Summer 8-2022

Brain Electrophysiology in the Presence of Acoustic Stimuli

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A thesis presented to
the McKelvey School of Engineering
of Washington University
in partial fulfillment of the
requirements for the degree
of Master of Science

August 2022
St. Louis, Missouri

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Terminology

For the purpose of this report, the following terminology has been defined and briefly explained to help introduce this research.

Alpha Rhythm: The alpha rhythm refers to the frequency of oscillations of the alpha band and is the focus of this research; the "alpha rhythm" is synonymous with the "Posterior Dominant Rhythm".

Acoustic Stimuli: Exogenous stimuli played during the trial used to manipulate natural neural oscillations; synonymously defined as "auditory stimuli" throughout this paper.

Binaural Tone: Tones applied to both ears but at different frequencies. The intent of binaural tones is to allow the brain to pick up a specific beat determined by the different frequencies provided to the ears.

Frequency Bands: There are five frequency bands: gamma, delta, theta, alpha, and beta. Each band represents a separate frequency range of electrical excitation happening in the brain (Table 1).

Electroencephalography: the measurement of the electrical activity in different parts of the brain. An electroencephalogram is the recording of the electrical activity in the brain, and the device used for the electroencephalogram fits on the scalp and is attached with a chin strap and reference electrodes fixed to the mastoid bone behind each ear.

Isochronic Tone: A tone applied at equal tone, pitch, volume, and frequency to each ear simultaneously. Typically played as an on/off tone such as sequential beeping.

Monaural Tone: A tone delivered either combined before reaching the ear or combined for one ear and not the other.

Neural Oscillations/Rhythm: electrical oscillations produced by the activity of brain cells

Peak Resonant Frequency (PRF): The Peak Resonant Frequency is the maximum frequency identified in the first power spectral density plot conducted during the testing performed as part of this research. This PRF value is used to determine the tones played back to the subject in subsequent trials.

Posterior Dominant Rhythm (PDR): an intrinsic neural rhythm that appears over the rear portion of the scalp when a subject is restful and closes their eyes. The PDR is known to exhibit individual variation, which is sometimes referred to as a person's peak resonant frequency.

Acknowledgments

To my family, friends, mentors, and professors who supported me in this journey, thank you! Without your support, this wouldn't have been possible.

ABSTRACT OF THE THESIS

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Neuroscience is the study of the structure and function of the brain. This is inclusive of neurochemistry, physiology, anatomy, molecular biology, computer science, engineering and many adjacent disciplines. While there have been a multitude of studies in this field, there are still so many questions left unanswered about the brain. One such question pertains to how animals and humans perceive stimuli from the environmental periphery, a neuroscience sub-area known as sensory processing.

By design, humans have neurological states and receptors which organically take in all of these stimuli and decipher them for everyday needs. Sensory input enables subjects to perform daily tasks fluidly and often subconsciously. Therefore, through sensory processing the brain is able to take in information and turn it into appropriate motor and behavioral responses [20]. As the brain naturally ages, sensory function can decline, which in turn reduces a subject's cognitive ability, including the ability to form short term memories. This decline can be steep and can lead to diseases, including Parkinson's, Huntington's, Dementia, and/or Alzheimer's [4, 21]. This inability for short-term memory formation is most apparent in the occipital region, or back, of the brain.

Research has shown that neural rhythms (electrical oscillations produced by the activity of brain cells) are associated with sensory functionality, and it has been hypothesized that these rhythms may be causal to function [5]. Understanding this begs the question of whether natural

neural rhythms could be exogenously controlled (e.g., in terms of their frequency or magnitude) and, subsequently, whether cognitive function would be affected.

Integrating neuroscience technology with engineering methodology allows this hypothesis to be tested. Specifically, in the current research, we examine exogenous control of the Posterior Dominant Rhythm (PDR), an intrinsic neural rhythm that appears over the rear portion of the scalp when a subject is restful and closes their eyes. The PDR is known to exhibit individual variation, which is sometimes referred to as a person's peak resonant frequency. Our hypothesis was that properly tuned sensory input in the form of acoustic stimulation, could modulate or control the PDR in individual subjects.

The objective is to study whether it is feasible to manipulate and alter a subject's natural neural frequency or specifically, the PDR with exogenous stimuli. Therefore, this research aims to prove that with specific stimuli it is possible to alter the natural neural frequency of a subject. Statistical analysis is used to further prove the objective and results from this study do show that exogenous stimuli alters subject neural frequencies.

Chapter 1: Introduction

This thesis describes current research and background on neural oscillations, a phenomenon in which populations of brain cells produce rhythmic, synchronous electrical activity that can be detected using non-invasive electrodes. Specifically, electroencephalography (EEG) is used to test the hypothesis that acoustic stimuli alter the natural frequency of a particular type of neural oscillation. Auditory isochronic tones will be described and used toward the goal of identifying those which could augment the brain's natural PDR, which appears over the occipital (or, posterior) region of the scalp during eyes-closed wakefulness. Since the PDR is unique to each subject, an algorithm is developed and implemented in MATLAB, which uses EEG data to identify the PRF associated with an individual's PDR. Isochronic tones are then designed and delivered to subjects, and the ensuing EEG recordings are analyzed to assess whether the PDR was significantly altered. The ability to alter brain rhythms through non-invasive sensory stimuli could provide a pathway to interact with the nervous system, which in turn could reveal novel ways of manipulating neurological diseases such as Parkinson's or Alzheimer's Disease.

Background

The PDR is typically described as a low amplitude, mixed frequency rhythm which is an example of the canonical EEG "alpha band" (8-12 Hz) of oscillations [2, 8]. The frequency of the PDR is unique to every individual based on a multitude of factors. For example, PDR frequencies change over the course of one's life span. From 6 months to about age 10, human PDRs increase from 6 – 10 Hz. As humans age, these frequencies tend to decrease steadily with age [8]. Individual differences in frequency are also dependent on the cognitive ability of each subject. It is believed that the PDR can be impacted by short-term effects in healthy individuals

(i.e. fatigue) and more long-term effects in those with various health complications such as Parkinson's, Alzheimer's, and/or Depression. Those with these neurological diseases have lower neural frequencies which are correlated to a greater inability to use short-term working memory [8]. Gender is also a variable that implicates these standard frequency ranges. Women tend to have higher neural oscillatory frequencies than men, and therefore, the natural frequency of their PDR could be much higher than a male counterpart [21]. Anxiety can also lead to higher PDR frequencies as well [8]. We will heretofore refer to an individual's PDR frequency as their peak resonant frequency (PRF).

A long-held goal in clinical neuroscience is the development of mechanisms by which to alter brain activity and subsequent behavior. In this regard, there is a history of using sensory stimulation (e.g., acoustic tones) as an external stimulating input (to the brain). However, even though there is research on this sensory stimulation, there is not yet a systematic way to pick and use the tones to optimize the PRF value [1]. Engineering and designing a process to select and play tones which coincide with the PRF value is part of the goal of this testing.

