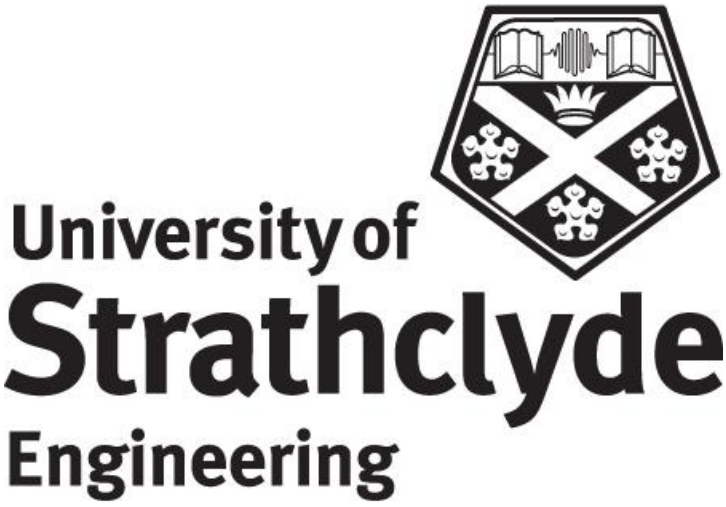
Development of EEG test for psychiatric disorder – 'Schizophrenia'



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August, 2011

This thesis is submitted in partial fulfillment of the requirements for the degree Master of Science, Department of Bioengineering, University Of Strathclyde, Glasgow.

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ABSTRACT

Schizophrenia is a long-term mental illness affecting ~1% of the population. Patients exhibit symptoms such as hallucinations, delusions, emotional flattening, as well as functional deficits. Structural and functional alterations in the prefrontal cortex and abnormalities in other brain regions have been reported.

Early diagnosis of Schizophrenia is challenging and there are few appropriate biomarkers to study associated brain abnormalities. Deficits in mismatch negativity (MMN), an electroencephalography (EEG) measure to distinguish rare deviant auditory stimuli, are specific to Schizophrenia, and associated with functional and cognitive disability. The MMN amplitude is reduced compared to healthy controls. Reduced auditory P300 amplitude also reflects deficits in cognitive processing in Schizophrenia.

This project measured five deviant MMNs and P300 in 13 healthy male controls and investigated increased inter-stimulus interval (ISI), allowing more data to be included in a novel frequency analysis. Time domain analysis was performed on raw and filtered data while time/frequency analysis was performed on MMN and P300 raw data in one of the subjects. With an ISI of 1 second, the latency of MMN deviants was found to be within the range reported in the literature with shorter ISIs of ~500 ms. Peak amplitudes were lower in group averages than values reported in the literature. However, the longer ISI increased the P300 amplitude for averaged raw and filtered data. A pilot study of 500, 750 and 1000ms epochs did not reveal any differences in the peak latency or amplitude. The time/frequency analysis showed increased power in the delta and theta bands in the majority of MMN deviants, perhaps reflecting unconscious stimulus processing. Increased gamma activity was seen in P300 standard, deviant and difference data, reflecting the conscious nature of the task and suggesting the presence of higher cognitive processing. The increased ISI improved frequency analysis, reduced amplitude and provided appropriate MMN latencies.

ACKNOWLEDGEMENT

*I dedicate this study to my younger sister **Surabhi** and my parents.*

The satisfaction and euphoria that accompanies the successful completion of the thesis would be incomplete without mentioning of the people who made it possible and whose constant guidance and encouragement have kept me going on.

*I am extremely thankful to our Head of the Dept. Bioengineering **Prof. Bernard Conway** for having given me this opportunity to carry out this thesis.*

*I express my heartfelt gratitude for my supervisor **Dr. Campbell Reid** as his guidance, suggestions, motivation and time devoted to each and every aspect of my thesis has made this study more fulfilling. I am thankful to clinical psychiatrist **Dr. Bob Hunter** for his visit and sharing his valuable thoughts on the study in spite of his busy schedule.*

I would also like to thank all my lab mates in the neurophysiology lab Ange Tano, Radhika Menon, Danial Kahani and Bahman Nasseroleslami who have been very inspiring and helpful during the study period.

I am very grateful towards all the participants who made their time for helping me conduct the study on them.

Without the support from friends, this project would not have been an enjoyable experience; I thank them for their love and faith in me.

***"There must be a beginning of any great journey, but its continuing to the end until it's thoroughly finished yields true glory." – Sir Francis Drake**.*

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Chapter 1: INTRODUCTION

1.1 Schizophrenia: The Disease

Mental illness is a seriously underestimated component of the 'global burden of disease' that is projected to increase at a rate greater than that of cardiovascular disease. Schizophrenia is one of the five psychiatric disorders that are in the top 10 causes of disability when measured against disease length (Murray & Lopez 1996). Schizophrenia is estimated to affect about 0.5% to 1% of the total world population (Weinberger & Harrison, Chapter: 1, Schizophrenia, 3rd edition). Schizophrenia is a mental disorder that makes it difficult for a person to differentiate between real and unreal experiences, to think logically, to experience and express normal emotions and respond to them, to behave normally in social situations and to interpret ongoing events and make predictions about future circumstances (Weinberger & Harrison, Chapter: 1, Schizophrenia, 3rd edition). It paralyses the patient by attacking the most crucial part of the body: the brain and its complex functioning. Schizophrenia affects men and women equally and affects all ethnic groups equally throughout the world (Schizophrenia Booklet 2009, National Institute of Mental Health).

The disease can have an early onset (as early as adolescence) and may persist chronically with considerable levels of severity that not only affects the patient greatly but also leaves the patient's family tormented for many years. In young people who develop Schizophrenia, the stage of the disease where they start to isolate themselves, withdraw from others and there is an increase in unusual thoughts and suspicions is referred to as the "prodromal" period (Schizophrenia Booklet 2009, National Institute of Mental Health). Schizophrenia is highly heritable and it is believed that genetic factors contribute as much as 80% towards developing the illness. Several environmental factors such as drug abuse (cannabis, cocaine), prenatal infection, social stress and obstetric and perinatal complications increase the likelihood of developing Schizophrenia (Tandon et al., 2008). There have been many studies conducted to determine its etiology and pathophysiology over decades now; yet accurately defining the illness remains a challenge to this day.

1.2 Symptoms

In 1919, Kraepelin came up with the concept of "Dementia Praecox" which led to the representation of Schizophrenia as an illness of early and progressive deterioration. The term Schizophrenia was coined by the Swiss scholar Eugen Bleuler; from the Greek Schiz meaning fragmenting or splitting and phren meaning mind. His idea was to highlight that fragmented thinking was one of the most crucial feature of this disease and he also believed that the disease had a chronic and deteriorating effect. Psychiatrist Kurt Schneider emphasized psychotic symptoms like delusions and hallucinations which were referred to as first rank or Schneiderian first rank symptoms. (Weinberger & Harrison, Chapter: 1, Schizophrenia, 3rd edition). For decades researchers have believed Schizophrenia to be a heterogeneous disease.

The heterogeneous nature of the condition has made diagnostic criteria difficult to define. Since the early 1980s Schizophrenia has been characterized by two broad categories of symptoms which are based on phenomenological profiles (Kay et al., 1987):

- Type I or Positive symptoms: These symptoms represent an excess or distortion of normal functions:

    1. Delusions: These are false beliefs or thoughts which are not based on reality. For example, the person might feel that other people are talking to him or that messages are being communicated to him through radio, TV or any other media.

    2. Hallucinations: Seeing, hearing, tasting, smelling or feeling things that do not exist. The patient might hear imaginary voices which command them.

    3. Catatonic motor phenomena and bizarre movements: Catatonia is a movement disorder, in which the person remains still, does not move at all or respond to others. A person might repeat a movement several times and portray a very agitated body movement (Schizophrenia Booklet 2009, National Institute of Mental Health).

4. Positive formal thought disorder: This describes inexplicable language; either speech or writing that reflects thinking. Tangential and incoherent speech is considered to impair communication, and is a clear positive symptom according to Diagnostic and Statistical Manual of Mental Disorders IV (DSM_IV). The person might also make up meaningless words (neologism).

- Type II or Negative symptoms: These symptoms reflect a reduction or loss of normal functions. They might exist during the stage of low or no positive symptoms in the patients. They are not easy to diagnose as these symptoms are not as clearly bizarre as the positive symptoms.

    1. Cognitive deficits: These are deficits related to concentration and memory. Cognitive impairments are believed to be present prior to the onset of psychosis and tend to persist throughout the course of illness (Tandon et al., 2008).

    2. Affective flattening / Blunting: The range and intensity of the emotional expression is diminished which includes facial expressions, tone of voice, eye contact and body language.

    3. Alogia or poverty of speech: There is reduced fluency in speech and productivity which reflects slow or blocked thoughts.

    4. Attention impairment: This along with lack of content of speech is considered to be closely related to disorganized or neurocognitive symptoms of Schizophrenia.

    5. Avolition: This is the reduction or inability to initiate and persist in goal-directed behavior. It is believed to have a direct impact on functional

outcomes in Schizophrenia. It indirectly influences neurocognitive dysfunction.

