CORTICAL HABITUATION OF ACOUSTIC STARTLE REFLEX

A Master's Thesis

Presented to

The Faculty of the Department of Biomedical Engineering

University of Houston

In Partial Fulfillment

of the Requirements for the Degree

Master of Science

in Biomedical Engineering

by

Trac Duy Nguyen

May 2015

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ABSTRACT

The study of acoustic startle reflexes (ASR) has recently shown promising potential in augmenting the recovery of voluntary movement in patients who undergo neuro-rehabilitation. However, these ASRs have been associated with the decrease or inhibition of startle responses over successive stimulation, known as habituation. This study hypothesizes an acoustic startle pathway that involves the dorsolateral prefrontal cortex is inhibiting the ASR. To do this investigation, three paradigms have been developed in conjunction with EEG recordings. Independent component analysis has been implemented to minimize the intrinsic motion artifacts in the acquired data. The results show possible anti-correlation between the EMG startle signal and the activity located along the frontal midline suggesting possible habituation. However, no solid conclusion can be made whether the dorsolateral prefrontal cortex is part of the habituation process in the acoustic startle pathway.

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List of Abbreviations

AEP	Auditory Evoked Potential
CNS	central nervous system
dB	decibel, the unit of sound intensity
DLPFC	Dorsolateral prefrontal cortex
EEG	Electroencephalogram
EKG	Electrocardiogram
ERP	Event Related Potential
Hz	Hertz
IIR	Infinite impulse response
LL	lateral lemniscus
N1	N100 – negative deflection at 100 ms
P2	P200 – positive deflection at 200 ms
OSHA	Occupational Safety and Health Administration
RPC	Reticularis pontis caudalis
SCI	Spinal cord injury
SCM	Sternocleidomastoid
VCN	Ventral cochlear nucleus

1. Introduction

1.1 Motivation

The number of individuals requiring neuro rehabilitation is steadily increasing. Stroke patients alone seeking these services make up more than 530,000 people each year [1], whereas individuals with spinal cord injuries (SCI) compose up to 12,000 [2]. Other impairments to the central nervous system (CNS) such as multiple sclerosis, Parkinson and neuromuscular disorders further increase the need for a better and faster treatment. However, the repair of function of the CNS is very difficult [3], resulting in limited success. New neurological protocols have therefore been developed to expand the tools for rehabilitation medicine. One of the newest tools is the acoustic startle response which has shown promising potential in augmenting voluntary movement [4]. It is non-invasive and easy to modulate, yet it is not well understood. It is therefore in our interest to investigate the acoustic startle response and its mechanism to contribute to the field of neuro rehabilitation.

1.2 Acoustic Startle responses

Acoustic startle responses have been used as a tool in a variety of fields to assess substance abuse, neurological diseases, psychiatric disorders, employed therapies, or pharmacological assays [5]. The most dominant field to deploy the acoustic startle response to date, however, has been cognitive sciences, where cerebral processes associated with behavior are evaluated. One of the earliest research to utilize the acoustic startle response as a tool was led by Robert B. Malmo, who was interested in

pathological anxiety in psychiatric patients. He concluded that the acoustic startle response can be used as an indicator for “behavioral arousal” [6]. Many other groups have since then used the acoustic startle response as a tool to evaluate other mental disorders such as schizophrenia, autism, obsessive-compulsive disorder [7]. In contrast to the field of cognitive sciences, neural rehabilitation just recently started to utilize the acoustic startle response. It was first used to track the progress and improvement of patients; however, was recognized later by J. Valls-Sole and his group that the mechanism of the acoustic startle response could possible augment “voluntary movement in the clinical rehabilitation of patients with SCI” [4]. The transformation of using the acoustic startle response as an indicator for various conditions to a possible clinical treatment is intriguing.

1.3 Physiology and Acoustic Startle reflex

1.3.1 Reflexes

Comparable to all reflexes, the acoustic startle reflex exhibits an involuntary and rapid movement when an intense and unexpected auditory stimulus is presented. Once the acoustic startle reflex is activated in the brainstem, a stereotypical body posture can be observed [8] which is also known as the acoustic startle response. Tactile and vestibular stimuli can also trigger an acoustic startle response; however, this study solely focuses on the acoustic startle response elicited by the acoustic startle reflex. It is important to note that the abbreviation “ASR” will be reserved for the term acoustic startle reflex instead of the acoustic startle response. The ultimate goal of the ASR is to protect the individual from possible harm and danger [5]. To better understand the

mechanism of the ASR, the current models and pathways will be discussed in the following section.

1.3.2 Auditory Startle Pathway

The current auditory startle pathway will be established in this section. As the auditory stimulus enters the cochlear, a large number of nuclei and connections are involved in eliciting the startle response. However, there are three key brain structures as shown in Figure 1, which are the ventral cochlear nucleus (VCN), lateral lemniscus (LL), and reticularis pontis caudalis (RPC) [9].

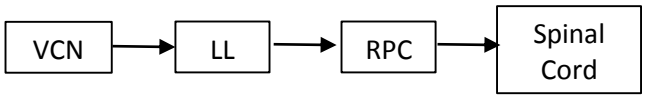

Figure 1: Auditory Startle Pathway involving three key brain structures

Each of these structures are located in the brainstem and can be activated independently to excite the RPC which then induces a startle response. The RPC is connected with the spinal cord by which motor neurons can initiate the startle movement. This model is well described by Davis et al. in 1982 and has been cited numerous times [9]. In 2002, Henn suggested that this pathway consists of only a few central synapses that can mediate the response fast and efficiently. Since reflexes have to be mediated and executed fast, the activation of either one of these nuclei located in the brainstem is enough to elicit the startle. In other words, the cortex is believed to be circumvented by this pathway [9] and plays little role in mediating the acoustic startle response.

1.4 Habituation

A well-known phenomena in the studies of ASR is habituation. Habituation refers to the decrease in muscular startle magnitude with successive presentation of auditory startle stimuli [9]. This process is believed to be decremental and affects the acoustic startle response in both latency and amplitude [10]. This observation has been well studied in animal models and findings suggest that the habituation process is an intrinsic property involved in non-associative learning processes [11]. In other words, habituation is associated with neural plasticity and short –term memory. If the individual is exposed to the startle stimulus and does not perceive it to be threatening or harmful, sensory filtering reduces the individual's response for the next incoming stimulus [11].

1.5 Dorsolateral Prefrontal Cortex

As described in the previous section, shaping the temporal flow of information is important in anticipating stimuli. One area known to exhibit this temporal integration of events is the prefrontal cortex [12]. Furthermore, Duncan J. (2001) believes that the structure is also involved in working memory, attention and control to ultimately exercise adaptive neural coding [13]. A sub structure, known as the dorsolateral prefrontal cortex (DLPFC) has recently shown to specifically inhibit unwanted reflexive saccades. To be able to suppress this unwanted reflexes, the DLPFC must have short-term memory regarding the previous reflex and inhibit the next predicted saccade. This mechanism is proposed by Pierrot-Deseilligny C. in 2003 [14]. From these findings, it is therefore interesting to investigate whether the inhibition of acoustic startle reflexes also occur in the DLPFC.

