitive actions to simplify tedious tasks, such as filter application. A second Macro was written to apply Badblocks and Artifact Reduction. For artifact reduction, data outside the range of -50μv to + 50μv would be removed to reduce artifacts due to eye-blink, movement, and electrode pop. The specific settings were as follows: Lower/Upper threshold were ± 50μv with respect to channel VEOU, pre-stimulus was 100ms and post-stimulus was 100ms with a refractory period of 500. This ensured eye-blink and eye-motion artifacts which occurred within the timeframe of the ERP had been reduced and would not alter the ERP. A badblock filter was applied to the data following the artifact reduction. A badblock filter works in a similar manner to artifact reduction but it marks spans of time where the data is noisy even after artifact correction. When CURRY averages the ERP, any

condition epochs which contain a badblock filter will be excluded from the averaging process. The conditions for the badblock filter were set as follows: Lower threshold 0 µv, upper threshold 50 µv, with respect to channel VEOU, pre-stimulus -100ms and post stimulus +100ms. After filtering/ artifact reduction and badblocking the data was then epoched.

Epoching is the process of taking the continuous data and breaking it into smaller intervals. Each epoch contains the 1000ms immediately after the stimulus presentation, in order to include the first 500ms of the brain's response. This epoching strategy allowed capture of the ERP associated with the stimulus, as well as the beginning of cognitive thought. Using CURRY's epoching tool and the event related averaging tool, the data was broken into multiple 1000ms intervals associated with the three stimuli. The epochs were then averaged to obtain the ERP for each participant and for each stimulus. The averaged epochs were examined for discontinuities in the waveform. If there were a discontinuity it would indicate the badblock filter missed an artifact and it was included in the epoching and averaging processes. In order to correct for discontinuity, the epochs included in the averaging were examined individually. Then the offending epoch(s) were removed and the epochs were re-averaged to produce a smooth continuous ERP. After confirming that the averaged ERP was continuous, the averaged epochs were saved in a separate folder for that condition (i.e. Condition P, U, or N epochs). A separate folder was also created to save each participant's average ERP for each stimulus.

3.5 Averaging and Conversion

The participants had 10 usable epochs on average per stimulus group. These epochs were averaged, participant by participant, to obtain the ERP per participant per stimulus resulting in 21 ERPs per stimulus type, 11 high-stress and 10 low-stress. The newly epoched data was exported for use in LORETA. The default export file extension of CURRY is "Curry Raw Float Format"

then was changed to "Curry Raw ASCII Format" to be opened in the NeuroScan program. Direct import of CURRY files into the LORETA program resulted in an import error that prevented further progress. The work around was to export the data from CURRY in ASCII format, then open the file in NeuroScan and resave as a NeuroScan .avg file. After all of the epochs were saved as .avg files the extension was changed to .txt. Doing this made it possible to import the averaged participant ERPs into the LORETA program.

3.6 LORETA Preparation

The electrodes used in the data collection were entered into the electrode module in the LORETA software, to prepare the LORETA software for analysis. The electrode array used was a modified 10/10 electrode array in which the A1 and A2 electrodes were excluded from the array. Once the array was saved, the transformation matrix was generated and saved for use in later steps.

3.7 EEG/ERPs to LORETA Conversion

The previously converted ERP files, now with the extension .txt, were converted to LORETA files. From the utilities page of the LORETA program the tab "EEG/ERPs to LORETA" was selected. There the ERPs to be converted were selected, along with the transformation matrix based off the modified 10/10 electrode layout. The newly generated LORETA files could then be viewed in the viewer module, which allows one to see the estimated current distribution within the brain with respect to time (or the data could be entered into the statistics module to perform statistical tests).