6. Anhedonia: This is defined as a diminished capacity to experience any pleasant emotions or having trouble in experiencing pleasure or interest. Though a Schizophrenia sufferer has impairment in outward emotional expression, findings suggest that they do not lack in the internal experience of emotions. (Referred from Diagnostic and Statistical Manual of Mental Disorders IV, Schizophrenia Booklet 2009, National Institute of Mental Health)

- Mixed Schizophrenia: Both positive and negative symptoms are prominent or neither. Both positive and negative symptoms are not mutually exclusive; they both can be present at the same time (Kay et al., 1987)

There is a close relationship between the negative symptoms and prefrontal hypometabolism, specifically in the right dorsolateral convexity (Wolkin et al., 1992). The study also revealed that this relationship was not an artefact of age or cerebral atrophy and was independent of the severity of positive symptoms (Wolkin et al., 1992).

Positive symptoms are related to hyperdopaminergia suggesting that Schizophrenia is a neuroleptic responsive disorder while the negative symptoms occur due to stable neuro-developmental deficits. Also, the correlation and multiple regression analysis done by Kay et al indicates that positive symptoms are believed to be strongly associated with unusual thought content and anxiety while negative syndrome reflect motor retardation, lack of judgment and insight and active social withdrawal (Kay et al., 1987).

1.3 Types of Schizophrenia

There are four common types of Schizophrenia that are extensively recognized. These types are based on the symptoms exhibited (Diagnostic and Statistical Manual of Mental Disorders IV).

1. Paranoid Schizophrenia – A person feels extremely anxious, suspicious, persecuted, or grandiose, or experiences a mixture of these emotions. They often get angry and argumentative and falsely believe that others are trying to harm them or their loved ones.

2. Disorganized Schizophrenia – A person is often incoherent in speech and thought. They have problems with expressing their ideas clearly and may exhibit childlike behavior, show little emotion but do not have any delusions.

3. Catatonic Schizophrenia – A person is negative, withdrawn, mute, and often displays a constant state of unrest with very unusual body positions and odd facial expressions.

4. Residual Schizophrenia – A person no longer experiences delusions or hallucinations, but has no motivation or interest in life.

1.4 Methods of Diagnosis and Treatment

The diagnosis of Schizophrenia is still considered to be challenging. The first few years after the diagnosis are distressing and may lead to higher suicidal tendencies among patients (Tandon et al., 2008). Usually, a Schizophrenic will go through a series of assessments to correctly diagnose the symptoms and severity of the disease. No single symptom is enough for the diagnosis. The diagnosis of Schizophrenia includes a pattern of symptoms along with any impairment in social and occupational function. In European countries the International Classification of Disease criteria (ICD-10, Classification of Mental and Behavioral Disorders, World Health Organization, Geneva, 1992), which emphasizes first rank symptoms, are used in diagnosis, while the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) are widely used in the US and the rest of the world. According to the DSM-IV, it is a requirement for a patient to display two or more evident positive symptoms for at least a month.

A large number of assessment tools have been devised to help aid diagnosis. The following are the most commonly used scales of assessment:

1. Scale of Assessment of Negative Symptoms (SANS)
2. Scale for the Assessment of Positive Symptoms (SAPS)
3. Global Assessment of Functioning Scale (GAF)
4. Positive and Negative Symptom Scale (PANSS)

Most of the scales follow a similar pattern of assessment. Usually a series of interviews are conducted to assess different criteria in the patients to categorize their various symptoms and severity based on a particular scale. In general, there are different phases of interview which are intended to obtain information regarding the current life circumstances and history of the patient. Personal interviews are then conducted to determine any postural impairment, deficits in thought processing, speech and content, affective and motor responses, the state of mood, anxiety and reasoning ability through a series of questions. Also assessed are any peculiar symptoms such as delusions, hallucinations, grandiosity and its severity, presence of any social behavior beyond the limits of testing and susceptibility to disorganization and various other factors. Depending on the results, a point rating is evaluated to represent the level of psychopathology in the patient (Kay et al., 1987).

The onset of psychotic symptoms in Schizophrenia is usually during adolescence or early adulthood. However, the age of onset is earlier in males and they have a prolonged period of untreated illness with much more severe negative and cognitive symptoms (Tandon et al., 2008).

Recent neuroimaging studies have found that there is an increase in the subcortical release of the neurotransmitter dopamine during phases of positive psychotic symptoms while a reduced level of dopamine release is noticed in the pre-frontal cortex during negative symptoms and cognitive impairment (R. Weinberger, J. Harrison, Schizophrenia, Chapter:15, 3rd edition).

Anti-psychotic medication has been used to treat Schizophrenia from a long time. The medication is given in order to either reduce or eliminate the symptoms in patients. The anti-psychotic drugs such as Chlorpromazine (Thorazine), Haloperidol (Haldol), Perphenazine (Etrafon, Trilafon), Fluphenazine (Prolixin), which have been used since the 1950s are called "typical" antipsychotics. The medications used presently are called second generation or "atypical" antipsychotics such as Risperidone (Risperdal), Olanzapine (Zyprexa), Quetiapine (Seroquel), Ziprasidone (Geodon), Aripiprazole (Abilify), Paliperidone (Invega) (Schizophrenia Booklet 2009, National Institute of Mental Health).

People with newly diagnosed Schizophrenia are treated with oral antipsychotic medications (NICE clinical guideline). Dopamine-2 antagonists are believed to be the only effective antipsychotic agents at present. Clozapine (Clozaril) is one of the widely used antipsychotics. Antipsychotic drugs help cure positive symptoms to a greater extent though they have limited efficacy on negative symptoms and cognitive deficits and do not make an effective difference in "real-world" functioning (Tandon et al., 2008; Light & Braff, 2005). The negative symptoms have a stronger relationship with the cognitive deficits and the functional outcome in a patient (Light & Braff, 2005). Recently, there has been a significant effect on neuroleptic-refractory positive symptoms and suicidality with the use of Clozapine which is an atypical antipsychotic drug (Tandon et al., 2008). Although, Clozapine has been very effective for patients who do not respond to any other antipsychotic medication, the use of Clozapine can sometimes cause agranulocytosis (loss of white blood cells) in patients (Schizophrenia Booklet 2009, National Institute of Mental Health).

Though these antipsychotic drugs are effective in curing the symptoms, these drugs have the potential to cause metabolic effects including weight gain, higher risks of cardiovascular disease, diabetes and other adverse effects depending upon the drug used (NICE clinical guideline). Extrapyramidal side effects have also been a common side effect. These affect the nerves and muscles which control and coordinate movements. The long-term usage of typical anti-psychotics may lead to an extrapyramidal side effect

called tardive dsykinesia which causes uncontrollable muscle movements in the patient, commonly seen around the mouth region (Schizophrenia Booklet 2009, National Institute of Mental Health).

Patients in an acute phase may require hospitalization to help monitor suicidal tendencies, problems with drugs or alcohol abuse and to treat severe delusions or hallucinations. Cognitive behavior therapy is a psychotherapy that deals with thinking and behavior. One of the common symptoms in patients of Schizophrenia is to hear voices which are not real. This therapy helps them to find ways to deal with these voices. Also, it may help reduce cognitive deficits and reduce psychotic symptoms by helping patients to determine reality more clearly (Schizophrenia Booklet 2009, National Institute of Mental Health).

It is very difficult for patients to get back to their normal life while they are still on medication. It is necessary for family members to understand the requirements of the patient and provide them with care and support. Psychosocial education is necessary for both family members and the patient to cope with the problems together so that it can help the patient to slowly get back to a normal life and thus reduce the chances of a relapse occurring (Tandon et al., 2008; Schizophrenia Booklet 2009, National Institute of Mental Health).

1.5 Biomarkers in Schizophrenia

According to various studies, the higher-order cognitive deficits and psychosocial behavior in patients of Schizophrenia have shown a correlation to the dysfunction in the neural activity at the pre-attentive and early attentive levels of information processing (Light et al., 2010). For decades now, neurophysiological methods have been widely studied and used in the research of Schizophrenia. These measures have many advantages that have led to them being researched as potentially useful biomarkers for developing new drug therapies and improving the overall functional outcome in patients. These neurophysiological methods can be adapted into passive paradigms where a subject does not have to engage himself in a task or pay any attention. This is

advantageous in testing on younger patients and also in the patients who are difficult to engage in behavioral studies. Usually these methods provide a wealth of information on the underlying neuronal activity in the cortex which relates to the perceptual and cognitive dysfunction in Schizophrenia. Moreover, the high temporal resolution of the neurophysiological biomarkers is useful in tracing the flow of information from the regions of auditory cortex to the association areas where the auditory data is interpreted and processed. This helps in determining any impairment in auditory information processing at an earlier stage of the disease (Javitt et al., 2008). Also, these neurophysiological processes can be used to determine any pathway from genetic predisposition to the illness and help in identifying susceptible genes as well, thus proving to be useful as biological markers or 'endophenotypes' of the disease (Hall et al., 2006).

1.6 Aim of Study

Functional and structural brain abnormalities are present at the onset of the illness (Tandon et al., 2008). Many researchers have indicated that electrophysiological assessment using electroencephalography (EEG) can be used as prospective biomarker for detecting the onset of Schizophrenia at an early stage.