1.6 EEG

Electroencephalography (EEG) is a technique that measures and records the electrical activity on the scalp due to the voltage fluctuations [15]. These voltage fluctuations are the result of the communication between the neurons of the brain. In fact, the neurons communicate with each other by producing very small amount of electrical signals, called impulses. Consequently, signal generated by the neurons is recorded for a short period of time by placing multiple electrodes on the scalp [16]. This technique can be used to diagnose different disorders such as epilepsy, cerebral infarction, or edema. This is possible due to the different EEG signatures that exists in each disorder or abnormality. However, EEG is not the only imaging modality that can be utilized effectively. Nowadays, there are many new technologies that help the study of brain function, including near-infrared spectroscopy (NIRS), magnetoencephalograhy (MEG), positron emission tomography (PET), nuclear magnetic resonance spectroscopy, functional magnetic resonance imaging (fMRI), single photon emission computed tomography (SPECT), magnetic resonance imaging (MRI) [17]. Even though EEG has a low special resolution, it holds multiple advantages over other technologies. For example, EEG has a very high temporal resolution. EEG can measure activity in the order of milliseconds which makes it ideal for the study of rapid and short lasting activities, such as reflexes. Furthermore, this technology is non-invasive and can be deployed more cost-effectively compared to many other modalities.

2. Methods

2.1 Subjects and Experimental Conditions

Ten Subjects for this study were chosen without any prior preferences. However, all subjects were between the ages 20 to 35. Among the ten subjects were two females. No known conditions such as hearing loss or auditory related diseases along with mental disorders were reported. All participants were briefed on the purpose of the experiment without giving them detailed information about the paradigms. The experiment was carried out in an enclosed room with an ambient noise level of ~ 40dB. To reduce visual activation, the lights were turned off. However, participants were asked to have their eyes remained open for the following two reasons. First, to reduce the prominent alpha oscillation when eyes are closed [18] and second, to minimize the chance of falling asleep.

2.2 Paradigms

To test the hypothesized auditory startle pathway, three paradigms have been developed. All paradigms were carried out under similar conditions as described in the previous section. Each of these paradigms involve the presentation of auditory stimulus which lasted 0.5 seconds. In this study, one trial is defined as one presentation of the auditory stimulus. One the other hand, one trial block consists of 30 trials. A more detailed explanation and purpose of each paradigm will be discussed, following the figure below.

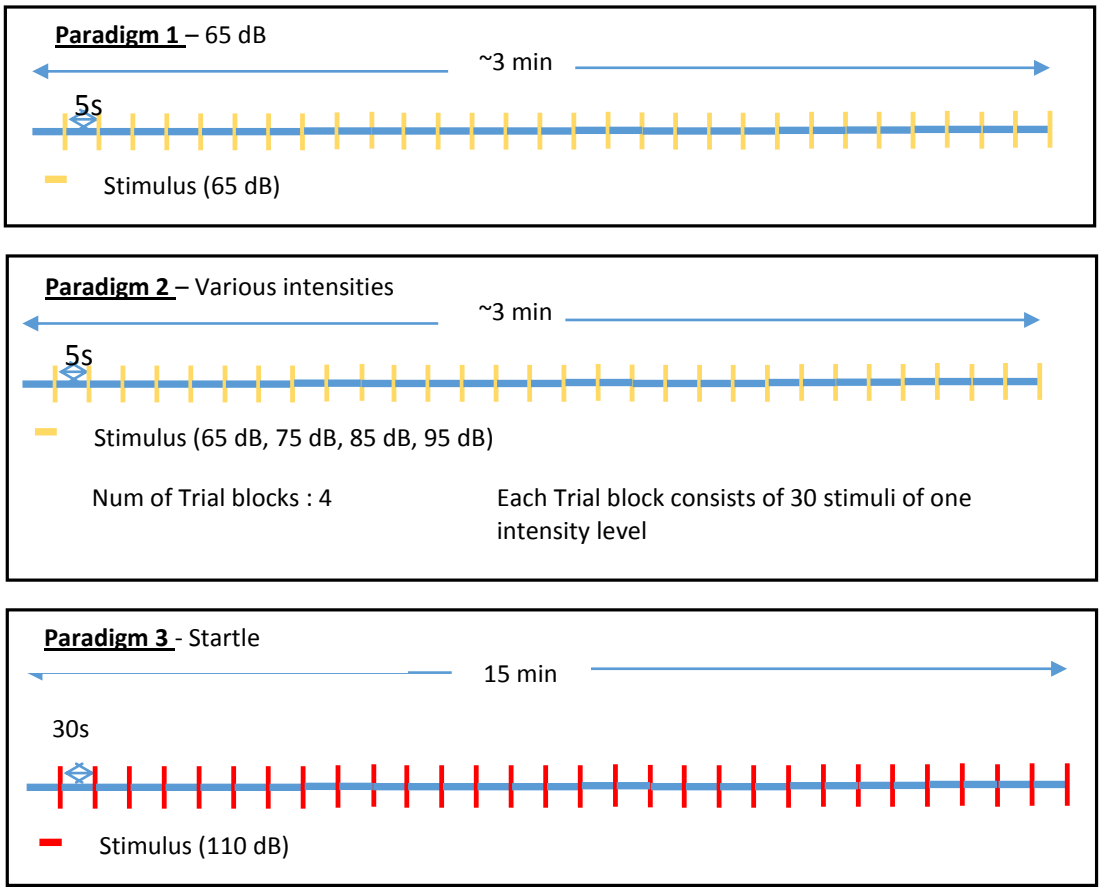

Figure 2: Three Paradigms developed to test the hypothesized habituation of acoustic startle reflex

2.2.1 Paradigm 1

Paradigm 1 is to test whether an auditory evoked potential (AEP) can be seen in the primary auditory cortex when a stimulus of 65dB is presented. The AEP has been well studied by various groups and can be considered a standard event related potential (ERP) paradigm. Each trial will yield a constant response that is time locked to the onset of the stimulus [19]. A time interval of 5 seconds was given between each trial. Paradigm 1 lasted about 5 min. The purpose of this paradigm is to test the validity of the hardware and software set up as by replicating a well-known phenomenon.

2.2.2 Paradigm 2

Paradigm 2 is essentially an extension of paradigm 1, where a trial-block of 30 auditory stimuli was presented to the participant at each stimulus intensity level. The first trial block utilized an auditory stimulus intensity of 65 dB, the subsequent trial blocks were then varied to 75dB, 85 dB, and 95 dB respectively. The inter-trial time interval remained the same as paradigm 1. This paradigm would essentially characterize the AEP with increasing sound intensity. This would answer the question whether an increased stimulus intensity is positively correlated with an increase AEP. The intensity level were capped at 95dB at which individuals reach a threshold for the elicitation of startle reflex.

2.2.3 Paradigm 3

Paradigm 3 was designed to test the activation of the dorsolateral prefrontal cortex. As described above, the DLPFC has been associated with the inhibition of unwanted reflexes. By presenting the participant with 110dB sound level for 30 times, an increased activation in the DLPFC is expected. The inter-trial time interval has changed from 5 seconds to 30 seconds. This adjustment was made to allow enough time for the participant recover from the startle movement. Furthermore, a compromise between Occupational Safety Health Administration safety sound exposure guidelines, as seen in Table 1, and extracting a large sample size was made. The participant was exposed to 110db for 15 minutes which was still considered safe while still able to extract 30 trials for further analysis.