3.8 Statistical Analysis

The LORETA software includes a statistical analysis module that can be used to test for the dissimilarities between different testing conditions given ERPs themselves or LORETA results. The participants were split into the two respective categories: high-stress (HS) and low-stress (LS), and stimulus type: unpleasant (U), pleasant (P) and neutral (N). Seven statistical tests were performed: HS-U vs. LS-U, HS-P vs. LS-P, HS-N vs. LS-N, HS-U vs. HS-N, HS-P vs. HS-N, LS-U vs. LS-N, and LS-P vs. LS-N. These seven tests were chosen because they provide the proper comparisons within and between conditions to ascertain the relationships between the stress conditions and stimuli. H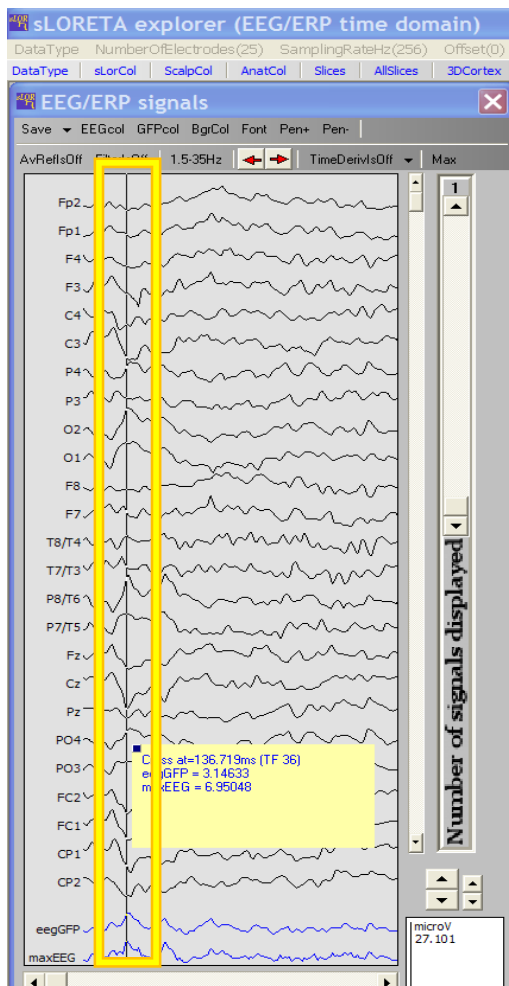igh-stress vs. low-stress comparisons were made to examine differences in current density distributions between stress groups. Comparisons within stress groups were done to examine the differences in current densities within the same stress group. The LORETA statistics module uses statistical non-parametric mapping (SnPM) which operates outside any standard distribution using the data on hand to compute statistical values [21]. This was necessary because the EEG/ERP data may not follow any standard distribution. The statistics module also has methods to address and reduce family-wise errors which can occur when working with large sets of data. Both ERP and LORETA current source reconstruction were used to analyze the data in order to include both changes in

Figure 5. Identification of significant time frame

ERP, which can be attributed to different current sources, and direct comparisons of LORETA current reconstructions.

The first step in the statistical analysis was to identify key time windows within the ERP that vary significantly from the neutral stimulus. The time windows correspond to a dissimilarity in current source location between the conditions being compared, seen as the yellow square in Figure 5. The identified time window was then examined in the LORETA current source reconstruction comparison. If no significant results were found in the ERP alone, then the entire LORETA current reconstruction was then tested. All participants had their pleasant and unpleasant epochs grouped and compared to their neutral stimulus counterpart. Using the ERP identified time windows makes it easier to identify the time of significance based on ERP data alone. T-tests were performed on the LORETA data as well because analysis of ERPs only indicates there is a statistical difference in the time domain while there can still be a statistical difference in the current density distribution. After running the statistical analysis on the current density data the statistics module output a table of critical t-values and the associated p-values, as well as a visible solution mapped on the brain which can be viewed in the viewer module Figure 6. The output maps specify the difference between the two compared data sets as a distributed color scale within the cortex. The color scale is the statistical t-value with the yellow indicating dataset 1 showing more activity than data set 2 while the blue indicates the opposite.
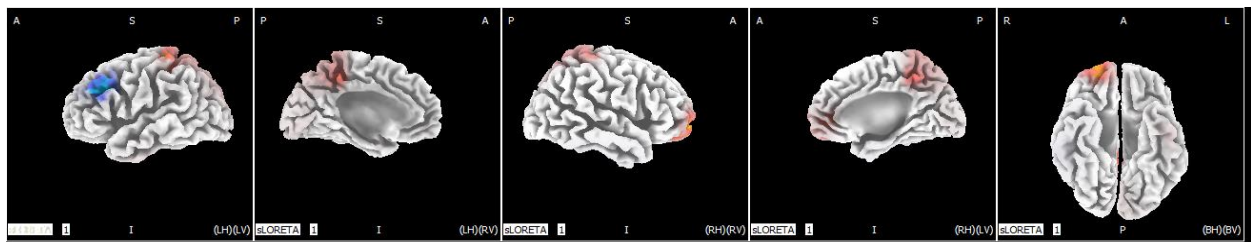