Mismatch Negativity (MMN) is an event related potential (ERP) which is defined as a "pre-attentional change detection response" which has been repeatedly shown to be reduced in patients with Schizophrenia (Bodatsch et al., 2010). In patients with Schizophrenia, MMN can be used to characterize the integrity of sensory network dysfunction (Keshavan et al., 2008). It is believed that the MMN is not affected by IQ or medication. MMN can be elicited in the absence of attention, which makes it a useful assessment tool for young and impaired patients (Duncan et al., 2009). There has been evidence of a reduction in amplitude of the duration deviant MMN and intensity deviant MMN early in the course of illness, whilst the frequency deviant MMN deficit correlates to the length of the disorder. The deficits in mismatch negativity correlate with the severity of negative symptoms and impaired functional outcome in chronically ill patients (Javitt et al., 2008). Also MMN deficits have been noticed in the unaffected first

degree relatives of patients with Schizophrenia. The sensitivity of different types of deviant to different features of the disease, the specificity of deficits to Schizophrenia and the genetic links to unaffected family members make it a preferable choice as a biomarker (Michie et al., 2000).

The auditory evoked P300 is an ERP which is elicited by infrequent, task-related audio stimuli. It is useful in analyzing the conscious reaction to stimuli, as the participant must indicate whether a stimulus is rare or frequent by pressing a button. Usually, an auditory P300 is elicited using an oddball paradigm in which low-probability rare stimuli are intermixed with high-probability frequent stimuli. The latency and amplitude of P300 is delayed in patients of Schizophrenia in comparison to healthy controls (Tandon et al., 2007).

There has been an established relationship between mismatch negativity and P300 with higher order cognitive measures and psychosocial functional outcome (Hermens et al., 2010). Although P300 deficits are not exclusively related to Schizophrenia, the fact that P300 is a measure of conscious processing of stimuli makes it an ideal measure to use in conjunction with MMN which is a measure of subconscious processing. The two measures may then reflect a combination of underlying cortical pathology seen through both negative and positive symptoms.

The aim of the study is to measure MMN and P300 in healthy male controls. The onset of the disease is earlier in males, making a male control group selected from the available student population easier to match to a typical average onset age. Developing the experimental protocol and analysis techniques on healthy control data is vital before tests are carried on patients and compared. In addition to the traditional time domain analysis, a time/frequency analysis is planned to obtain a better insight into MMN and P300 data, which has not been extensively studied in the literature. The incompatibility of traditional time series data processing methods (filtering) with the time/frequency data processing requirements will be investigated. A suitable compromise between providing sensitive and repeatable time domain measures with unfiltered, high

bandwidth data for the frequency analysis will be demonstrated. This study aims to provide a key foundation to establish MMN and P300 as potential biomarkers, and additional information provided by the frequency domain analysis will further improve the chances of the biomarkers being useful in the early detection of Schizophrenia in the future.

Chapter 2: LITERATURE REVIEW

2.1 Electrophysiology

The brain activity of patients with Schizophrenia was first determined using an electrophysiological technique called the Electroencephalogram or EEG, which records the electrical activity of neurons from the scalp. Neurons are the functional units of the nervous system. Each neuron communicates with the other neurons at a synapse which involves the release of chemicals called neurotransmitters. Each synapse involves a pre-synaptic cell which transmits the message by the release of neurotransmitters and the post-synaptic cell that receives the message. The synapse might occur on a dendrite, the cell body of the neuron or along the length of the axon. A typical stimulus produces an electrical impulse called the action potential which travels along the axon membrane of the pre-synaptic cell and due to the synaptic activity; the neurotransmitter released from the pre-synaptic cell binds to the receptors on the post-synaptic cell. In response to the neurotransmitter, a graded potential is produced in the post-synaptic membrane referred to as the post-synaptic potential. These post-synaptic potentials can be excitatory or inhibitory depending upon the nature of the receptor. The EEG field is a voltage (usually in microvolts), which measures a summation of simultaneously occurring postsynaptic potentials obtained from the dendritic fields of a group of cortical neurons from the different regions of the cortex, which are either directly perpendicular or oriented radially (producing a strong field potential) to the surface of the scalp under an electrode (Weinberger & J. Harrison, Schizophrenia 3rd edition; Martini & Nath, Fundamentals of Anatomy and Physiology 8th edition).

Electroencephalography has many advantages in studying Schizophrenia and has been useful in the process of obtaining a biomarker for the detection of the disease at an early stage. EEG is a non-invasive and radiation free recording which can be repeated without doing any harm to the subject. Changes in the alertness and cognitive functioning can be well detected using this technique. It is an appropriate method to observe and monitor the effects of drugs on the brain in pharmacological studies rather than measuring blood levels (Keith et al., 2000).

To analyze the power spectrum of EEG, the time domain signal is decomposed using the Fourier Transform into the relative power contribution of each component frequency to the signal as a whole. EEG frequency data is grouped into specific frequency bands associated with different cortical functions: delta (<4Hz), theta (4-8Hz), alpha (8-12Hz) beta (12-31Hz) and gamma bands (31-100 Hz). Though EEG records the surface cortical activity only, it can be used to look at the time, frequency, spatial and temporal aspects of the brain activity (Keith et al., 2000).

2.2 Event Related Potentials

Event-Related Potentials (ERPs) are the various positive or negative potentials which are obtained from the time-locked activity of the brain. They are evident when EEG epochs that are time-locked to a repeated stimulus or response event are averaged in time. When a large number of epochs are averaged, the increase in the number of trials increases the signal to noise ratio of the time-locked activity and effectively cancels out the random non time-locked activity in the EEG. The averaged epochs reveal various components of ERP with peak latencies and amplitudes corresponding to the various underlying sensory and cognitive processes which develop over time in response to a particular event, but are too small to see in single trials due to the inherently noisy nature of the EEG signal (Roach & Mathalon, 2008).

The auditory cortex in the temporal lobe is involved with conscious perception of auditory stimulus. An auditory ERP (Figure 2.1) starts with a positive-polarity response at 50ms, followed with a series of responses labeled as P or N depending upon their positive or negative polarity and their latencies (Keith et al., 2000). The typical peaks in an ERP begin with sensory registration (N1, ~100ms post-stimulus), automatic change detection (mismatch negativity, ~100-250ms post-stimulus), the orienting shift in attention (P3a, ~300ms post-stimulus), attention-dependent processes (P3b, ~300ms post-stimulus) and reorienting of attention (RON) following the deviant stimuli (Light et al., 2010). The ERP components occurring up to 200ms after the onset of deviant stimuli are called exogenous or stimulus driven components as they reflect the physical properties of the stimulus applied. The components after 200ms are referred to

endogenous/concept-driven or context-dependent components as they are based on the task performed by the subject in response to the stimulus (Keith et al .,2000).

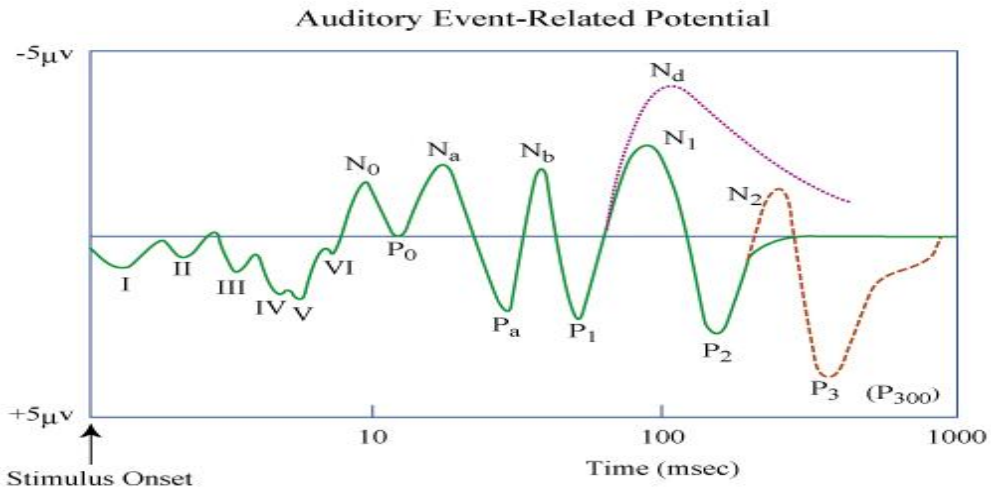

Figure 2.1: The various auditory event-related potentials. (Taken from: http://www.flickr.com/photos/mitopencourseware/4812734673/sizes/o/in/photostream/)

ERP measures are useful in quantifying the neuronal processes associated with cognitive and sensory events along with excellent temporal resolution in milliseconds (Light & Braff, 2005).

2.3 Time-Frequency Analysis

Time-frequency analysis of EEG decomposes the EEG signal into its magnitude and phase information for each frequency present in the time-domain signal and characterizes their change over the time in regard with a particular event. The neural synchrony or oscillations in neuronal communication are not obvious in an on-going EEG while the time frequency analysis provides information about which frequencies have the most power at specific points in time and space and how their phase angles synchronize over time and phase. Time-frequency analysis has the potential to determine parallel processing of information with oscillations across various frequencies at spatially discrete cortical locations which reflect neural processes underlying information processing (Roach & Mathalon, 2008).