2.3 Safety Sound Exposure

As described in the last section, participants were exposed to various sound intensities. To ensure their health and safety, a guideline from the Occupational Safety and Health Administration from the United State Department of Labor was consulted. The sound intensities in the paradigms varied from 65dB to 110dB. The stimulus length was 0.5 seconds and were presented 30 times. This exposure level was well below the proposed threshold at which permanent damage may occur. In Table 2, a sound intensity for 110dB had a safety threshold of 30 minutes per day [20]. That is the maximum time an individual can be exposed to 110dB continuous sound.

In general, the normal conversation has the power of 65 dB while the hand drill generates the sound with the power of 98 dB in which hearing may be damaged. The table 1 has shown some examples of different type of environment noise and its related power. In addition, as the power of sound increase, the time for one person to be exposed in that power will be decreased [21].

Table 1: OSHA Safety Exposure Level

Duration per day (hours)	Power of sound (dB)
8	90
4	95
2	100
1	105
0.5	110
.25 or less	115

Table 2: Various acoustic sound intensity levels

Environment Noise	Power of sound(dB)
Normal conversation at 3 inches	60-65
Telephone dial tone	80
Level at which sustained exposure may result in hearing loss	90-95
Jackhammer at 50 inches	95
Subway train at 200 inches	95
Hand Drill	98
Power saw at 3 inches	110
Pain begins	125
Pneumatic riveter at 4 inches	125
Even short term exposure can cause permanent damage	140

2.4 Experimental Setup

The experiment was carried out at the University of Houston in an enclosed room. The room was prepared with the necessary hardware as seen in Figure 3 below.

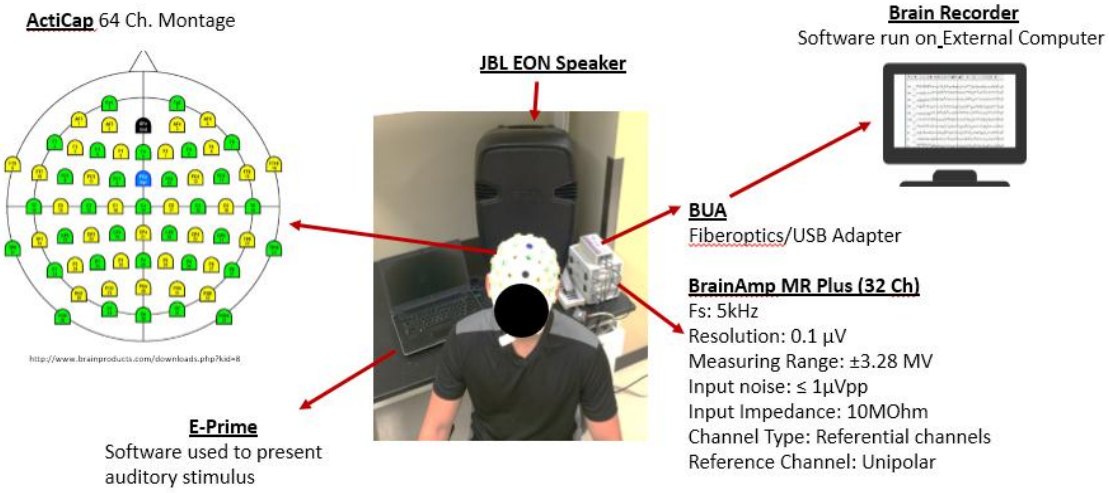

Figure 3: Experimental Setup in an enclosed room

Two computers were utilized where one presented the auditory stimulus while the other was dedicated for the data acquisition. E-Prime 2.0 is a specialized software

application for writing and presenting the auditory stimulus on the first computer. A feature of the software is the ability to mark the onset of the presented stimulus. However, a certain stimulus delay was introduced by the software and will be discussed at a later point. The laptop was then connected to a JBL EON speaker to deliver the auditory stimulus at various gain. The same laptop was also used to record the participant from the back to trace back

2.5 EEG Recording

2.5.1 EEG Cap

The EEG hardware equipment was purchased from Brain Products. A whole scalp 64-channel acti-Cap was deployed for each of the ten subjects (actiCap system, Brain Products GmbH, Munich, Germany). The cap's montage has been included in the appendix and followed the extended 10-20 international label system. Acti-Caps result in higher signal quality since they are fitted with active electrodes with impedance conversion. Electrode impedance were maintained below 10 kΩ for signal quality. These electrodes have a bigger profile and are not as comfortable for individuals laying down [22, 23]. However, for this particular study, participants are asked to sit down in a chair which would not compromise their comfort.

2.5.2 EEG Amplifier

The signals recorded from the electrodes are then amplified and digitized with BrainAmp MR Plus. These amplifiers were designed to be used in the MRI environment; however, they are compatible with Brain Products Acti-Cap. Each amplifier unit can be

utilized with 32 channels. The analog to digital converter has a resolution of 16 bit and a sampling frequency of 5000 Hz was chosen to record the data. The resolution was set to 0.1 µV per bit with an operating range of ±3.28 mV [23]. The output of the amplifiers were then send through fiber optic cables a second computer dedicated to acquire the signals. This computer was located outside the room where the signal has been live monitored through BrainVision Recorder software 1.10.

2.6 Data Analysis

2.6.1 Pipeline

The acquired recording for each subject underwent a series of data analysis. The following pipeline as shown in Figure 4 was used for signal processing. The first software to be utilized is Brain Analyzer where raw data is preprocessed. Brain Analyzer offers a wide range of simple tools such as filtering, segmentation, and EKG removal. However, the most intuitive tool to analyze event related potentials (ERPs) is to be able to easily see and manage the stimulus markers. Once the data was finished, the data was exported into MATLAB. EEGLAB is a MATLAB toolbox that specializes in EEG data and has been used extensively for independent component analysis (ICA), event related potential mapping (ERP), and short time fourier transform mapping (STFT).