Standard tones used as acoustic stimuli include, isochronic, binaural, and monaural tones. Isochronic tones are delivered as on/off tones at the same frequency to both ears. They are unique since they provide a consistent sound at the same frequency to both sides of the brain. The tone is not subject to the brain generating the specific effect of the sound because it is implicit within a specific frequency [3]. This means that that the response by excitation can be recorded without the implication of a person's perception of the sound (i.e. listening to music and soliciting an emotional response to it) which otherwise could solicit activity in a different frequency [3]. Separately, binaural tones are continuous and provided to each ear at slightly different frequencies. The intent of binaural tones is to allow the brain to pick up a specific beat

determined by the different frequencies provided to the ears. While the slightly different frequencies of the same tone are provided by binaural tones, monoaural tones are delivered either combined before they reach the ear or combined for one ear and not the other. Most often binaural tones are used to study those with Attention Deficient Hyperactivity Disorder (ADHD) or those with anxiety, and/or migraines [3]. For this study, isochronic tones are administered since the intent is to play the same tone to both ears at the same frequency which will only vary depending on the subject in test.

Chapter 2: Human Studies Design

Frequency Analysis

Acoustic stimuli used to manipulate neural oscillations presents promising research that could allow for a breakthrough in how to understand, predict, and control neural activity. Research already shows that sound can have an immense, positive impact on an individual's motor response [16]. While numerous tests have been conducted focusing on predictive, pre-determined stimuli during sleep [6], which includes theta and gamma frequencies, there is limited research for applying stimuli which is personalized to each test subject's unique PRF using only acoustic stimuli. A subject's normal EEG contains posteriorly dominant, symmetrical, and reactive alpha rhythms [2]. Since this frequency activity is separated into different bands, each band serves a different function at different frequencies (Table 1). Thus, the background noise taken in by the brain is processed as part of the PDR alpha rhythm. Since the PDR alpha rhythm is the main focus of this study, the threshold frequency assessed will be those frequencies between 8-12 Hz only.

Frequency Band	Frequency	Description
Gamma (γ)	>35 Hz	Concentration
Beta (β)	12 - 35 Hz	Anxiety dominant, active, external attention, relaxed
Alpha (α)	8 - 12 Hz	Very relaxed, passive attention
Theta (θ)	4 – 8 Hz	Deeply relaxed, inward focused
Delta (δ)	0.5 – 4 Hz	Sleep

Table 1: Standard EEG Frequency Bands [17]

The alpha band is most apparent in the occipital region of the brain which is known for memory formation and is typically detectable when a subject experiences a low level of stimulation during wakefulness [2, 18]. Steady, symmetrical oscillations are also to be expected with voltage ranges between 20-100 μV and a main frequency around 10 Hz [12]. EEG data is

recorded for this test initially without any application of acoustic stimuli to assess a subject's natural PRF between 8 – 12 Hz.

Tone Depiction

Since the PRF varies subject to subject, the optimal tone to alter the PRF will also vary between participants. For this test, the tones should be simple and should only vary in frequency.

As mentioned previously, isochronic tones provide an input which is controllable and allow for only the frequency of the sound to be changed depending on the subject's natural PRF. Isochronic tones are used in the study because they provide the same level of sound to each ear and therefore, the only delta between trials will be the frequency of the delivered tone. These frequencies will be delivered by the tone which is most similar to that of the natural PRF of the participant (within 0.3 Hz of the natural PRF). It's important to note that the range of frequencies for alpha and all other bands can vary slightly. This is due to how that the neural activity of every individual is unique and therefore, identifying optimal frequencies and identifying optimal tones for playback to solicit a measurable response becomes much more difficult but necessary for this experiment. For this test tone playback was +/- 0.3 Hz from the subject's PRF. Long-term, it would be ideal for the algorithm to identify from the subject the appropriate range to play tones, but for this experiment tone values were fixed around the subject PRF.

Chapter 3: Technical Methodology & Components

Methodology

There is an immense amount of research dedicated to studying neural activity, the effect of visual and acoustic stimuli on working memory, and neural activity during sleep. However, there is very little research that has been conducted on individuals which are awake during a study and/or who are only being tested with acoustic stimuli for a limited duration. This study aims to test the potential to control neural activity using only acoustic stimuli and aims to prove that alpha band frequencies can be altered with the long-term intent to improve short-term working memory.

The experiment methodology was designed to be straightforward and streamlined to minimize the opportunity for human error to occur while facilitating the test. The test algorithms aided this by creating a process for interpreting, predicting, and optimizing a subject's PRF. This was first accomplished by using an algorithm written in MATLAB to take the EEG data and then plot it in a readable format, a .mat file. Initially, the data is plotted as EEG micro-voltage (μV) versus time (t) and then plotted again using spectral estimation in MATLAB. Applying a bipolar montage to the data would be typically done in order to localize the data over the entire scalp. However, since the alpha rhythm is most prominent in the occipital region located on the backside of the brain, one electrode would be sufficient for the study. In this study, data from three electrodes was used and compiled into a new data set.

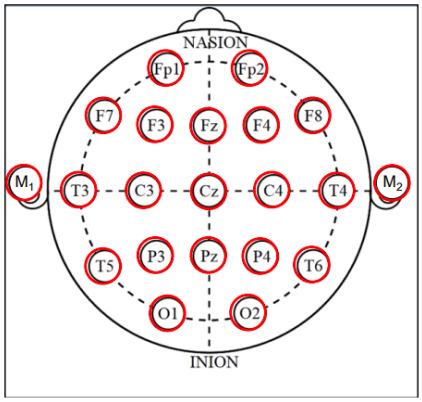

Figure 1: 10-20 Assignment of the Scalp EEG Device [15]

A subject's normal neural activity was then averaged, and Fourier Transforms were used to identify the power of the dataset. As an experimental study, the test could yield two conclusions. The first conclusion would be that the initial control data gathered from the subject, a normal EEG recording with no isochronic tone, is similar to the data gathered during the trials with tones. Statistical significance would not be identifiable from the data and thus, there would be no way to prove that the tones altered the PRF of the subject even if it seemed like there was a difference between trials. The second conclusion would yield the initial control response and then responses unique to the frequencies which have statistical significance. The response would showcase that the neural activity of the subject is receptive to the tone provided by MATLAB. The second choice would prove the first portion of the trial to be true: isochronic tones can alter alpha rhythms if played at a frequency which is personalized to the subject's PRF. If the tones solicit a unique response, then it is possible to alter neural frequencies using personalized tones.

Chapter 4: Planning, Procedure, & Set Up

Integration of Neuroscience & Engineering for Test

It is imperative to understand the integration required between Neuroscience and Engineering for this experiment. Both are integral to building and designing the experiment and to testing the objective of whether acoustic stimuli could manipulate a subject's PRF. Up front, the research for this testing revolved around Neuroscience and understanding the functionality of the brain. However, it became clear that engineering would be needed to orchestrate and design the test apparatus.

Initial code was written by John-Harry Wagner, a masters student who had worked initially with the EEG device and who had written code which interfaced between the device and MATLAB. This code was immensely useful in this study as it provided the team with the resources to record the data from each subject.

Recording data was certainly a very critical aspect of the testing. However, the type of data being recording is just as important. For this testing, two types of data would be recorded:

1) A control data set with no application of tones
2) Subsequent data sets with the application of different tones

Wagner's code could already record the control data for the subject for any duration needed. The question became, how could tones be designed in MATLAB and then played to the subject at the appropriate frequencies. Subsequent scripts of code had to be written to identify the PRF from the control file and designed to play tones at different frequencies.