2.4 Mismatch Negativity

ERPs play a very important role in studying various aspects of information processing. Mismatch Negativity (MMN) (Figure 2.2) is a negative voltage component of the ERP which is automatically elicited when a train of uniform auditory stimuli (standard tones) are presented, randomly interspersed with rare deviant stimuli (typically less than 20% probability) which differ in sensory characteristics like frequency, duration or intensity (Keshavan et al., 2008). MMN is an early auditory ERP which has been determined as an index of an automatic, pre-attentive alerting mechanism which stimulates an individual to respond to unexpected environmental events (Gené-Cos et al., 1999). The MMN response is a difference waveform which is obtained by subtracting the standard stimuli ERP from the deviant stimuli ERP. The standard and the deviant stimuli are usually separated by a short Interstimulus interval (ISI) of 300ms to 1s. MMN amplitude increases with shorter ISIs. A short ISI is used to prevent memory decay of the representation of the standard stimuli. If the ISI is greater than 3 seconds then no MMN is elicited. This is due to the memory trace of the standard stimulus not being maintained between deviant stimuli (Näätänen et al., 2007).

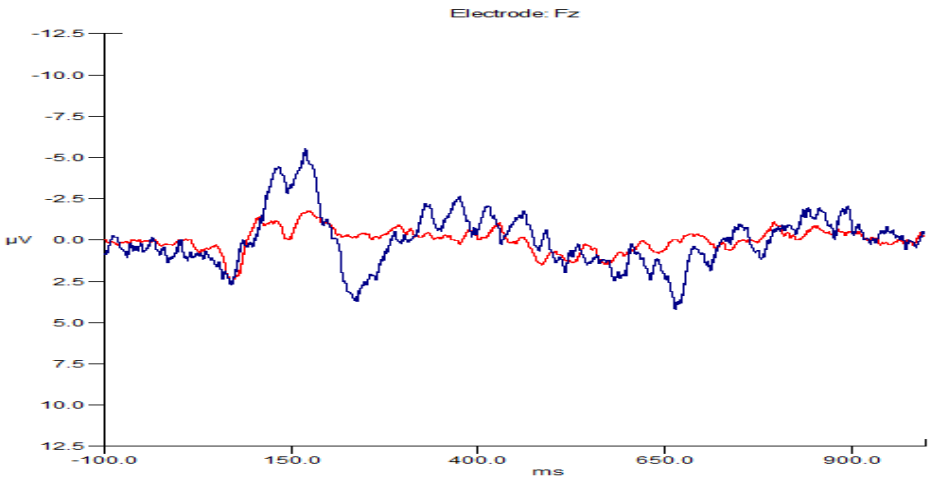

Figure 2.2: Frequency MMN (filtered) elicited by frequency deviant (represented by the blue waveform) to the standard tone (represented by the red waveform) at Fz electrode in Subject 5.

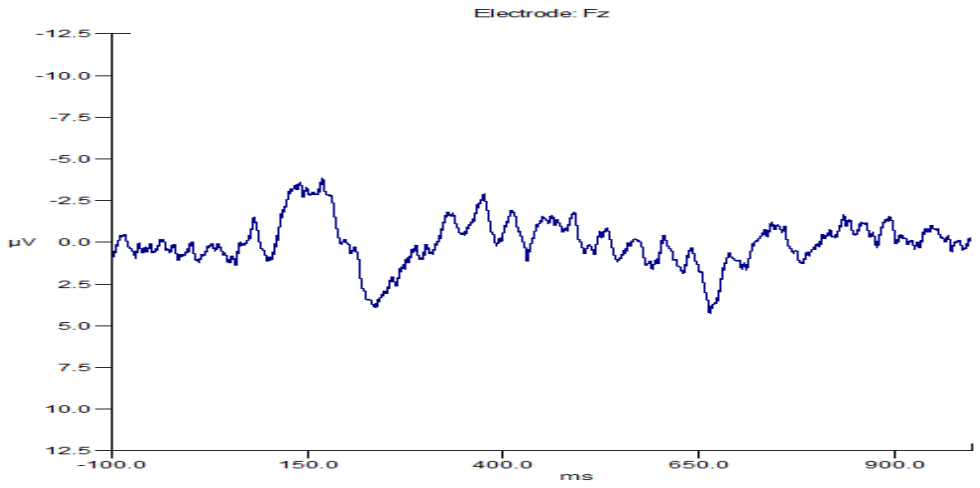

Figure 2.3: Difference wave (filtered) showing the Frequency MMN at Fz in Subject 5 with a latency of 170ms and amplitude of -3.774μVolts. Data was epoched from -100ms (pre-stimulus) to 1000ms.

To generate the memory trace of the standard stimuli the central auditory system must be able to form a representation of the repetitive aspects of auditory stimulus before the deviant stimulus is introduced. This has been termed as an "echoic memory trace" (Michie et al., 2000). This indicates the dependence of the MMN on the presence of a short-term memory trace in the auditory cortex, and the ability of the deviant stimuli to differ enough from the standard stimuli to elicit a substantially different ERP. The amplitude of MMN can be increased by increasing the probability of the deviant stimuli. But if the interval between these deviant stimuli is short, then there are chances that a memory trace of these deviant stimuli might interfere with the generation of MMN altogether (Näätänen et al., 2004). However, MMN is elicited irrespective of conscious attention, leading to the idea that it is a subconscious alerting mechanism, intended to shift the attention to an unexpected stimuli (Näätänen et al., 2004). To prevent any attention-dependant ERP components that might overlap with the MMN participants in experiments usually have to attend to another stimulus such as a familiar (muted) video (Michie et al., 2010).

MMN amplitude increases and peak latency decreases with an increase in the magnitude of the deviation from the standard. MMN is thus referred to as "pre-attentive" to emphasize the fact that it differs from the N100 ERP and is associated with an attention

switching mechanism caused by the changes in the auditory stimulus. N100 is an auditory ERP which is produced in response to repetitive standard stimuli (Javitt et al., 2008). The peak latency of the MMN usually occurs after the sensory N100 component. This can be supported by the fact that the MMN generated by deviant sounds among standard sounds and the enhanced N1 response to deviant sounds occurring with no intervening standard sounds are generated by different neuronal populations. This was shown by a study conducted by Kropotov et al., in 2000. It was found that an area of temporal cortex responded differentially in the MMN latency range when deviants of 1300Hz among standards of 1000Hz tones were presented. There was no such differential response noted when these tones were individually presented in a sequence or when only the standard tones were presented at a fast or slow rate. This clearly showed that N1 is a frequency and rate specific cortical afferent response which originates from a different group of afferent neurons (Näätänen et al., 2007).

The Mismatch Negativity response usually peaks within 150-250 ms of stimulus onset. The MMN amplitude increases with an increase in the number of standards preceding a deviant. When two deviant occur one after the other, the MMN generated by the second deviant is smaller in amplitude than the first. However, the Mismatch Negativity generated by the second deviant does not attenuate when the two consecutive deviants differ from each other as well as differing from the standard (Näätänen et al., 2007). Mismatch Negativity is typically seen as a negative displacement, specifically at the frontocentral and the central electrodes with respect to a mastoid or a nose reference electrode. But a reversed polarity is generally observed at the mastoids (positive displacement) when a nose reference is used (Näätänen et al., 2007).

Mismatch negativity generators are located bilaterally in the supratemporal region which contributes to pre-perceptual change detection. The MMN generators located in the right frontal hemisphere are believed to be responsible for the involuntary attention shift caused by the auditory change. The right frontal generator is more sensitive than the temporal generator, producing large MMN amplitude at the frontal region (Näätänen et al., 2007). MMN generation is related to activity of the glutamatergic N-methyl-D-

aspartate (NMDA) receptor system (Javitt et al., 1997). This receptor plays a vital role in cortical excitation and brain development (Weinberger, J. Harrison, Schizophrenia 3rd edition). NMDA receptors are one among several excitatory amino acid glutamates which is released in the auditory cortex after sensory stimulation (Javitt et al., 1997). A reduction in the MMN amplitude related to the dysfunction of NMDA receptors involved in the auditory memory trace formation has been found (Duncan et al., 2010).

2.5 Mismatch Negativity in Schizophrenia

The reduction of MMN amplitude has been consistently replicated in Schizophrenia. In a study of 25 Schizophrenia patients who met DSM-IV criteria, it was found that the patients had significantly reduced MMN amplitudes when compared with age, sex and education matched controls (Light & Braff, 2005). A recent meta-analysis of 32 studies involving Schizophrenia patients demonstrated a significant effect on temporal processing due to neuropathological changes underlying MMN. The study reported that duration deviant MMN deficits were significantly larger than frequency deviant MMN deficits (Duncan et al., 2009). This suggests that duration deviant processing is comparatively more impaired than frequency deviant processing (Michie et. al, 2000).

There is also evidence that the length of illness is related to the MMN. In the early stages of illness the MMN deficit to duration deviants is evident while the MMN deficit to frequency deviants increases with length of illness. A reduction in the intensity MMN deficit is also seen at the early onset of the disease (Duncan et al., 2009). A reduction in the amplitude MMN deficit has also been observed in first-degree relatives of patients with Schizophrenia (Michie et al., 2000). Increases in the deviant are associated with reduced MMN latencies in healthy controls. However, MMN latency does not seem to play an important role in patients with Schizophrenia: longer latencies have been associated with improved symptom expression in patients treated with anti-psychotic drugs (Horton et al., 2010).