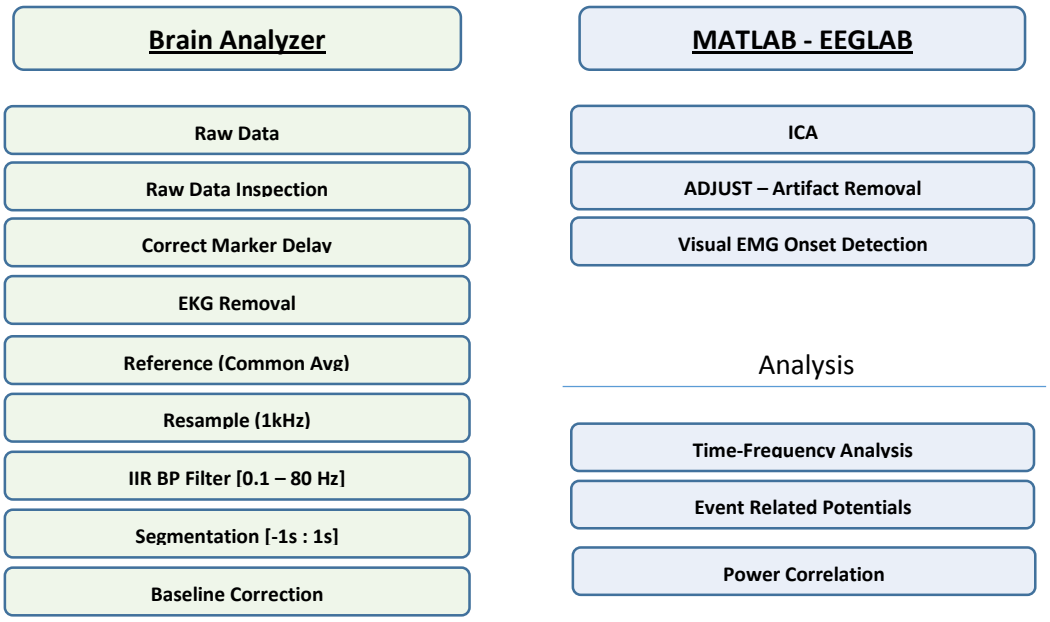

Figure 4: EEG Data Analysis Pipeline

2.6.2 EEG Pre-processing

Continuous raw EEG data was acquired by Brain Recorder with the specifications mentioned in the previous section. The data was then exported to Brain Analyzer which enabled intuitive visualizations of the raw data. Each dataset was first scanned for simple motion artifacts and bad electrodes, which were then thrown out. As E-Prime presented the stimulus and it was placed on the raw EEG data, the timing of the acoustic onset was tested once with an external microphone. A delay introduced by E-Prime was found which consistently placed the stimulus marker 114ms before the actual onset of the sound. A simple offset was then applied to all markers to correct the offset.

Brain Analyzer offers cardio ballistic (EKG) artifact removal that are present in the EEG. By placing an EKG electrode on each participant's back, the algorithm was able

to extract an EKG template. Using that template, each EEG channel was then subtracted by the template. This step minimized any activity unrelated to the auditory evoked potentials and was necessary to yield clean data for single trial analysis. It is important to mention that the acoustic startle response is accompanied by an increase in heart rate which sustains over several cycles. The EKG artifact removal was able to account for the change in heart rate as well. Afterwards, EEG electrodes were re-referenced against their common average. EMGs and EKG channels were excluded in re-referencing process.

The frequency interest of the AEP and startle AEP ranged from theta to upper gamma frequencies. It was therefore not necessary to keep the data at the sampling frequency of 5 kHz. Therefore, the data was down sampled to 1 kHz. An infinite impulse response (IIR) bandpass filter was applied to the data with a lower and upper bounds of 0.5 Hz and 80Hz, respectively. A 60H notch filter was also implemented to reduce electrical noise. Afterwards, each trial was segmented into epochs [-1s : 1s] with respect to the stimulus marker. The pre-processing step is then finalized by taking a baseline correction of each epoch.

2.6.2 Independent Component Analysis

EEG recordings from the scalp through electrodes is believed to be the summation of potentials in many different locations. Each electrode records signals from many different sources such as brain activity and muscle activity [24]. Independent Component Analysis (ICA) can be used to differentiate these underlying sources by separating the independent components. This tool can be used in a variety of

applications and field. However, for this particular study, ICA was used to mainly remove eye blinks which have been heavily correlated as startle reflexes [9].

In order to utilize ICA, certain assumptions were made as referenced in the book *Independent Component Analysis* by Hyvarinen A., Karhunen J, Oja E. The independent components (1) are assumed to be statistically independent, (2) must have non gaussian distributions, and lastly (3) where we assume the unknown mixing matrix is a square [24].

EEGLAB is a MATLAB toolbox that implements the ICA algorithm. Having 64 channel electrodes, ICA is able to compute 64 independent components. However, by excluding the EMG and EKG channels, only 61 independent components were calculated. An EEGLAB extension toolbox called ADJUST was then utilized to aid with the rejection of eye movement artifacts. ADJUST specializes in statistically determining the spatial and temporal variability of eye movement artifacts [25] as shown in the following figure. The following descriptions of the ADJUST statistics have been taken from the ADJUST Tutorial [25].

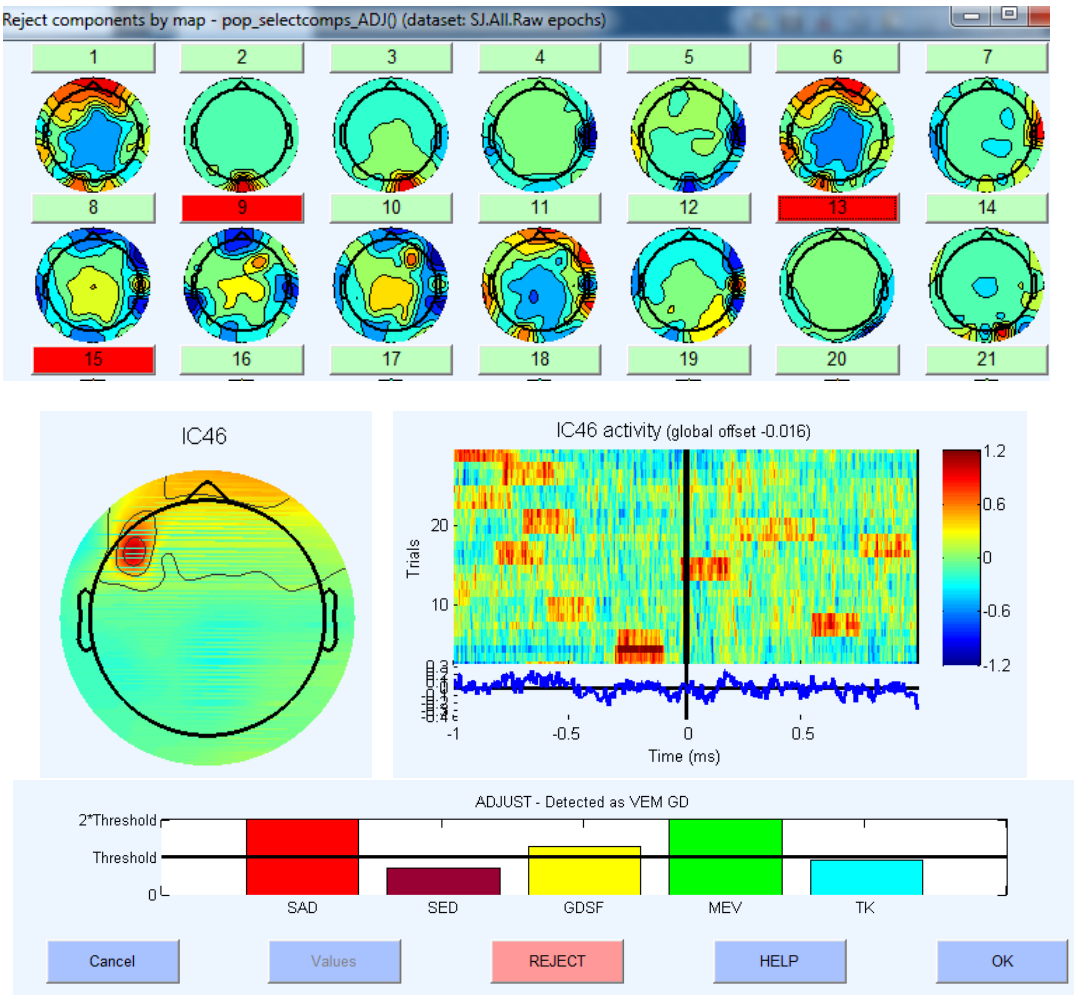

Figure 5: Eye movement artifact removal after calculating the independent components from ICA