Figure 6. Orthogonal views of LORETA result; This is the statistical comparison between two different conditions. Red indicates an increase in activity while blue indicates a decrease in activity.

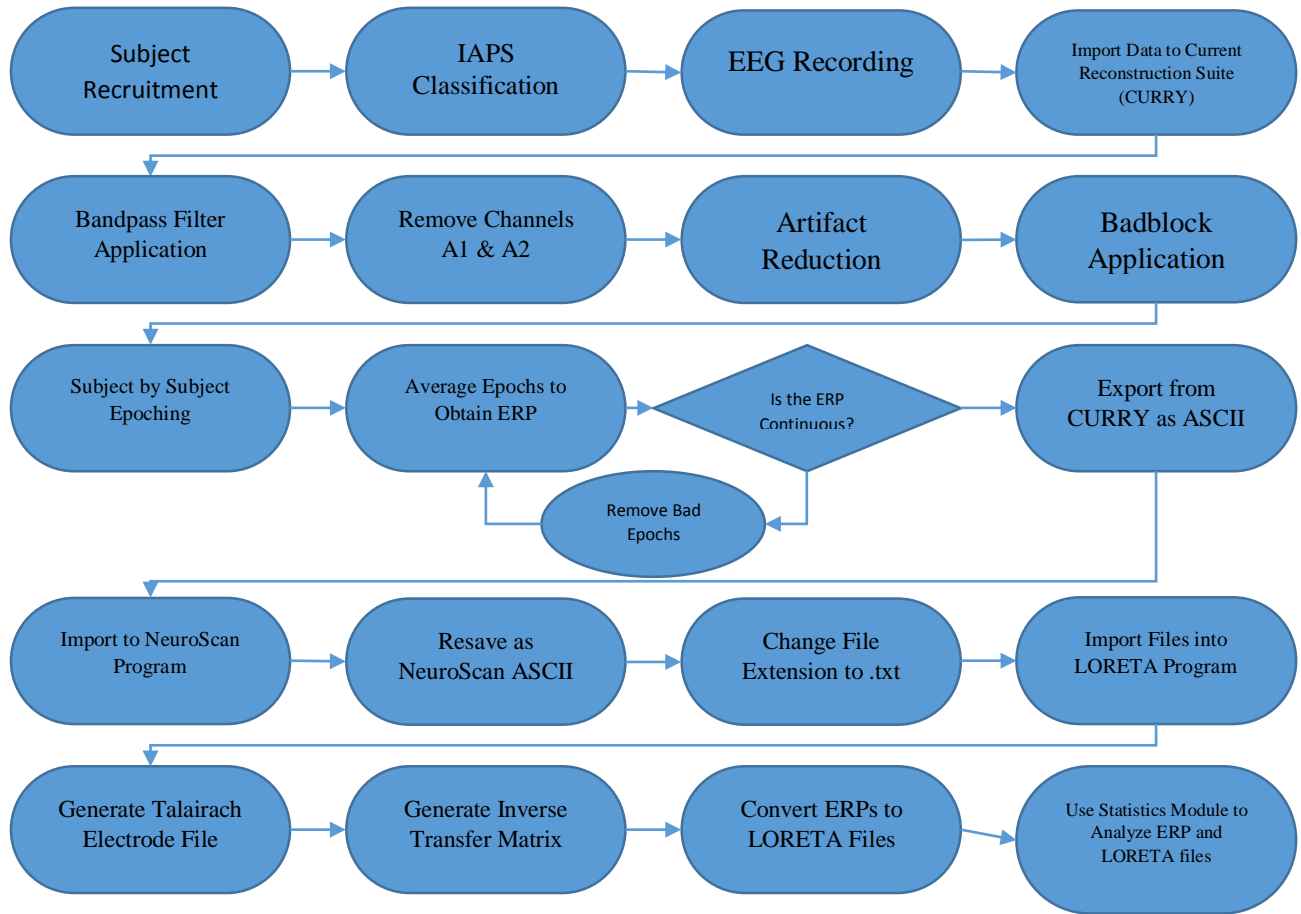Figure 7 is a step by step flowchart of the entire experimental procedure.

