The Effects of Binaural Beats on the Brain's Functional Connectivity

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ABSTRACT

Binaural Beats (BB) are rhythmic tones used to alter one's mental state; BB are an illusionary, perceived third tone. This study aims to answer if BB stimulation can elicit unique functional connectivity (FC) patterns in the brain by utilizing electroencephalography (EEG) data and graph theory analysis (GTA) in analyzing FC between anatomically separate regions of the brain. The hypothesis is that a singular 10-minute session of BB listening through earphones will modulate the brain's FC. Noticeable changes between subjects pre- and post-BB EEG recording sessions are seen; changes in state of balance are assessed using GTA. Results show distinguished effects due to different types of BB intervention. A pattern of connectivity is determined for a singular type of BB within this study.

The Effects of Binaural Beats on the Brain's Functional Connectivity

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LIST OF ABBREVIATIONS

BB	Binaural Beats
BC	Betweenness Centrality
DC	Direct Current
EEG	Electroencephalography
FC	Functional Connectivity
FCA	Functional Connectivity Analysis
FFR	Frequency Following Response
FT	Fourier Transform
GCC	Global Clustering Coefficient
GTA	Graph Theory Analysis
ICA	Independent Component Analysis
IFT	Inverse Fourier Transform
PLI	Phase Lag Index
PLV	Phase Locking Value
wPLI	Weighted Phase Lag Index

Chapter 1. Introduction

Music and rhythmic tones have been used historically to alter one's mood and improve cognitive performance [1,2]. The use of sound for physical and mental healing can be traced back to ancient civilizations in India and China. Sanskrit chants are documented in ancient Hindu scriptures and sound therapy is a Chinese medical tradition. A notable early discovery in music used for healing is Solfeggio frequencies. Also known as ancient frequencies or healing frequencies, they originated from Western European music in the 11th century and are commonly used in sound therapy. For centuries, various cultures have used Solfeggio frequencies for their supposed healing properties. These frequencies are based on an ancient system used in medieval music that assigns a specific syllable to a specific musical note i.e., tone. Tones are used differently, as some tones are believed to treat specific ailments. There is still some controversy on the use of Solfeggio frequencies in alternative medicine and sound therapy, but research on frequency effects has helped support their effectiveness. Based on the properties of the different frequencies that comprise the Solfege scale and their assumed effects, Solfeggio frequencies claim to activate cellular repair at deep levels and help treat chronic depression, anxiety, and pain [3].

Recently, there has been an increase in neuropsychology research relating music to emotion. Many musical components and reactions to musical components have been investigated, including melody, harmony, modality, rhythm, timbre, and specific musical cues [2]. Music can have many effects on the human body and mind, and has been proven to help with stress management. Faster music tends to be associated with feelings of alertness and increased concentration, while slower music is associated with relaxation and calming of the mind. Researchers at Stanford University have determined that the effects of listening to music can change functions in the brain to the same extent as medicine [4].

A relatively new application of rhythmic tones being used to treat stress and improve mental performance are called Binaural Beats (BBs). A beat is defined as the regular pulse of music [2]. Binaural beats are an illusionary, perceived third tone created by the brain when two different but similar pure tone frequencies are played in separate ears through earphones (i.e left ear 350hz and right 320hz) [1,5,6,7]. This difference in tones is small, between 2-30 hz, and cannot be transcribed by the brain so it is tuned instead, making a completely new and additional third tone - BBs. Previous literature has shown BBs being used successfully to improve cognitive functions, memory, mood, creativity, alertness and vigilance. As well as reducing stress, anxiety, pain, and increasing levels of hypnotic susceptibility. The use of BBs as a noninvasive stress management tool has the potential to be extremely valuable; however, the use of BBs for mood regulation are still highly speculated within the scientific community, as many researchers have found inconclusive or contradicting results. One notable result that is inconclusive or contradicting throughout previous studies is the ability to determine if BBs stimulation causes changes in the brain's Functional Connectivity.

Chapter 2. Thesis Hypothesis and Significance

This thesis aims to answer if BBs stimulation can elicit unique functional connectivity patterns in the brain by utilizing EEG data in the analysis of the functional connectivity between different regions of the brain after being subjected to Binaural Beats (BB) stimulation, and by applying GTA to examine the sensitivity level for detecting subtle changes in the brain network due to BBs stimulation. Graph Theory is a well-known neuroimaging tool used to study complex networks through its representation of functional connectivity between brain networks. Different imaging modalities can be used to construct the functional connectivity between networks such as, MRI, fMRI, PET, diffusion tensor imaging (DTI), and EEG [48]; however, non-invasive methods such as EEGs are preferred. The hypothesis is stated below:

The hypothesis is that a singular 10-minute or longer session of listening to BBs at the proposed alpha (8 hz) or beta (30 hz) frequency band values through earphones can modulate the functional connectivity of the brain.

After BB stimulation, changes in the brain's network and thereby the graph theory model's state of balance should be expected. There should be indications of decreased cognitive performance in the group of subjects exposed to 8 Hz BB frequency, and indications of increased cognitive performance in the group of subjects exposed to 30 Hz BB frequency. These changes in state of balance are assessed using GTA. Instead of comparing wPLI results channel-by-channel, the traditional way, GTA will be applied to wPLI FC results to assess changes in the brain's network.

Chapter 3. Background

3.1. Stress

Everyone experiences stress, as it is a natural reaction of the human body to internal or external stimuli, commonly referred to as stressors. Every individual experiences and reacts to stress differently. The body is designed to experience and react to stress, as small amounts of stress can be positive and help individuals stay alert and motivated throughout the day. However, too much stress, especially over time without periods of rest or relaxation, can cause both physical and mental health problems [8,9]. It is important to note that stress is subjective, and only the person experiencing it can determine how intense it feels and the emotions it elicits. When stress is present, it can affect one's ability to concentrate or relax, provoking strong emotions like frustration, angst, and irritability. That is why learning coping methods that suit your body and lifestyle to help manage stress is important [8,9]. Without stress management, individuals can develop chronic stress and related medical conditions.

3.1.1. Effects of Stress

Stress can affect every system in the human body. Although the human body is equipped to deal with stress in small amounts, long-term/chronic stress and frequent acute stress can have serious negative health effects on the body [8,10,11]. Chronic and acute stress can cause ailments such as tension headaches, migraines, muscle pain, changes in breathing, panic attacks, nausea, gastrointestinal discomfort, and much more. However, chronic and acute stress are best known for exacerbating pre-existing underlying medical conditions [8,10]. Acute stress is short-term and onsets quickly; it causes an increase in heart rate, forcing the heart to work harder, and is accompanied by the release of stress hormones — adrenaline, noradrenaline, and cortisol — which act as messengers for the human body flight or fight response. Once an acute stress episode is over the body will return to its normal state. On the other hand, chronic stress

is experienced for prolonged periods of time which causes the body to maintain a state of increased heart rate, high blood pressure, and elevated hormones. Persistent chronic stress and repeated acute stress take a toll on the human body and increase the risk for hypertension, heart attack, stroke, and other medical conditions. While stress affects all systems of the body, the Endocrine and Nervous system are most strongly connected to the hormonal changes that chronic and acute stress induce [10].

The Endocrine system houses the hypothalamic-pituitary-adrenal (HPA) axis, which is responsible for the stress response of the endocrine system. When an immediate threat is perceived by the brain, the hypothalamus — what connects the brain to the endocrine system, and part of the HPA axis — activates the pituitary and adrenal glands, causing an increased amount of steroids called glucocorticoids, which includes cortisol, to be produced. Cortisol provides energy, and is normally produced at a high level in the mornings after waking up and will slowly decline throughout the day; this is what provides the human body with its normal daily energy cycle. However, when the body remains in an elevated state of stress over time it will continue to produce glucocorticoids, resulting in excess amounts of cortisol in the body. Persistent chronic stress has been shown to impair the communication between the body's immune system and its HPA axis. Over time, this impaired communication can lead to the development of various mental and physical health conditions like chronic fatigue, depression, immune disorders, and metabolic disorders.

The autonomic nervous system is housed in the peripheral division of the Nervous system. It is divided into the sympathetic nervous system (SNS) and the parasympathetic nervous system (PNS), both of which play a direct role in the human body's physical response to stress. The SNS largely facilitates in the body's flight or fightresponse, as it signals for

adrenal glands to produce adrenaline and cortisol when the body becomes stressed as a reaction to perceived threat. Whether the brain perceives something as a threat or not is largely controlled by the central nervous system. The hormones produced from the adrenal glands in combination with the actions of autonomic nerves cause an increase in heart rate and breathing rate, cause blood vessels in the extremities to dilate, and cause an increase in glucose levels and digestion rates in order to supply the body with enough energy to deal with a high-stresssituation for a short amount of time. Once the stressful event has passed, the PNS aids in the recovery of the body to its original unstressed state. Typically the PNS has the opposite effects as the SNS, but if the PNS is overly active it can cause stress effects onthe body, such as excessive vasodilation or bronchoconstriction. Continuous activation of the autonomic nervous system and the physical reactions it provokes in the body will wear the human body down over time, corrupting the integrity of other bodily systems. Stress effects on the immune system can compromise the entire body, making itvulnerable to infection.

3.1.2. Stress Management Methods

There are many ways individuals can cope with and manage their stress. For those who experience high amounts of stress on a regular basis, it is typically recommended to make appropriate lifestyle changes. General recommendations include getting a proper amount of sleep, keeping a daily routine, eating a balanced diet, drinking enough fluids, exercising regularly, taking time for one's self, and spending time with friends and family [8,9,11]. Allowing the body nutrients and rest it needs can help anyone feel better physically, but goal setting and keeping a personal agenda can help an individual mentally to feel in control of their time and energy [8,9]. It is also highly recommended to limit screen time, especially for those who heavily follow news and social media. Screen time is an inactive way to manage stress, as activities like watching tv, playing video games, or scrolling through social media are distracting and may seem

relaxing, but given time can lead to increased stress [8,11].

There are various programs that can help people feeling overwhelmed; they can be found online, in smartphone apps, at local community centers and gyms, as well as with a mental health professional. If seeking help from a therapist is intimidating it is recommended to try and talk about overwhelming feelings with a trusted and close friend or family member [8,9]. While trying to manage stress it is important to remember that not everything can be controlled and to acknowledge good moments from your day or life [9]. Also, try to avoid tobacco, nicotine, and illegal substance use, as well as excessive amounts of caffeine and alcohol since all of these can increase anxiety and exacerbate current emotions [11].

There are also numerous activities that can be incorporated into daily lifestyles to help reduce stress and manage it when it arises. Any type of exercise can help with relief of stress that is persistent or has onset quickly. Some common relaxation activities include taking a walk, meditation, yoga, muscle relaxation and breathing exercises, participating in a hobby, or listening to music [8,9,11]. Historically, there have been many types of music and rhythmic tones used to alter people's mood [1,2].

3.2. Binaural Beats

Binaural beats (BBs) are an illusionary, perceived third tone created by the brain when two different but similar pure tones, a.k.a. frequencies, are played in separate ears through earphones (i.e left ear 350hz and right 320hz) [1,5,6,7]. It is important to use earphones to ensure the two original tones are being played separately in each ear and not being combined. If the two original tones are captured by both ears, then then no third tone (BB) will be perceived. It is also important that the difference between the original tones be small, generally

between 2hz and 30hz, so the two separate tones are not captured by both ears [5,7]. This difference in tones cannot be transcribed by the brain but is tuned, making a completely new and additional third tone (BBs). Two original tones, one in the left at 350 Hz and the right at 320 Hz, the brain uses to create the perceived third tone at 30hz. This perceived third tone, the BB, has an amplitude that oscillates at a frequency equal to the difference between the two original tones, commonly referred to as the modulation frequency [5,6]. BBs have a modulation frequency and a fundamental frequency; in the example above the modulation frequency is calculated by taking the absolute value of the difference between the two original tones (350-320 = 30hz), and the fundamental frequency is the frequency at which the entire sinusoidal wave of the BBs oscillates, calculated by averaging the two separate tones (350+320/2 = 335hz) [5]. To better understand fundamental frequency, consider the following example: a person is sitting in the middle of a room with a speaker to their left and a speaker to their right. Simultaneously, the speaker on the right begins playing a pure 320 Hz tone and the speaker on the left begins playing a pure 350 Hz tone. The sound the person sitting in the middle of the room hears is an average of the two frequencies being played aloud. This average, 335 Hz, is the fundamental frequency, and is being heard by both ears as a singular tone in this scenario. When headphones are used in the same scenario instead of speakers, the brain receives two separate tones and is forced to make sense of them. However, during normal functions the brain does not oscillate higher than 60 Hz, so instead of the brain trying to average to two tones at 335 Hz, the brain takes the difference between the two tones and attunes to their difference of 30 Hz, i.e. modular frequency, instead of their average. Previous research that has tested the effects of BBs in different formats suggest that the fundamental frequencies used for the original tones be between 200-900 hz. To maximize the probability of detecting effects of BB, fundamental frequencies around 500 hz should be used [5], and the maximum fundamental frequency used should not exceed 1000 hz [5,7].

3.2.1. Effects of Binaural Beats

Previous literature on the effects of BB shows varying results that overall cannot make any definitive claims about the effects of BB on the brain's functional connectivity (FC). Some research has made broad claims, such as brainwave oscillation and FC may change under BB stimulation [5]. While others have made more narrow claims, such as delta and theta BB stimulation leading to network changes in the temporal regions [9]. So far, research has been able to show that delta (1.5-4 Hz), theta (4-8 Hz), and gamma (30-60 Hz) modulation frequencies produce some change in brainwave amplitude as a response to BB stimulation [7,12]. Multiple studies that focused on effects of gamma BB found that subjects had an increase in focus after BB stimulation, and that performance speed could be increased while listening to gamma BB. This increase in focus and performance speed was evaluated using numerous attentional tasks. Studies that focused on delta and theta BB found that subjects had impaired performance after stimulation, which can be expected as delta and theta frequency bands are associated with sleep and drowsiness. A comparison of beta BB stimulation to delta/theta BB stimulation concluded that beta BB may potentially increase vigilance [6]. In general, recorded effects of BB have been variable due to the fundamental frequencies and modulation frequency chosen, exposure time, other differences in methodologies, and difference in analysis techniques [1,7]. Remaining within suggested fundamental frequency ranges and modeling methodologies from successful experiments will help produce consistent resulting effects of selected BB stimulations.

3.2.2. Brain Entrainment

Brain entrainment is a reaction to audio or visual stimuli by the brain that causes it to oscillate at the same frequency as the stimuli [13]. However, since BB stimuli are two different frequencies, the brain produces this third illusionary BB tone that the brain

oscillates at. Depending on the modulated frequency, it can have different effects on the brain's network. There are four main groupings of brain frequencies, known as frequency bands; they are: Delta (1.5-4 hz), Theta (4-8 hz), Alpha (8-12 hz), and Beta (12-30 hz) [6,14,15]. EEG brain waves oscillate in cycles per second, commonly known as hertz (Hz). Brain waves have different rates of oscillation that are associated with various cognitive functions [12]. The pattern of brain waves over time is instrumental to exploring the conscious state of the brain and its effects on the body and mind. The slowest, delta waves, are associated with deep sleep [7,10]. Theta waves are associated with deep relaxation, meditative states, day-dreaming, and drowsiness [6,10]. Brain waves in the alpha band are associated with relaxed/passive states of consciousness, attention, alertness, memory, and focus. Alpha waves are typically associated with meditation and yoga. The fastest of the four main frequency bands, beta waves are associated with alert states of consciousness, such as thinking, concentrating, memorizing, decision making, and sensory processing information [6,12,14]. Several studies have been conducted regarding the effects that different BB frequencies have throughout the brain's network; none fully successful in characterizing BB through the auditory pathway i.e., from subcortical responses to functional connectivity (FC) [1].

Through studies of brain entrainment, it is known that BB originates in the brainstem; specifically, that the first nucleus to receive bilateral input is the subcortical medial nucleus of the superior olivary complex [1]. Brain entrainment is commonly characterized using Frequency Following Response (FFR), as it is assumed BB elicits a FFR — indicative of potential behavior change. FFR is a neural response commonly used to study the human auditory system. It is a steady-state evoked potential that can be recorded using an EEG. The exact origin of the FFR in the brain is unknown. However, it is suggested that the FFR may stem from converging input from overlapping

neural generators starting in the brainstem [7,16]. There is evidence that varying portions of the cortical and subcortical areas contribute to the FFR depending on what frequency is used for stimuli [16]. However, there may be a better measure of brain entrainment, as certain research suggests that prolonged exposure to BB causes the brain to oscillate at the same frequency as the stimuli which would make identifying an FFR in the EEG signal near impossible as time progresses. It is unknown how widespread electric cortical changes in the brain are after exposure to BB stimuli, due the high number of inconsistencies in previous literature. Some researchers have reported varying amplitude changes in pre and post-stimulation EEG recordings, while others with very similar experiments failed to find any evidence of change [7].

3.2.3. Binaural Beats Effect Monitoring

The majority of studies previously performed to explore the effects of BB stimulation used resting EEG recordings to compare before and after results. In these recordings, researchers look for an increase or decrease in frequency amplitudes in each frequency band to determine if the BBs had an effect on the brain. Other studies that used EEGs to record effects of BB chose to track EEG changes over time [5], and track EEG changes during BB stimulation [7]. In addition to EEG recording, varying self-reports for stress, anxiety, and concentration levels of participants were used for pre and post-BB stimulation comparison purposes. Some studies even measured patients heart rates, looking for an overall decrease, to see if BBs stimulation could provide stress relief [6].

3.3. Electroencephalogram (EEG)

One of the most common non-invasive methods for measuring brain activity is using Electroencephalography (EEG). EEGs use the principle of differential amplification in

combination with small metal discs attached to the scalp, commonly referred to as electrodes, to record electrical activity on the surface of the brain [14,17]. It is a safe approach that can be used to study the human brain in almost any mental state, as it essentially detects and measures brain waves over the different approximate frequency bands: delta (1.5-3 hz), theta (4-7 hz), alpha (8-12 hz), beta (13-30 hz), and gamma (31-60 hz) [6,14]. In this study, resting-state EEGs will be utilized. Resting-state EEG paradigms measure brain activity that is not associated with specific sensory modalities, and do not require the subject to actively participate in any tasks or use any thought [18]. Their purpose is to detect spontaneous neural activity that can be helpful in determining states of connectivity [19]. Subjects are usually stationary with their eyes open or closed during resting-state EEG and not actively doing anything.

The ability for EEGs to detect and record electrical activity from the surface of the brain is reliant on neurophysiological principles, and more specifically, a specific type of neuron known as pyramidal neurons. There are a copious amount of pyramidal neurons in the cortical layer of the brain; they have apical dendrites that extend out of the top of them, through the cortical tissues towards the surface of the scalp, which allows excitatory and inhibitory postsynaptic potentials (EPSP/IPSP) to reach the scalp's surface as they spread through the apical dendrites. The electrical potentials generated in pyramidal neurons can be modeled by dipoles, whose electric field flows from the negative direction towards the positive. The dipolar currents produced by simultaneous magnetic and voltage field alternations are strong enough to be detected by noninvasive measurement tools, such as an EEG [20]. However, EEGs cannot detect individual neuron activity. Rather, they provide strong recordings of the potential alterations taking place in a small, specific region of the brain [17]. The structure of a pyramidal neuron is represented in Figure 1 [21].