In a study conducted by Salisbury et al., (2007) on a cohort of Schizophrenia patients, bipolar disorder patients and psychiatrically healthy controls, there was no notable

difference in the amplitude of frequency MMN among the 3 different groups at initial hospitalization. The Schizophrenia patients showed a progressive reduction in the MMN amplitude 1.5 years post-hospitalization. Magnetic Resonance Imaging (MRI) volumetric measurements revealed a reduction in left-hemisphere Heschl's gyrus gray matter volume in Schizophrenia patients. Heschl's gyrus contains the primary auditory cortex and some of the secondary auditory cortex. The finding suggests the possibility of a pathological process linked to a reduction in gray matter volume of Heschl's gyrus (Duncan et al., 2009, Weinberger & Harrison, Schizophrenia, Chapter 15). Another finding suggests that the generation of MMN depends on the intracortical inhibition due to the inhibitory activity of the Gamma-amino butyric acid (GABAergic) interneurons present in the auditory cortex (Javitt et al., 2008).

According to a study by Nakamura et al., (2007), progressive post-onset changes in both the temporal and pre-frontal cortex have been noticed. The pre frontal cortex (PFC) co-ordinates information relayed from the association areas over the entire cortex. Association areas are the regions in the cortex which interpret information or coordinate a motor response. Pre- Frontal cortex performs abstract intellectual functions, interprets ongoing circumstances and predicts future consequences. Feelings such as anxiety, tension and frustration are evoked in the PFC. In a recent MMN source analysis, altered electromagnetic activity in the pre-frontal cortex of schizophrenics was found. Reductions in the amplitude of mismatch negativity could be linked to mechanisms in the PFC which also contributes to switching involuntary attention (Baldeweg et al., 2004) and controls the direction of attention (Näätänen et al., 2007). Schizophrenia patients do not have an effective attention switching adaptability. This could be a potential reason as to why there is a diminished activity noticed in the pre-frontal cortex when a patient is involved with attention-dependent tasks (Javitt et al., 2008). Additionally, this may be a contributing factor in the asociality and social withdrawal symptoms exhibited in the patients (Duncan et al., 2007). The temporal lobe processes the auditory information and Heschl's gyrus is located within the temporal lobe. In patients of Schizophrenia, there is a reduction in MMN amplitude due to a frequency

deviant which is related to the gray matter reduction in the bilateral Heschl's gyrus as well as motor and executive regions of the frontal cortex. A reduction in the right gray matter in the Heschl's gyrus is correlated to reduced MMN amplitude due to duration deviant (Light & Braff, 2010). Thus, the temporal lobe can be associated with pre-perceptual change detection and the pre-frontal cortex and frontal lobe with involuntary switching and higher-order cognitive processes. Moreover, deficits in verbal memory and attentional switching are correlated to reduction in temporal and frontal mismatch negativity (Hermens et al., 2010).

To establish a significant contribution of MMN to the research and treatment of Schizophrenia, it is essential to be able to predict patients' functional outcome and provide an endophenotype for genetic studies (Duncan et al., 2009). However, MMN is considered to be an automatic and preattentional probe for deviance detecting which correlates with "downstream" real world functioning, and seems to be a useful target for developing drugs which would improve the cognitive and functional deficits in Schizophrenia (Light & Braff, 2005).

2.6 P300

The P300 is a large amplitude, long lasting, positive ERP that occurs in response to a rare stimulus when consciously detected and processed by a subject. The P300 (also known as P3) has two forms: the P3a which is frequently observed over the frontal lobe in response to rare stimuli that are not task related and P3b which is more evident over the parietal lobe and is task related. P300 (Figure 2.4) occurs approximately 300ms after the deviant tone is presented (P3a occurs slightly earlier), and is one of the most studied ERPs (Duncan et al., 2009; Weinberger & Harrison, Schizophrenia, Chapter: 15).

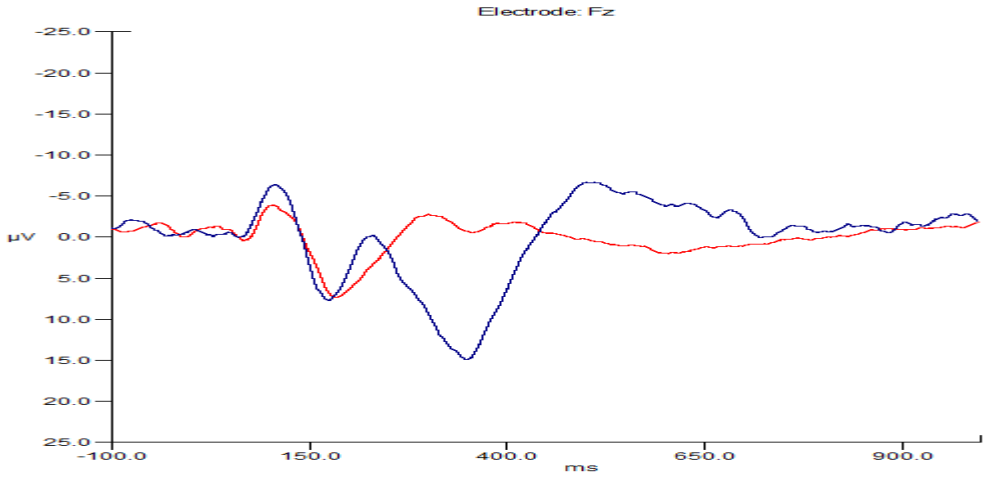

Figure 2.4: P300 (filtered data) elicited by the deviant tone at Pz (shown by the blue waveform) and the standard tone (represented by the red waveform) in the pilot subject.

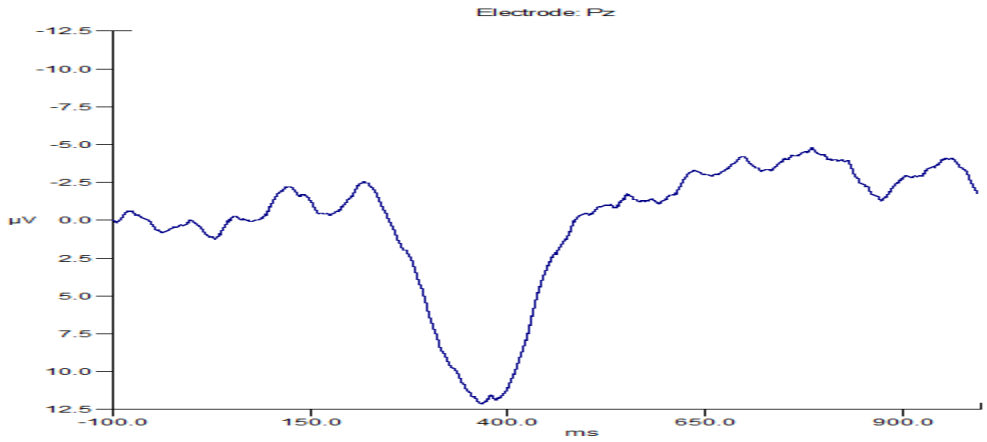

Figure 2.5: P300 Difference waveform (filtered) at Pz in the Pilot subject with a latency of 364 ms and peak amplitude of 12.039µV. Data was epoched between a -100ms (pre-stimulus) to 1000 ms.

P300 or P3b is usually elicited using an oddball paradigm which consists of a target stimulus with 20% probability and a standard stimulus with 80% probability. Subjects are asked to either count the number of target stimuli or press a button in response to each stimulus (Umbricht et al., 2003). If proper attention is not given to the target stimuli, then no P300 will be elicited (Duncan et al., 2009). A visual P300 can also be elicited using continuous performance tests which are designed to assess sustained and focused attention in individuals with neural damage using visual stimuli such as letters or, digits (Light & Braff, 2005).

The brain generators responsible for P300 ERPs are widely distributed over the cortex forming a network of multiple and relatively independent bilateral generators. Areas such as the inferior parietal lobule and posterior superior temporal gyrus correspond to the scalp-recorded P3b, while areas such as the dorso-lateral pre-frontal cortex and anterior cingulated cortex correspond to the scalp-recorded P3a. Although deep brain sources such as the hippocampus, amygdale and thalamus may also display P300-like responses, they are unlikely to be detected by scalp recorded EEG. However, the overt behavioral response (i.e. reaction time) not only depends on the level of activity occurring in the cortex but also on the distribution of activity between the network of participating generators (Duncan et al., 2009; Weinberger & Harrison, Schizophrenia, Chapter: 15).

The amplitude of the P300 is proportional to the amount of attention given to the task and the component magnitude is linked to working memory (Jeon & Polich, 2003). The amplitude is also inversely proportional to the probability of a target stimulus; the lower the probability the larger the P300 amplitude (Duncan et al., 2009). In addition, the interstimulus interval (ISI) affects the P300 amplitude. A short ISI elicits smaller amplitude P300 while a longer ISI generates a larger amplitude response (Duncan et al., 2009). The P300 amplitude depends not only on the ISI but also on the interval between successive target stimuli. If the ISI exceeds 6 seconds, the effect of the stimulus may reduce, and if the interval between the subsequent target stimuli exceeds 8 seconds then the effect will be completely eliminated. The expectation generated by the sequence of standard stimuli occurring before the deviant stimuli also affects the P300 amplitude in a similar manner to the MMN: successive repetitions of deviant stimuli lead to a reduction in the P300 amplitude (Duncan et al., 2009). If the target and non-target stimuli occur with the same probability, then target stimuli that require a response generate larger P300s than non-target stimuli (Duncan et al., 2009).

P300 latency is associated with how well a subject can classify the stimulus as standard or deviant and is independent of behavioral response time and the response selection process. In other words, the P300 latency represents the stimulus evaluation time (Hall et

al., 2006). Neuropsychological tests for attentional resource allocation indicate that shorter P300 latencies are associated with better cognitive performance and peak timing is thus negatively correlated with mental efficiency (Jeon & Polich, 2003). The latency of P300 becomes longer with increasing stimulus complexity, varying in the range of 250 to 1000ms (Duncan et al., 2009).