The first script was simple conceptually but designing the code was tedious. Initially, the plan was to integrate these two latter scripts into Wagner's main recording script.

Various multi-functions within the code prevented this, so the scripts actually had to be run between two laptops for test execution. The first script identifying the PRF was designed to take in the full control data set and create a new vector of values from the three electrodes 29, 31, and 32 or PO3, PO8, and OZ nodes, respectively. A separate time vector was also created by dividing the number of samples taken for the trial by the sample frequency, 250 Hz. The number of samples taken was determined by the length of the data set. Two plots were created from this script:

1) A Time History Plot
2) A Power Spectral Density Plot

The time history plot was used as a measure to assess if the data was similar to that of the plots created live during the recording. If the plots were vastly different, it would be apparent that translation between the recording and the scripts was incorrect and therefore, the PSD could be inaccurate.

The PSD compared the frequency and power of the data. The PRF was identified by the peak value between the frequencies of interest: 8-12 Hz. Part of the script isolated the frequencies within this range by assigning them with a “1” and all others with a “0”.

Aside from looking visually at the plot, the maximum was identified and denoted in the legend so that there were no inaccurate PRF estimates during testing. More information on each part of the algorithm used for the analysis is detailed in subsequent sections. An example of the PRF plot can be seen in Figure 8 and Figure 9.

Even this initial identification of the PRF, which seems simple, is important because it drives the design and purpose of the testing. Careful planning had to be performed to ensure that the correct value was identified during each test. In addition, research on the brain had to be

conducted to be able to understand what the first step was to find the PRF and then understanding why it is important once found. Second, outlining the process to find this value and then using it to optimize tone playback to solicit a specific, unique response showcases the need to integrate Neuroscience and Engineering for this experiment. For this experiment both are imperative to the success of the testing performed in subsequent sections.

Approval to Test

Studying the brain and how it works is imperative to learning about its functionality. Similarly, bridging this with engineering to manipulate neural frequencies in the brain is even more critical to uncovering and answering questions we still know so little about. However, conducting research involving human subjects does run some risk and required immense preparation to perform. Prior to any testing, an initial 'green light' had to be received by the myIRB board. MyIRB is a data management system used for research protocols involving human subjects. Since the experiment involved using human subjects, all test equipment, recruiting material, population information, and experiential material had to be approved through the university myIRB system. The approval ensures the experiment was performed and executed humanely by approving all material and processes used by the research team. The approval process involves consolidating all of this material and providing it to a board for approval. Also required by myIRB was a human studies training which requires researchers to understand and assess the magnitude to which participants will be involved in the experiment. All of these factors must be considered to ensure that participants are informed and prepared to participate prior to testing.

Test Set up

The number of human participants planned for this experiment was fifteen, which was based on the average of participants who previously participated in studies focusing on acoustic stimulation during EEG experiments [10, 13-14]. Subjects were recruited voluntarily to participate in this study. All of the students attend Washington University in St. Louis, MO and/or are above eighteen years of age. Each participant completed an initial consent form to participate. The form included a liability waiver which released the trial team and the university from any and all liability; it also made subjects aware that their participation was voluntary and could be terminated at any time. Subject data will be kept for future analysis, and subjects were made aware of this before starting the trial. Each subject was independently scheduled to participate in a one-hour trial; the first thirty minutes were dedicated to a brief introduction, the consent form, and an opportunity for questions. The rest of the time was used to facilitate the formal test. Subjects were briefed on the intent of the trial. They were asked to stay calm and relaxed which is consistent with the frequencies being tested (i.e. the alpha-band frequency requires subjects to have minimal stimulation in their environment). Recall that the alpha band is of interest because it is responsible for short term working memory, specifically memory formation located in the occipital region of the brain. As subjects work harder to retain information, frequencies increase. As frequencies increase, there is potential that this shift would push the frequencies out of the alpha band range [9].

The test was conducted in Dr. ShiNung Ching's Lab at Washington University in St. Louis, Missouri. An isolated room within the lab was set up with two chairs and one desk. The test setup included one EEG device, two laptops, and one set of headphones. Headphones initially purchased for the experiment were unreliable and were not used after issues during the

first subject's test. Due to this dilemma, participants used their own headphones or sound was projected in the trial room. Headphones may sacrifice some sound quality and allow for some erroneous signaling to the brain (i.e. white noise in the background), however, the team does not believe that this impacted the test results.

Each subject was in the lab for their entire one-hour trial. They were seated in a chair with their feet flat on the floor at the desk. Both laptops were set up and easily viewable by the primary investigator and the subject. Next, the EEG device was placed on the subject's scalp. The device cap, seen in Figure 2, must be firmly attached to the scalp for proper contact with the skin. This includes pulling the cap over the entire scalp and attaching the chin strap from the left to the right ear. It also requires the reference nodes to be attached to the mastoid bone behind each ear. As an example, Figure 2 shows the yellow wire which was used to attach the reference node behind the left ear on the mastoid bone. If the cap is not fixed properly on the head and the reference nodes attached incorrectly, the data recorded will not be accurate.

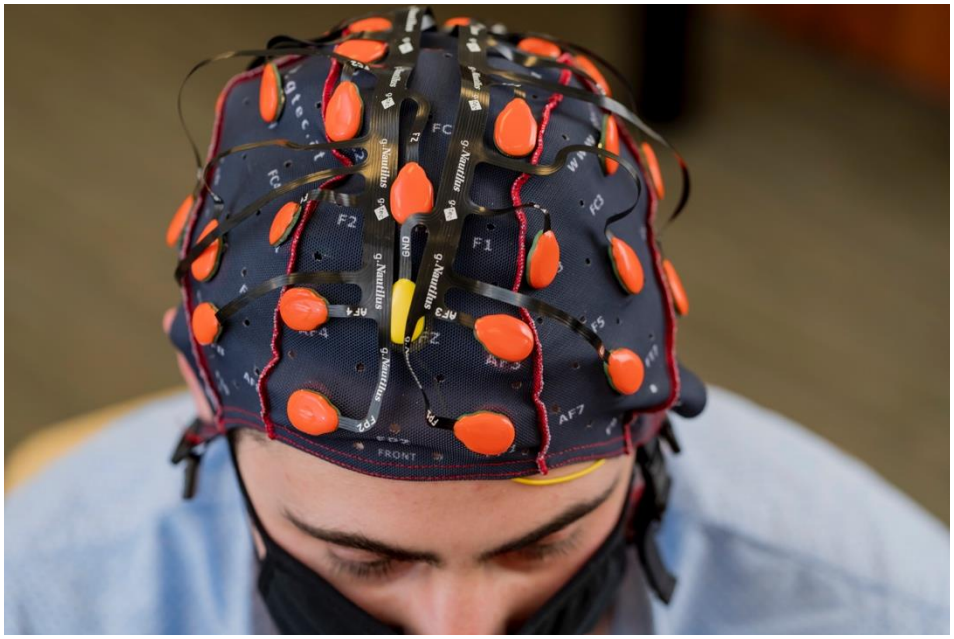

Figure 2: The EEG Device On the Scalp

Once the EEG was situated a “zero” trial was performed to ensure the system was functional and recording data accurately. Data from this trial was not used as part of the results. Next, the headphones were plugged into the laptop which the tones would be played from. The subject was asked to confirm they were firmly inserted and comfortable. Following this, a set of control data was recorded to use as the baseline for the tones played back to the subject.