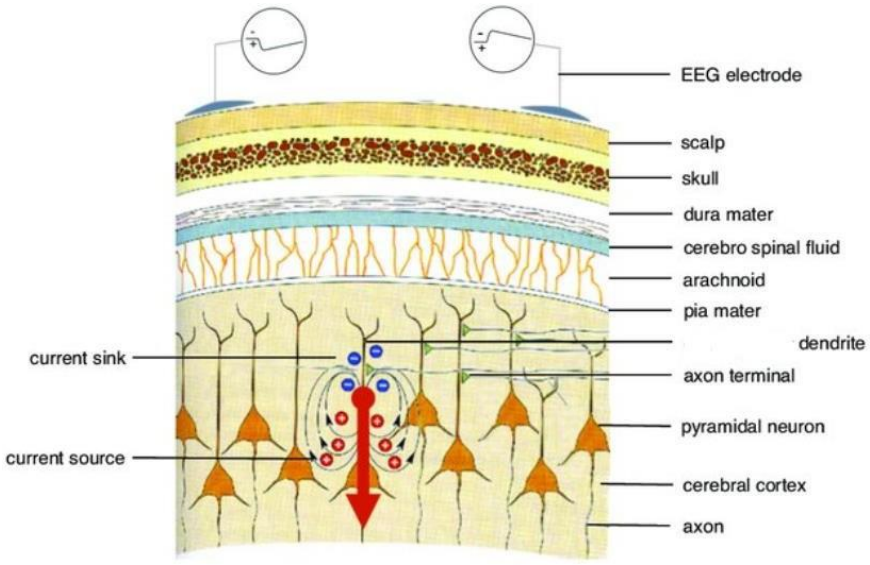

Figure 1: A representation of Pyramidal neurons and their Apical dendrites. As well as the electric potentials, modeled by the dipole.

Many studies that have researched the effects of BBs have chosen to use EEGs as an instrument in their data collection for varying reasons. EEGs allow for a graphical representation of the voltage differences experienced in varying brain locations over time [6]. EEG signals are visually represented as oscillatory waves and typically separated into groups according to their frequency, commonly known as frequency bands. The small, specific regions of the brain where potential alterations occur, mentioned earlier, are referred to as neuronal sights. It is common for different frequency bands to compete for dominance in a neuronal sight, as different frequency bands are associated with different brain states. However, it is possible for multiple rhythms to coexist and interact temporarily, which allows multiple processes to occur in the brain while multiple frequency bands are active at the same time [6]. Using an EEG allows for analysis of different frequency bands at varying time points, which ultimately gives researchers the ability to test new analysis techniques and gain a better understanding of the brain's networks. Previous literature has used resting EEG signals to study short-lasting FC changes in

the brain due to BB stimulation [1,5,7,12]. Some studies were more successful than others at showing an elicited change in FC or brain entrainment, however most studies focused mainly on the physical effects the subjects felt following their BB stimulation sessions. It is suggested that earlier studies of brain entrainment caused by BB stimulation that failed may have neglected to use a proper control condition and standard normalization practices in their EEG analysis [1]. In order to properly normalize EEG data using a control condition, all data must be in the same scale. The human EEG spectrum has a 1/f power scaling which can be used to help disentangle background noise and task-unrelated dynamics in recorded EEG signals [1]. While utilizing an EEG to analyze FC it is important to use appropriate control conditions to ensure their results are not due to surrounding stimulus properties, which are addressed more in the FC description.

3.4. Functional Connectivity

Functional Connectivity (FC) can be described as the mathematical measure between correlated temporal activation patterns in response to neurological events in an anatomically separate region of the brain. FC is a mathematical concept that is dependent on measures of correlation, coherence, covariance, or phase locking to detect and show distinct and distant regions of the brain actively processing information at the same point in time [22], i.e. to show the functional connectivity of the working network and the high-dimensional data sets it yields. Specifically, the theory of FC states that different regions of the brain must elicit a signal with the same phase or power to exchange information [23]. These estimates of correlation can be measured in the time or frequency domain of the corresponding recorded signal [24]. Distant regions of the brain are considered to be functionally connected if there is a mathematical measure of synchronized activity between them during an event [23], given that that detected connection is not caused by an external factor.

Problems quantifying and interpreting neuronal interactions have been seen with non-invasive methods of recording, including EEG. Specifically, a common problem that can arise during functional connectivity analysis (FCA) is artificial coherence or phase-locking caused by volume conduction artifacts. Volume conduction refers to the strong flow of currents in the tissue surrounding active neuronal sights. It is conceptually understood as the spatial spread of electromagnetic fields generated from pyramidal neurons, which can cause one sensor to pick up activity from multiple neuronal sights [25]. If this were to happen, the presence of FC between two regions would be caused by surrounding stimulus activity, which is not true neuronal activity. In the frequency range, the effects of volume conduction artifacts are instantaneous and can be visualized through the display of EEG oscillations from different brain regions [6,25]. For example, if an active neuronal sight is detected by two different sensors at the same time then the phase observed in both sensors' EEG signals at that time point will be the same (or opposite at 180 degrees). With this information, connectivity measures can be used to remove instances in the recorded EEG signal where instantaneous phase values of 0 or 180 degrees occur, since they represent artificial coherence and phase-locking [25]. Another reason quantifying neuronal interactions remains challenging is the overwhelming number of quantification methods for signal interactions. Each quantification method has its advantages and disadvantages which makes it difficult to determine and justify the best method to use in varying circumstances. This limits the interpretation of FCA, as its results may not always be straightforward and can be susceptible to over-interpretation [25].

Functional Connectivity (FC) is an important biomarker used in a broad range of studies aiming to understand the underlying mechanisms of brain disorders, including Alzheimer’s disease, Parkinson’s disease, schizophrenia, and frontotemporal dementia [23,26]. Although stress and anxiety are not classified as brain disorders, the effects of stress and anxiety exhibit similar internal responses in the body that some brain disorders may trigger as well. FC

provides a different approach to analyzing resting EEGs by evaluating the synchronization of different brain regions after the influence of BB stimulation. Depending on the FC measure used to approach this analysis, network-wide alterations may be seen throughout the brain. The FC measure chosen for a given data set should be dependent on its required level of sensitivity and specificity. Strong consideration should be put into choosing an appropriate FC measure, as conclusions regarding the strength of connection between two neural sights will be determined by the results of the approach that was used; it is commonly known that different approaches yield different outcomes, especially since each FC measure comes with its own sensitivity and specificity issues [27].

FC measures are phase-based or power-based. The amount of phase stability between distinct signals over time, also known as inter-site synchronization, can be estimated using phase-based connectivity measures. Many types of analyses that are phase-based use cross correlation, spectral coherence, phase lag index (PLI), and phase locking value (PLV) measures since they have been proved to be effective in accessing information on brain network connectivity [27]. Previous BB literature has been successful in using PLV combined with Hilbert Transform to show cross frequency patterns at long and short ranges elicited by BB stimulation [1]. So far, the use of FC measures to analyze EEG signals over time has allowed researchers to determine certain frequency bands for a BBs modulated frequency are more effective in altering the brains activity in the temporal regions [7], but no definitive FC pattern has been identified as a result of BB stimulation [5].

3.4.1. Cross-correlation and Coherence

One of the first measures used to evaluate phase synchronization between two signals in the time domain was Cross-correlation [28]. Cross-correlation is based on Pearson's correlation coefficient, which measures linear connectivity through linear

analysis of two signal's similarities. Linear analysis of similarities between signals is determined by applying a sliding dot product technique to both signal vectors at each time point throughout the signals [25]. A representation of this technique is summarized in Figure 2 [29].

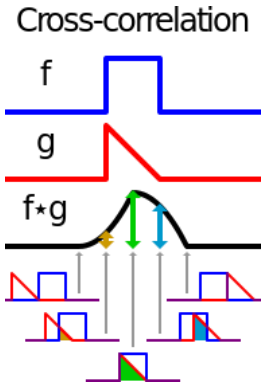

Figure 2: A representation of the sliding dot product technique used for linear analysis between two signals (f and g).

Pearson's correlation coefficient treats each signal as a random variable, which neglects to account for the temporal structure of a signal. In doing so, the resulting estimated connectivity after cross-correlation measures will be the same whether the time series is in a consecutive order or a random order, which is a major downfall to this method of measuring phase-synchronization [25]. However, if the cross-correlation function, computed by applying the sliding dot product technique to two signals, is evaluated as a function of time lag then the resulting data will account for the signal's temporal structure. Although cross-correlation can be used in general applications for detecting similarities and linear connectivity between two signals, most neural signals are bivariate and non-linear which makes it difficult to use this measure for identifying neural connectivity patterns [25].

On the contrary, coherence is basically the frequency domain equivalent of the cross-correlation function. Coherence is commonly referred to as the cross-spectral

density, since it is the spectral analysis of EEG signals used to compare signal power. Coherence is calculated by applying frequency-wise multiplication from a signal in the frequency domain to a complex conjugate of a second signal [25]. The conjugate of the second signal can be found by making its imaginary component negative, which is represented below in Figure 3 where the phase angle component (x+iy) becomes (x-iy).

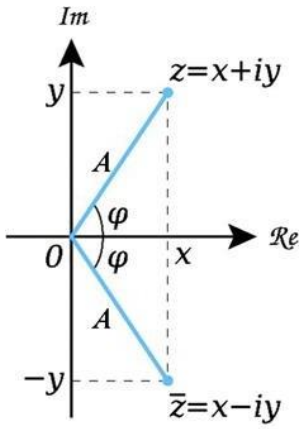

Figure 3: A representation of complex signals in a cartesian plane. The upper vector represents the original signal and the lower vector represents the conjugate. [30]

The result of frequency-wise multiplication is 2D cartesian axes, where the angle associated with the real axis represents the phase difference between two signals and the vector represents the product of the two signals magnitude. Figure 4 below shows a representation of the cross-spectrum between a signal and the conjugate of a second signal [25]. In this example, the vector length can be found by multiplying the amplitudes of the signal and the conjugate, and the phase difference is determined by finding the angle between the two vectors.

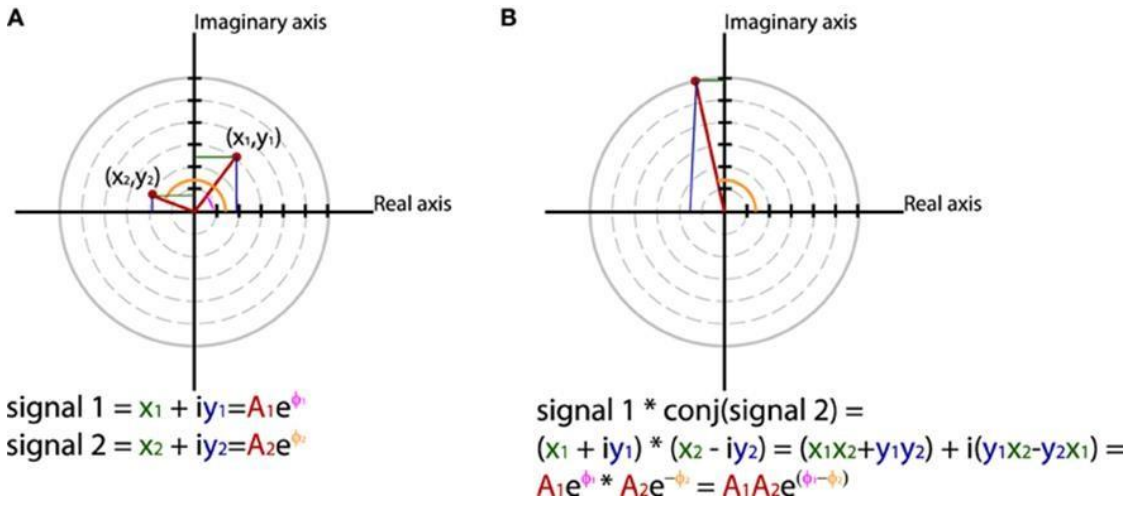

Figure 4: (A) Frequency domain representation of two signals. (B) The phase and amplitude components of two signals. [25].

One benefit of using coherence to measure phase synchronization is that it is possible to obtain a relatively accurate estimate of phase synchronization from a short segment of data [31]. Another benefit is that processing signals in the frequency domain eliminates the temporal structure problem seen with cross-correlation measures. However, coherence measures between two EEG signals are susceptible to their own problems as well. The common reference problem is typical, and can be described as the contamination of multiple electrodes with the same voltage component by a singular reference electrode that is creating signal fluctuation/noise. This results in artificial coherence between the regions contaminated by the reference electrode [32]. There are various computational methods that can be used to address the common reference problem. Assigning multiple references to each EEG electrode has been shown to notably enhance the spatial scale in terms of where connectivity can be considered [33]. Another problem seen with measures of phase synchronization is the volume conduction problem mentioned earlier. Volume conduction is another common artifact seen in EEG recordings, caused by a singular signal source instantaneously projecting macroscopic

potentials towards multiple EEG electrodes. The potential field projected towards the electrodes attached to the scalp can also travel through the tissues in the skull and scalp to reach adjacent electrodes [34]. A representation of this potential field projection and spread is depicted in Figure 5.

Figure 5: A representation of volume conduction artifact. Multiple electrodes may pick up on the same source activity.

Artificial coherence can occur due to volume conduction artifacts and account for recorded patterns of synchronization. Such artifacts can lead to a misinterpretation of data if they are not removed, indicating false accounts of connectivity in the brain [34]. Looking for instantaneous synchronization, where the phase between two signals is zero, is an easy way to identify volume conduction artifacts. In addition to instantaneous synchronization, decreasing synchronization strength in conjunction with increasing distance can be used to find possible phase synchronization related to volume conduction. In the time domain, when multiple electrodes detect the same signal, but from opposite directions of the dipole, the phase difference between the two signals will be equal to 180 degrees/pi [25,34]. There are various methods of analysis that can be used to minimize and prevent volume conduction artifacts, including spatial filters and specific phase-lag based measures. Phase based measures, typically weighted measures, are unaffected by volume conduction activity. These specific phase based measures are commonly used to eliminate volume conduction artifacts [25]. Further

elaboration on the differences between phase-based measures is provided in the following sections.

3.4.2. Phase-based Measures

Two signals must be in their frequency domain representation for phase measures to be computed from them. By applying time-frequency transforms to epochs, a spectral representation of an EEG signal with its amplitude and phase components is obtained. In this spectral representation, $Ae^{i\varphi}$ is a phase format of a Cartesian format a+ib. Fourier transform (FT) of x(t) yields a series of complex-valued numbers X(jw), which can be represented both ways, i.e. where A = |a+ib| and $\varphi = \angle$ (a+ib). The analytic signal can be obtained by applying inverse Fourier transform (IFT) to the positive frequency portion of X(jw). In Figure 2 from the previous section, the amplitude is visually represented by a vector that connects to a non-zero point, and the phase is the angle the vector makes with the x-axis. In a graphical representation of complex signals in a 2D cartesian plane, a non-zero point is represented by (x+iy), where the distance between the real axis and the non-zero point is ‘x’ and the distance between the imaginary axis and non-zero point is ‘y’.

A. Phase-Locking Value (PLV)

Phase-Locking Value (PLV) is the most common phase-based measure. It measures the consistency of the numerical phase difference value between two signals over time or trial. Also known as Inter-Site Clustering (ISPC), PLV is mathematically represented by the following equation, where it is the absolute value of the average phase difference between two signals:

$$PLV = \left| n^{-1} \sum_{t=1}^{n} e^{i(\Phi^j - \Phi^k)_t} \right|$$

The resulting PLV value between two signals at a specific point in time is expressed as a complex unit length vector in a complex plane. Levels of synchrony between two signals can be estimated by calculating PLV at numerous time points over time and clustering them in a complex plane [35], as seen in Figure 6 below [36].

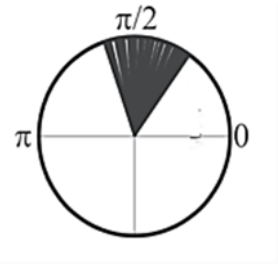

Figure 6: A representation of inter-site phase clustering in a complex plane i.e., PLV [37].

This process is referred to as inter-site phase clustering, and can help predict the possible number of neural interactions collected in the data between the two signals. It is typically one of the first measures obtained after pre-processing. However, if the phase angles between two signals are not consistent across trials it may not be possible for PLV measures to detect real phase synchronization. In this case, it is also possible for PLV measures to detect spurious connectivity due to volume conduction. That is why the following measure is incorporated to disregard indications of phase-locking centered around real values in the complex plane.

B. Phase Lag Index (PLI)

Phase Lag Index (PLI) is similar to PLV in that it estimates the clustering of complex unit length vectors obtained over numerous timepoints between the two signals

[25]. However, PLI is different because it eliminates any potential phase locking centered around the real axis of the unit circle on the complex plane i.e., values of 0, π, and 2π. It does this by measuring the consistency of the sign of the phase difference value (positive or negative) between two signals over time or trial. PLI is represented mathematically by the following equation:

$$PLI = \left| n^{-1} \sum_{t=1}^{n} sgn(Im \left[e^{i(\Phi^j - \Phi^k)_t} \right]) \right|$$

Euler's complex form is used to represent the phase difference over time between two signals in both the PLV and PLI equations. Both equations average the complex components, sum them, and return their absolute value. But, the PLI equation has two additional transformations: Im and sgn. The 'Im' represents an imaginary part of a complex number and it transforms the graph by projecting the cluster of resulting phase difference vectors onto the imaginary axis — typically the vertical axis. The second transformation is 'sgn', which stands for sign. This function converts imaginary components from the imaginary axis into a group of unit vectors with values between -1 and +1. The unit vector values are summed and averaged to obtain the phase lag index.

If the final PLI value is zero (or 2π) then instantaneous coupling, also known as artificial phase-locking, is occurring. The centering of a cluster of vectors about the real axis (0, π, 2π) indicates that two electrodes are detecting the same signal. Due to this volume conduction effect, the instances with average PLI values of zero (or 2π) will be rejected from the final estimated instances of coherence. A visual representation of clustering centered about the real axis can be seen on the left in Figure 7 below [36].

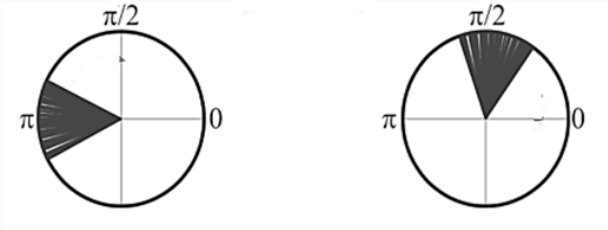

Figure 7: Examples of PLI instances. (Left) Centering of a cluster of vectors about the real axis — artificial phase-locking. (Right) Cluster of vectors displaying strong phase locking index — true phase-locking.