Initial genetic studies have shown a connection between P3b and genetic variations in dopamine receptor genes. However, genetic variations in the dopamine catalyzing enzyme 'catechol-O-methyltransferase' have been associated with variations in the fronto-central and centro-parietal P300 amplitude (Javitt et al., 2008; Weinberger & Harrison, Schizophrenia, Chapter: 15). Based on post-mortem findings in patients with Schizophrenia, Gamma-amino butyric acid (GABA) disturbances have been linked to a reduction in the number of parvalbumin (calcium-binding protein) expressing interneurons in the hippocampus and the frontal regions of the brain (Javitt et al., 2008). GABA release in the central nervous system appears to reduce anxiety (Martini & Nath, Fundamentals of Anatomy and Physiology, Chapter: 12). The application of GABAergic drugs reduces the amplitude of P300 and delays the latency. In contrast with GABAergic drugs, Acetocholine (ACh) and cholinergic drugs tend to increase the P300 amplitude while reduced amplitude has been observed with anti-cholinergic drugs. The abnormality in cholinergic transmission is linked to cognitive, memory and attentional deficits among the schizophrenics. Thus, P300 appears to be a suitable biomarker to detect these pathophysiological abnormalities in the patients of Schizophrenia (Javitt et al., 2008).

2.7 P300 in Schizophrenia

Many studies have repeatedly shown the reduction in the amplitude of P300 in chronically ill patients with Schizophrenia (Weinberger & Harrison, Schizophrenia, and Chapter: 15). P300 amplitude is heritable in 70-80% of the patients, making it a potential trait marker for the genetic risk of developing Schizophrenia. 68.3% (1 standard deviation) of chronically ill patients show a reduction in the P300 amplitude compared with healthy controls. Though there is a positive correlation between heritability of P300

deficits and family members of the patient, there is no significant evidence for the same in the offspring of Schizophrenia sufferers (Javitt et al., 2008). However, the reduction in amplitude is not due to a lack of motivation in patients: the deficit remains even when task performance is taken in to account. The rate of P300 latency increase was greater with increasing age among patients than among healthy controls implying that the disease has an affect over and above that of normal ageing. The complexity of the stimulus affects the latency among the patients in a similar manner to the healthy controls, but the deficit in amplitude is always present (Weinberger & Harrison, Schizophrenia, Chapter: 15).

Schizophrenia alters attentional resource allocation among patients when P300 is elicited using a simple auditory oddball paradigm, and that the generation of the P300 is affected by the experimental protocol and recording methods as well as characteristics of the disease (Jeon & Polich, 2003).

In first episode patients, a broad reduction of P300 amplitude over both right and left hemisphere has been observed. In addition, smaller P300 amplitudes have been observed in the left temporal lobe compared to the right, in both the chronic and first episode patient group. The asymmetry of P300 reduction is positively correlated to a reduction in gray matter volume of the left posterior superior temporal gyrus, one of the P300 generators and the area associated with thinking and language processing. There is also a negative correlation between the gray matter volume of the left posterior superior temporal gyrus and the extent of psychopathology, evident through thought disorder, delusions and severity of auditory hallucinations (Weinberger & Harrison, Schizophrenia, Chapter: 15).

In the patients of Schizophrenia, anti-psychotic medication does not affect the amplitude of P300 although age of disease onset and length of illness does have an effect on amplitude reductions in patients when compared with controls. It is also believed that the severity of negative symptoms in Schizophrenia can be traced using the deficits in P3b (both over time and within sub-categories of the disease) (Javitt et al., 2008).

The measurement stability and heritability of P300 amplitude is as high as the duration deviant MMN. P300 amplitude reduction has been found in the family members of the patients of Schizophrenia suggesting that it could be used as a potential endophenotype. In contrast to P300, the attention independent mismatch negativity focuses on the various traits and states during the course of illness (Weinberger & Harrison, Schizophrenia, Chapter: 15). Thus, both P300 and MMN seem to be convenient biomarkers which can be studied together for enhanced results.

Chapter 3: METHODOLOGY

3.1 Auditory test Paradigm for MMN and P300

Auditory stimuli were presented binaurally using foam ear phones while the participants watched a pre-selected movie. The auditory stimuli for both mismatch negativity and P300 were generated using Stim^2 Gentask software. The oddball paradigm with an interstimulus interval of 1000ms was used to elicit mismatch negativity. It comprised of one frequent standard tone intermixed with five deviants namely duration, frequency, intensity, location and gap. 50% of the stimuli comprised of the standard tone and the remaining 50% comprised of the 5 deviants, each occurring with a probability of 10%. The deviants were presented pseudo-randomly with each deviant followed by a standard tone. A total of 1510 stimuli were presented with 760 standards and 150 stimuli of each deviant. The full details of the paradigm are shown in table 1, and closely follow the optimal paradigm determined in Näätänen et al 2004.

The oddball paradigm for P300 consisted of one standard tone and one target tone. 80% of the stimuli were standard tones and the remaining 20% were the deviants. The participants were required to focus their attention on the stimuli and press a button on the response pad corresponding to each type of stimulus. Details of the P300 paradigm are given in table 2, and again follow the paradigm specified by Näätänen et al 2004.

A pilot study investigated the effects of the interstimulus interval on the latency and amplitude of the MMN by testing two modifications to the paradigm. In the first additional paradigm the interstimulus interval was 500ms and in the second it was 750ms. However, the probability of the standard tone remained the same, 0.50 and the probability for each of the five deviants was 0.10.

T**able 1: Optimal MMN experimental paradigm**

Recording conditions for Mismatch negativity *(adapted from Näätänen et al., 2004)*

Parameter	Comment
I. Stimulus factors	
A. Optimal Paradigm	One frequent standard, five rare deviant tones
Standard	Harmonic stimulus comprising 3 sinusoidal partials of 500, 1000, and 1500 Hz, with intensity of second and third partials 3 and 6 dB lower than the first partial.
Duration	75 ms, 5 ms rise/fall
Intensity	80 dB SPL
Interstimulus interval	1000 ms (fixed)
Location	Midline (binaural)
Deviants	
Duration	25 ms, 5 ms rise/fall
Frequency	Half of frequency deviants are 10% higher partials, half are 10% lower partials.
Intensity	Half of intensity deviants are 10 dB higher, half are 10 dB lower.
Location	Half of location deviants are perceived as having a spatial location 90° to the right and half 90° to the left of the midline by introducing an interaural time difference of 800μs.
Gap	Silent gap of 7 ms (including 1 ms rise/fall) in the middle of a 75-ms stimulus.
Probabilities	.50 (standard), .10 (each of the deviants), one standard between each deviant
II. Participant and task	
Position	Seated
Eyes	Open
Active or passive task	Participants are asked to watch a familiar muted video.
III. Electrophysiological recording	
Electrode sites	32 scalp electrodes (Cz, C3, C4, T7, T8, FC2, FC3, FC4, FT7, FT8, CPz, CP3, CP4, TP7, TP8, Fz, F3, F4, F7, F8, Pz, P3, P4, P7, P8, O2, O1, Oz, FP1, FP2) and left and right mastoid electrodes (M1 and M2).
Reference	Nose – with algebraic re-referencing to average of the mastoid
Ground	AFz
Bandpass of amplifiers	DC, 0-500Hz
Digitization rate	2000 Hz
Epoch length	1000 ms; with 100-ms prestimulus baseline only for the filtered data
Artifact reduction	Vertical and Horizontal EOG rejection or correction; ±50 μV for all EEG channels
Minimum # trials	150 of each deviant
Digital filtering	1–30 Hz
IV. Quantification	
Average ERPs	Average ERP waveforms and difference waveforms are presented for each condition.
Difference waveform	Deviant average ERP minus Standard average ERP
Latency	120–250 ms
Amplitude	Peak amplitude in a latency window in difference waveforms.
Scalp distribution	Maximal at Fz;

Table 2: P300 experimental paradigm

Recording conditions for P300 *(Adapted from Näätänen et al., 2004)*

Parameter	Comment
I. Stimulus factors	Two categories of stimuli, one rare, one frequent
Auditory	Tones
Frequency	Harmonic stimulus comprising 3 sinusoidal partials of 500, 1000, and 1500 Hz, with intensity of second and third partials 3 and 6 dB lower than the first partial.
Duration	Standard 75ms, target 25ms duration, 5 ms rise/fall
Intensity	80 dB SPL
II. Participant and task	
Position	Seated
Eyes	Open
Active or passive task	Attention to stimuli required; Participants are asked to press button 1 for the non target stimulus and press button 2 for the target stimulus.
III.Electrophysiological recording	
Electrode sites	32 scalp electrodes (Cz, C3, C4, T7, T8, FC2, FC3, FC4, FT7, FT8, CPz, CP3, CP4, TP7, TP8, Fz, F3, F4, F7, F8, Pz, P3, P4, P7, P8, O2, O1, Oz, FP1, FP2) and left and right mastoid electrodes (M1 and M2)
Reference	Nose reference
Ground	AFz
Bandpass of amplifiers	DC, 0-500 Hz.
Digitization rate	2000 Hz
Epoch length	1000 ms; with a prestimulus baseline of 100 ms for the filtered data.
Artifact reduction	EOG rejection or correction, and rejection of trials with voltages ±50μV for all EEG channels
Minimum # trials	50 artifact-free
Digital filtering	1-30 Hz bandpass filtering.
IV. Quantification	
Average ERPs	Average ERP waveforms, excluding trials with incorrect behavioral responses for all subjects and conditions.
Latency	Typically peaks in the 300–400 ms range but varies according to the difficulty of categorizing the stimuli and age.
Amplitude	Peak amplitude in a latency window (e.g., 280–420 ms) relative to prestimulus baseline.
Scalp distribution	Parieto-central (Pz electrode) in the participants.