Figure 7. Flow chart outlining methodologies.

CHAPTER 4: RESULTS

4.1 Tests and Thresholds

For ERP and LORETA, both two-tailed t-tests and one-tailed t-tests were performed. Two-tailed t-tests were used to identify whether the groups being compared showed any statistical significant differences from one another. Appendix A contains the data of all of tests and their results. One-tailed t tests were performed after to determine which group showed an increase or decrease in activity. Seven statistical tests were performed. Out of the seven statistical tests performed only three met the set threshold of statistical significance. The statistical significance threshold chosen for this study was a p-value of 0.10. This was chosen because working with a small population of EEG based data multiple studies recommended using a lower threshold to identify statistically different populations. With larger populations and a higher number of stimuli the threshold can be increased.

4.2 High-Stress Neutral and Low-Stress Neutral

The first statistical analysis was performed between the high-stress neutral (HS-N) and low-stress neutral (LS-N) to determine if there was a statistical difference between the high-stress and low-stress conditions for the neutral stimuli. The result indicated there was no statistical difference between high-stress and low-stress individuals in either the ERP or LORETA current density distributions with two-tailed p-values of 0.5120 and 0.6628 respectively. This indicates that there was no statistical difference between HS-N and LS-N. P-values for the one tailed t-tests for HS-N > LS-N and HSN < LS-N yielded similar results indicating neither test group was more or less active than the other. Based on this, the high-stress

and low-stress groups can be equivocally compared against their respective neutral conditions, on the basis that their reaction to the neutral stimuli were statistically similar.

4.3 Low-Stress Pleasant vs Low-Stress Neutral

LS-P vs LS-N showed no statistical difference in ERP with a two-tailed t-test p-value of 0.5448, but did yield a LORETA current density two-tailed t-test p-value of 0.0168, signifying a difference between groups. Table 2 shows the results of the statistical tests performed on LS-P vs. LS-N. 'A' refers to low-stress pleasant and 'B' refers to low-stress neutral. The highlighted cells indicate the tests which were of significance with a p-value < 0.10.

Table 2. Tests and results for low-stress pleasant vs. low-stress neutral.