As seen in Figure 7, a phase difference of π is also possible. This occurs when electrodes detect the same signal but from opposite sides of the same dipole. This is still considered false connectivity and is represented by strong negative correlations in the time domain. On the other hand, PLI values of 1 represent a true interaction between two neural networks originating between two signals and indicate some functional correlation between networks. An upside to using PLI is that it rarely results in type II errors i.e., false positive connectivity. PLI provides researchers with information on interregional communication in the brain's network, however this is one pitfall. While PLI eliminates all indications of false connectivity, this includes getting rid of true neuronal interactions that may have occurred simultaneously [37].

C. Weighted Phase Lag Index (wPLI)

The one downside to PLI is it may eliminate true phase locking if the phase difference approaching zero or 2π is very small. This effect is due to PLI's high sensitivity to volume conduction and noise, which is attributed to the discontinuity that occurs in its calculation [38]. PLI is a better estimate of phase leads and lags between two signals because of the small perturbations that occur in the brain that turn leads into lags and vice

versa. In order to account for the possibility of true neuronal interactions simultaneously occurring at the same time false connectivity is detected, a newer analysis method known as weighted phase lag index (wPLI) is applied. Like PLI, wPLI calculates the imaginary components ($\Im\{X\}$) of the cross-spectrum. However, wPLI weights the influence of phase leads and lags by the magnitude of their imaginary component, taking away weight centered about the real axis [38]. The formula to calculate wPLI is given below:

$$\Phi = \frac{|E\{|\Im\{X\}|sgn(\Im\{X\})\}|}{|E\{|\Im\{X\}|\}}$$

The numerator of this formula scales the sign of the angle by the magnitude of the imaginary component ($\Im\{X\}$). This means that when a cluster of vectors is near a real axis, the vector values closer to the imaginary axis will be weighted more, pulling the center value of the cluster closer to the imaginary axis; this will have a greater effect on the estimated connectivity. The denominator helps to normalize the phase leads and lags by eliminating noise and small perturbations using the magnitude of the imaginary components. Figure 8 visually represents the difference between PLI and wPLI graphs used to estimate phase differences.

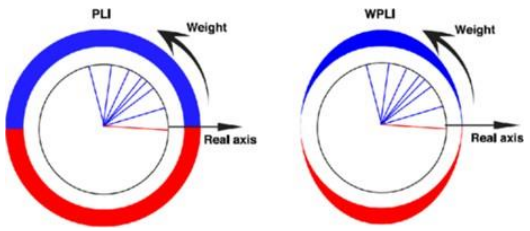

Figure 8: A visual representation comparing Phase Lag Index (PLI) and weighted Phase Lag Index (WPLI) unit circles. Blue lines represent the phase differences between signals. (Left) Phase differences contribute equally to the synchronization value. (Right) Phase differences far from the real axis make a larger contribution to the synchronization value.

Due to the weighting of components that wPLI employs, its calculations are less susceptible to noise now that the sign of the phase is not the controlling factor. Many studies have demonstrated the effectiveness of utilizing wPLI instead of PLI. wPLI is preferred in data analysis because it allows the highest level of sensitivity and specificity while also maintaining as much data as possible [38].

3.5. Graph Theory

In addition to using phase-based measures to quantify levels of synchrony between two signals, techniques that can model the brain's functional connectivity have been developed and employed in different types of research. Graph Theory Analysis (GTA) is a statistical method used to quantify aspects of a given graphical representation of a working network. Over the past decade, GTA of connectivity measures has been widely studied and is now able to offer insights into the functionality and structure of different networks in the brain [39]. Graph theory is used to help topographically illustrate the brain by visually displaying groups of nodes and their pairwise interconnections, a.k.a. edges. Nodes represent distinct neural elements, while edges that connect nodes indicate levels of synchronization between them at varying points in time. A connection matrix, i.e., an adjacency matrix, is used to mathematically summarize nodal and edge connections within a network. Row and column labels of the n x n adjacency matrix denote a specific node, with n being the label/number of the node. Weighted adjacency matrices, constructed from weighted directed graphs, are used to quantify graph measures. By using a weighted adjacency matrix, edges are assigned a weight of zero or a positive non-zero value that is indicative of the edge's connection strength between two nodes. Many measures are quantified between this weighted adjacency matrix and its associated constructed brain network that are eventually used to assess the topology and efficiency of the network [40].

During the construction of the graph, before evaluating the network, the adjacency matrix must be thresholded. By thresholding the weighted adjacency matrices, only the set of edges with the largest weights are retained to construct the final graph, which is represented by binary matrices. There are two common methods of thresholding applied to weighted adjacency matrices: Absolute thresholding and Proportional thresholding. Both methods have different influences on the weaker connections in a graph. In absolute thresholding a threshold value is selected and any connections with a weight less than the threshold value are set to zero, while those with weights greater than the threshold are set to 1. Due to the large impact that weak connections have on overall brain network organization when present, the elimination of weak connections is an advantage to absolute thresholding. However, this method of thresholding is not suitable for comparison of patients to control groups. Research has shown evidence of unstable network measures due to inconsistent density across, i.e., the number of connections, across graphs in studies that used this method to compare patients to control groups [41]. On the other hand, proportional thresholding uses a selected percentage value to eliminate weak connections. The difference in the number of edges between systems that causes the inconsistent density problem is avoided by using the same percentage of the strongest connections from each network to construct its graph, allowing for equal density across network graphs. The downside to this method is that it keeps some of the less reliable connections to maintain a consistent density across graphs, which introduces artifacts to the constructed network [42]. There is no general consensus on which method will help obtain the closest true representation of the brain’s anatomical network, especially in studies that compare independent groups. Many researchers have found that applying different thresholding methods to the same graph data yields different results, which heavily impacts the reproducibility of a study's results [43].

Graph Theory is mainly the utilization of varying measures to quantify network features used in the construction of nodal, global, and topological graphs. The properties of global graphs help quantify neural network's functional segregation, the integration between distant brain networks, centrality, and assortativity. These quantifiable properties are explained in the following sections.

3.5.1. Segregation

Within networks, nodes form localized communities that share a specific function or purpose. Segregation is a measure used to describe the relative strength of the nodes in a localized community and between communities. Figure 9. A is a visual representation of the two most common measures used to quantify levels of segregation in brain networks: clustering coefficient and modularity. The clustering coefficient measure shows local connectedness relative to a singular common node. This is done by picking a node and finding how many of its neighboring nodes are connected to each other [44]. These functionally connected areas in brain networks are referred to as modules [39]. Modularity quantifies the strength of division between modules [45]. This is done by grouping nodes with similar degrees together to create interconnected modules. Brain networks with high modularity will have modules with densely connected nodes inside a module with few connections between nodes in other modules.

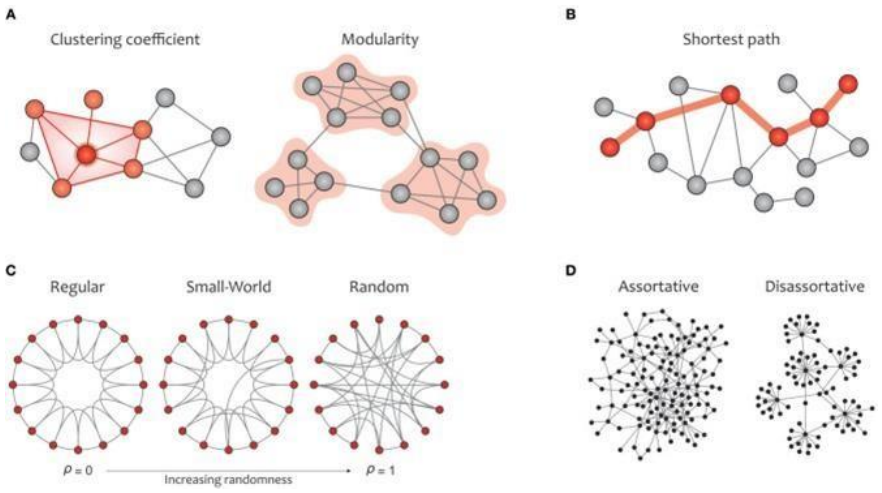

Figure 9: A representation of the 4 global graph measures. (A) The two fundamental properties of segregations — clustering and modularity. (B) The integration in brain networks represented by path length. (C) Centrality (Small world-ness). (D) The level of network resilience against damage represented by assortativity [39].

Global clustering coefficients (GCC) are used to quantify and evaluate levels of segregation in a brain's network. Three types of GCCs are used in this study's statistical analysis: Nodal, Global type 1, and Global type 2. Nodal Clustering Coefficient (NCC) measures local connectedness in a hub or module relative to a singular common node. Global Clustering Coefficient 1 (GCC1) is a cluster detection method based on the number of triangle loops, i.e. the number of connected triples present in a network. Global Clustering Coefficient 2 (GCC2) uses the NCC value to calculate a clustering coefficient base on the average of local clusters. GCC2 is currently the most common way to evaluate global clustering across a network. GCC values range from 0-1. Clustering coefficients with low values indicate a network with short average path lengths and minimal clustering. This type of network is typically referred to as a random network. Clustering coefficients with high values indicate an organized network with long average path lengths and high levels of clustering. This type of network is typically

referred to as a regular network. Both random and regular networks have their drawbacks, and neither are the most efficient network configurations for the brain to use. A Small-World network provides the ideal balance of segregation and integration needed for optimal transfer of information within a network. This occurs when relatively short path lengths are present, which are indicative of a network with a high integration level and high efficiency, and maximum functional segregation is present. GCC's with mid-range values, closer to 0.5, represent a Small-World network.

3.5.2. Integration

Integration is the quantified probability of a node from one region occupying the same module as other nodes from a different region at the same time. It helps determine how efficiently information is traveling between neural networks, which describes the capability of scattered neural networks combining specialized information quickly to perform highly complex tasks. The efficiency or performance speed of network connections is quantified by path length measures, which are represented in Figure 9.B. A path is a sequence of nodes connected to each other by edges. Path length is the average distance between one node and the rest of the nodes in its path. High integration is denoted by short path lengths, so the shorter the path length the higher the integration between neural networks [1].

3.5.3. Centrality

Centrality, or small world-ness, of networks provides information on properties representative of an ideal balance of segregation and integration within a network. Optimal transfer of information within a network occurs when relatively short path lengths, indicative of a high integration level and high efficiency, and maximum functional segregation are present; the extent of their presence is measured using centrality. A visual

representation of centrality is given in Figure 9.C. Centrality is crucial to graph theory, as it indicates the efficiency of information processing on a local and global scale. Assessment of these scales can describe changes in the cognitive state. There are various measures of nodal centrality that indicate the importance of a selected node in a graph, including: degree, closeness, betweenness, and Eigenvector centrality [46]. Each measure is used to describe a different characteristic of a node, and together they are used to identify provincial hubs and connector hubs, shown below in Figure 10. In this figure, a module represents a group of densely clustered nodes. Provincial hubs are used to connect groups of nodes within a module, while connector hubs help connect numerous modules within the same network. Within a module, the node with the greatest number of connections to adjacent nodes is deemed the provincial hub. In surrounding modules, nodes on the perimeter that form connections with nodes in adjacent modules represent the connector hub. The connector hub contributes largely to the global efficiency of a network. Applying different measures of nodal centrality helps identify how heavily individual nodes contribute to local and global connections with a network. This is crucial in the use of nodal centrality to construct graphs from distant, non-neighboring nodal clusters that can connect via the shortest path length available [39].

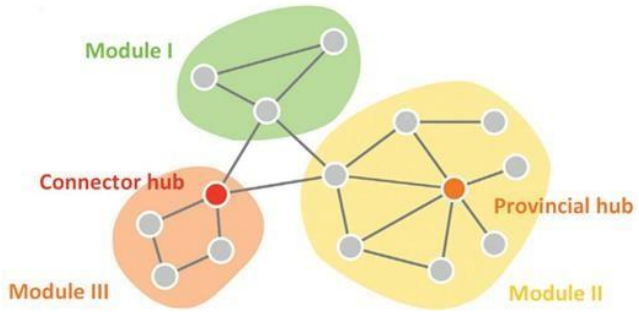

Figure 10: A representation of how modules within a network are connected using Provincial and Connector hubs [47].

Degree centrality is used to assess how important a node is to its local module. The level of importance a node has to its local module is determined by its number of edges, as shown in Figure 11 below. Degree centrality is mathematically defined as the ratio between the total and possible number of connections that could be made with nodes in the same module [46]. It should be noted that degree centrality is the most basic centrality measure, and in order to fully understand the overall degree centrality and influence a node has within a network, an absolute measure of the node's significance within the network on a macro level is needed [48].

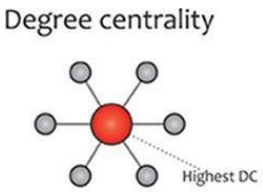

Figure 11: A singular node with six edges. Degree centrality is the measure of a node's number of edges [40].

In global centrality, the two most commonly used measures are closeness and betweenness centrality. Closeness centrality is a measure of proximity between a selected node and the other nodes in its network. It is a quantification of how close one node is to all the others in the same network, which is mathematically represented as a reciprocal of the total distance between a selected node and any nodes it's connected to; the smaller the distance, the more central the node is in a network [46]. This resulting value is an indication of a node's communication speed with other connected nodes. Closeness centrality within a neural network is depicted in Figure 12 below.

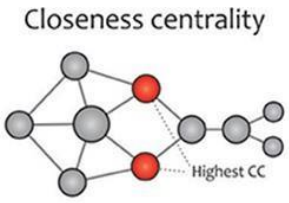

Figure 12: A representation of Closeness centrality; high closeness centrality (CC) indicates easy and fast access to other nodes [36].

On the other hand, Betweenness centrality is a measure based on the shortest path between nodes. It helps indicate which nodes are critical, as they function as connector hubs or bridges between groups of nodes, which allows information to travel through networks more efficiently. Betweenness quantifies how many times a node is used for a bridge or connector hub along the shortest path between two other nodes. A node will have a high betweenness value if it occurs frequently in the shortest path lengths between varying couplings of nodes [46]. Figure 13 shows a visual representation of a node with high betweenness centrality, being used as a bridge.

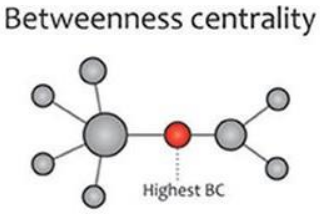

Figure 13: A representation of a node with high Betweenness centrality (BC) being utilized as a bridge between two separate modules [40].

The last measure of nodal centrality is Eigenvector centrality, sometimes referred to as prestige centrality. This measure is used to describe how important a node is based on the other nodes it is connected to and their relative importance to their neural network. Relative values are assigned to nodes in a network based on the assumption that highly connected nodes, i.e., a node with a high degree of connectivity, have a higher contribution to their neural network [48]. A node with a higher value indicates it is connected to a node with high connectivity, and vice versa with nodes that are given a low value. A visual representation of Eigenvector centrality is shown in Figure 14.

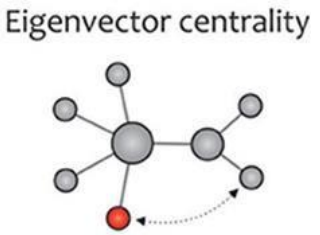

Figure 14: Eigenvector centrality describes the significance of a node by its connection to a particular node within a neural network. This measure does not account for the number of connections between a node and other nodes [40].

The context of a research study is important in determining which measures should be used in quantifying a node's ability to influence other nodes in its network. There is not

one method that is favored over others, so characteristic(s) of interest in the brain network that will be analyzed should be the determining factor for the measures chosen. This study focuses on global features of networks throughout the brain and the overall differences that will be seen pre and post stimulation. Some of the nodal centrality measures discussed may be beyond the scope of this study, and therefore not utilized in future analyses.

3.5.4. Assortativity

Assortativity is a measure that quantifies a network's resilience to intentional or random damage of its main components. In an assortative network, this means that if a node is damaged, or just fails to function properly, another node with a similar function will easily take over for the failed node. In a disassortative network, if a significant node were to fail, it could cause problems or failure throughout an entire module or neural network depending on the significance of the node. Networks are classified as assortative or disassortative based on node's degrees and the level of connection to other nodes with similar functions in the same network, as can be seen in Figure 9.D. As mentioned earlier, Hubs determine the creation of local clusters. The significance of a hub, based on its overall function and contribution to network resilience, is represented by a participation coefficient. A low participation coefficient is indicative of a provincial hub, while a high participation coefficient indicates a connector hub [39]. Global networks are measured using degree assortativity, with resulting values ranging from -1 to 1 [47]. Assortativity is very important, as it is used to quantify the functional ability of a network given the presence of a brain dysfunction. So far, research has used this property to assess network functions in subjects with brain dysfunction caused by physical damage and psychiatric disorders. However, other disorders that would relate more to this study, such as major

depressive disorder (MDD) and anxiety disorders have not been investigated as much using applied graphs [39].

Chapter 4. Materials and Methods

The following sections explain the required steps needed to collect and process EEG data and to test the research hypothesis. Necessary steps include data collection, processing, and analysis, as well as statistical analysis of the resulting data.

4.1. Subject Recruitment

All subjects were healthy college students between the age of 18-35, who passed the exclusion criteria for this study. College students were recruited for this study due to convenience. Subjects were recruited based on the following exclusion criteria: (1) No smoking or heavy alcohol consumption in the 48 hours prior to recording. (2) No recent history of medication from the following subgroups: Benzodiazepines, Non-benzodiazepine sedatives, Opiates, Anticholinergics, and Antipsychotics/Mood-stabilizers; these subgroups of medications are known to slow the brain down and dampen brain functions [49]. (3) No recent history of an acute illness. (4) No hearing impairments. (5) No history of diabetes, endocrine disorders, or mental illness. (6) No braided hair or hairstyles that will prevent the EEG cap from fitting to the subject's head correctly; an EEG cap whose electrodes do not have proper contact with the scalp will experience poor signal quality. Since all necessary recordings took place in a single session, the criteria for the pre- and post-BB listening sessions remained constant.

Prior to being selected for this study and used in its data collection, subjects were asked to fill out the Medical History portion of the BB Questionnaire. If they met the requirements of the study, they were randomly assigned to one of the two frequency groups. In total, 20 subjects were recruited for this study randomly split into two groups, with 10 subjects per group. The

procedure for this study and the informed consent documents that subjects received were approved by the University Medical Center Institutional Review Board (UMCIRB) prior to any data collection from the subjects. A clear explanation of the study and expectations for the subject were explained prior to any data collection, and each subject was informed that they may choose to opt-out of the study at any time if desired.

4.2. Data Collection

Once the subject arrived at the lab on the day of data collection, they were asked to complete the recent 24-48 Hours section of the BB Questionnaire, an informed consent document, and a Perceived Stress Scale (PSS-10). They were also asked to remove any metal jewelry/accessories and wearable electronics from their person before participating. Metal and electronic items can have negative impacts on EEG recordings, as they may contaminate the signal or the accuracy of a signal near electrodes. Prior to being fit with the EEG cap, the subject was given a set of noise-cancelling earbuds and was asked to place them in their ears to provide feedback on the volume level. Approximately 5 seconds of BB were played for the subject to gage if the volume was satisfactory as is, too loud, or too quiet. The earbud volume was set between the 45-55% range of the available maximum volume. A volume level was deemed too loud for an individual if they felt the volume level used during the earbud setup would cause them discomfort, ear pain, or a headache after listening for a prolonged period of time, and a volume level was deemed too quiet if the individual felt they were straining or intently focusing on the sound to try and hear it clearly. Once the earbud volume was at an appropriate level for each subject, the earbuds were placed on the table next to where the subject was sitting for quick and convenient access during the experiment.