ADJUST Toolbox

SAD: Spatial Average Difference

- Difference in amplitude between frontal and parietal lobes

SED: Spatial Eye Difference

MEV: Maximum Epoch Variance

- Computes max value over epochs of temporal variance (higher sensitivity than kurtosis)

GDSF: Generic discontinuities spatial features

TK: Temporal kurtosis

- Outliers in amplitude distribution, typical eye blink signature

3. Results

The following section will encompass the results for the three paradigms established in the Methods section. Each paradigm was designed to address a specific research question which ultimately leads to the proposed auditory startle pathway. Paradigm 1 was used to replicate an auditory evoked potential and verify the signal processing as well as hardware system. Paradigm 2 focused on the stimulus intensity dependence of the AEPs and its characterization. Finally, paradigm 3 tested the involvement of the DLPFC as a possible source of habituation of the acoustic startle reflex.

3.1 Paradigm 1

The auditory stimulation at 65dB successfully elicited an auditory evoked potential (AEP) which has shown characteristic waveforms comparable to previous literature works [26, 27]. The recorded data in Figure 6a represents an Event Related Potential (ERP) mapping of the Fz channel with time and trial number on the x- and y-axis, respectively. Each trial is represented by a colored horizontal line beginning with trial 1 at the bottom to trial 30 at the top. The average of all 30 trials can be seen in the same figure below the colored ERP mapping. The peaks in the averaged ERP was identified as typical N1 and P2 AEPs. The recorded AEP waveform was compared with previous literature work. The auditory evoked potentials were successfully measured, with waveform characteristics agree well with previous literature works. Figure 6a demonstrates the measurement and visualization of AEP

(auditory stimulus at 65 dB), where there appears apparent P1, N1, P2 peaks. Figure 6a depicts an event related potential mapping where the x-axis represents time

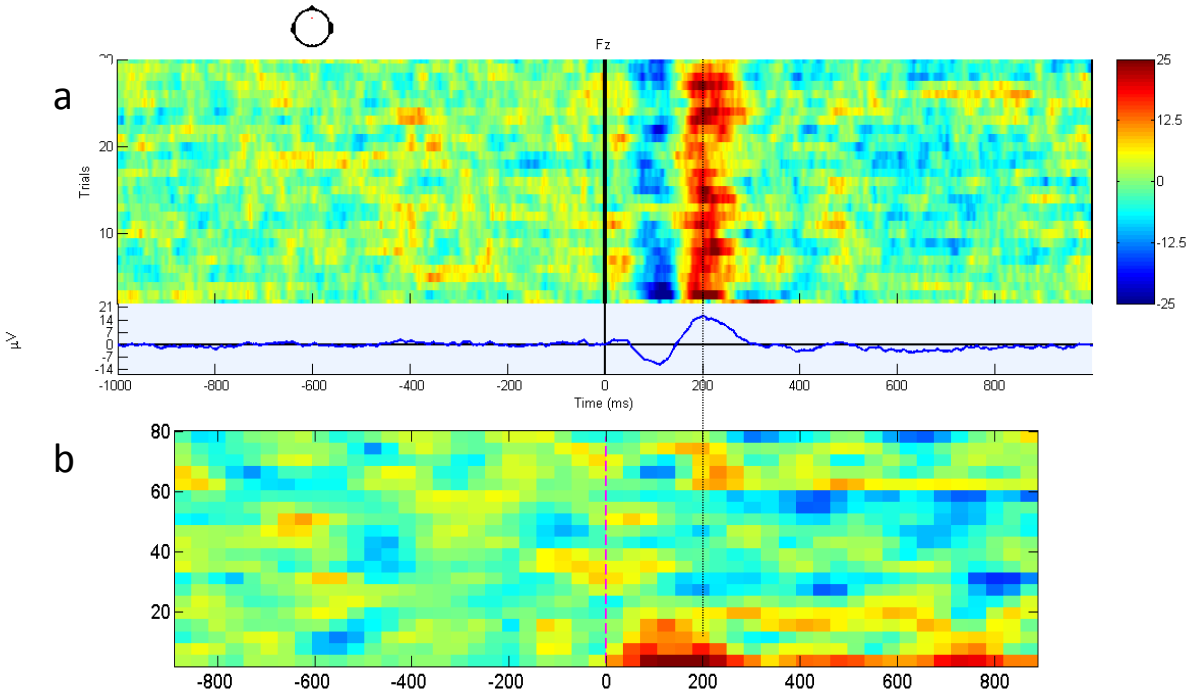

Figure 6: Auditory Evoked Potential of the Fz Channel at 65dB.

while the y-axis indicate the trial number. Activity averaged across all 30 trials yield in the typical auditory waveform. The frequency of the AEP has been computed with the short time Fourier transform which can be seen in Figure 6b. Most of the activity is concentrated below 18 Hz within a time window of 0ms to 250ms.

3.2 Paradigm 2

Detailed investigation of the AEP was conducted with the paradigm design that varied across sound intensities. A total of five intensities [65dB, 75dB, 85dB, 95dB, 110dB] was used, where each intensity constituted one trial block. Similar to Paradigm 1, the average of each intensity in the Fz channel was computed across trials and are shown in Figure 7A. Figure 7B depicts a closer representation of the first four stimuli

intensities. As this parameter increased, a higher P2 amplitude can be observed. This observation has been validated with previous work from Irene Neuner et al. where they also studied the variability of different sound pressure levels affecting the AEP in a fMRI environment [29]. Moreover, an earlier N1 and P2 onset seemed to be correlated with a higher sound intensity (see Figure 7b).However, the observed trend in both latency and amplitude only held between the stimuli intensities of 65dB to 95dB in which no startle

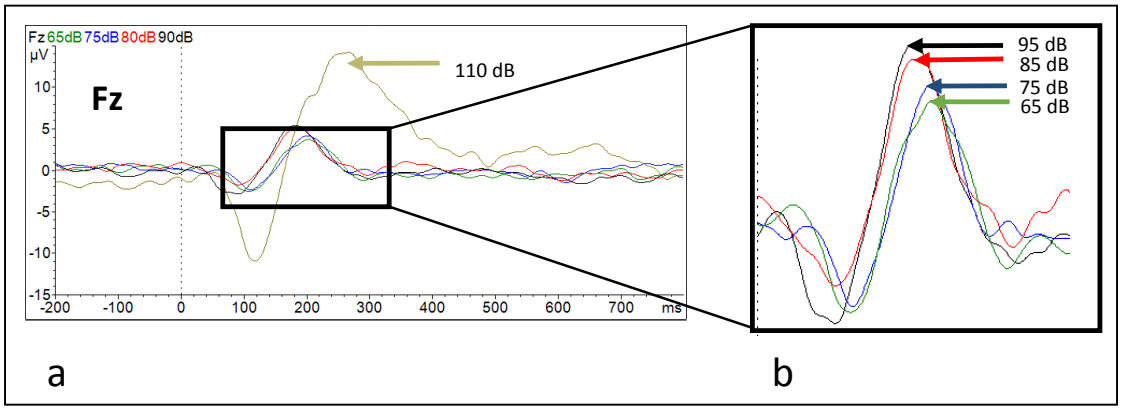

Figure 7: Averaged AEPs at various stimulus intensities

responses have been observed.