3.2 Event-related potential Recordings

Electroencephalographic recordings for both mismatch negativity and P300 were acquired continuously with Neuroscan 4.5 Acquire software (Compumedics) and SynAmps^2 amplifiers from 32 scalp locations located according to the international 10/20 system (Cz, C3, C4, T7, T8, FC2, FC3, FC4, FT7, FT8, CPz, CP3, CP4, TP7, TP8, Fz, F3, F4, F7, F8, Pz, P3, P4, P7, P8, O2, O1, Oz, FP1, FP2, left mastoid (M1), and right mastoid (M2), see Figure 1 for details). The ground electrode was located at AFz and the reference was placed on the nose. A nose reference helps to visualize and measure both the negative aspects of the MMN waveform at the frontal lobe and the positive aspects at the mastoids. Two bipolar channels were used to monitor eye movements and blink artefacts: left vertical electro-oculogram (VEOG) and horizontal electro-oculogram (HEOG). Electrode impedance was kept below 5 kΩ. The recorded electrical activity was amplified and digitized using Neuroscan SynAmps^2 at a sampling rate of 2000 Hz.

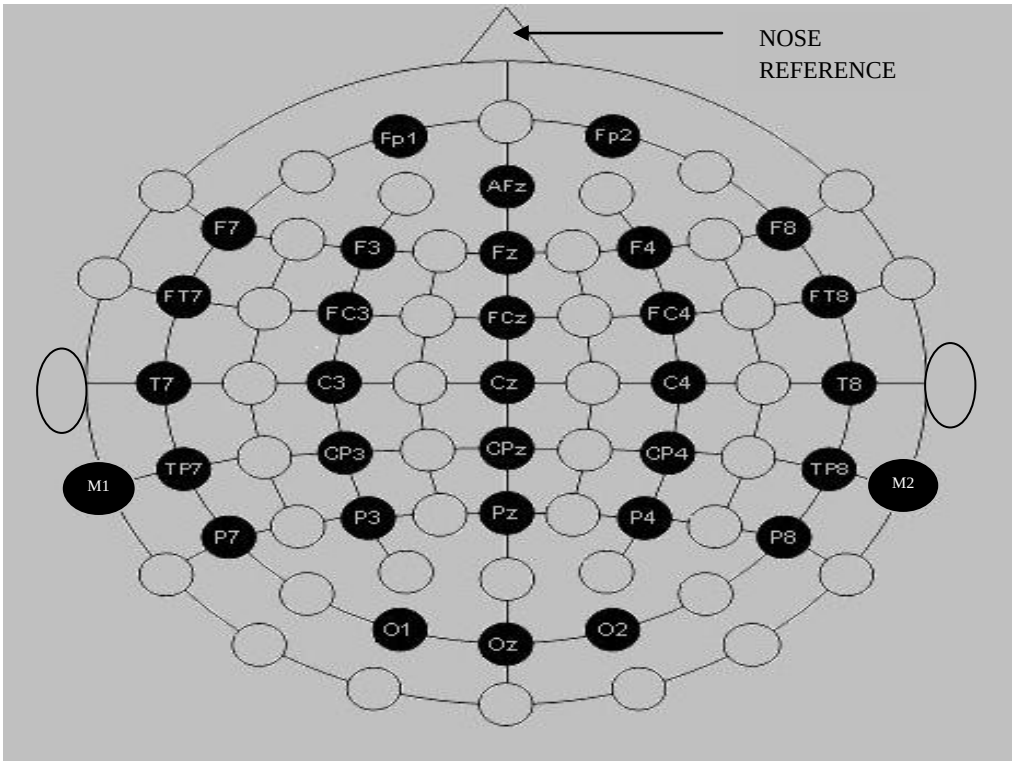

Figure 3.1: EEG montage showing the electrode configuration (Taken from- http://www.easycap.de/easycap/e/electrodes/05_M03.htm)

The auditory stimuli were generated by Stim^2 Gentask software and the triggers were interfaced with the NeuroScan 4.5 Acquire module using the Stim^2 hardware.

Epochs were generated from the continuous data with an interval of 0ms to 1000ms post-stimulus in both MMN and P300, based upon the triggers from Stim^2. Epochs were then baseline corrected, using the whole epoch as a reference. Artefact rejection to remove noisy epochs or epochs contaminated by eye movements was conducted using the frontal channels closest to the eyes: FP1, FP2, HEOG, VEOG, F7, and F8. The rejection criterion was based upon amplitudes exceeding +/- 50 μV. The remaining artifact free epochs were linearly detrended to remove slow DC potentials and then averaged. Averaging is done to improve the signal to noise ratio of the ERP components. Mismatch negativity ERPs were obtained by subtracting the averaged standard epochs from the averaged deviant epochs. P300 ERPs were created from the correctly identified averaged deviant epochs where the response occurred between 200 and 900 ms post-stimulus, and a difference waveform was also created by subtracting the averaged deviant epochs from the correctly identified averaged standard epochs. The peak amplitude and the latency at which it occurred were determined for all the MMN deviants and the P300 deviant and difference waveform. MMN measures were obtained from the Fz electrode and P300 was assessed from the electrode Pz.

The raw data was then re-referenced to the average of the two mastoid channels (M1 and M2) and a bandpass filter of bandwidth 1Hz-30Hz applied using NeuroScan edit. The filter was applied to observe the spectral data within the specified frequency range. The filtered and re-referenced data was then epoched and processed in the same manner as the raw data and the latency and amplitude measured and tabulated again.

3.3 Time-Frequency Analysis

Time-frequency analysis of the raw data grand averages of the 5 deviant MMNs and the P300 difference wave were investigated using the EEGLAB software toolbox in MATLAB (The Mathworks, Inc.). The average files obtained from the NeuroScan 4.5 edit were imported into the EEGLAB toolbox and were used to process the averaged EEG data. Event-related Spectral Perturbation (ERSP) measures the mean event-related

changes in the power spectrum over time in a broad frequency range at a specific data channel (Delmore and Makeig 2004).

The ERSP is calculated by computing the power spectrum over a sliding latency window across the averaged data. ERSP was calculated for the collected grand averages in the raw data. To determine the ERSP in the MMN deviants and P300, the latency window was specified from -100 to 900ms. The color at each image pixel indicates the power (dB) at a given frequency and latency relative to the time locking event. To compute the spectral estimate of the trial, Fast Fourier Transform (FFT) was used. This transform performs a Fourier transform using a fixed duration time window which is applied to all the frequencies in the EEG signal to characterize its power and phase over time. However, the uniform time window applied across all the frequencies results in optimal characterization of temporal changes in low-frequency signal while compromising on the temporal resolution of high-frequency signals. Significance of deviation from the baseline power is assessed using a bootstrap method. A bootstrap significance level of 0.05, with 400 time points and padding ratio of 4 was used. The mean event-related changes in spectral power at each time during the epoch and at each frequency in the range of 1-50 Hz were observed. To investigate the spectral power in the averages for mismatch negativity due to the various deviants, raw data from subject 7 (having one of the cleanest data sets) was analyzed. Mismatch negativity due to the various deviants were collected from channel number 16 which recorded the signal from the electrode Fz, and for P300 channel number 21 was used which recorded the signal from electrode Pz (Roach & Mathalon, 2008).

3.4 Procedure

The study was conducted at the Neurophysiology lab, Bioengineering Unit, University of Strathclyde. 11 healthy male controls within the age group 22-30 yrs were recruited for the study. One pilot subject was recruited to test the effect of stimuli with 500 ms and 750 ms epoch length. Another pilot subject was used to test the paradigms for mismatch negativity and P300, before they were used on the control group.

Subjects were asked to sit on a chair and the electrode cap (EasyCap 56 channels) was fitted. Two electrodes were placed near the outer canthus of each eye to record horizontal eye movements and two were attached above and below the left eye to record vertical eye movements. A single electrode was attached near the tip of the nose to provide a reference channel. Alcohol swabs (PDI Electrode Prep Pad) were used to clean the skin under these electrodes, and the electrodes were attached to the skin using double-sided adhesive O-rings (manufactured by EasyCap). Abrasive gel (Nuprep ECG & EEG Abrasive and Prepping Gel) was applied using cotton-tipped applicators (Dukal Corporation Non-sterile Cotton Tipped applicators) to achieve the target impedance of 5kΩ. Then conductive gel (Electro-Gel, Brain Vision UK) was inserted into the electrodes using a syringe (BD 10 ml Syringe, Luer-Lok^{TM} Tip) with a blunt-nosed needle (SRS needles).

The subjects were asked to insert specially designed foam earphones, which consisted of a small channel for the sound to pass through with the sound levels maintained to precise standards.