In this study, the 8 Hz and 30 Hz BB mp3 files used for listening were created using the audio compilation app, Audacity. For 8 Hz modulation frequency, 493.88 Hz was played in the

Left ear and 502.25 Hz was played in the Right ear. To create the 30 Hz modulation frequency, the same frequency was played in the left ear and 523.25 Hz was played in the right ear. Taking into consideration tones that sound generally pleasant to the human auditory system, original tones were chosen based on their similarity with standard musical notes. The frequency 493.88 Hz is equivalent to the musical note B4, and 523.25 Hz is equivalent to the note C5. There is not a musical note equivalent to 502.25 Hz, but this frequency is best represented by the note B4 plus 29 cents (can be thought of as B4 plus 29/100ths). The choice of these three original tones ensured that the 8 Hz modulation frequency was a minimum of 8 Hz (actual = 8.37 Hz) and the 30 Hz modulation frequency was as close to 30 Hz as possible without exceeding it (actual = 29.37). These choices are consistent with recommendations from prior research studies on the best way to maximize the probability of detecting effects of BB.

Data collection consisted of 2 separate EEG recording sessions; the first was a pre-BB recording session that lasted approximately 5 minutes. During this recording session the subject was asked to sit comfortably in a chair, with their feet flat on the ground and their hands resting in their lap or on their legs. They were asked to close their eyes and remain as still as possible during the recording, to avoid ocular and muscular artifacts. To remove surrounding stimuli from the environment and promote sensory deprivation, the overhead lights in the lab were turned off and white noise was played. The g. SAHARA EEG cap was placed on the subject's head and each dry electrode was checked for good impedance quality before recording was started. In accordance with the 10/20 international system shown in Figure 15, 32 electrodes were placed throughout the frontal, temporal, central, parietal, and occipital regions, with reference and ground electrodes placed behind the ears over the mastoid process region. During the EEG recording sessions, the first 1-2 minutes was used as a waiting period to allow the subject to adjust to their environment and the request to stay as still as possible, as well as to let the EEG cap settle and adjust to its' new environment. Once all 32 EEG channels were relatively calm,

i.e. not picking up clear activity from anything/anyone other than the subject, a 2 minute and 33 second EEG recording was started. The pre-BB recording was a resting EEG recording that was used as each subject's control data. After the pre-BB EEG recording, a 30 second to one minute break was taken to transition to the BB stimulation session. During the short break, the subject was asked to open their eyes, was given a brief moment to move and stretch if needed, and then instructed to place the earbuds in their ears.

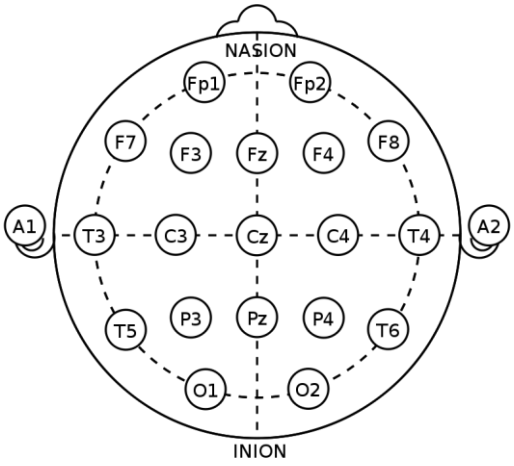

Figure 15: A representation of the 10/20 international system used for EEG electrode placement [49].

During the BB stimulation session, subjects listened to BBs through earbuds for 10 minutes. One of two different modulation frequencies were used: 8 Hz or 30 Hz. Participants were randomly assigned to one of two groups, both of which listened to one specific modulation frequency continuously for 10 minutes. Participants listened to the selected BBs through a pair of noise-cancelling earbuds provided by the lab, which were cleaned and disinfected between subjects. Using the same earbuds and audio setup for each subject allowed the volume of BBs to be controlled and maintained at a consistent level for all participants. The average volume considered safe for a daily exposure of at least eight hours of listening is 70 dB, which is

approximately the volume of a normal conversation between two people [50]. However, for those who prefer a louder volume it is recommended to limit exposure of 100 dB or more to less than 15 minutes at a time to minimize damage to the ears [51]. The earphone volume used in this study was kept between 55 and 80 dB, which is approximately 45-55% of maximum volume on standard earphones [50,51]. Prior to listening to the BB stimuli, the subject was informed that they would be tapped lightly on the shoulder once every 2 minutes to ensure they were awake, and to give a thumbs up when they felt the shoulder tap to verify their state of consciousness. The purpose of the shoulder taps was explained to the subject prior to the BBs listening session. They were also informed that since the EEG would not be recording data during the BB listening session that they did not need to stay completely still for the 10 minutes of listening. During the BB stimulation session, subjects were allowed to adjust their bodies as needed to stay comfortable. Once the subject was ready, they closed their eyes and began listening to BBs. After the BB stimulation session, another 30 second to one minute break was taken to transition to the post-BB EEG recording. During this short break, the subject was asked to open their eyes and place the earbuds back on the table before the post-BB EEG recording was started.

The second EEG recording session was a post-BB session that followed the same process and guidelines as the pre-BB session. Since the EEG cap remained on the subject's head during the BB stimulation session, the impedance of the electrodes was double-checked again prior to starting the second EEG recording session to ensure a good quality connection, as some electrodes could become misplaced between EEG recording sessions. After the 5-minute post-BB EEG recording session, the data collected from the pre and post-BB EEG recording sessions was transferred to BCI2000. BCI2000 is software that was used in conjunction with MATLAB to store and process recorded EEG data for this study. Subjects also

completed a second PSS-10 form after the post-BB EEG recording, to assess their post-BB self-evaluated stress levels.

The flow of the data collection process is summarized in Figure 16.

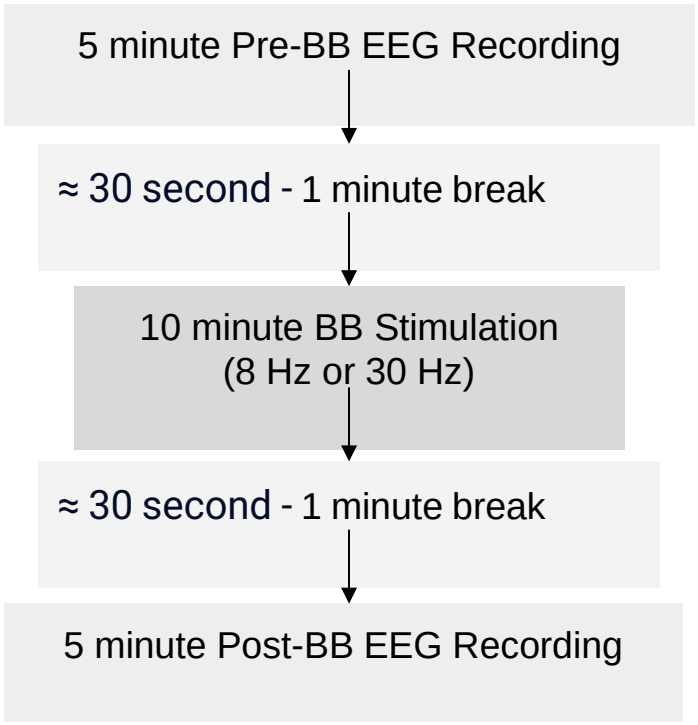

Figure 16: A representation of the process followed for data collection

4.3. Pre-processing

Raw data was processed using an EEGLAB toolbox built-in to MATLAB. Unwanted noise, artifacts, and Direct Current (DC) offsets were removed using a bandpass filter with cut-off frequencies of 4 hz and 30 hz and spatial filters. As mentioned earlier, spatial filters are an analysis method that can be used to minimize and prevent artifacts. Spatial filters, specifically those that employ independent component analysis (ICA) methods, are used for data analysis. ICA is a machine learning technique that produces subcomponents that are statistically

independent and spatially localized [52]. ICA methods help with identification of mixed signals, and aid in their separation into independent subcomponents [53]. Figure 17 shows a representation of ICA being used to unmix signals from an EEG recording and separate their frequency components. The separation of components helps identify the source of each signal [54].

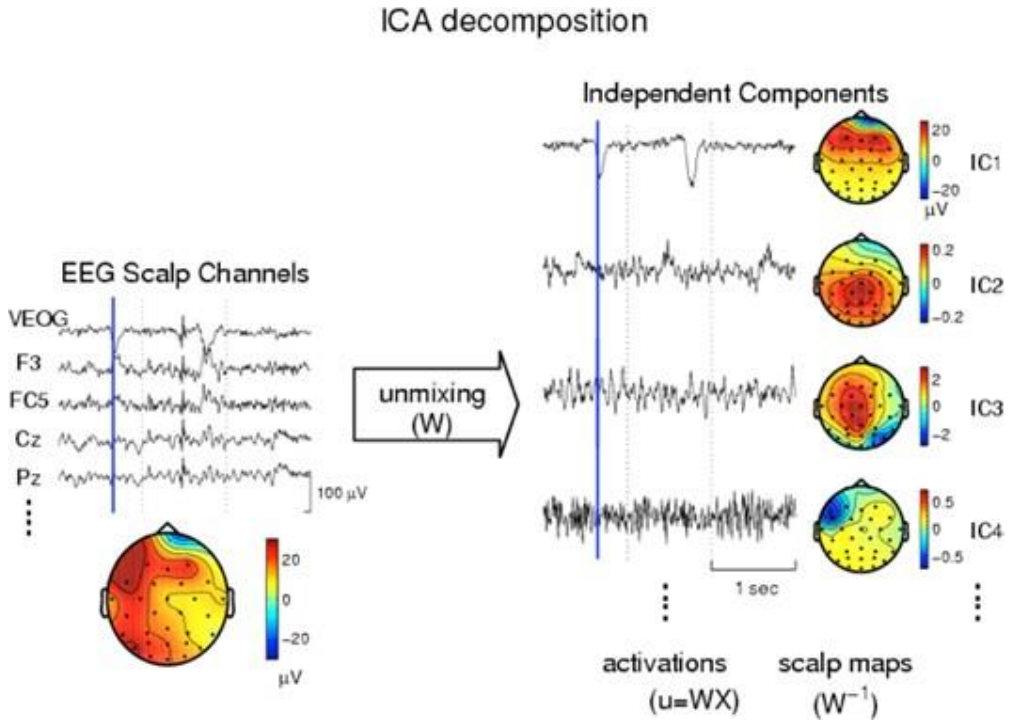

Figure 17: A representation of EEG signal recording separation using ICA techniques. (Left) The topographical map shows a mixed signal recorded from an EEG. (Right) The topographical maps show the results after applying ICA technique. [54]

Then, each 2 minute and 33 second EEG recording was segmented into 1,000 epochs, roughly 10 ms each. Given that each participant had a 2 minute and 33 second EEG recording session for both pre and post BB stimulation, each participant had a total of 2,000 pieces of data for both EEG recording sessions; 1,000 for pre-BB stimulation EEG and 1,000 for post-BB

stimulation EEG. These epochs were used to assess and compare the changes in resting EEG frequency for each subject before and after the BB stimulus was applied. By extracting epochs, longer EEG recordings can be analyzed using a reduced number of data pieces and prevents any loss of data that would result from averaging a signal over time. As a reminder, it is hypothesized that there will be a small but noticeable difference between pre and post stimuli EEG recordings, and an obvious difference between the alpha group and beta group.

4.4. Functional Connectivity Analysis

The weighted phase lag index (wPLI) is computed using a MATLAB toolbox created by East Carolina University's BEACON Lab (Biomedical Engineering Analysis and Cognitive Neuroscience). In this toolbox, Morlet wavelet transform is used to break down the obtained averaged epochs into their frequency components. After an epoch is sorted into its respective frequency groupings time-frequency analysis is applied. By combining a complex Morlet wavelet with a pure time series signal a complex (analytic) signal is created. This resulting analytic signal can then be used to extract phase information at any given time point.

To allow for phase differences to be extracted between signals at each desired time point in each frequency band, time-frequency cross-spectrum information between channel pairs is needed. To achieve this, time-frequency transformation is used to calculate the cross-spectral data for each channel pairing by multiplying each channel with the conjugate of its paired channel. This creates a 3D matrix now with information on the time-frequency cross-spectrum information of a channel pairing and its frequency, in the data structure: channel x channel x frequency. In order to compare phase synchronization levels over time before and after the BB stimulation, FC must be computed over the time dimension. Then, this data can be sorted into four distinct frequency bands: delta (1.5-3Hz), theta (4-7Hz), alpha (8-12Hz), and

beta (13-30Hz). Data from each frequency band will be averaged and consolidated into a 2D matrix of the following structure: channel x channel.

For each frequency band wPLI will be calculated over 1,000 total epochs. A Monte Carlo simulation will be performed at each epoch based on 75% of the epochs randomly selected out of the 1,000 total epochs. These resulting epochs will be averaged and used in the wPLI calculation. wPLI will be calculated for each epoch in the corresponding 2D matrix of channel x channel. Now, the output will be the wPLI value from every channel pairing obtained over time for every individual epoch. These wPLI values will be further averaged over all the epochs, resulting in a 2D matrix with the data structure: channel x channel. This resulting 2D matrix is an output of the average wPLI from all channel pairings in a 32x32 matrix over time. A visual representation of the changes in the data structure for each step of FCA is provided in Figure 18. In total, there should be 1 pre-BB 8hz 32x32 matrix and 1 post-BB 8hz 32x32 matrix, or 1 pre-BB 30hz 32x32 matrix and 1 post-BB 30hz 32x32 matrix in each frequency band for each subject.

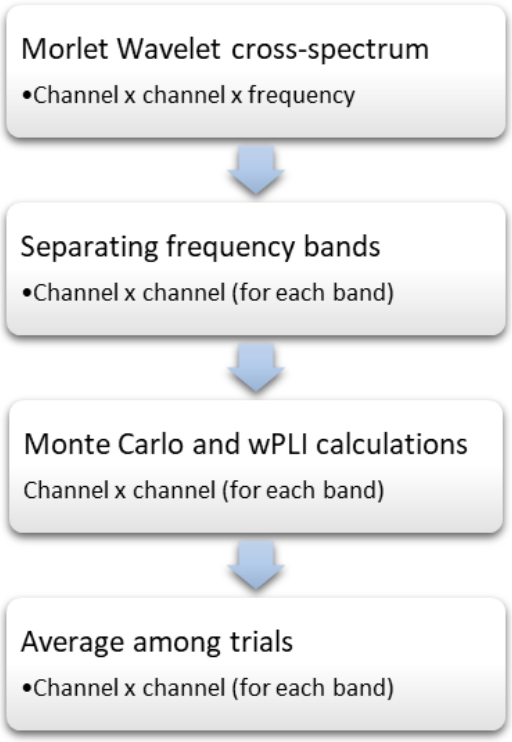

Figure 18: A representation of the data structure and matrix size used during FC analysis.

4.5. Graph Theory Analysis

Adjacency matrices (n x n) will be constructed using wPLI results ranging from 0 to 1, as those values will be the weights of the edges obtained over the period for the averaged resting EEG. In order to eliminate unreliable connections and allow for edge density of neural networks pre and post-BB to be compared, an absolute threshold of 50% will have to be applied to construct adjacency matrices. Adjacency matrices can be undirected for this analysis since causality will not be assumed in the FCA. In terms of efficiency of information exchange, different aspects of the brain connectivity can be characterized in the pre and post-BB brain networks. This can be done by quantifying certain measures. The first measure that must be calculated is the global efficiency, and is done by quantifying the inverse of the characteristic path length using the following formula:

$$L = \frac{1}{n}\sum_i \frac{\sum_{j\neq i} d_{ij}}{(n-1)}$$

In this formula, the total number of nodes in the graph is denoted by ‘n’, ‘i’ and ‘j’ represent the matrix row and column respectively, and the shortest distance between nodes is denoted by ‘d_{ij}‘. In the numerator, the shortest path length of each node to all the other nodes is summed (i.e. the Weiner index). This value is then divided by n x (n-1), which is the total number of nodes multiplied by the distance to all other nodes. This global efficiency measure provides information on neural network integration, which describes how efficient communication is across a global network. A short average path length is ideal, as it indicates high global efficiency and many integrated modules throughout a network.

The function of a module is described using network segregation. To evaluate network segregation, another measure must be calculated; the clustering coefficient, which can be calculated using the following formula:

$$C_i = \frac{\sum_j \sum_k w_{ij}\, w_{jk}\, w_{ki}}{(\sum_j w_{ij})^2 - \sum_j w_{ij}^2}$$

In this average local clustering coefficient formula, ‘j’ and ‘k’ denote immediate neighboring nodes of ‘i’ in the graph exclusively, while notations of ‘w’ are weights corresponding to their respective subscript label. An alternative way to describe this formula is the sum of clustering coefficients will be calculated for each individual node, which will then be averaged together and divided by the total number of nodes present in the network. A resulting value that is low indicates a network with minimal clustering and short average path lengths, which is ultimately considered a random network. Alternatively, a high value indicates an organized network with a high amount of clustering and relatively long average path lengths.

Another measure used to evaluate network segregation is modularity. Modularity is a type of statistic that quantifies the number of subdivisions a network can have by identifying groups of graph nodes that are highly connected to each other and have a low connection to other surrounding nodes. The simplest formula to measure a network's ability to divide into two subgroups is as follows:

$$Q = \frac{1}{2m}\sum_{ij}\left[A_{ij} - \frac{k_i k_j}{2m}\right]\delta(c_i, c_j)$$

In this equation, K_i and K_j represent the degrees of their nodes and '2m' represents the total possible number of edges in the network. The ratio of nodal degrees (K_iK_j) to the total number of edges (2m) results in the probability of an edge being the connection between nodes 'i' and 'j' in a randomized network. The mathematical definition of modularity is represented by this subtraction term, as it is essentially the difference between the number of edges in a graph being used to connect nodes and the number of possible connections that could form in a random network. The ratio of nodal degrees to the total possible number of edges is subtracted from the actual number of edges between nodes 'i' and 'j', denoted by A_{ij} — an adjacency matrix. The 'c' variables in the above equation denote the class of nodes 'i' and 'j', while the Kronecker delta term in front of them returns a value of zero or one for each class. When the classes are equal, a value of one is returned. Otherwise, a value of zero is returned which will inherently cause the subtraction term for all nodes not in the same class to be zero. When these values are calculated over an entire network, the resulting value is the modularity (Q) of the network [55]. Values of 'Q' range from -1 to 1, where a negative value is indicative of fewer edges between nodes, compared to a random network, and a positive value is indicative of more edges between nodes when compared to a random network community. If the modularity is zero exactly, then the network is random.