Chapter 5: Analysis & Subsequent Characterization

There are three formal parts of the trial. The first part includes a control reading of neural activity from the subject. The second part assesses the PRF of the subject from the initial control recording taken in Part 1. The third part includes playing the tones to the subject and includes the same script used in Part 1. Each part is necessary for trial completion and is explained in subsequent sections.

Part 1

Part 1 includes the algorithm previously mentioned which was written by John-Harry Wagner. This algorithm collects and saves data from participants taken from the Nautilus EEG device. For this test, each set of data was one minute in length. The first and last twenty seconds of data were removed from the data set due to initial buffering of the system and to simplify the data set being analyzed. Initial skewing occurred from the buffering leading to inaccurate PRF values during initial draft testing. Electrode data from the recording is stored in columns with a sampling frequency of 250 Hz/sec. For a sixty second test, there were 15,000 data points and thirty-four columns of data (Figure 3). Each column corresponded to one electrode from the EEG device.

$$data\ points = sample\ rate\ [Hz] * time[s]$$

$$250\ Hz * 60\ s = 15{,}000\frac{Hz}{s}$$

$$\frac{15{,}000\frac{Hz}{s}}{3} = 5000\frac{Hz}{s} per\ 20sec. recording\ interval$$

Figure 3: Range of Interest Calculation

For this test, all electrode data was recorded, but only three electrodes were used in the assessment. EEG data was recorded and saved to a folder with the Subject's Number determined

by their order of participation. Figure 4 shows three channel time history plots (time vs. μV) from one sample trial.

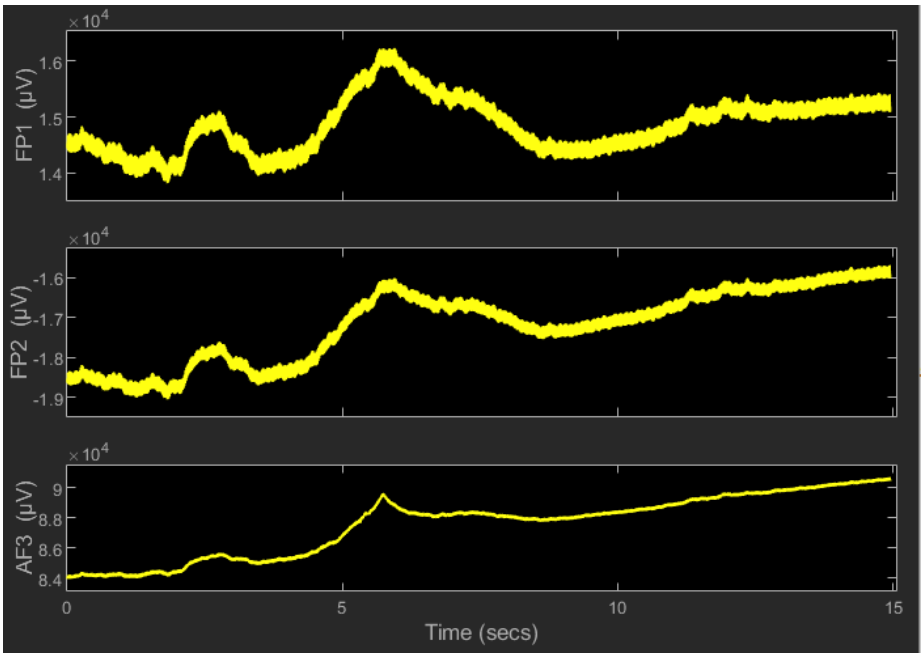

Figure 4: Sample Time Histories

The Time History data from Figure 4 is consistent and symmetric implying good contact from the reference nodes and from those on the scalp. In a poor recording there would be minimal contact, a sharp downward slope in the data, and the sinusoids would be very low frequency and asymmetric. A recording which implies that no data is being recorded would be seen by a flat line on the screen. This issue most often seen when one of the reference nodes shifted out of place and it was easily remedied when re-affixed. There were instances in recordings where the device would momentarily lose contact with the scalp. When this happened, those trials were re-run and data was re-recorded.

Part 2

After the data was recorded and saved to the appropriate folder, it was loaded into Part 2, the second script written for this test. This portion of the code created a time vector based on the

sampling frequency and initial recording time from Part 1. The data obtained in Part 1 should be similar to the time history plotted in Part 2. Time history data plotted in MATLAB is an average of the data from the electrodes located closest to the occipital region of the brain.