As the 110dB stimulus was presented, the characteristic AEP no longer held. A startle response has been recorded at 110dB through two EMG electrodes as seen in Figure 8 in section 3.3 and the corresponding cortical response in Figure 7a. The recorded AEP at 110dB showed significant amplitude increase in the N1-P2 complex. Moreover, the P2 peaked at ~250ms compared to ~190ms when a stimulus at 95dB was presented.

As a summary, the sound intensity characterizes the AEP in both amplitude and latency of the N1-P2 complex. A trend can be seen from intensities 65dB to 95dB until a certain threshold is met and a startle response can be observed. At 110dB, the

characteristic AEP waveform is severely deformed and can no longer be analyzed similarly to the other stimulus intensities. The next section will present various results in more detail as the 110dB stimulus was presented.

3.2 Paradigm 3

As mentioned in the previous section, a startle response has been observed at 110dB. During this state, both the EEG and EMG were recorded to analyze and characterize the habituation of acoustic startle reflexes. Typical EMG startle responses from the sternocleidomastoid (SCM) and the Bicep can be seen in Figure 8a and 8b, respectively. Each of these EMG responses have been averaged across 30 trials. Dreissen

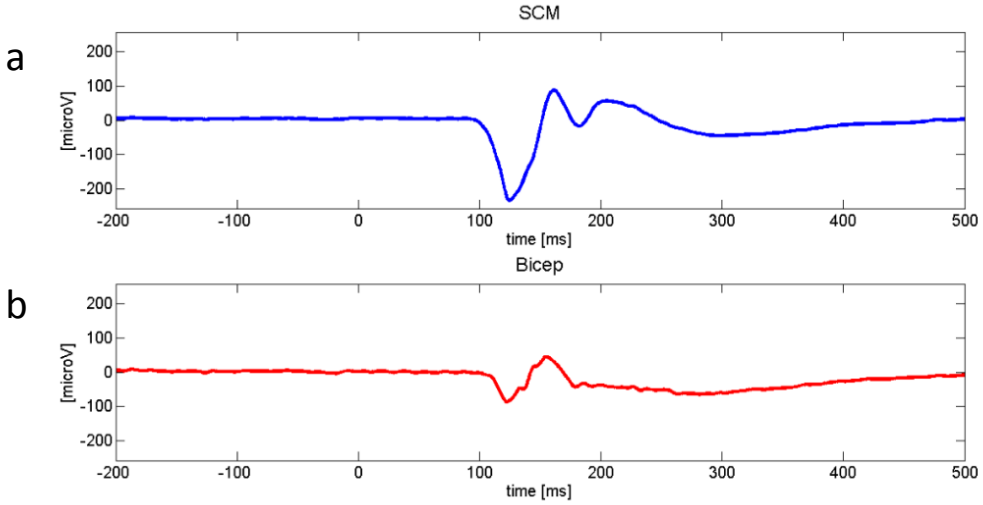

Figure 8: Averaged EMG startle recordings from the right Sternocleidomastoid (SCM) and right Bicep

et al. has reported a startle onset in the SCM at about 71ms to 75ms [28]. Our measurements show a consistent startle onset at the SCM of about 90ms to 110ms across all subjects. These findings do not contradict previous work and is believed to be reasonable. The onset of the Bicep activity lags the SCM onset response by 10ms to 25ms.

Focusing on the analysis of cortical activity involved in startle responses invoked by high intensity auditory stimulus, Figure 9 shows the hypothesized pathway once again, and Figure 10 presents analyzed results of cortical activity at several brain functional locations, for three subjects.

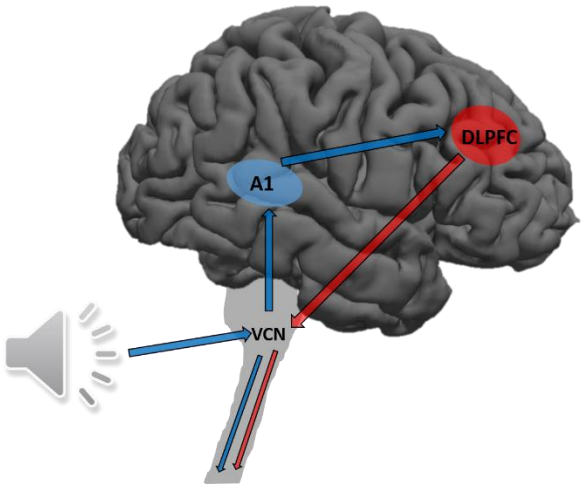

Figure 9: Hypothesized Pathway for the habituation of the Auditory Startle Reflex

Figure 10 depicts three different participants' cortical responses in ERP mappings. Each participants have been subjected to paradigm 3, where an auditory stimulus at 110dB has been presented for 30 times. Participants 1, 2, and 3 were chosen on the basis of the intensity of recorded EMG startle responses, ordered from high, moderate, and low, respectively. For each subject, three channels have been designated to represent the activity of interest. F3 is associated with the left DLPFC found on the left column of Figure 10, whereas F4 represents the right DLPFC located on the right column. The Fz channel is the frontal midline dividing both the F3 and F4 channels. From Figure 10, the most consistent activity has been found in the Fz channel. A positive peak

at 0ms to 100ms, prior to the high N1 activity show a magnitude increase with increasing trial number. To show whether this build up activity can be associated with a cortical process, the power of the positive activity between the stimulus onset and N1 has been computed for the Fz channel for each trial. To correlate this activity, the power of the EMG startle response between 100ms to 400ms has been computed as well. Both the EMG startle and Fz activity have been normalized against their respective maximum activity and have been plotted as shown in Figure 11.