The centrality of a node can be used to find the balance between two measures. There are multiple measures used to calculate centrality, including degree centrality — the simplest measure. Degree centrality measures the degree of a normalized node. The degree of a node is represented mathematically by the values in the row of a corresponding node in an adjacency matrix, or by the sum of values in a corresponding node's column in the same matrix. The values in these rows and columns represent the number of edges connected to a node. The formula for degree centrality calculation is as follows, with d(i) representing the degree of a node:

$$C_d(i) = \frac{d(i)}{n-1}$$

A node is normalized by using the denominator in the above equation, which ensures the resulting value is between zero and one. If the resulting value is close to one, then the degree centrality of that node will be high, and vice versa. As previously stated, the summed values from a specific node's matrix row and column are the same, implied from the symmetric adjacency matrix used from an undirected simple graph. However, it is important to know that in the above equation, when the degree of a node is in the numerator, node dependence on adjacent, directly connected nodes is automatically implied [56].

To describe the robustness and efficiency of a network a measure of assortativity can be calculated. Assortativity uses the degree of nodes to weight them and uses node's weights to associate them with similarly weighted nodes i.e., nodes with a high degree usually associate with other nodes of a high degree in a network, and vice versa [57]. However, it is possible for dissimilar nodes to associate with each other, which is called disassortativity. Disassortativity in a network can negatively impact its robustness. The index assortativity is measured on uses

Pearson's correlation coefficient, represented in the following formula, to return a value between -1 and 1 [57]:

$$r = \frac{\sum_{i=1}^{n}(X_i-\bar{X})(Y_i-\bar{Y})}{\sqrt{\sum_{i=1}^{n}(X_i-\bar{X})^2}\sqrt{\sum_{i=1}^{n}(Y_i-\bar{Y})^2}}$$

If the correlation coefficient is positive then a network has assortativity, and if the correlation coefficient is negative then a network has disassortativity. Any values close to zero describe neutral networks [57].

In this study, GTA measures will be quantified for each matrix 1,000 times. Indices for each matrix will be averaged over the total 1,000 simulations to obtain a mean value for each network index, at every time point in all four frequency bands.

4.6. Statistical Analysis

Differences between pre- and post-BB stimulation were analyzed using a paired t-test to evaluate their means in the applied global graphs created from pre- and post-BB matrices. Using a matched-paired t-test for both the pre- and post-BB groups was appropriate because each individual subject within a group was subjected to pre- and post-EEG recordings, to be compared for a difference after. This means the subject's BB EEG groupings are considered dependent. For the paired t-tests, the mean of a graph index is calculated for each subject over time and used to find the differences for each type of stimuli. This helps with testing if the mean difference between the pairs is nonzero. A difference of nonzero would indicate that BB stimuli played for subjects between EEG records significantly affected the brain network and had lasting effects long enough to be recorded during the 6 minutes following the end of BB stimulation. There are three important assumptions to meet before running a paired t-test: 1) the

groups used must be dependent, or 'paired', since the analysis is used to test an effect pre- and post-intervention on the same group of subjects. 2) The observations from individual subjects are independent, so one subject's experience does not affect the experience of any other subject in the same group. 3) Approximate normal distribution between the differences in values for both groups or a large sample size (>25) [58]. A representation of the data structure used during statistical analysis is provided in Figure 19.

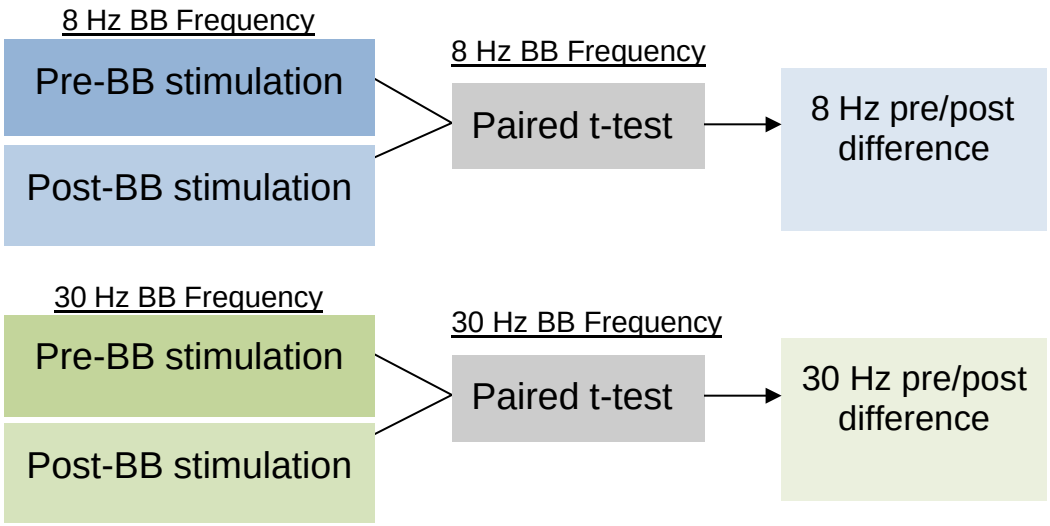

Figure 19: A representation of the data structure used for statistical analysis.

Chapter 5. Results

5.1. PSS-10 Results

The Perceived Stress Scale (PSS-10) used in this study is a stress assessment instrument that has been in use since 1983. It is used to gage, or quantify, how individual's feelings and their perceived stress are affected by different situations [59]. Many medical professionals use the PSS-10 form in their regular practices, as it has been proven to be a reliable, valid, and accurate tool for stress assessment [60]. PSS-10 results can range from 0-40and are defined by 3 levels of perceived stress: Low stress (0-13), Moderate stress (14-26), andHigh stress (27-40) [59]. The results from this study's PSS-10 forms differ between frequency groups. Individual score results for each subject are displayed in Figure 20 and Figure 21 below.The 8 Hz frequency groups perceived stress score pre-BB ranged from 1-20, and post-BB ranged from 0-21. For the 30 Hz group, their perceived stress score pre-BB ranged from 11-30, and post-BB ranged from 9-28.

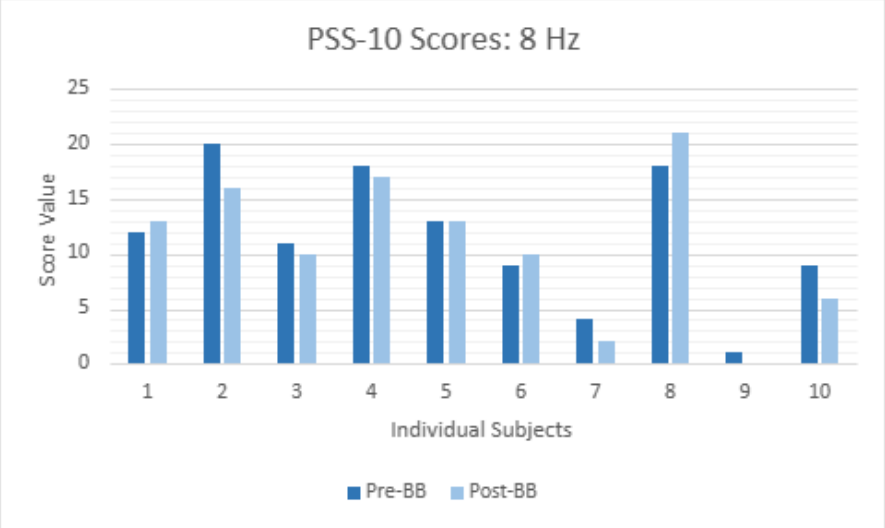

Figure 20: PSS-10 numerical score results pre- and post-BB for the 8 Hz frequency group.

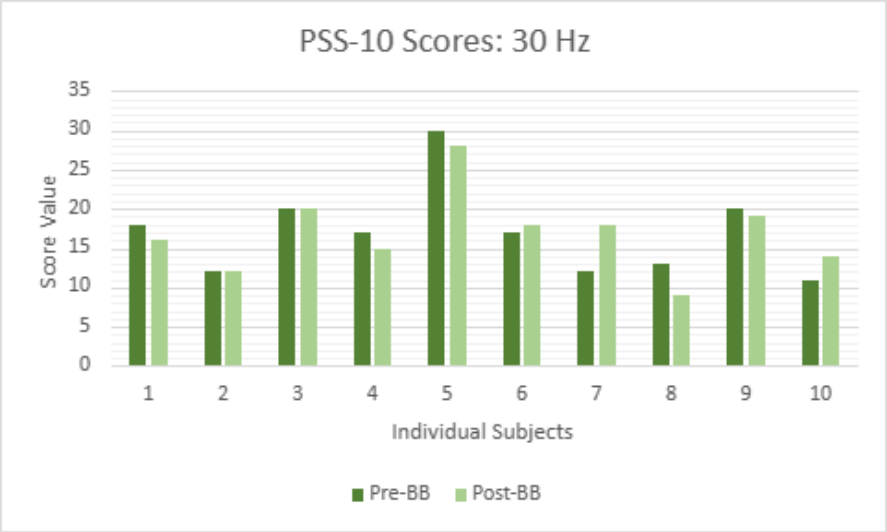

Figure 21: PSS-10 numerical score results pre- and post-BB for the 30 Hz frequency group.

The numerical change to each individual's PSS-10 score varies in direction and quantity, as can be seen in the above figures. In general, after listening to BB for 10 minutes the majority of subjects perceived stress scores decreased. Some subject's scores did not change, and other's scores increased. These results are summarized in Table 1 below.

Table 1: Numerical changes in PSS-10 scores after a 10-minute Binaural Beats listening session.

Numerical Change in PSS-10 Score	**Frequency Group**	
	8 Hz	**30 Hz**
Decrease	6	5
No Change	1	2
Increase	3	3

5.2. Thresholding

For this study, a value of 0.5 was used to threshold the weighted adjacency matrices that were used to assess changes in neural network synchronization levels pre- and post-BB intervention.

Varying groups of data samples were used to construct the absolute thresholded matrices included in this discussion. All matrices display results averaged over the frequency bands Alpha (Al), Beta (Be), Mid-Beta (MB), and High-Beta (HB), and over time. Each figure also includes a color bar to indicate the proportional matrix weight.

8 Hz Frequency Group: For the 8 Hz frequency band group, all 10 subject's data was averaged to construct pre- and post-BB adjacency matrices. Distinct differences in global synchronization patterns and connection weights were observed in the adjacency matrices and associated topological graphs due to the complete elimination of weak connections. Figure 22 illustrates the absolute threshold matrices for all subjects in the 8 Hz group pre- and post-BB intervention. Higher synchronization levels are found in the Pre-BB matrix when compared to the post-BB matrix. Higher levels of synchronization for the group averaged matrices are denoted by yellow- and orange-colored pixels, which range from 0.04-0.05, approximately. Individual subjects with connectivity patterns similar to the group average, pre- and post-BB, reported connection strength values as high as 0.8 in the Al and HB frequency bands.

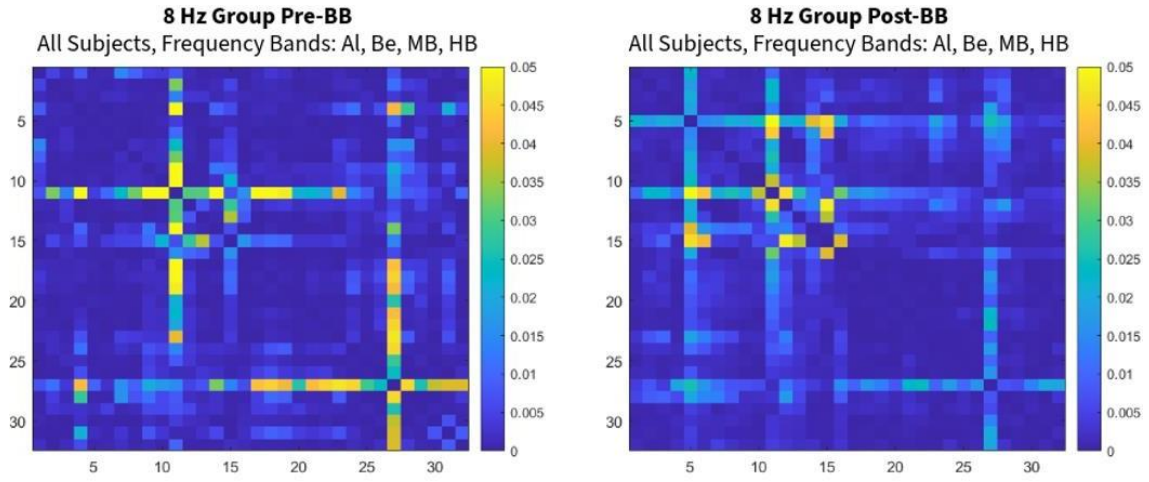

Figure 22: Thresholded Adjacency Matrices for 8 Hz BB Frequency Group; all 10 subjects averaged over Al, Be, MB, and HB frequency bands.

The matrices presented in Figure 22 were converted in MATLAB to topographic maps of nodes and edges, representative of the brain's networks. Figure 23 below illustrates this topology for all subjects in the 8 Hz group over the Al, Be, MB, and HB frequency bands averaged together. The maximum edge weights for the group average pre-BB are larger (0.07) than the post-BB (0.05) edge weights. Graph density does not significantly change, as very few, if any, edges were eliminated from post-BB networks after applying an absolute threshold of 0.5.

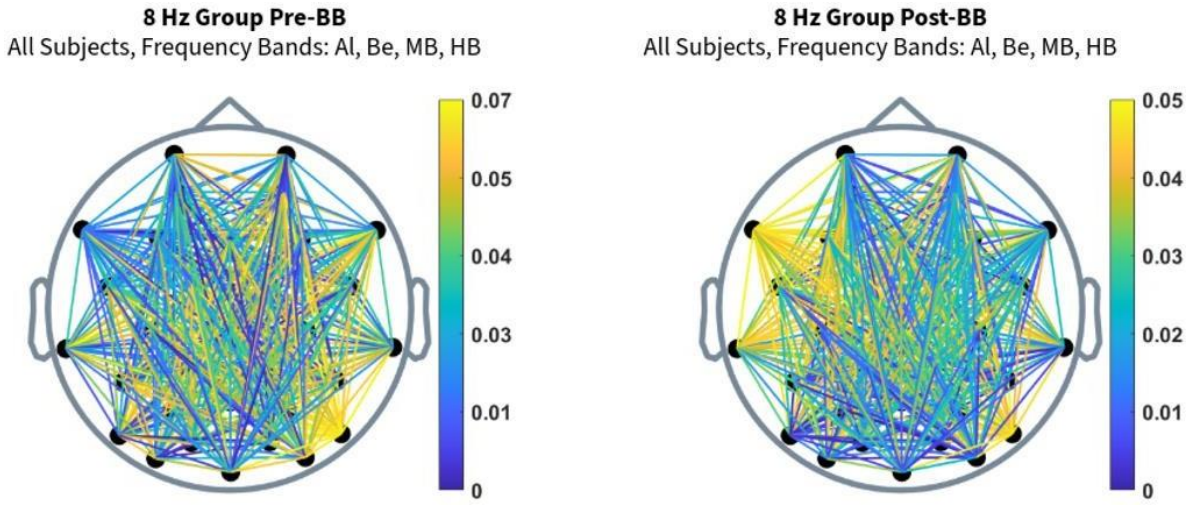

Figure 23: Topographic representation of thresholded measures for 8 Hz BB Frequency Group; all 10 subjects averaged over Al, Be, MB, and HB frequency bands.

As previously mentioned, select subjects demonstrated connectivity patterns similar to the group average, pre- and post-BB, but with much higher connection strength values. Subject 10's adjacency matrices were very similar in connection patterns and weight distribution among pixels when compared to the group average. Figure 24 below illustrates the absolute threshold matrices for Subject 10 in the 8 Hz group pre- and post-BB intervention. Many of the same distinct differences in global synchronization patterns and connection weights are observed in this subject's adjacency matrices and associated topological graphs as in the group averages.

Higher synchronization levels are also found in the Pre-BB matrix when compared to the post-BB matrix. However, higher levels of synchronization for Subject 10's matrices range from approximately 0.6-0.8, which is significantly higher than the levels of synchronization seen for the group average.

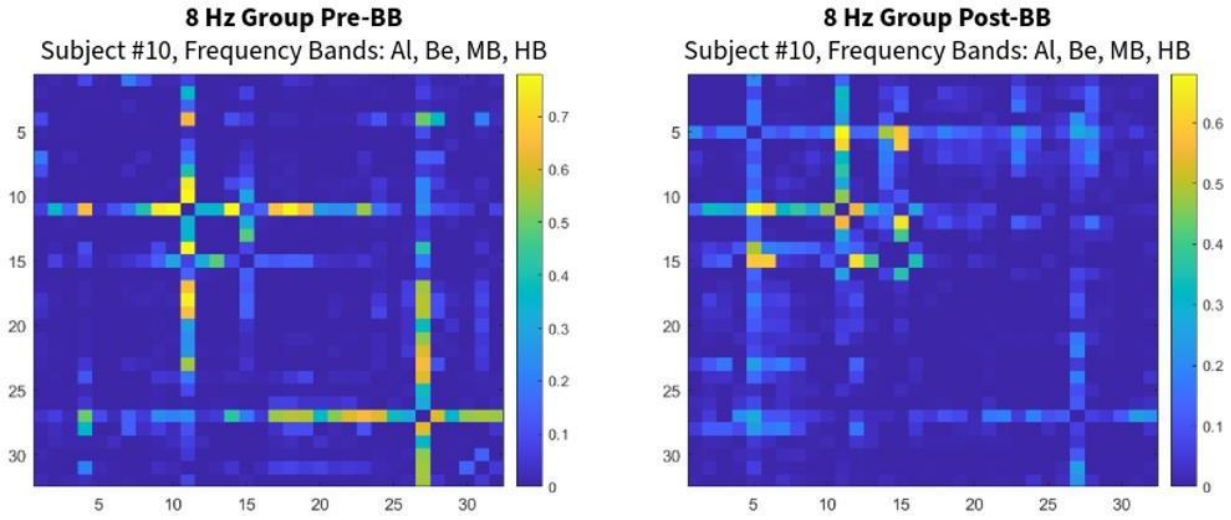

Figure 24: Thresholded Adjacency Matrices for 8 Hz BB Frequency Group Subject #10, averaged over Al, Be, MB, and HB frequency bands.

Subject 10's matrices presented in Figure 24 were converted into topographic maps of nodes and edges, representative of the brain's networks. Figure 25 below illustrates this topology for Subject 10 the same way as the group average. The maximum edge weights for Subject 10 pre-BB are larger (< 0.8) than the post-BB (< 0.7) edge weights. Similar to the group average, graph density does not significantly change, as very few, if any, edges were eliminated from post-BB networks.