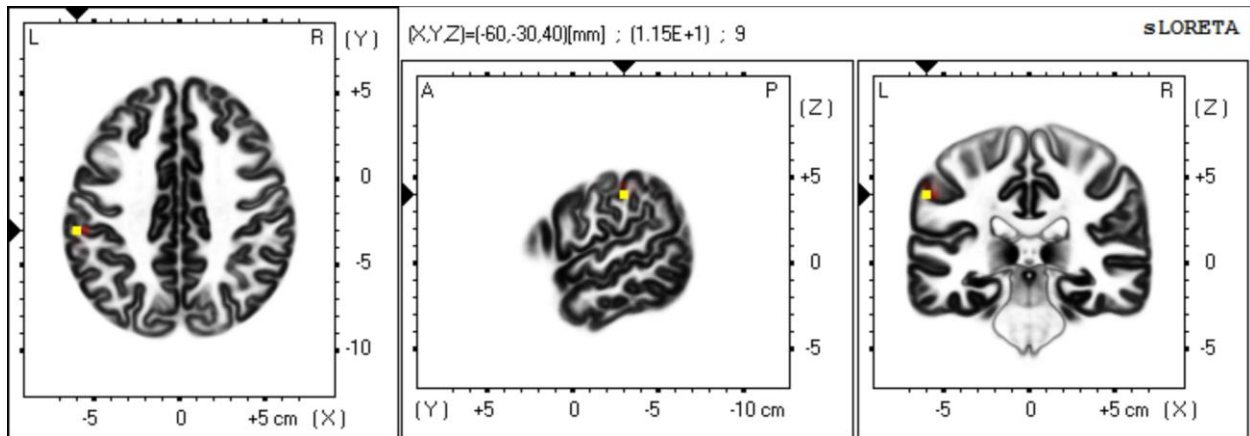| Data Type	Test	P-value
ERP	Two-Tailed (A ≠ B)	0.5448
	One-Tailed (A > B)	0.3554
	One-Tailed (A < B)	0.5688
LORETA	Two-Tailed (A ≠ B)	0.0168
	One-Tailed (A > B)	0.0088
	One-Tailed (A < B)	0.3278

Figure 8. LORETA map of low-stress pleasant vs. low-stress neutral

Performing one-tailed t-tests revealed that, with a p-value of 0.0088, LS-P > LS-N meaning that LSP was more active than LSN. LORETA provided that this significant difference occurred at

207ms within Brodmann Area 2 in the post central gyrus as seen in Figure 8. This is the brain map that shows the estimated current activity difference between low-stress pleasant and low-stress neutral. The figures from left to right are the transverse, sagittal, and coronal planes. The yellow indicates increased activity in the pleasant condition as compared to the neutral condition.

4.4 High-Stress Unpleasant vs Low-Stress Unpleasant

HS-U vs. LS-U showed no significant difference in ERP but did in the LORETA analysis. A two-tailed-t-test revealed that the LORETA analysis did differ between high-stress and low-stress participants when viewing unpleasant stimuli with a p-value of 0.096. Table 3 shows the results of the statistical tests for HS-U vs. LS-U. In Table 3, 'A' refers to high-stress unpleasant and 'B' refers to low-stress unpleasant.

Table 3. Statistical tests and results for high-stress unpleasant and low-stress unpleasant.

Data Type	Test	P-value
ERP	Two-Tailed (A ≠ B)	0.5102
	One-Tailed (A > B)	0.3138
	One-Tailed (A < B)	0.7772
LORETA	Two-Tailed (A ≠ B)	0.0960
	One-Tailed (A > B)	0.0456
	One-Tailed (A < B)	0.6072

Further one-tailed t-tests showed that HS-U > LS-U was statistically significant with a p-value of 0.0456 at a time of 506ms after stimulus presentation indicating greater activation in the high-stress group. The location of the current difference was located in Brodmann Area 31 in the cingulate gyrus seen in Figure 9. This is the brain map that shows the estimated difference in current activity. The figures from left to right are the transverse, sagittal, and coronal planes. The yellow indicates increased activity.
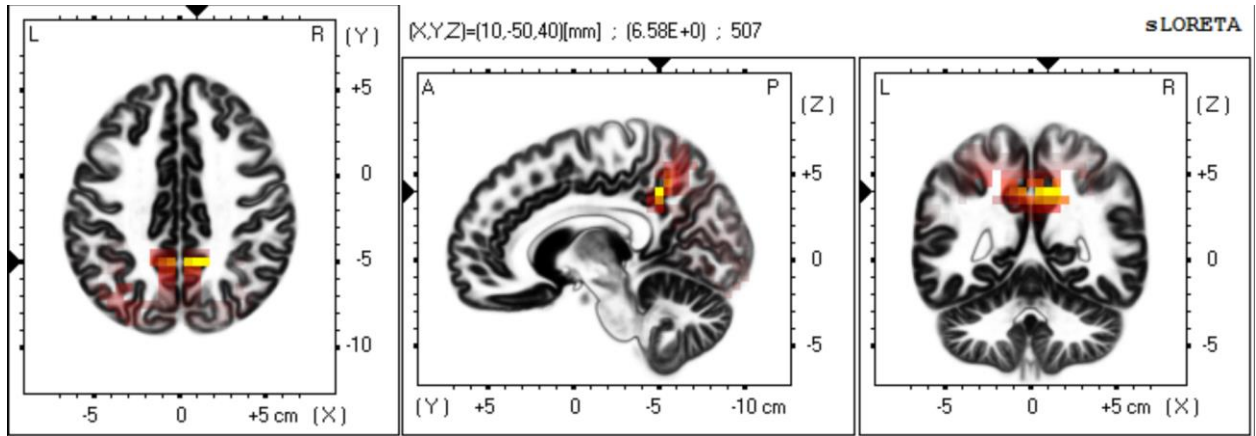
Figure 9. Brain map for high-stress unpleasant vs. low-stress unpleasant.