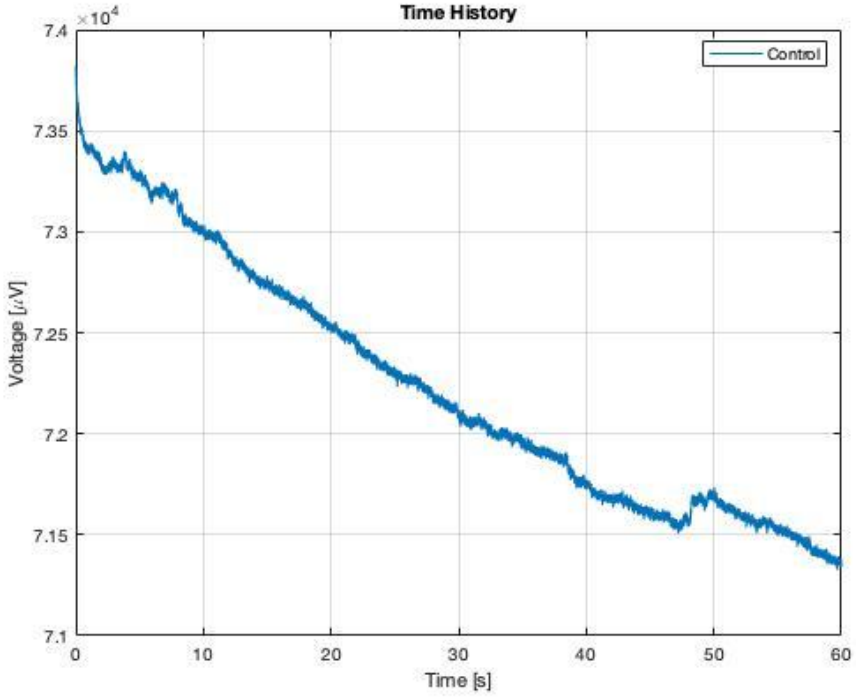

Figure 5: Subject 1 – Avg. Time History Recording

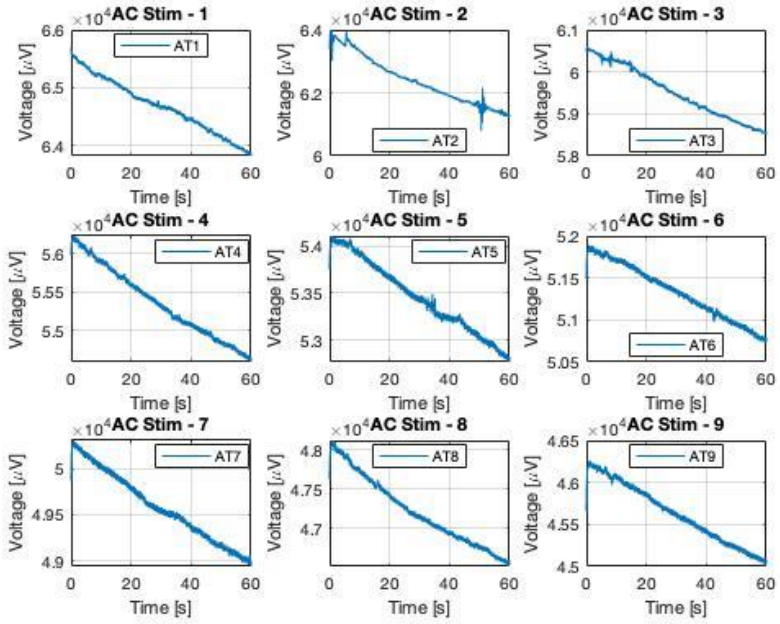

Figure 6: Subject 1 Recordings

The initial recordings of the data have no filtering which causes the downward slope seen in Figure 5 and Figure 6. For this test, we wanted to record the full range of frequencies upfront and filter for the range of interest after the recording.

As an example, Subject 1's data (control and those with acoustic stimuli) has been plotted in Figure 5 and Figure 6. It is apparent from the plots that the data is symmetrically dominant and consistent for the duration of the trial which is consistent with what was anticipated. It isn't surprising that the data between the control and the acoustic stimuli are similar since both sets are unfiltered.

Initial Analysis of the time histories was performed to ensure that the data captured was a proper representation of what was recorded in Part 1. Next, using spectral estimation in MATLAB, the function 'pwelch' was used to plot in the frequency domain. Since the data of interest was specifically from the occipital region of the scalp, electrode data was sufficient for the test. Plotting the Power Spectral Density (PSD) of the data commenced next, and the PRF

was identified using Fast Fourier Transforms over a new averaged vector of the electrode data mentioned previously. The max of the data set within the band of interest was taken and then published in the legend in the top right corner. This reduced the error by the user in trying to properly identify the PRF of the subject visually.

Part 3

The PRF identified from Part 2 was input into Part 3 to identify the tones played back to the subject. The tones, or exogenous acoustic stimuli, were designed in MATLAB. Each tone was played for one minute and mirrored the on/off isochronic rhythm previously mentioned. Subjects heard a series of beeping over one minute which only differed in frequency every three trials. The sample rate, tone duration, and pause duration were the same for each subject. Subjects listened to three tones of different frequencies three times during the test which equated to ten total recordings. The first recording was the initial control and the other nine were from the acoustic stimuli ($3\ tones\ x\ 3\ trials = 9\ total\ trials$). Tone thresholds ranged from 8-12 Hz and oscillated between $+/-0.3$ Hz to that of the PRF. Since these frequencies were so low they were magnified by 100 Hz to ensure they could be heard by the subject. Volume was adjusted for the subject as needed. There was no concern on volume variation between subjects, and for comfort, subjects were asked prior to the recording if the volume was too loud or too soft.

Analysis

Fifteen subjects were initially scheduled to test during this experiment. Hardware issues with the EEG device prevented the last three from participating. For the twelve participants who were able to complete the experiment, there were four women and eight men who made up the sample population. Women had a slightly higher average peak resonant frequency than men (9.8

Hz vs. 9.6 Hz). The average for the entire population was 9.7 Hz which is only slightly lower than the anticipated average of 10.0 Hz (Table 2).

Participant Population		Avg. PRF
General	12	9.7

Table 2: Upfront PRF Averages & General Population

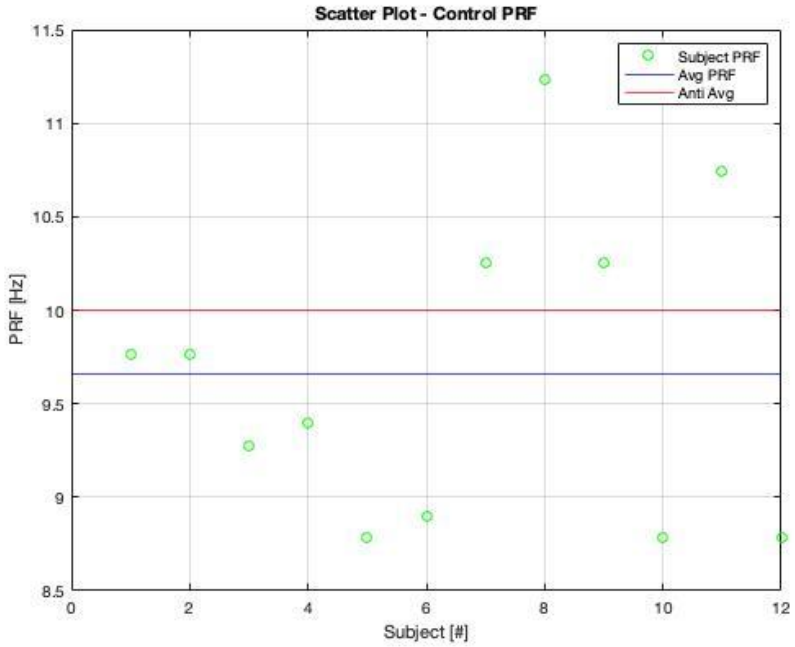

Figure 7: Scatter Plot – Subject PRFs

Figure 7 shows the PRF values for each subject and how they relate to the respective averages. Initial PRF values were found from the control recording taken at the beginning of the test. This data was input into Part 2 of the algorithm which selected the tones the participants would hear for the rest of the study from Part 3. Analysis of the data was split between the initial Part 2 and what is considered informally, Part 4, of the trial. The latter part of the analysis includes consolidating all trial data and comparing subject specific data.

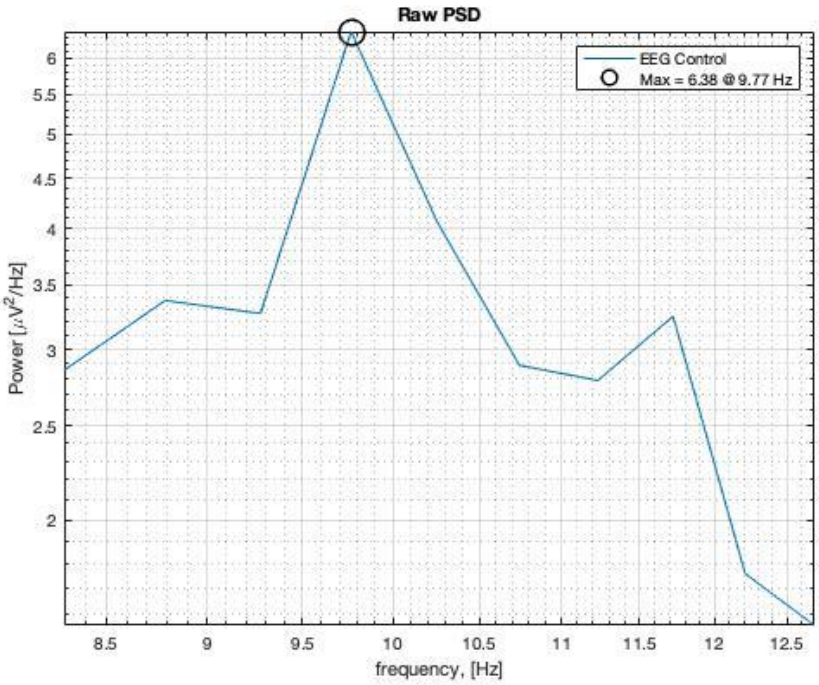

Figure 8: Part 2 – Subject PRF Analysis

Continuing to use Subject 1 as an example, initial control data yielded a PRF of 9.77 Hz as displayed both by the legend callout and the circle at the top of the screen. Since this is the highest peak of the PSD plot, it makes sense that the peak would be around 9.77 Hz for this subject. The y-axis, power, is irrelevant for this particular analysis. However, power is used to aid analysis in subsequent sections. What is important to note in this plot is the frequency of the measurement to understand the natural PRF of the subject.