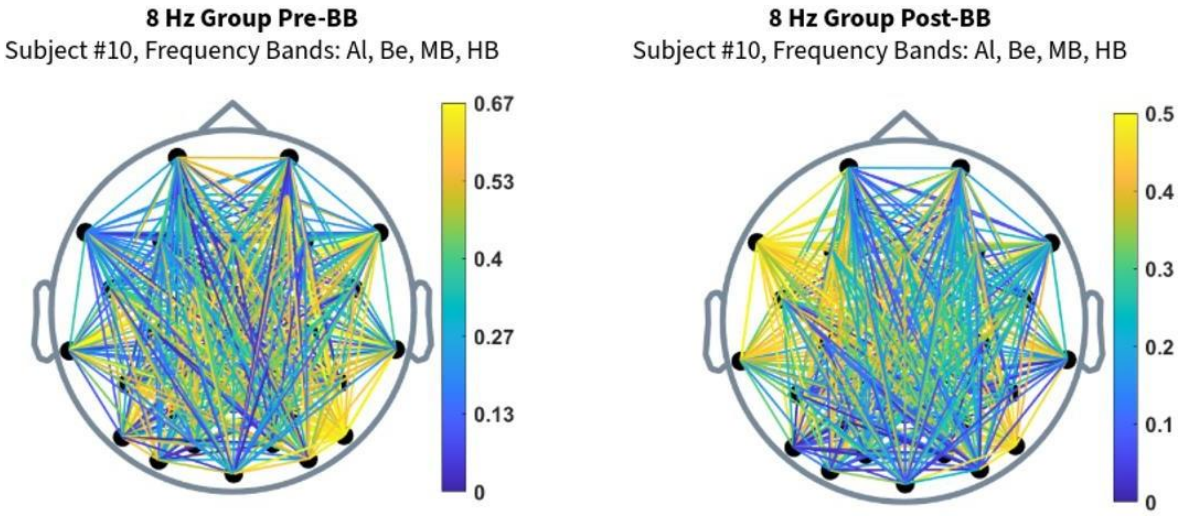

Figure 25: Topographic representation of thresholded measures for for 8 Hz BB Frequency Group Subject #10, averaged over Al, Be, MB, and HB frequency bands.

It should be noted that even though the graph density for the group average and the majority of individual subjects did not significantly increase or decrease as a result of BB intervention, the number of high edge connections present in certain regions of the brain were significantly altered after listening to 8 Hz BB for 10 minutes.

30 Hz Frequency Group: For the 30 Hz frequency band group, all 10 subject's data was averaged to construct pre- and post-BB adjacency matrices. Subtle changes in global synchronization patterns and connection weights were observed in the adjacency matrices and associated topographic representations. Figure 26 illustrates the absolute threshold matrices for all subjects in the 30 Hz group pre- and post-BB intervention. Slightly higher synchronization levels are found in the Post-BB matrix when compared to the post-BB matrix. Higher levels of synchronization for the group averaged matrices are denoted by yellow- and orange-colored pixels, which range from 0.016-0.02, approximately. Individual subject's connectivity patterns on

average increased in synchronization levels post-BB intervention. Some subjects reported connection strength values as high as 0.9 in the Be, MB, and HB frequency bands.

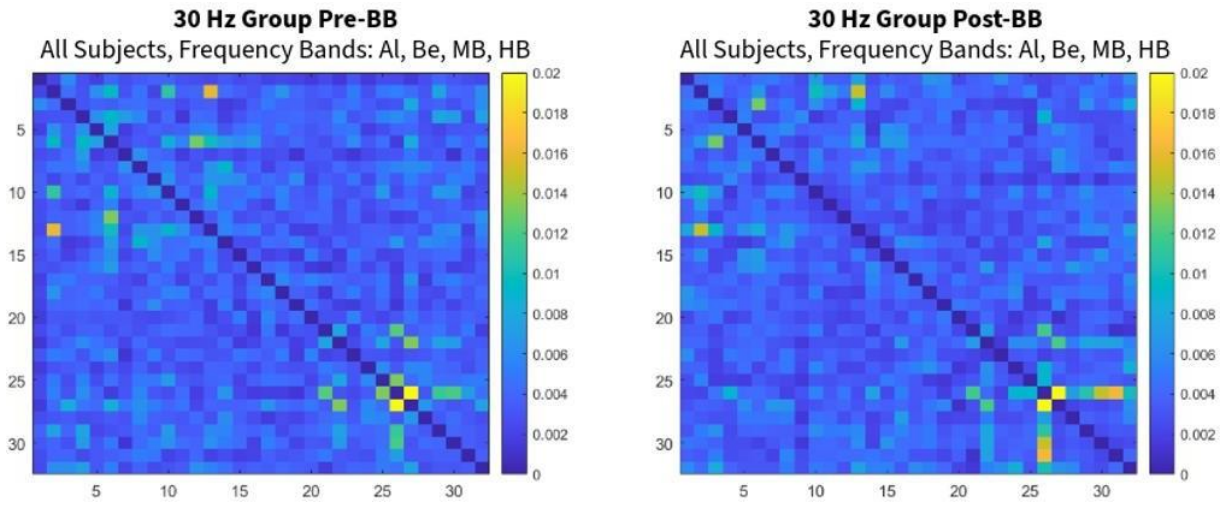

Figure 26: Thresholded Adjacency Matrices for 30 Hz BB Frequency Group; all 10 subjects averaged over Al, Be, MB, and HB frequency bands.

The matrices in Figure 26 were used to create topographic maps of nodes and edges. Figure 27 below illustrates this topology for all subjects in the 30 Hz group over the Al, Be, MB, and HB frequency bands averaged together. The maximum edge weights for the group average are 0.02 pre- and post-BB, and there is not a significant change in graph density for the group average after applying an absolute threshold of 0.5. In a similar manner as those who listened to 8 Hz BB, the 30 Hz group average shows obvious changes in where high edge connections are present in varying regions of the brain.

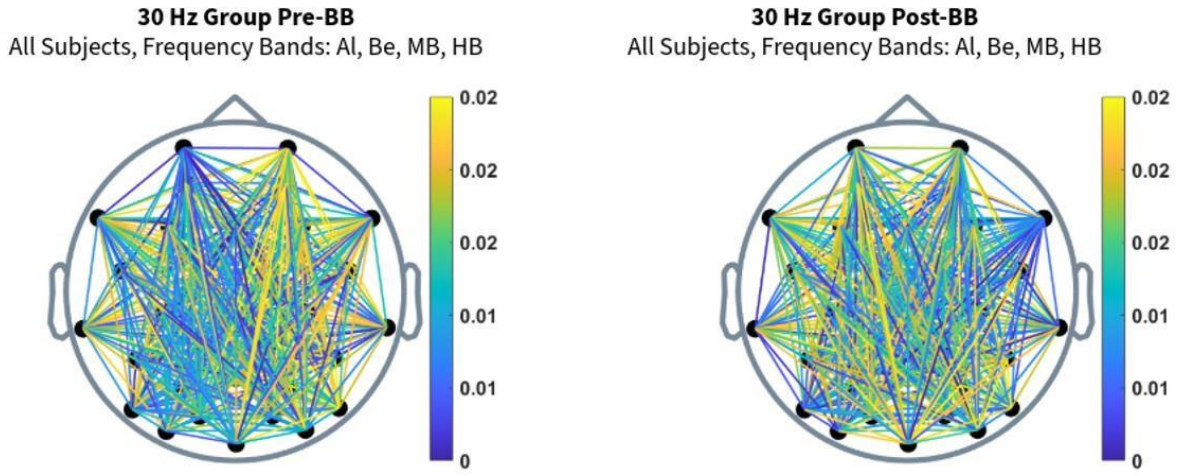

Figure 27: Topographic representation of thresholded measures for 30 Hz BB Frequency Group; all 10 subjects averaged over Al, Be, MB, and HB frequency bands.

As mentioned, most subjects had much higher connection strength values in pre- and post-BB data when evaluated individually. Between the 10 subjects in this frequency group, two main types of alterations were seen in subjects' neural networks pre- and post-BB. The first type of alteration is a significant change in high connection edge patterns, which is best demonstrated by Subject 6's thresholded adjacency matrices and topological graphs averaged over the Al, Be, MB, and HB frequency bands. Subject 6's post-BB adjacency matrix shows distinct changes in connection patterns, weight distribution among pixels, and graph density when compared to the pre-BB adjacency matrix. Figure 28 below illustrates the absolute threshold matrices for Subject 6 in the 30 Hz group pre- and post-BB intervention. Compared to the graphs for the 30 Hz group averages, higher synchronization patterns and connection weights are observed in this subject's adjacency matrices and associated topological graphs. Higher synchronization levels are still found in the post-BB matrix when compared to the pre-BB matrix. However, high levels of synchronization for Subject 6's matrices range from approximately 0.7-0.9, which is significantly higher than the levels of synchronization seen for the group average (0.02).

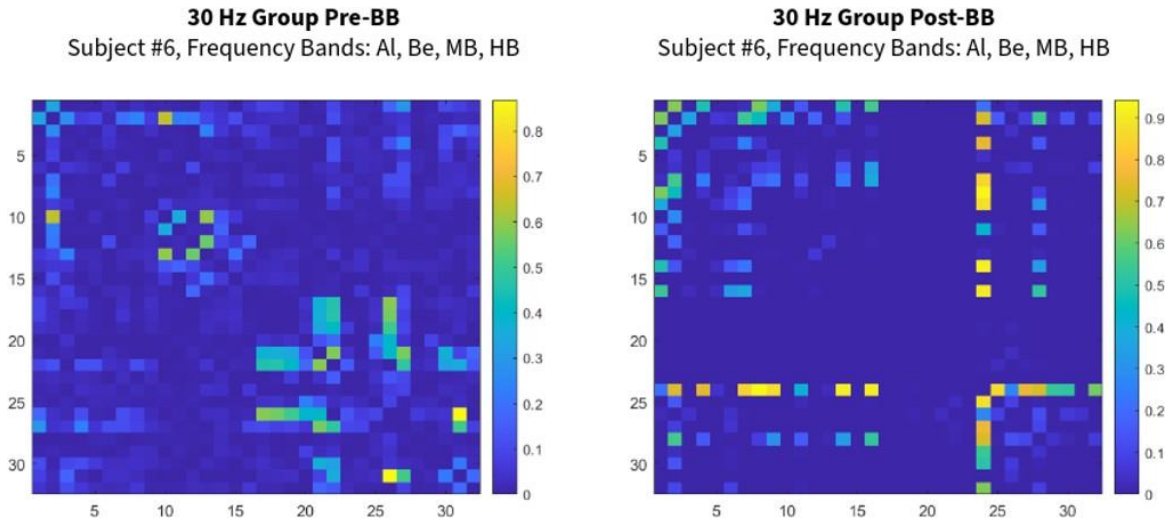

Figure 28: Thresholded Adjacency Matrices for 30 Hz BB Frequency Group Subject #6, averaged over Al, Be, MB, and HB frequency bands.

Subject 6's matrices from Figure 28 were used to create topographic maps of nodes and edges, representative of the brain's networks. Figure 29 below illustrates this topology for Subject 6 the same way as the group average. The maximum edge weights for Subject 6 pre-BB are slightly smaller (< 0.8) than the post-BB (< 0.83) edge weights. There is a subtle decrease in graph density, but not many edges were eliminated from post-BB networks after thresholding.

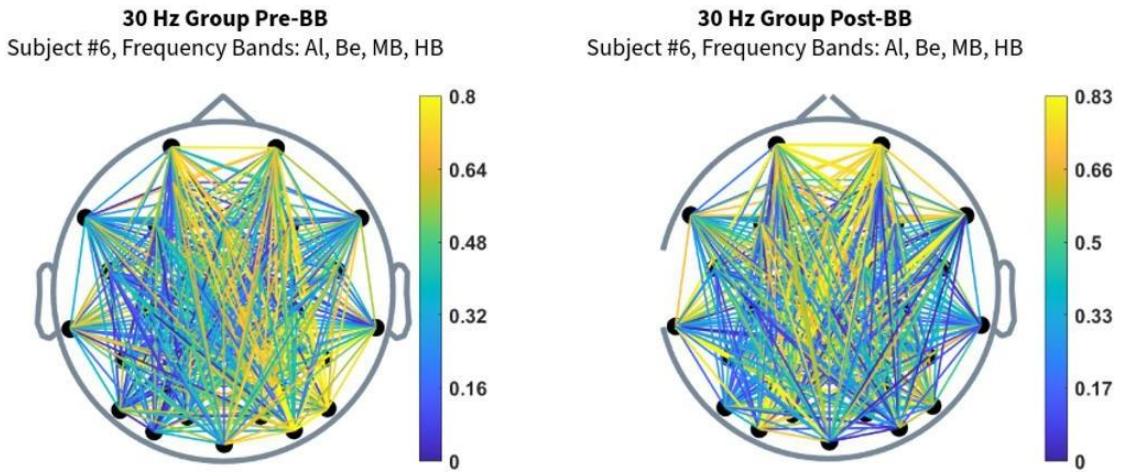

Figure 29: Topographic representation of thresholded measures for 30 Hz BB Frequency Group Subject #6, averaged over Al, Be, MB, and HB frequency bands.

The second type of alteration is a significant change in high connection edge values, which is best demonstrated by Subject 4's thresholded adjacency matrices and topological graphs averaged over the Al, Be, MB, and HB frequency bands. Subject 4's post-BB adjacency matrix shows distinct changes in edge connection weight distribution among pixels and graph density when compared to the pre-BB adjacency matrix. Specifically, distinct changes to the weights of edge connections that were present pre-BB are seen. Figure 30 below illustrates the absolute threshold matrices for Subject 4 in the 30 Hz group pre- and post-BB intervention. Compared to the graphs for the 30 Hz group averages, higher synchronization patterns and connection weights are observed in this subject's adjacency matrices and associated topological graphs. Consistent with other subjects and the group average, higher synchronization levels are found in the post-BB matrix when compared to the pre-BB matrix. However, high synchronization levels for Subject 4's matrices range from about 0.5-0.8, which is still much higher than the synchronization levels seen for the group average (0.02).

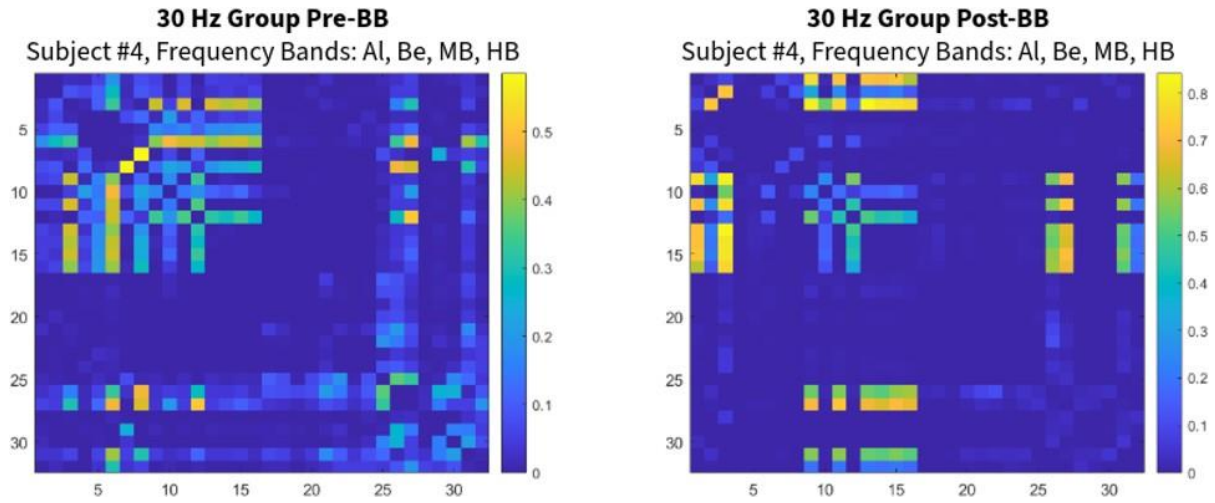

Figure 30: Thresholded Adjacency Matrices for 30 Hz BB Frequency Group Subject #4, averaged over Al, Be, MB, and HB frequency bands.

Subject 4's matrices from Figure 30 were used to create topographic maps of nodes and edges, representative of the brain's networks. Figure 31 below illustrates this topology for Subject 4 the same way as the group average. The maximum edge weights for Subject 4 pre-BB are smaller (< 0.6) than the post-BB (0.8) edge weights. Similar to Subject 6, there is a subtle decrease in graph density, but not many edges were eliminated from Subject 4's post-BB networks after thresholding.

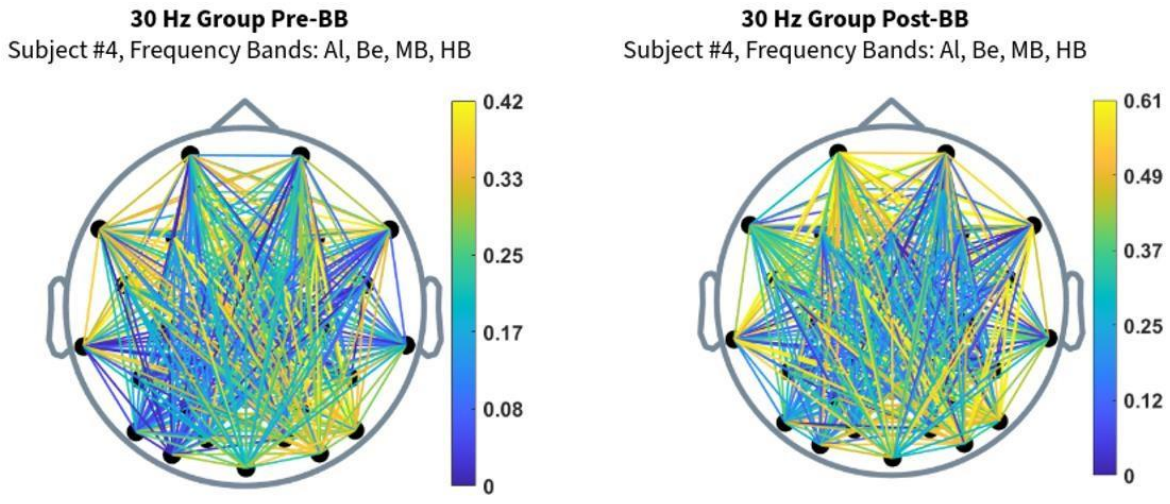

Figure 31: Topographic representation of thresholded measures for 30 Hz BB Frequency Group Subject #6, averaged over Al, Be, MB, and HB frequency bands.

It should be noted that although the group averaged results did not show significant changes in graph density, many individual subjects showed substantial decreases in graph density after listening to 30 Hz BB for 10 minutes. The resulting figures used in this section have depicted the qualitative differences between pre-BB and post-BB stimulation. These results address if a clear change in the brain's network could be seen after BB intervention. The following section addresses the significance of those changes and the graph measures that were influenced the most.

5.3. Numerical Results

By utilizing knowledge of the graph measures deemed significant from the statistical analysis and values associated with graph measures quantified from the connectivity matrices, further interpretations can be made regarding the local and global effects of the BB stimuli. The following results displayed in Table 2 are those needed for further network change interpretation.

Table 2: Descriptive statistics results from statistical analysis significant graph indices.