4.5 High-Stress Pleasant vs High-Stress Neutral

The final result was from HS-P vs. HS-N. Testing of the ERP revealed a time point at 256ms after stimulus presentation which was significant with a p-value of 0.0944. Table 4 shows the results of the statistical tests performed on HSP vs. HSN. In Table 4, 'A' refers to high-stress pleasant and 'B' refers to high-stress neutral.

Table 4. Statistical tests and results for high-stress pleasant vs high-stress neutral.

Data Type	Test	P-value
ERP	Two-Tailed (A ≠ B)	0.1728
	One-Tailed (A > B)	0.0944
	One-Tailed (A < B)	0.5716
LORETA	Two-Tailed (A ≠ B)	0.2564
	One-Tailed (A > B)	0.6052
	One-Tailed (A < B)	0.1334

This time segment was examined in the LORETA viewer module and it was noted that the brain volume was more activated in Brodmann Area 10 of the post central gyrus seen in Figure 10. This is the brain map that shows the estimated difference in current activity. The figures from left to right are the transverse, sagittal, and coronal planes. The yellow indicates increased neural
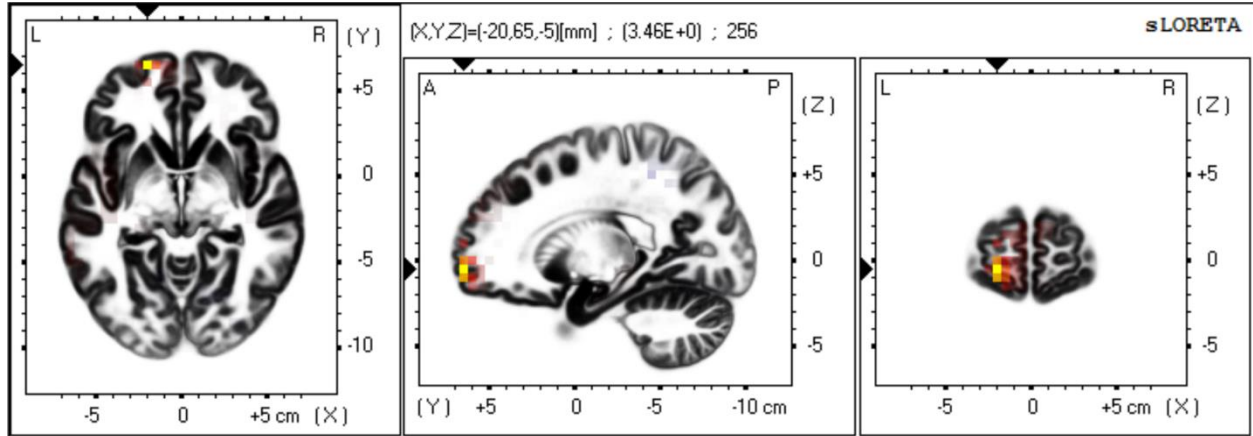
activity.