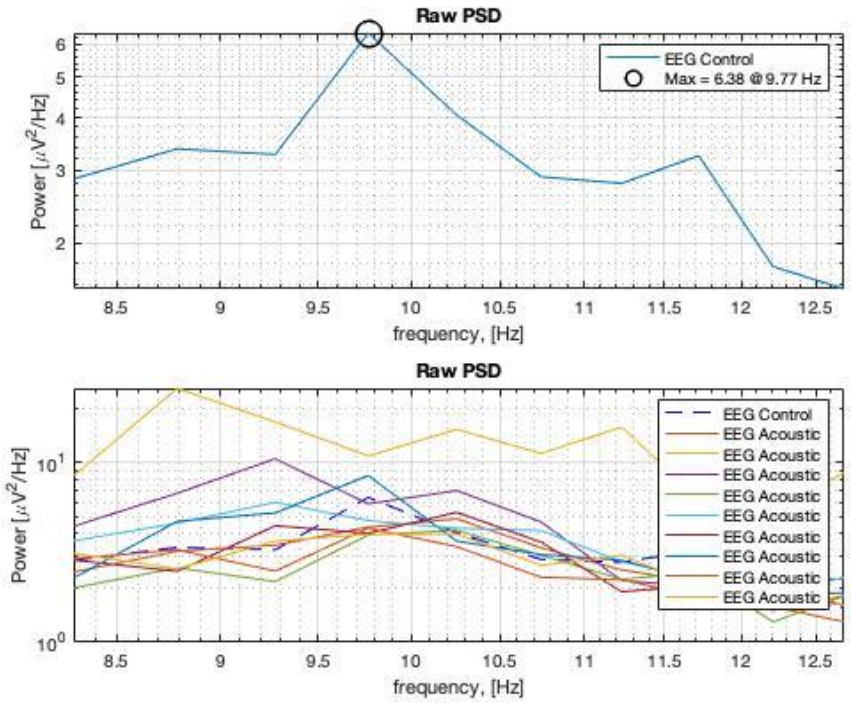

Figure 9: Part 4 – Subject PRF and Tone Analysis

For the first six subjects, a control set of data was recorded but the acoustic stimuli for the subsequent nine trials were the same (i.e. the subject only listened to one unique set of tones instead of three). This was an error within the code that was remedied prior to the seventh participant. The code now iterates for the nine trials between three unique sets of tones for the subject as initially intended. Even though this implication with the code changed the parameters of the experiment, this data was used to understand whether there was a change in frequency response over the course of each trial.

For this analysis, Figure 9 denotes first, the initial control analysis also displayed from Figure 8. The latter plot exemplifies the control versus all nine acoustic stimuli trials. Looking at the subject's individual data, there visually appear to be a defined increase in frequency during the third and fourth acoustic simulations. There seem to be peaks around 10.25 Hz and 10.75 Hz which represents a 5-10% increase in frequency from the control.

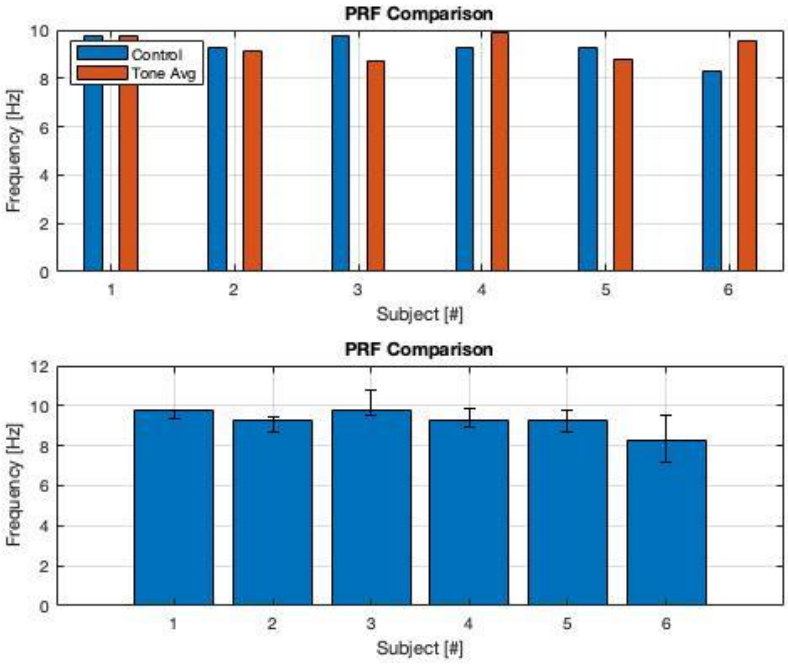

Figure 10: Subjects 1- 6 PRF Comparison

An initial comparison of Subject's 01 – 06 were also compared visually (Figure 10). It's apparent from the first plot that the peak resonant frequencies obtained from each subject were within the range of interest for this test, 8-12 Hz. The tone averages across the trials were also relatively close to that of the control recordings which is reassuring. This implies that at the very least the tones did not on average lower the peak resonant frequencies of the subject over the course of the experiment for 50% of the subjects. 33% of subjects actually saw a higher PRF with the tones than without. Subjects 04 and 06 both respectively saw an increase in PRF by 6% and 13% (Table 3).

Subject (#)	Control (PRF)	Avg. Acoustic (PRF)	Percent (%)	High Error	Low Error
01	9.7656	9.7656	100.00	0.00	0.37977
02	9.2773	9.1146	98.246	0.16276	-0.59679
03	9.7656	8.7348	89.444	1.0308	0.27127
04	9.2773	9.8741	93.956	-0.59679	-0.32552
05	9.2773	8.7891	94.737	0.48828	0.59679
06	8.3008	9.5486	86.932	-1.2478	1.1393

Table 3: Subjects 01 – 06 Similarity per PRFs

Visually analyzing the plots and the data in Table 3, however, is not enough to conclude that the subject's PRF has been manipulated. In addition, the question remains of whether or not these tones played back to the subject answer the objective and are statistically significant. To measure the statistical significance of the data, a Z-test was performed for each subject to identify the respective P-value [7, 22]. If the P-value is less than the defined confidence interval, 0.1 or 10%, the null hypothesis can be disproven which would imply that the tones would have influenced the subject's PRF response.