Graph Index	Frequency Group	Median		Maximum		Minimum		Data Range (Max-Min)		Median Difference
		Pre-BB	Post- BB	Pre-BB	Post- BB	Pre-BB	Post- BB	Pre-BB	Post- BB	
Global Clustering Coefficient 2 (GCC2)	8 Hz	0.01321	0.00684	0.02489	0.01833	0.00000	0.00000	0.02489	0.01833	0.00637
	30 Hz	0.00966	0.00564	0.02928	0.01255	0.00167	0.00000	0.02760	0.01255	0.00402
Nodal Efficiency (NE)	30 Hz	0.12377	0.05963	0.28268	0.12746	0.01563	0.00000	0.26706	0.12746	0.06414
Nodal Betweeness Centrality (BC)	8 Hz	6.27870	3.90970	8.50160	4.90120	2.59990	2.30620	5.90170	2.59500	2.36900
	30 Hz	5.21180	3.47310	9.15140	7.20000	3.72780	2.64190	5.42360	4.55810	1.73870
Global Assortativity (GA)	30 Hz	-0.35885	-0.18866	-0.14050	0.19353	-0.71186	-0.62536	0.57136	0.81889	0.17019

Each graph index has its own set range of values. Global clustering coefficient (GCC) and nodal efficiency (NE) measure values range from 0-1. GCC values for the 8 Hz frequency group pre- and post-BB median are 0.0132 and 0.0068, respectively. For the 30 Hz frequency group, the pre- and post-BB median values are 0.0097 and 0.0056. For the GCC index, both frequency groups median value decreased by approximately ½ after BB intervention; these changes occurred in the HB band for the 8 Hz group and the MB band for the 30 Hz group. A boxplot of these results are shown in Figure 22 below.

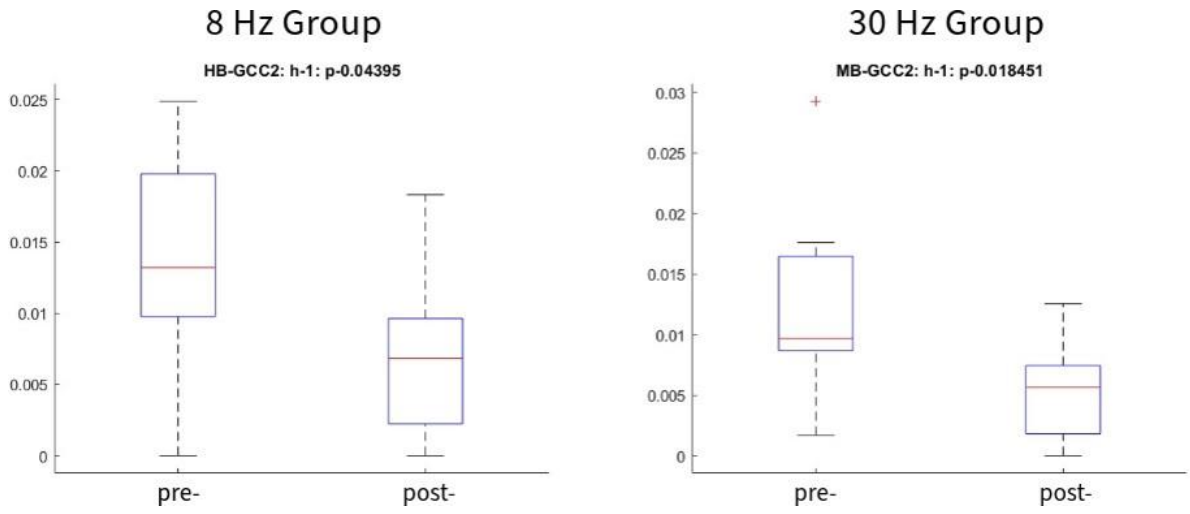

Figure 32: Boxplots of GCC2 graph index for both frequency groups, pre- and post-BB.

The NE median value decreased by more than ½ after the 30 Hz BB listening session. The pre- and post-BB NE median values in the MB band are 0.1238 and 0.0596, respectively. Also, note the overall range of data for GCC and NE values in each frequency group decreased by approximately half after the subjects listened to BB for 10 minutes. A boxplot of these results are shown in Figure 23 below.

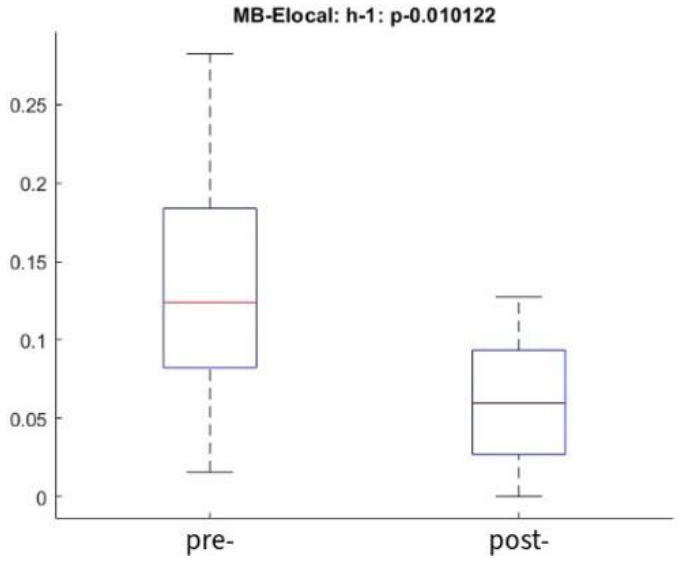

Figure 33: Boxplots of NE graph index pre- and post-BB for the 30 Hz frequency group.

Betweenness Centrality (BC) values range from 0-10. For the 8 Hz frequency group, the pre- and post-BB median values in the Al band are 6.2787 and 3.9097, respectively. In the MB band, the pre- and post-BB median values for the 30 Hz frequency group are 5.2118 and 3.4731. The data range for the 8 Hz group reduces in size by more than ½ post-BB. A boxplot of these results is shown in Figure 24 below.

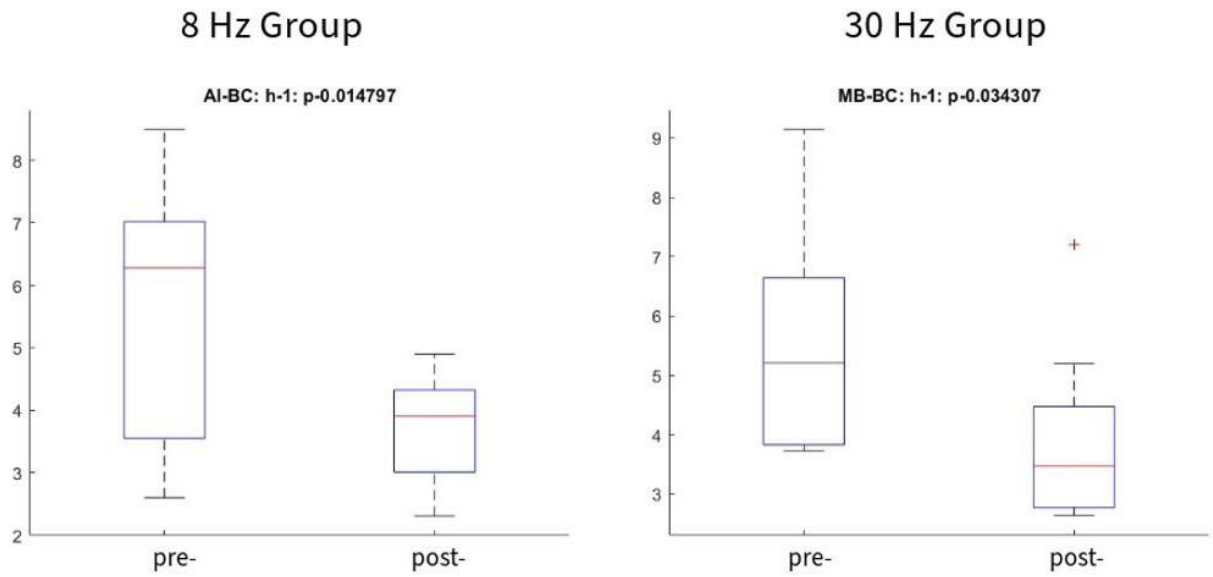

Figure 34: Boxplots of BC graph index for both frequency groups, pre- and post-BB.

Global Assortativity (GA) values range from –1 to +1. For the 30 Hz group, the pre- and post-BB median values in the Be band –0.3589 and –0.1887. The median value increases by ½ after BB intervention. The data range also increases and includes positive values post-BB. A boxplot of these results is shown in Figure 25 below.

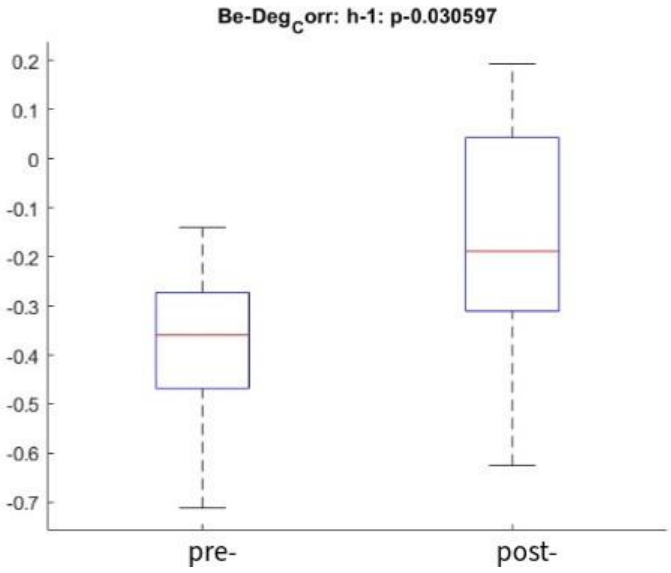

Figure 35: Boxplots of GA graph index pre- and post- BB for the 30 Hz frequency group.

5.4. Statistical Results

In this study, two matched-paired t-tests were performed to analyze the statistical significance of the various FCA and GTA values that resulted from their associated MATLAB codes. To perform the t-tests, graph measures were quantified from the connectivity matrices used during FCA and GTA and plotted over time. These graph measures were averaged individually for each subject's pre-BB and post-BB data. A paired t-test was performed for each measure, a.k.a. graph index, in each frequency band for both frequency groups. The resulting graph indices show a significant influence post-BB in the Alpha (Al) and Beta (Be) frequency bands, specifically the Alpha, Beta, Mid-Beta (MB), and High-Beta (HB) bands, as can be seen in Table 3 below.

Table 3: Paired t-test statistical results for select graph indices and their frequency bands.

Graph Index	Frequency Group	Al		Be		MB		HB	
		8-12 Hz		12-30 Hz		15-20 Hz		20-30 Hz	
		p-value	St. Dev.	p-value	St. Dev.	p-value	St. Dev.	p-value	St. Dev.
Global Clustering Coefficient 2	8 Hz	0.71446	0.00810	0.85171	0.00650	0.16491	0.00520	**0.04395**	0.00680
(GCC2)	30 Hz	0.56233	0.00830	0.10048	0.00860	**0.01845**	0.00610	0.34776	0.03410
Nodal Efficiency	8 Hz	0.70697	0.08750	0.82178	0.07730	0.12390	0.05870	0.09769	0.08220
(NE)	30 Hz	0.33106	0.09920	0.10756	0.07500	**0.01012**	0.06140	0.73074	0.08200
Nodal Betweeness Centrality	8 Hz	**0.01480**	1.61350	0.61766	2.23420	0.19555	1.25240	0.39319	2.08620
(BC)	30 Hz	0.35401	5.26720	0.32889	6.50800	**0.03431**	1.64810	0.90857	5.95520
Global Assortativity	8 Hz	0.42200	0.19210	0.51252	0.20310	0.21983	0.13670	0.18297	0.13460
(GA)	30 Hz	0.93324	0.23010	**0.03060**	0.21860	0.09038	0.18940	0.72391	0.25020

Every graph index was influenced by the BB stimulus in most of the frequency bands. The Alpha and Beta frequency bands house the most significant changes in network features, where $p \leq 0.01$ in two of the graph measures, and $p < 0.05$ in the other two measures. In the Alpha, Mid-Beta, and High-Beta bands, the global clustering coefficient (GCC) and betweenness centrality (BC) measures were significantly affected in both the 8 Hz and 30 Hz frequency groups. Additionally, the nodal efficiency (NE) and global assortativity (GA) measures were significantly affected in the Beta and Mid-Beta bands, respectively, for the 30 Hz BB group. The majority of significant p-values were found in the Mid-Beta band, and in contrast, there were no significant changes in graph measures found in the Low-Beta (12-15 Hz) and Theta (4-8 Hz) frequency bands.

Chapter 6. Discussion

To recap, Graph theory is used to help topographically illustrate the brain by visually displaying groups of nodes and their pairwise interconnections, a.k.a. edges; where nodes represent distinct neural elements, and edges that connect nodes indicate levels of synchronization between them. Graph Theory's quantifiable properties are typically classified into one or more of the following categories: neural network functional segregation, the integration between distant brain networks, network centrality, and network assortativity. Graph Theory Analysis (GTA) is used for the analysis of BB functional connectivity (FC) results because it provides a comprehensive view of the brain network alterations that occurred. The repetitive and tenacious nature of FC, assessing phase synchronization between channels by comparing them one at a time, allows for ample material to be used in GTA.

6.1. Thresholding & Adjacency Matrices

The use of functional connectivity results to create adjacency matrices allows for the evaluation and comparison of neural network features during different cognitive states. In accordance with previous brain research studies that have used thresholded graph-based analysis, an absolute threshold of 0.5 was applied to all adjacency matrices. A higher threshold value was considered, but when tested on the average of all 10 subjects in a group, it removed smaller significant connections that are primarily seen in the 30 Hz frequency group, refer to Figure 26. By thresholding the data, weaker connections within networks were removed for easier analysis of higher, more significant connections. To clarify, any pixel in the thresholded adjacency matrices that is dark blue and corresponds to zero on the color bar is a connection that was below the initial threshold value of 0.5.

A. 8 Hz Frequency Group

Approximately half of the subjects in the 8 Hz frequency group had pre- and post-BB edge connection patterns similar to the group average edge connections seen in Figure 22. The low edge connection values (0.05-0.07) seen in the group average adjacency matrices and topological graphs are due to the vast difference between subjects, both pre- and post-BB Although the group average adjacency matrices are relatively accurate results for the majority of subjects within the 8 Hz group, with a group size of 10 subjects it is likely that averaging and thresholding individuals' data together caused some loss of significant changes present in each individual's adjacency matrices. However, since most subjects did have FC results similar to the group average, a general claim can be made about the connectivity patterns in this study's 8 Hz frequency group subjects pre- and post-BB. For the group average and Subject 10, high edge connections exist between medial frontal nodes and throughout the occipital lobe prior to any auditory stimulus. After BB intervention, there is a decrease in edge connection weight between medial frontal nodes and throughout the occipital lobe, with an increase in high edge connections in the left temporal lobe. With a larger sample size, it may be possible to determine if this is a generalized pattern of connectivity change induced by listening to 8 Hz BB for 10 minutes.

B. 30 Hz Frequency Group

All 10 subjects in the 30 Hz group had vastly differing pre-BB connection patterns and weights in their individual adjacency matrices. Although most subjects post-BB data fell into one of two trends in how connectivity differed after BB intervention, the individual subject matrices also varied greatly in their connectivity patterns and weights. It is likely that the averaging and thresholding of all 10 subjects for pre- and post-BB adjacency matrices diminished, i.e. filtered out, any individual significant results. Although the group

average can show any collective similarities between subjects, the individual connectivity results of subjects bests describes the changes that BB intervention can have on the brain. The two types of distinguished change seen in subjects who listened to 30 Hz was a significant change in high connection edge patterns and a significant change in high connection edge values. Subject 6 showed distinct changes in nodes that had high connections, or any connections at all for the matter, pre- and post-BB. In Subject 6's adjacency matrices, there are multiple nodes that go from an edge connectivity value of zero in the pre-BB matrix, to a high or maximum edge connectivity value in the post-BB, and vise-versa. This is not the case for Subject 4, who experiences an increase in edge connection values post-BB to the nodes that were already moderate-highly active during their pre-BB recording. For subject 4 this indicates an improvement in efficiency and assortativity to their neural network but does not indicate any significant connectivity pattern changes between nodes or brain regions. In the topological graph for the group average, edge connection values increase between nodes in the frontal lobe and throughout the left side of the brain after BB intervention. A similar increase in edge connection values between frontal lobe nodes occurs for Subjects 6 and 4 as well. Subject 6 experiences a pattern of change where existing high edge connections between medial frontal nodes and right brain occipital nodes are altered to high edge connections between medial and lateral frontal nodes and left-brain occipital nodes after BB intervention. Subject 4's pattern of change shows a general increase of high edge connections between frontal and temporal lobes on both sides of the brain, but more significantly in the left medial frontal node after BB intervention. Between subjects and within the group average, edges connected to temporal lobe nodes show indications of high levels of connectedness but do not show any common patterns of change throughout all 10 subjects.

Regarding the low connectivity values for the averaged group adjacency matrices, 8 Hz group (0.05) and 30 Hz group (0.02); larger subject pools are needed to see high values like in the individual subject results. Averaging all subjects together that listened to the same type of stimuli is a good way to see any overlapping similarities, but with each subject's experience being unique to them a much larger sample size would be needed to make any definitive claims on the overall general effect of a 10-minute BB listening session.

6.2. Statistical & Numerical Significance

A. Segregation & Integration

Global Clustering Coefficient (GCC2) results from both the 8 Hz and 30 Hz groups indicate a significant change in network clustering after listening to BB for 10 minutes. Comparison of the change in median values between pre and post BB listening fo each group shows that both frequency groups experienced a decrease in clustering of networks and became more randomized due to the BB stimuli. A decrease in network clustering demonstrates longer path lengths, and networks working at a lower level of integration with less efficiency. This suggests the subject feels more relaxed, as the brain is not working as hard as it was prior to listening to BB. Comparison of the p-values for the two different frequency groups indicates that the 30 Hz frequency BB may have had a stronger overall effect on subjects' level of clustering.

Nodal Efficiency (NE) results from the 30 Hz frequency group indicate a significant change in a nodes' efficiency and ability to transmit information with other nodes after listening to BB for 10 minutes. NE values range from 0-1, with high values representing the presence of a hub, i.e. a node with high capability to share information with other nodes. Comparison of the change in median values between pre and post BB listening

in this frequency group shows a decrease in the MB band nodal efficiency due to the 30 Hz BB stimuli. A decrease in efficiency represents less demand for information transmission [61]. This suggests that subjects who experience a decrease in nodal efficiency have also experienced an increase in their feelings of relaxation and a decrease in their stress levels.

B. Network Centrality

Betweenness Centrality (BC) results from the 8 Hz and 30 Hz frequency groups indicate a significant change in the strength of network control after listening to BB for 10 minutes. Comparison of the change in median values between pre and post BB listening in both frequency groups shows a decrease in overall network control due to the BB stimuli. A decrease in network control indicates that less information is passing through nodes that previously had high degrees [62]. This suggests the subject feels more relaxed, as the brain is processing significantly less information than it was prior to listening to BB. Comparison of the p-values for the two different frequency groups indicates that the 8 Hz frequency BB may have had a stronger overall effect on subjects' brain network control.