Figure 10. Brain map for high-stress pleasant vs. high-stress neutral.

CHAPTER 5: DISCUSSION

5.1 Low-Stress Pleasant vs Low-Stress Neutral

The low-stress pleasant condition showed an increase in current localization occurred within Brodmann Area 2 (Ba2) in the post central gyrus in the parietal lobe. Brodmann areas 1, 2, and 3 are located in the primary somatosensory cortex within the parietal lobe of the brain. Neurons in Ba2 process both tactile and proprioceptive stimuli. Proprioceptive stimuli are defined as stimuli that are produced and perceived within an organism, especially those connected with the position and movement of the body [22]. The proprioceptive processing of Ba2 helps determine one's own location in space and where their limbs and other parts are in relation to the rest of them. Low-stress individuals, as opposed to high-stress individuals, showed more activity in this region of the brain when compared against their neutral conditions. This may be because low-stress individual's brains may process the visual stimuli in a method by which they can relate to what is being seen, or they can figuratively place themselves in a scenario or have experienced a scenario like the one they are seeing.

5.2 High-Stress Unpleasant vs Low-Stress Unpleasant

Independent comparisons were performed to directly compare the high-stress and low-stress populations. When comparing the LORETA results for unpleasant stimuli it was observed that high-stress individuals had a stronger response approximately 500ms after stimulus onset. The time of approximately 500ms is close to what is considered the time interval between the precognitive reaction (ERP) and cognitive thought which could correspond to a delayed P300. Ba31 has been linked to spatial memory, learning, and avoidance learning. It has also been shown that Ba31 shows increased activity when autobiographical memories are successfully recalled, specifically personal memories that affect the individual or someone they know. This

area of the brain did not show activation if recall was unsuccessful meaning the individual either had no related memory or could not relate to the stimulus [23]. An fMRI study by Sprengelmeyer *et al.* examining neural structures associated with emotions had a similar finding, reporting activation of Brodmann area 31 when participants were shown angry-faces [24]. The high-stress group may have exhibited activation of this area within the brain because they could relate to the unpleasant stimulus was being presented, recalling a personal memory.

5.3 High-Stress Pleasant vs High-Stress Neutral

Only one ERP analysis identified a time point which was statistically different when examining the ERP as was the case with high-stress pleasant HS-P vs high-stress neutral HS-N. The timeframe range tested was 236-276ms post stimulus. This 40ms window was selected because of the timeframe identified from the result of the t-test on the ERP. The results yielded from the LORETA computation indicated that the identified time point corresponded to an increase in current density brodmann area 10 in the superior frontal gyrus in frontal lobe Figure 9. Brodmann area 10 (Ba10) is at the most anterior region of the frontal lobe of the brain and its function, although not fully understood [25]. One theory as to the function of Ba10 is cognitive branching, which is similar to parallel processing in computers where a previous task or thought is maintained while a new one is beginning [26]. Another theory is that Ba10 is influenced by the limbic system through the ventromedial cortex. A meta-analysis by Gilbert *et. al* found it may be involved in memory recall and multitasking [27]. The HS-P vs HS-N result may indicate that the brain of an individual under high-stress may be trying to make connections utilizing a parallel processing approach as opposed to their low-stress counterparts. The remainder of the tests were based solely on comparisons of current source reconstructions.

5.4 Classification Border Participants

The Penn State Worry Questionnaire (PSWQ) was used to classify the participants into low-stress and high-stress categories based on their response to the survey. There were three individuals who were classified as border cases, meaning their responses to the PSWQ indicated that they could not simply be classified as either high-stress or low-stress. To see if these mild stress individuals would affect the results of the statistical tests, all tests were re-run with these mild stressed individuals removed from the data pool. Examination of the resulting tests with the removed individuals revealed that mild stress individuals had no statistical impact on the results. These individuals were thus included in the analysis to increase population size.