To determine this, a Z-test was performed:

Variable Definitions:

$x = control\ data\ set\ per\ Subject$
$\mu = average\ of\ the\ acoustic\ data\ set\ per\ Subject$
$s = std.deviation\ of\ \mu$
$n = sample\ population\ number\ (which\ is\ one\ when\ assessing\ per\ subject)$
$\sigma = estimated\ std.deviation\ of\ the\ entire\ sample$

$$\sigma = \frac{s}{\sqrt{n}} \qquad (1)$$

$$z - score = \frac{x-\mu}{\sigma} \qquad (2)$$

Figure 11: Z-Score Calculation

Solving the equations from Figure 11 in order, the Z-score was calculated by first determining the σ value, standard deviation, for the sample data set. This value was then used to solve for the Z-score. Z-scores were then found in the standard normal distribution table. The corresponding value, based on confidence interval, becomes the P-value for the data set. The P-value determines the likelihood that the null hypothesis can be proven or disproven. For this

experiment, the null hypothesis, H_0 , was the hypothesis that the tones played to the subject had no effect on their PRF. The alternative hypothesis, H_1, says that tones did alter the control PRF in some way. Disproving H_0 implies that H_1 is true [7]. Therefore, if $H_1$is true, it can be assumed that within the confidence level for this testing (10%) the tones did alter the subject's PRF. A confidence interval of 10% was chosen for this test to give the test the best chance of disproving the null hypothesis. Standard confidence intervals typically range between 1-10%.

Subject (#)	**Z-Score**	**P-Value**
01	0.00	0.51990
02	0.32909	0.63680
03	2.0843	0.98420
04	-1.2067	0.10560
05	0.98728	0.85310
06	-2.5231	0.005400

Table 4: Subjects 01 – 06 Z-Test & P-Value Estimate

Based on the values in Table 4, Subjects 04 and 06 do disprove the H_0 . Both P-values are approximately < 0.1, the confidence interval, which suggests that there is less than a 10% chance that the null hypothesis is true. Therefore, with certainty, the null hypothesis can be disproven, and the tones can be assumed to have altered the PRF of Subject 04 and Subject 06 during test. This result is consistent to the results denoted also in Table 3 and Figure 10.

Shifting focus to the latter half of the subjects, these participants did receive three unique tones over the course of their testing. To reiterate, these tones were +/- 0.3 Hz from the PRF from the control recording.

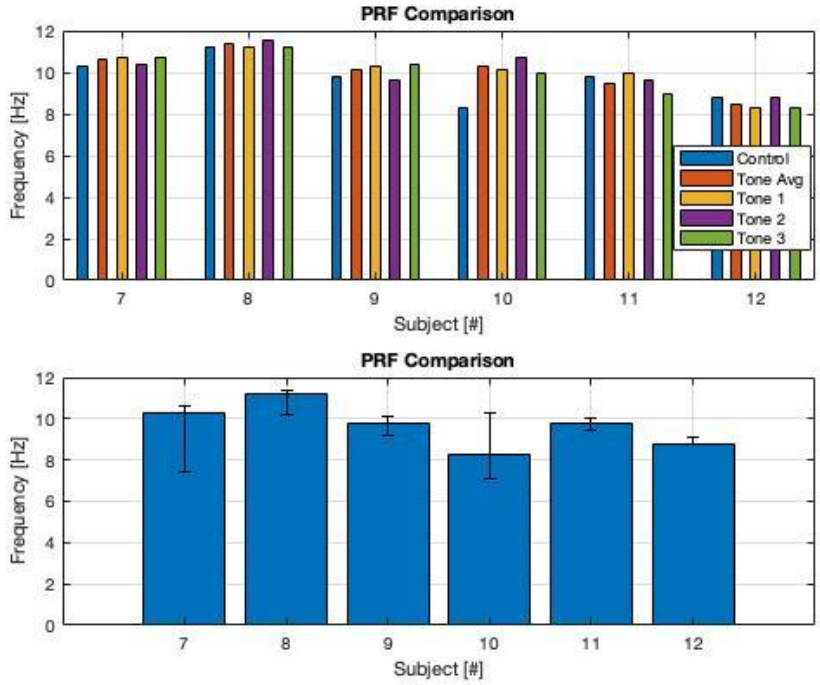

Figure 12: Subjects 07 – 12 PRF Comparison

From Figure 12, 83% of Subjects 07 through 12 saw the same or an increase in PRF between control and acoustic stimuli trials. Only Subject 12 saw a decrease in PRF from the acoustic stimuli provided. Four bars per subject are presented in the first subplot to showcase the control, the average frequency across all tone trials, and then the average across each individual set of tones. The second subplot of Figure 11 shows the control versus the error between the control and the average of all tones.

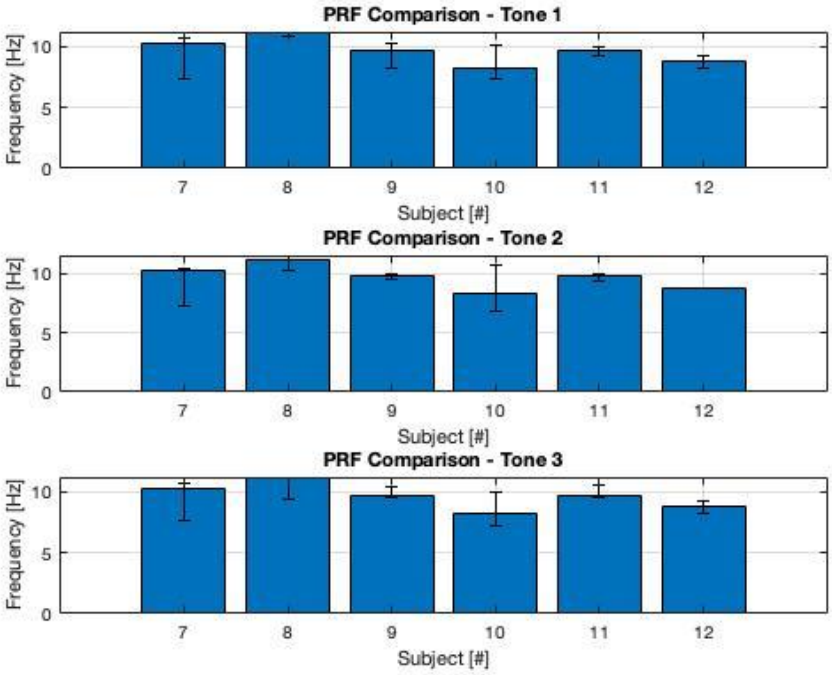

Figure 13: Subjects 07 – 12 PRF Comparison per Tone

Similar to the second plot of Figure 12, Figure 13 shows the error between the control and each average of the tones played back. The biggest discrepancy in tones vs. control can be seen by Subject 10. This is consistent with the representation of Subject 10's data displayed also in the first subplot of Figure 12.