C. Assortativity

Global Assortativity (GA) results from the 30 Hz frequency group indicate a significant change in the assortativity of networks after listening to BB for 10 minutes. Assortativity values range from –1 to +1, with negative values representing disassortative networks and positive values representing assortative networks. Comparison of the change in median values between pre and post BB listening in this frequency group shows an increase in the overall network assortativity due to the 30 Hz BB stimuli. An increase in assortativity represents an increase in a network's resilience

to damage and its' ability to function properly when damage or dysfunction is present. An increase in assortativity suggests "healing" of the brain, which occurs when networks and their components are able to rest and rejuvenate due to structural reorganization brought on my stimuli or lack of stimuli. It should also be noted that some of the subjects who listened to 30 Hz BB had GA levels as high as 0.1935 post-BB, indicative of a completely assortative network.

6.3. PSS-10 Significance

The PSS-10 results have varying indications. It is unusual that the range of perceived stress between groups was so varied prior to BB intervention. The subjects randomly assigned to the 8 Hz frequency group consistently rated their perceived stress with much lower values than those randomly assigned to the 30 Hz group. For both pre- and post-BB stress assessments the 8 Hz group did not have any subjects who perceived High stress, whereas subjects in the 30 Hz group did. For this study, the PSS-10 results indicate that over half of the subjects (11/20) felt a significant enough amount of change to the way they perceived their stress, that when they re-took the same PSS test after listening to BB their perceived stress score decreased, indicating their feelings of stress decreased. Taking into consideration the other 9/20 subjects who reported no change in their stress levels or a perceived increase, there are many factors that could have been attributed to this. A large factor to take into consideration is answer bias from the subject. In general, certain types of people can be intimidated by tests and/or personal questions, and the PSS-10 form is a combination of both, which has the possibility to negatively affect an individual's emotions while taking it. In contrast, it is possible that subjects answered the PSS-10 questions without putting much, or any, thought into their answers. In the case of the subjects whose perceived stress did not change, the possibility that the post-BB PSS-10 form was filled out based on memory of answers from the pre-BB PSS-10 form should not be ruled out. Other factors that could have influenced PSS-10 results include

gender and personality. It is known that females tend to perceive stress at higher rates than males, and on average report higher levels of stress than males [63,64]. Although, in this study the gender split between groups was approximately equal, with 6 males and 4 females in the 8 Hz group and 5 males and 5 females in the 30 Hz group. Each individual's personality also plays a role in the level at which they perceive stress. More specifically, a person's level of susceptibility can affect their ability to entrain to the BB stimulus as intended. People who are generally more susceptible, or easy to influence, may be able to entrain to the BB stimulus faster and with more ease than others.

6.4. Limitations

In this study, there are limitations that still need further consideration. Due to technological limitations, the effects of BB were not recorded during the actual BB listening session. In future studies, using proper equipment that could prevent the BB sound from interfering with the electrodes detected signals would allow for the analysis of how BB effects the brain's functional connectivity during listening, and the analysis of brain entrainment. Results from such analyses in combination with further study could answer the questions: "How long do the effects of BB last?" and "Will listening for longer prolong the effects?". Future studies interested in utilizing BB as a form or stress reduction should take gender into consideration, and the differences between the perception of stress associated with both genders. In regard to graph measurements, this study took into consideration global measures and their overall effect on the brain. Future studies should also consider local measures and their associated spatial alterations to investigate separate regions of the brain pre-, post-, and mid-BB listening; as well as collecting larger pools of data. This can be done by using a 64 or 128 electrode EEG cap, which would provide a better and more accurate understanding of brain functions and features, and/or by recruiting more subjects.

A much larger sample size is needed for each group in order to provide more meaningful statistical results and to find a more distinguishable pattern of connectivity change over time due to the BB stimuli. For future research based on this study, utilizing effect size and statistical power is recommended. Effect size represents the magnitude of the difference between groups being compared after intervention. Although p-values are informative on if an effect exists, they are not indicative of the size of the effect an experiment had. Effect size should be used to calculate the minimum number of subjects needed in a study to avoid type II error, i.e. instances of false positive results. When calculating a sample size, Effect size can be estimated using pilot study results, i.e. the results from this study [65]. In addition to this study, more studies on Alpha and Beta band BB frequencies are needed to determine which modulation frequency produces optimal levels of stress reduction and focus for the general population. Furthermore, more studies are needed that analyze the effects of BB using functional connectivity and graph theory. This would allow for a more standardized experimentalpractice, as researchers determine the most optimal thresholding and modulation frequency values to use.

Chapter 7. Conclusion

FCA and GTA have proven to be very useful in detecting levels of change in a brain's networks due to auditory stimulation. Specifically, the topographic representations created from GTA graphs provide a holistic view of brain networks in a way that allows for easy interpretation of where connections are taking place anatomically throughout the brain. Compared to functional connectivity results individually calculated between channel pairs, GTA results provide more information about an individual's or group's cognitive state at certain points in time. Used correctly, FCA and GTA results regarding a brain's network can be used to detect and monitor changes over time due to the BB stimuli.

After listening to BB for 10 minutes, subjects reported a decrease in perceived stress levels. Functional connectivity and GTA data indicate that while both modulation frequencies were adequate in changing the brain's functional connectivity, they had different effects. The 8 Hz BB was most effective at decreasing network centrality and clustering and changing significant nodal connection patterns after intervention. This led to an overall relaxing or sleepy feeling for subjects, as their neural networks required less demand, i.e. critical or logical thought, from the brain. Contrarily, the 30 Hz BB was most effective at increasing network assortativity and decreasing nodal efficiency after intervention. These network changes are consistent with feelings of increased focus or attention, and while this does not cause a direct feeling of relaxation, an increase in focus can cause individuals to feel more in control of themselves, and therefore more relaxed.

Overall, decreases in network control, efficiency, and clustering are all found to be results of stress reduction. Changes in network features were most significant in the Beta band (12-30 Hz), reflecting the impact of listening to BB for a prolonged period. A distinguishable pattern of connectivity change was found for the 8 Hz BB group, where a significant decrease in

edge connection weight between medial frontal nodes and throughout the occipital lobe occurred after BB intervention, and an increase in high edge connections in theleft temporal lobe developed post-BB. The GTA used in this study provided crucial information relative to the subtle neural network changes in the brain that occurred during resting EEG recordings and was successful at analyzing the effects of binaural beats on the brain's functionalconnectivity.

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Appendix A: IRB Approval

EAST CAROLINA UNIVERSITY
University & Medical Center Institutional Review Board
4N-64 Brody Medical Sciences Building· Mail Stop 682
600 Moye Boulevard · Greenville, NC 27834
Office **252-744-2914** · Fax **252-744-2284** ·
rede.ecu.edu/umcirb/

Notification of Initial Approval: Expedited

From: Biomedical IRB
To: Sunghan Kim
Date: 9/19/2023
Re: UMCIRB 23-000605
THE EFFECTS OF BINAURAL BEATS ON THE BRAIN'S FUNCTIONAL CONNECTIVITY

I am pleased to inform you that your Expedited Application was approved. Approval of the study and any consent form(s) occurred on 9/18/2023. The research study is eligible for review under expedited category # 4 and 7. The Chairperson (or designee) deemed this study no more than minimal risk.

As the Principal Investigator you are explicitly responsible for the conduct of all aspects of this study and must adhere to all reporting requirements for the study. Your responsibilities include but are not limited to:

1. Ensuring changes to the approved research (including the UMCIRB approved consent document) are initiated only after UMCIRB review and approval except when necessary to eliminate an apparent immediate hazard to the participant. All changes (e.g. a change in procedure, number of participants, personnel, study locations, new recruitment materials, study instruments, etc.) must be prospectively reviewed and approved by the UMCIRB before they are implemented;

2. Where informed consent has not been waived by the UMCIRB, ensuring that only valid versions of the UMCIRB approved, date-stamped informed consent document(s) are used for obtaining informed consent (consent documents with the IRB approval date stamp are found under the Documents tab in the ePIRATE study workspace);

3. Promptly reporting to the UMCIRB all unanticipated problems involving risks to participants and others;

4. Submission of a final report application to the UMICRB prior to the expected end date provided in the IRB application in order to document human research activity has ended and to provide a timepoint in which to base document retention; and

5. Submission of an amendment to extend the expected end date if the study is not expected to be completed by that date. The amendment should be submitted 30 days prior to the UMCIRB approved expected end date or as soon as the Investigator is aware that the study will not be completed by that date.

The approval includes the following items:

Name	Description
Binaural Beats Questionnaire	Surveys and Questionnaires
Email Template	Recruitment Documents/Scripts
Informed Consent Document	Consent Forms
Perceived Stress Scale (PSS-10)	Standardized/Non-Standardized Instruments/Measures
Thesis Proposal	Study Protocol or Grant Application

For research studies where a waiver or alteration of HIPAA Authorization has been approved, the IRB states that each of the waiver criteria in 45 CFR 164.512(i)(1)(i)(A) and (2)(i) through (v) have been met. Additionally, the elements of PHI to be collected as described in items 1 and 2 of the Application for Waiver of Authorization have been determined to be the minimal necessary for the specified research.

The Chairperson (or designee) does not have a potential for conflict of interest on this study.

Appendix B: Informed Consent Document

East Carolina University

ECU

Informed Consent to Participate in Research

Information to consider before taking part in research that has no more than minimal risk.

Title of Research Study: The Effects of Binaural Beats on the Brain's Functional Connectivity

Principal Investigator: Sunghan Kim (Person in Charge of this Study)
Institution, Department or Division: East Carolina University Department of Engineering
Address: 216 Slay, Greenville, NC 27858
Telephone #: (252) 737-1750

Researchers at East Carolina University (ECU) study issues related to society, health problems, environmental problems, behavior problems and the human condition. To do this, we need the help of volunteers who are willing to take part in research.

Why am I being invited to take part in this research?
The purpose of this research is to study the differences between the brain waves of people before and after they have listened to binaural beats. These brain waves can be recorded from the surface of the scalp using an EEG. This research study aims to determine neural network differences by analyzing the small differences in the subjects' response to binaural beat stimuli. You are being invited to take part in this research because you are a cognitively healthy volunteer of at least 18 years of age. The decision to take part in this research is yours to make. By doing this research, we hope to learn how binaural beats stimuli affects the way the brain structures itself and processes information.

If you volunteer to take part in this research, you will be one of about 20 to 40 people to do so.

Are there reasons I should not take part in this research?
You should not take part in this research if you (1) are under the age of 18 or over the age of 35, (2) Do not speak English, (3) or cannot provide your own transportation to the research lab.

What other choices do I have if I do not take part in this research?
You can choose not to participate. No alternative involvement is available.

Where is the research going to take place and how long will it last?
The research will be conducted at ECU Biomedical Engineering Analysis of Cognitive Neuroscience (BEACON) laboratory (Science and Technology Building 134). You will need to come to the examination room once during the study. You will be asked to volunteer for approximately 30 to 45 minutes.

What will I be asked to do?
You will be asked to do the following: A graduate student researcher will have you wear a cap with 32 electrodes while you are seated comfortably in a chair. Those electrodes will make direct contact with your scalp but should not cause any pain. You will sit comfortably in a chair in the lab. You will be asked to self-evaluate your stress before an initial EEG recording is taken, then listen to binaural beats for approximately 10 minutes, followed by another EEG recording and self-evaluation stress test.

Appendix B: Informed Consent Document

What might I experience if I take part in the research?
We don't know of any risks (the chance of harm) associated with this research. Any risks that may occur with this research are no more than what you would experience in everyday life. We don't know if you will benefit from taking part in this study. There may not be any personal benefit to you, but the information gained by doing this research may help others in the future.

Will I be paid for taking part in this research?
We will not be able to pay you for the time you volunteer while being in this study.

Will it cost me to take part in this research?
It will not cost you any money to be part of the research.

Who will know that I took part in this research and learn personal information about me?
ECU and the people and organizations listed below may know that you took part in this research and may see information about you that is normally kept private. With your permission, these people may use your private information to do this research:

- Any agency of the federal, state, or local government that regulates human research. This includes the Department of Health and Human Services (DHHS), the North Carolina Department of Health, and the Office for Human Research Protections.
- The University & Medical Center Institutional Review Board (UMCIRB) and its staff have responsibility for overseeing your welfare during this research and may need to see research records that identify you.

How will you keep the information you collect about me secure? How long will you keep it?
All individuals working on this study have received special training on protecting your privacy and keeping you research and health information confidential. You will be assigned a special identification number for this study instead of your name. No printed forms that have your name, aside from this consent form, will be kept by the research study team. All printed forms will be shredded 6 years after study closure to protect your personal health information.

What if I decide I don't want to continue in this research?
You can stop at any time after it has already started. There will be no consequences if you stop and you will not be criticized. You will not lose any benefits that you normally receive.

Who should I contact if I have questions?
The people conducting this study will be able to answer any questions concerning this research, now or in the future. You may contact the Principal Investigator at (252) 737-1750 (Monday through Friday, 9:00 am - 5:00 pm).

If you have questions about your rights as someone taking part in research, you may call the University & Medical Center Institutional Review Board (UMCIRB) at phone number 252-744-2914 (days, 8:00 am-5:00 pm). If you would like to report a complaint or concern about this research study, you may contact the Director for Human Research Protections, at 252-744-2914.

Is there anything else I should know?
Your information or biospecimens collected as part of the research, even if identifiers are removed, will not be used or distributed for future studies.

I have decided I want to take part in this research. What should I do now?

Appendix B: Informed Consent Document

The person obtaining informed consent will ask you to read the following and if you agree, you should sign this form:

- I have read (or had read to me) all of the above information.
- I have had an opportunity to ask questions about things in this research I did not understand and have received satisfactory answers.
- I know that I can stop taking part in this study at any time.
- By signing this informed consent form, I am not giving up any of my rights.
- I have been given a copy of this consent document, and it is mine to keep.

_____ _____ _____

Participant's Name (PRINT) **Signature** **Date**

Person Obtaining Informed Consent: I have conducted the initial informed consent process. I have orally reviewed the contents of the consent document with the person who has signed above and answered all of the person's questions about the research.

_____ _____ _____

Person Obtaining Consent (PRINT) **Signature** **Date**

Appendix C: Medical History Questionnaire

Questionnaire

Title of Research Study: The Effects of Binaural Beats on the Brain's Functional Connectivity

Principal Investigator: Sunghan Kim (Person in Charge of this Study)
Institution, Department or Division: Department of Engineering, East Carolina University
Address: East 5th Street, Greenville, NC 27858
Telephone #: (252) 737-1750
Study Coordinator: Emily Fenton
Telephone #: (252) 737-1750

This is a questionnaire designed to gather information about you that can allow us to conclude whether you are fit for the study, whether the study involves risks for you to participate in, or whether the data we gather from you will be of sufficient quality. For each question below, please circle the answer that fits your description (YES or NO). If you do not believe that a question can be answered with a simple yes or no, write in your response beside the field labeled "other:".

Medical History

Have you ever been diagnosed with any hearing disorders/impairments?

YES NO Other:_____

Have you ever been diagnosed with diabetes?

YES NO Other:_____

Have you ever been diagnosed with any endocrine disorders?

YES NO Other:_____

Have you ever been diagnosed with any psychiatric or neurological diseases?

YES NO Other:_____

Do you have any form of cognitive impairment?

YES NO Other:_____

Appendix C: Medical History Questionnaire

Have you recently taken any medications from the following sub-groups: Benzodiazepines, Non-benzodiazepine sedatives, Opiates, Anticholinergics, and/or Antipsychotics/Mood-stabilizers?

YES NO Other:_____

Is there anything else that the principal investigator and study coordinator should be aware of? (if so, please write it in the "other" section)

YES NO Other:_____

Recent 24 -48 Hours

Have had any acute illness?

YES NO Other:_____

Did you sleep at least five hours the previous night?

YES NO Other:_____

Do you feel awake and alert?

YES NO Other:_____

Have you smoked or heavily consumed alcohol?

YES NO Other:_____

Are you under the effects of any stimulants or medications intended to enhance focus (example: amphetamines for those diagnosed with attention deficit hyperactive disorder)?

YES NO Other:_____

Are you under the effects of any substances or medications that may dull the ability to focus or concentrate (example: certain pain medications or barbiturates)?

YES NO Other:_____

Appendix D: PSS-10 Document

Perceived Stress Scale

A more precise measure of personal stress can be determined by using a variety of instruments that have been designed to help measure individual stress levels. The first of these is called the **Perceived Stress Scale**.

The Perceived Stress Scale (PSS) is a classic stress assessment instrument. The tool, while originally developed in 1983, remains a popular choice for helping us understand how different situations affect our feelings and our perceived stress. The questions in this scale ask about your feelings and thoughts during the last month. In each case, you will be asked to indicate how often you felt or thought a certain way. Although some of the questions are similar, there are differences between them and you should treat each one as a separate question. The best approach is to answer fairly quickly. That is, don't try to count up the number of times you felt a particular way; rather indicate the alternative that seems like a reasonable estimate.

For each question choose from the following alternatives:
0 - never 1 - almost never 2 - sometimes 3 - fairly often 4 - very often

_____ l. In the last month, how often have you been upset because of something that happened unexpectedly?

_____ 2. In the last month, how often have you felt that you were unable to control the important things in your life?

_____ 3. In the last month, how often have you felt nervous and stressed?

_____ 4. In the last month, how often have you felt confident about your ability to handle your personal problems?

_____ 5. In the last month, how often have you felt that things were going your way?

_____ 6. In the last month, how often have you found that you could not cope with all the things that you had to do?

_____ 7. In the last month, how often have you been able to control irritations in your life?

_____ 8. In the last month, how often have you felt that you were on top of things?

_____ 9. In the last month, how often have you been angered because of things that happened that were outside of your control?

_____ 10. In the last month, how often have you felt difficulties were piling up so high that you could not overcome them?

Appendix D: PSS-10 Document

Figuring Your PSS Score

You can determine your PSS score by following these directions:

- First, reverse your scores for questions 4, 5, 7, and 8. On these 4 questions, change the scores like this:

  0 = 4, 1 = 3, 2 = 2, 3 = 1, 4 = 0.

- Now add up your scores for each item to get a total. **My total score is** _____.

- Individual scores on the PSS can range from 0 to 40 with higher scores indicating higher perceived stress.
  - ► Scores ranging from 0-13 would be considered low stress.
  - ► Scores ranging from 14-26 would be considered moderate stress.
  - ► Scores ranging from 27-40 would be considered high perceived stress.

The Perceived Stress Scale is interesting and important because your perception of what is happening in your life is most important. Consider the idea that two individuals could have the exact same events and experiences in their lives for the past month. Depending on their perception, total score could put one of those individuals in the low stress category and the total score could put the second person in the high stress category.

***Disclaimer**: The scores on the following self-assessment do not reflect any particular diagnosis or course of treatment. They are meant as a tool to help assess your level of stress. If you have any further concerns about your current well being, you may contact EAP and talk confidentially to one of our specialists.*

State of New Hampshire
Employee Assistance Program