5.5 Limitations

The population size for the experiment was only 21 individuals. The population was essentially split in half after the participants had been classified into the high-stress and low-stress categories. In future experiments a larger test population is desired. With the current participant population the threshold for significance was 0.10 which is acceptable for human data studies regarding LORETA, but with a larger population more definitive answers could be reached and the threshold for significance could be changed to 0.05 giving the statistical tests more power. A series of 20 images for each stimulus group was selected from the IAPS. Eye-blink artifacts contaminated on average half of the EEG epochs. This reduced the number of epochs that were used to calculate the ERP, resulting in an ERP that was not as well defined as one would see in textbooks. Ideally, in future studies the number of stimuli will be increased to counteract epoch contamination.

CHAPTER 6: CONCLUSION

The results of the experiment show stress does effect the brains pre-conscious response to emotional visual stimuli. Analysis of the ERPs of high-stress and low-stress individuals' revealed one instance of statistical difference between high-stress and low-stress individuals. ERP analysis barely indicated any statistical difference between stimuli presenting the limitations of lone time-domain ERP analysis. LORETA exhibited a higher sensitivity in identifying statistical differences between conditions and stimuli. The results of this study have shown that it is possible identify differences between high-stress and low-stress individuals using LORETA. More importantly LORETA can localize where the differences occurred within specific regions of frontal and parietal lobe of the brain. This allows for a deeper insight into which cortical mechanisms are active between differing participant groups. Stress has a detrimental effect on a person's wellbeing and current testing/monitoring methods do not offer a view of the changes occurring within the brain. This research is a step towards developing noninvasive diagnostic stress tests.

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Table 6. This table contains all of the tests performed in the study as well as their t-test results.

Group	Data Type	Test	P-value
HSP vs HSN	ERP	Two-Tailed (A ≠ B)	0.1728
		One-Tailed (A > B)	0.0944
		One-Tailed (A < B)	0.5716
	LORETA	Two-Tailed (A ≠ B)	0.2564
		One-Tailed (A > B)	0.6052
		One-Tailed (A < B)	0.1334
HSU vs HSN	ERP	Two-Tailed (A ≠ B)	0.5754
		One-Tailed (A > B)	0.5560
		One-Tailed (A < B)	0.3538
	LORETA	Two-Tailed (A ≠ B)	0.6630
		One-Tailed (A > B)	0.5960
		One-Tailed (A < B)	0.4002
HSP vs LSP	ERP	Two-Tailed (A ≠ B)	0.3872
		One-Tailed (A > B)	0.2318
		One-Tailed (A < B)	0.4568
	LORETA	Two-Tailed (A ≠ B)	0.6134
		One-Tailed (A > B)	0.3544
		One-Tailed (A < B)	0.9360
HSN vs LSN	ERP	Two-Tailed (A ≠ B)	0.5012
		One-Tailed (A > B)	0.3172
		One-Tailed (A < B)	0.5018
	LORETA	Two-Tailed (A ≠ B)	0.3806
		One-Tailed (A > B)	0.5098
		One-Tailed (A < B)	0.6628
LSP vs LSN	ERP	Two-Tailed (A ≠ B)	0.5448
		One-Tailed (A > B)	0.3554
		One-Tailed (A < B)	0.5688
	LORETA	Two-Tailed (A ≠ B)	0.0168
		One-Tailed (A > B)	0.0088
		One-Tailed (A < B)	0.3278
LSU vs LSN	ERP	Two-Tailed (A ≠ B)	0.3152
		One-Tailed (A > B)	0.1776
		One-Tailed (A < B)	0.7416
	LORETA	Two-Tailed (A ≠ B)	0.6290
		One-Tailed (A > B)	0.7770
		One-Tailed (A < B)	0.3848
HSU vs LSU	ERP	Two-Tailed (A ≠ B)	0.5102
		One-Tailed (A > B)	0.3138

		One-Tailed (A < B)	0.7772
	LORETA	Two-Tailed (A ≠ B)	0.0960
		One-Tailed (A > B)	0.0456
		One-Tailed (A < B)	0.6072