Performing a similar analysis to what was done for the first six subjects, the next step is to determine whether the increase in PRF aligns with the null or alternative hypothesis. Using the same H_0 and H_1 hypothesis from the first assessment, the same confidence interval, 0.1, and the same Z-testing calculation, the results were as follows:

Subject	**Z-Score (Avg.)**	**Z-Score (Tone 1)**	**Z-Score (Tone 2)**	**Z-Score (Tone 3)**
07	-0.38477	-0.48925	-0.16537	-0.43853
08	-0.10994	0.0000	-0.33074	0.00
09	-0.32981	-0.48925	0.16537	-0.58471
10	-1.9788	-1.7939	-2.4806	-1.4618

Subject	Z-Score (Avg.)	Z-Score (Tone 1)	Z-Score (Tone 2)	Z-Score (Tone 3)
11	0.27484	-0.16308	0.16537	0.73088
12	0.32981	0.48925	0.0000	0.43853

Table 5: Z-Scores Subjects 07 – 12

Subject	P-Values (Avg.)	P-Values (Tone 1)	P-Values (Tone 2)	P-Values (Tone 3)
07	0.3300	0.2912	0.4040	0.3246
08	0.4400	0.5199	0.36320	0.5199
09	0.3369	0.2912	0.55960	0.2578
10	0.0207	0.03220	0.05400	0.0600
11	0.6368	0.4404	0.5987	0.734
12	0.6736	0.7088	0.5199	0.6738

Table 6: P-Values Subjects 07 – 12

From both Table 5 and Table 6, it can be agreed that the Z-score and P-values for Subjects 07 - 09 and 11 and 12 do not disprove the null hypothesis, H_0. However, for Subject 10, H_0 can be disproven since the P-value for the average and all three tones < 0.1. This again implies that there is less than a 10% chance that the null hypothesis is true. Therefore, the alternative hypothesis H_1 would be true for this test and therefore implies that the tones provided to the subject did alter their PRF. For the other subjects, there cannot be a concrete determination that the tones altered the PRF of the subjects during testing. Thus, H_0 is not rejected and the influence of the tones on the PRF could have been coincidental.

Chapter 6: Conclusion

Conclusion

The initial objective of the test was to understand whether the application of acoustic stimuli would vary a subject's PRF. Varying a subjects PRF would insinuate that there is opportunity to alter neural frequencies, with the potential to increase them, which could help prevent the onset of debilitating diseases like Huntington's, Parkinson's or similar diseases.

From the results of twelve subjects, it is seen that exogenous acoustic stimuli can alter subject PRF values. Since the first six subjects were inundated with the same tone over nine subsequent trials, the tones were played back to the subject intermittently for approximately 10-15 minutes. From the Z-test performed on the results, Subject 04 and Subject 06's P-values rejected the null hypothesis and thus prove that the tones did alter their PRF. Separately, Subject 10 was also able to disprove the null hypothesis, therefore 25% of the subjects did experience a variation in PRF from the tones played by MATLAB. Therefore, the acoustic stimuli did alter the PRF of the subjects during testing.

Three main takeaways from the testing include:

1. The length of tone playback could play a part in altering the subject response
2. Aligning tones close to the PRF can manipulate the subject response
3. It is possible to personalize tone playback to alter frequency responses based on the initial subject PRF.

These findings and conclusions then answer the objective initially posed by this research: yes, it is possible to manipulate a subject's frequency response with exogenous acoustic stimuli.

Since subject responses can be manipulated using stimuli, the next step would be further refining the algorithm designing tones for the subjects. As denoted previously, a subject's

response is unique to themselves exclusively. Therefore, even for those subjects who were not affected by the tones in this test, there are tones which could alter their neural frequencies. Ideally, an algorithm would be able to perfectly predict what would alter any subject's PRF, and design tones appropriately to increase PDR frequencies.

Applications which could identify and increase frequencies for those who are pre-dispositioned, diagnosed, or at higher-risk of degenerative diseases like Parkinson's or Huntington's, could be greatly aided by this research. By being able to manipulate and specifically increase frequencies, subjects would be able to improve their sensory functionality and their cognitive capability. Long term, this could reduce neural frequency loss in patients which would otherwise be severely impacted by Alzheimer's, Dementia, Parkinson's, or Huntington's Disease.

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Appendix A: Matlab Scripts

If interested in the MATLAB scripts used for this experiment, please reach out to Kristen Howorka.

Appendix B: Test Procedure

ESE 599: Test Steps / Set up

Purpose: To test a subject's neural activity using acoustic stimuli (i.e. measuring and predicting changes based on different isochronic tones)

Subject Requirements/Criteria:

- Subjects 18+ (15 Participants)
- Student at Washington University in St. Louis (or non-student 18+)
- No known hearing impairments

Testing Location:

- Washington University in St. Louis – Dr. Ching's lab – Green Hall 0102

Test Set up:

Step #	Step Description	Notes	Completed (Y/N)
1	The Test administrator will relay testing specifics to subject's individually for ten-twenty minutes prior to test start. This includes providing subjects with the consent form. - Introduce the test Q: How will the test be conducted (i.e. the participant will wear X headphones and hear simulated noise at different intervals)	Allow the subject to ask questions about the test and testing process in a private room. Subjects are allowed to leave the test at any time. Note: The purpose of the test will not be disclosed until after the test is complete. There will be some overlap between the alpha and beta bands. However, there is no concern that this will obscure data.	
2	Ask the student to sign a pre-defined waiver / informed consent form, releasing the team and university of liability	See Step 1.	
3	Ask the participant to sit in a pre-defined chair comfortably (both feet on the ground, arms on the arm rest)		
4	The test administrator should help the participant place the EEG device and audio head phones on the subject		

Step #	Step Description	Notes	Completed (Y/N)
	comfortably; ask the subject to confirm that they are comfortable		
5	Turn on the encephalography (EEG) device and ensure that it is tracking accurate, normal brain activity.	Ensuring the EEG device is on and functioning will occur with the lab laptop and pre-written EEG device MATLAB code. This code will be used for the duration of the test to run and store EEG data as a .m file locally on the laptop.	
6	Play test sounds through the head set to ensure that no outside, white noise can be heard.	No data will be recorded at this time. This test is solely used to ensure that the audio coming through the headphones can be heard by the participant.	

Facilitating the Test:

Step #	Step Description	Notes	Completed (Y/N)
1	The EEG device is now hooked up and the MATLAB code is running and recording data correctly. Create a local folder on the laptop which will store all of the test data Label each recording as "Subject X – Control" or "Subject X – Test" to distinguish between the control data and data with tones	No recording of test should have taken place by this step	
2	Record **1 minute** of control data (a normal EEG reading) from the subject. Save the data to the respective folder		
3	Load the data into the second MATLAB file used for Power Spectral Density Analysis. Double check the peak resonant frequency with visual inspection (identifying on the PSD the peak amplitude of the plot between 8 and 12 Hz). There will be a peak at 60Hz which is from the AC current from the EEG device. Disregard this peak.	There should only be one subject recording at this point Note: Ensure the plots look clean (no steep drop offs)	
4	Based on the mean and PSD of the data, select the appropriate threshold of tones to play back to the subject. Part 3 of the code will ask the engineer what thresholds of tones to play (a popup will appear when the code runs. Based on the PSD from Step 3, enter this value into the window). Then click play.	Tones should be played for **1 minute.** Data should be recorded and saved simultaneously during tests.	
5	The code will ask if you are ready to run the next test and/or if you want to rerun. After confirming the data has been saved, run the next tone.		
6	Repeat step 4 and 5 until 9 acoustic stimuli trials have been completed		
7	This concludes the test; ask the participant if they have any questions prior to leaving and compensate them with a gift card.		