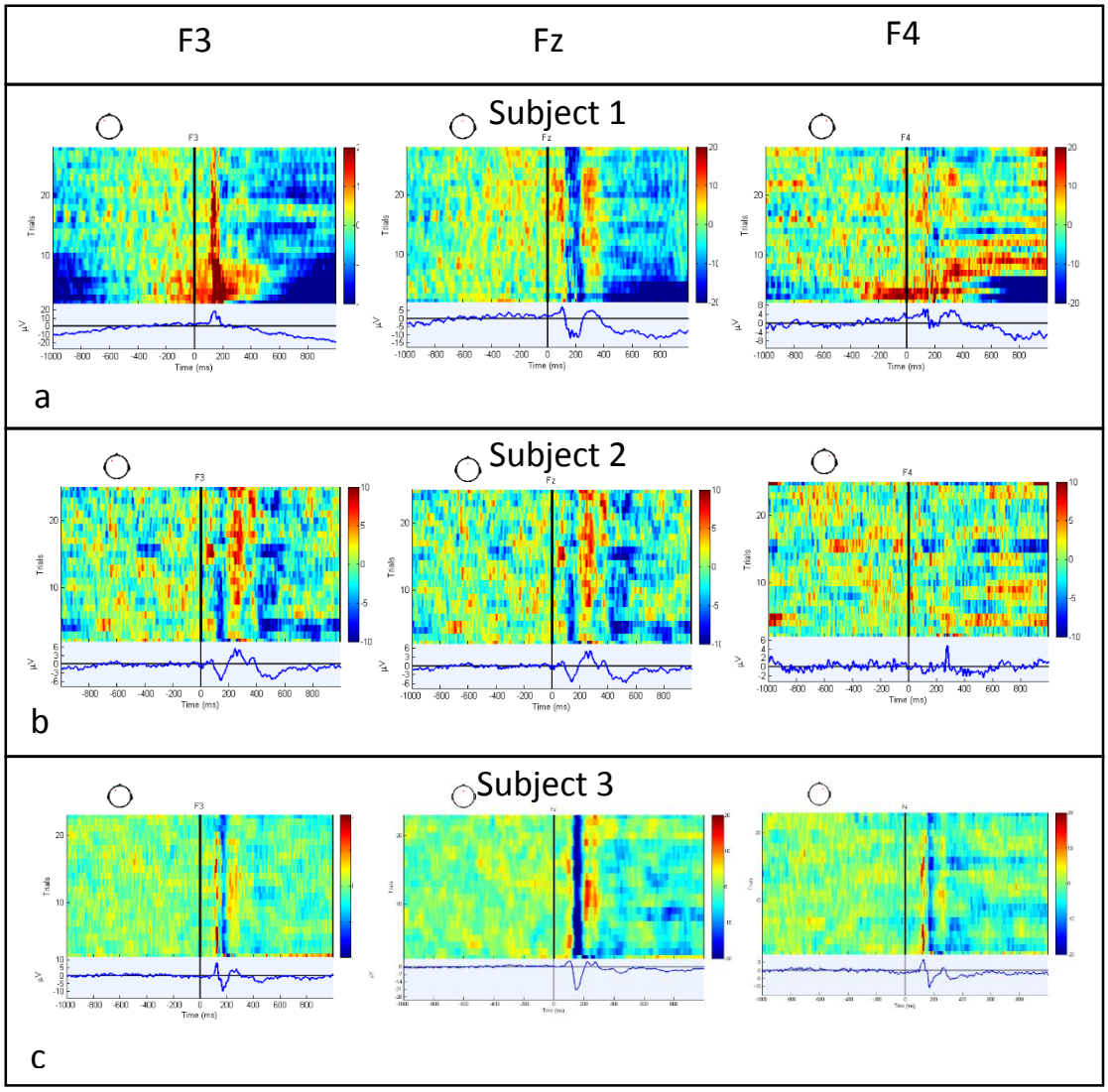

Figure 10: Evoked Related Potential Mapping of F3, Fz, F4 Channels

The most anti correlation was seen in Subject 1 at the Fz location. As the EMG startle power decreased slowly throughout the presentation of stimulus, activity in the Fz channel slowly increased.

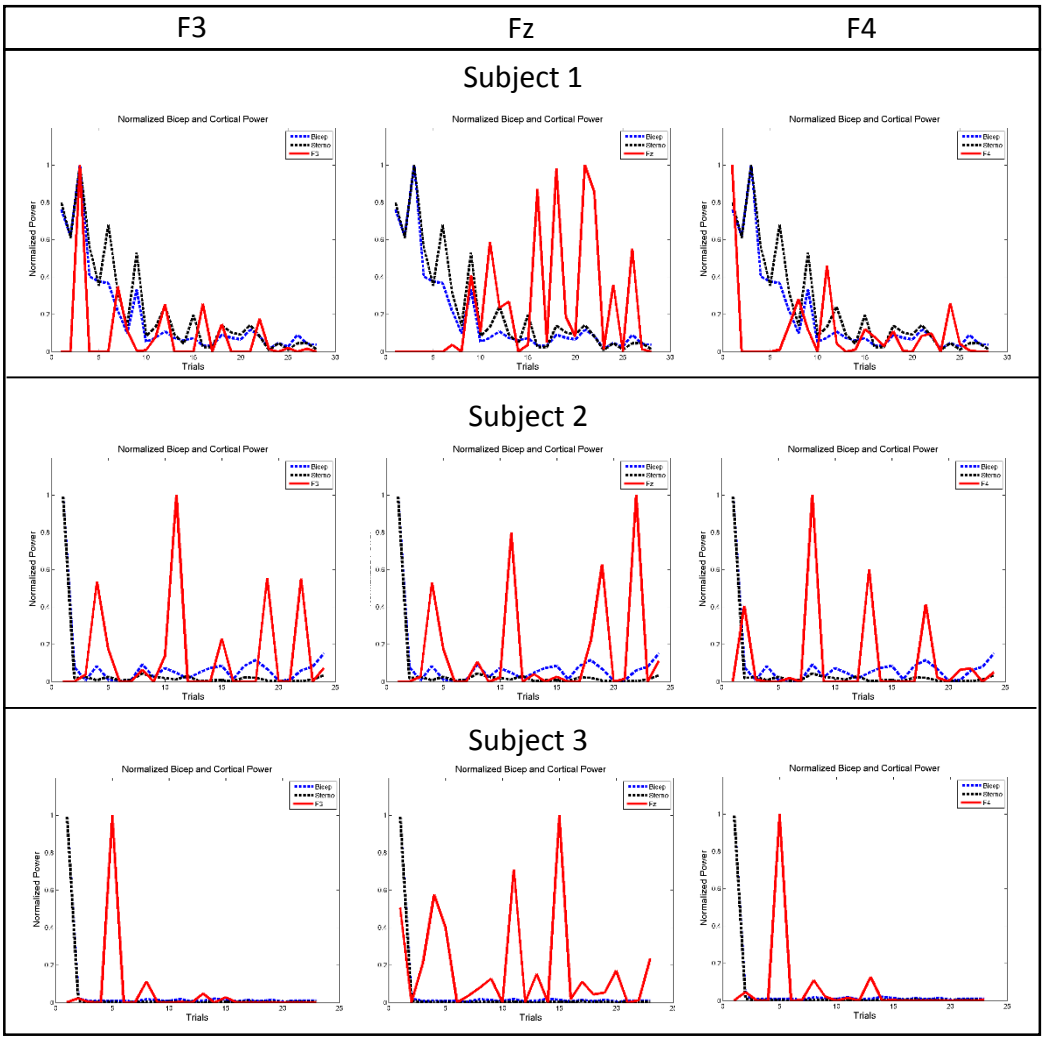

Figure 11: Comparison of Cortical and EMG signal power across trials.

4. Discussion

First, it is necessary to stress the importance of this study before a number of considerations are made regarding the study's results. Up to date, the majority of ASR studies involve the diagnostics of certain mental states and disorder. However, ASR has recently been recognized as a potential clinical treatment in the rehabilitation process. J. Valls-Sole and his group in Spain believe that ASR can be used to augment voluntary movement in patients who suffered from SCIs [4]. Moreover, the field of neuro rehabilitation medicine could expand the treatment for SCI to any other neuromuscular impairments to the central nervous system such as stroke. However, to push ASR into the clinical setting, its mechanism must be investigated carefully. The coupling of decreased startle responses with successive presentation of ASR has been widely observed. Nevertheless, this process called habituation is not well understood. It is therefore in our interest to investigate this process by proposing a pathway that could explain the documented observations.

Three paradigms have been implemented to test the hypothesized acoustic startle pathway which is believed to be habituated through the dorsolateral prefrontal cortex (DLPFC). As the startle response ceases, an increase in the cortical activity can be observed at the frontal midline. The frontal midline is represented by the Fz channel and is located between the left and right DLPFC denoted as F3 and F4, respectively. This anti-correlation between the EMG and cortical activity is best represented by Subject 1 as shown in Figure 11. However, Subject 2 and 3 did not have similar findings which do not fully support the consistency of the new results. Furthermore, the left and right DLPFC

do not exhibit similar cortical activity as the frontal midline. The next few paragraphs will discuss the results in detail and suggest considerations for future studies.

4.1 Paradigm 1

Paradigm 1 has successfully shown the auditory evoked potential following the presentation of a 65dB auditory stimulus. This result is supported by numerous literature and validates the first condition of the hypothesized startle pathway. As the stimulus is presented at 65dB, the sound pressure travels through the ear canal and its signal is transmitted to the cochlear nuclei. Since 65dB does not meet the threshold to elicit a startle response, the cochlear nuclei does excite the startle pathway in the brainstem [7]. The signal travels through the midbrain towards the cortex and excites the primary cortex instead, which results in the auditory evoked potential. By successfully replicating this response with the correct latencies, it was possible to validate the hardware setup as well as the signal processing.

4.2 Paradigm 2

4.2.1 Amplitude

Having confident that the AEP in paradigm 1 could be replicated, paradigm 2 was implemented to further characterize the AEP in more detail. By presenting various stimulus intensities, the amplitude and latencies have changed accordingly. A higher intensity was associated with a higher AEP amplitude suggesting a positive relationship of both the N1 and P2 peaks to stimulus intensity. The AEP waveform was consistent between 65dB to 95dB in which no startle has been observed. At 110dB, a startle

response has been recorded and a deformed AEP became apparent in the Fz channel as shown in Figure 5a and 10. Therefore, it is possible to correlate the change in AEP at 110dB with the startle response. Examining the prolonged AEP, it is possible to identify a small P2 peak as indicated in Figure 10.

4.2.2 Latency

Similar to the amplitude changes when the stimulus intensity increased, the N1-P2 complex latencies reveal a particular pattern. With higher stimulus intensity, a shorter N1 and P2 onset can be observed which could have been the result of the order of stimulus intensity presentation which had the following order: 110dB, 65dB, 75dB, 85dB, 95db. As discussed in the method section each intensity level was presented in a trial block. By subjecting the participant with the 110dB trial block first, the acoustic startle response can be maximized due to no prior acoustic exposure. However, for the very same reason, the subject could have been acoustically primed for the latter trial blocks without exhibiting a startle response. This shortened latency with repetitive stimulation may be due to sensitization rather than habituation as described by Pilz and Schnitzler [10].

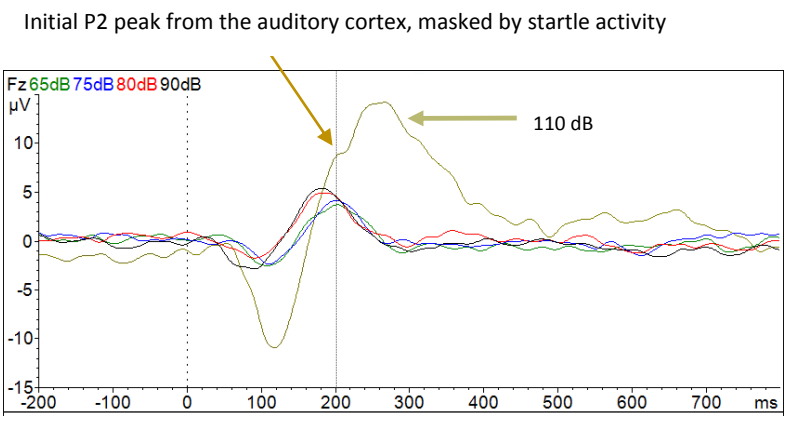

Figure 12: Averaged AEPs at various stimulus intensities

4.3 Paradigm 3

As described in the previous sections, DLPFC is a brain region that has been associated with the inhibition of reflexive saccades [14]. This paradigm tested whether the DLPFC can be linked to the inhibition of acoustic startle reflexes. As presented in Figure 8, three participants have been analyzed. The right and left DLPFCs are associated with the F3 and F4 channels; however no consistent activity could have been concluded. Subject variability was too great that it was difficult to extract repetitive information. However, the limited analysis revealed a possible activation between 0ms and ~100ms that could be associated with habituation, especially in the Fz channel. In subject 1, an increasing positive activity (red color) can be seen from trial 10 to 25. To better visualize the relationship between the EMG power and cortical power at particular channels across trials, Figure 9 has been computed. In subject 1, the EMG power has a steady and slow decrease in power across trials while the Fz activity slowly gains in power. Therefore, a clear anti correlation can be seen in this particular subject. The hypothesis suggests that the DLPFC (represented by F3 and F4) should display similar pattern, yet this trend is not apparent.

Subject 2 and subject 3 display very different EMG startle behavior compared to subject 1. A very sharp decrease in EMG power after the first startle stimulus exposure can be observed. This suggests that the habituation process occurred too fast; which inhibits the startle response dramatically after the first stimulus. This subject variability raises an important question. Is it better to study the habituation process in certain participants then others? Is it possible that subject 2 and 3 habituated the startle

response so fast that activity after trial 3 in their respective Figure 9 is irrelevant? These questions have to be addressed and considered in future studies.

5. Conclusion

Three paradigms have been developed to test the involvement of the DLPFC in the habituation of acoustic startle reflex. Paradigm 1 has been successfully implemented and the results were verified with published literature. Paradigm 2 achieved the results as expected as well and a threshold to elicit the startle response has been confirmed. The AEPs induced by startle and non-startle stimuli have distinguished features and separate signal processing methods must be used, respectively. The non-startle AEP is considered to be stationary and elicits the same response with each stimulus. Minimum motion artifacts exists and averaging across trial is an effective tool to reveal the N1-P2 complex. On the other hand, startle AEP are masked with motion artifacts which reduces the signal to noise ratio. Moreover, this constraint also limits the time window at which signal processing can effectively be applied. The signal is non-stationary as each stimulus given, a new response is presented. Single trial analysis is therefore necessary to explore the data.

Paradigm 3 tried expose the underlying habituation process which is the driving force for the varying responses across trials. This process suggests that the inhibition generally builds up over successive number of trials. Paradigm 3 also established that each person has a different habituation rate to the acoustic startle stimulus. Participants who have a slow habituation rate show more anti-correlation between the EMG startle

activity and activity at the frontal midline, Fz. This consideration may play an important role for future studies. In conclusion, however, our results do not support the hypothesis at this time which states that the dorsal lateral prefrontal cortex is associated with the habituation of acoustic startle response by successively inhibiting the acoustic startle reflex.

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