Before commencing the tests, the subjects were asked to blink, move their eyes in right, left, up and down directions, clench their jaw and swallow. This was done to make a note of these artefacts affecting the recording. Subjects were asked to avoid doing these actions as much as they could and stay relaxed during the recording.

Task A: Mismatch Negativity

During the MMN task participants were asked to listen to a series of tones which comprised of standard tones of 75ms duration intermixed with deviant tones which were altered in features such as frequency or intensity as detailed in table 1. The subjects were asked to watch a muted video of their choice without paying any attention to the tones, while they listened to the tones. This task lasted for 25 minutes.

Task B: P300

For the recording of P300, participants were asked to listen to the standard and target tones (see table 2 for details) and press button 1 on the response pad (Compumedics NeuroScan Stim System Switch & Response Pad) when they heard a standard tone and press button 2 if they heard a deviant tone. They were asked to fix their eyes onto a green dot on the computer monitor while they paid attention to the tones and carried out the task. This task took 13 minutes.

The whole experiment lasted for 1.5-2 hours including preparation time. The tasks A and B were randomly selected by the subjects before the recordings were conducted.

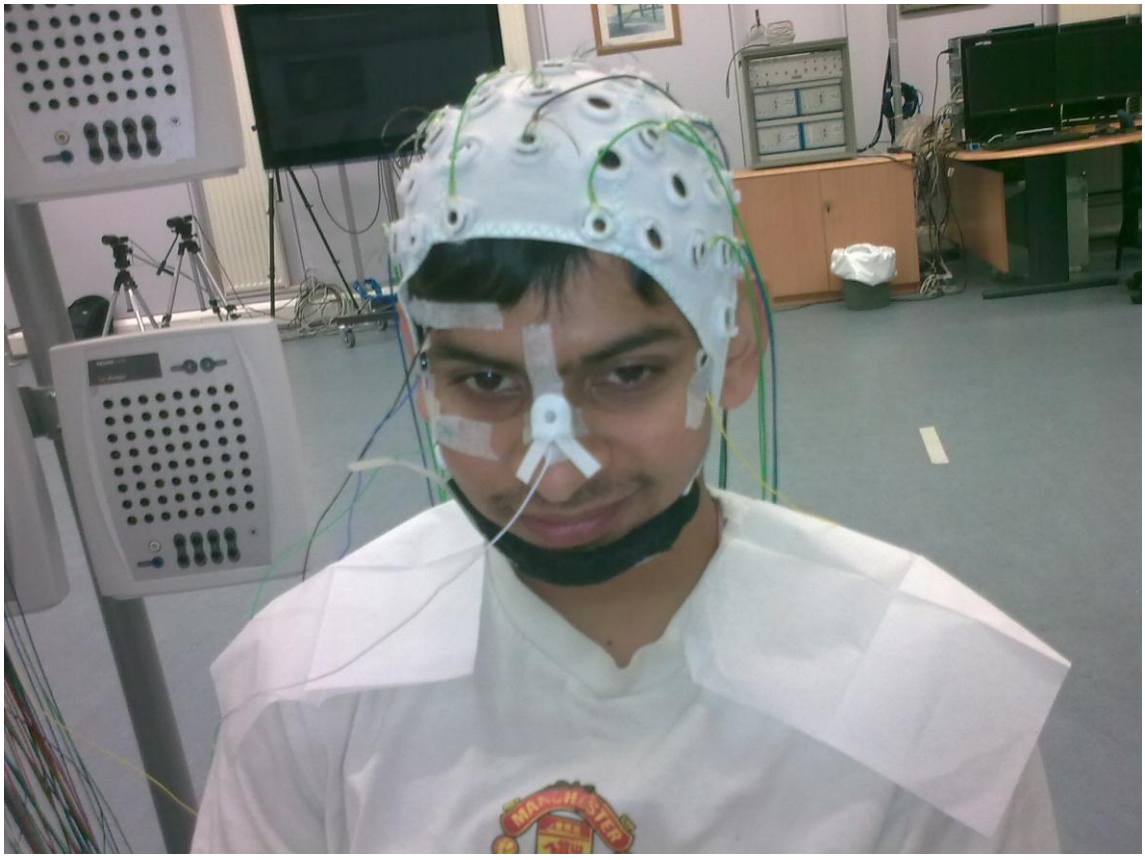

Figure 3.2: Pilot subject with the electrode cap, the nose reference and vertical and horizontal electrodes which were used to capture the artefact due to movement of eyes.

Chapter 4: RESULTS

4.1 Time-domain Results

NeuroScan 4.5 Edit was used to determine the peak latency and amplitudes in the 5 deviants for MMN and P300 in the raw and filtered data. As discussed earlier in section 3.2, it is not feasible to obtain a good estimate of the latency and amplitude without having a sufficient number of good trials to obtain the average ERP. First the number of accepted sweeps was calculated after baseline correction and artefact rejection in order to have the maximum number of good trials. Subject 4 and Subject 11 were excluded from the calculation of grand averages of the five deviants for MMN as the accepted sweeps for the various deviants were below 15 and that for the standard tone was found to be below 100. The numbers of accepted sweeps in the raw and filtered data for the five MMNs across the subjects are shown in table 3.

After re-referencing and applying the bandpass filter (1-30 Hz) to the continuous EEG data, the number of accepted sweeps increased greatly. However, there was no obvious difference noticed in the peak latency and amplitude in the five MMN deviants for both raw data and filtered data due to the variability associated with each average. This can be reviewed in tables 4 and 5.

Mismatch negativity in healthy controls is believed to peak within a latency window from 100-250ms (Duncan et al., 2009). In the present study, with the interstimulus interval of 1 second, the latencies of the MMN deviants fall within the expected latency window. The grand averages in table 9 show the peak latency and amplitude of the five deviant MMNs and the P300 components. The MMN amplitudes are not very high in the grand averages when compared to values reported in the literature. For example, a gender- and age-matched study conducted by Umbricht et al., on healthy controls (N = 25), patients with Schizophrenia and bipolar disorder, reported an amplitude of -5.53+/-2.60 µV for the MMN due to frequency deviant while in the present study the grand average (N = 9) amplitude for frequency MMN was found to be -2.58µV. The increase in the probability of the deviant stimulus further leads to a decrease in the amplitude (Näätänen et al., 2008). This might be the contributing factor to the reduced amplitude

observed. As the difference between the standard and the deviant was more when compared to the standard paradigm, MMN response was accompanied by significant sensory response N1 for all the deviants.

Table 3: Total number of accepted sweeps in the raw and filtered data for Mismatch negativity in the subjects

Subject	**Accepted Sweeps for Mismatch Negativity Raw Data and Filtered Data ( )**					
	Standard	Duration	Frequency	Intensity	Location	Gap
Subject1	238(374)	54(76)	39(70)	52(85)	51(75)	53(71)
Subject2	241(303)	49(64)	49(60)	42(53)	53(57)	42(58)
Subject3	238(575)	44(111)	45(100)	47(118)	46(111)	46(103)
Subject4	71(489)	12(100)	10(83)	12(100)	7(101)	9(88)
Subject5	104(342)	55(71)	48(59)	59(74)	54(67)	35(58)
Subject6	273(387)	60(74)	47(54)	49(86)	47(77)	32(67)
Subject7	328(538)	75(107)	67(96)	78(115)	57(110)	61(102)
Subject8	319(486)	66(98)	33(79)	45(108)	40(96)	44(98)
Subject9	94(462)	25(113)	17(74)	17(87)	24(90)	18(86)
Subject10	344(389)	73(92)	75(98)	75(103)	78(114)	62(87)
Subject11	61(473)	19(100)	20(74)	24(102)	17(100)	7(85)
Mean	210(448)	48(91)	40(77)	46(94)	43(91)	37(82)
Standard Deviation	107.9(84.4)	21.4(17.3)	20.1(16)	21.2(18.1)	20.2(19.2)	19.2(16.4)
Pilot Subjects						
Pilot Subject1	143(203)	50(36)	44(40)	63(50)	43(44)	46(33)
Pilot Subject 2 (500ms ISI)	385(552)	77(126)	76(102)	75(112)	70(107)	61(103)
Pilot Subject 2 (750ms ISI)	102(515)	21(103)	24(105)	23(100)	29(116)	14(89)

Table 4: Peak latency in the five mismatch negativity deviants and standard tone for the raw and filtered data recorded at the electrode Fz

Subject	**Latency Mismatch negativity Raw Data and Filtered Data ( ) [in milliseconds]**					
	Standard	Duration	Frequency	Intensity	Location	Gap
Subject1	260.0(263.0)	210.5(216.0)	168.0(169.0)	170.0(154.0)	159.0(232.0)	151.0(234.5)
Subject2	266.0(266.5)	162.0(174.0)	172.5(176.0)	200.5(206.0)	156.5(164.0)	217.5(144.0)
Subject3	295.0(296.5)	154.5(154.0)	164.5(169.0)	179.0(183.5)	151.0(151.5)	146.5(281.5)
Subject5	166.0(176.5)	242.0(147.5)	183.5(170.0)	171.0(172.5)	251.0(151.5)	143.0(147.5)
Subject6	290.5(297.5)	191.5(182.5)	182.5(169.0)	173.5(185.0)	157.5(141.5)	159.0(143.0)
Subject7	284.0(306.5)	174.5(186.0)	164.5(164.0)	188.0(190.0)	174.5(177.5)	175.5(181.0)
Subject8	280.5(248.0)	155.5(171.5)	195.0(154.0)	153.0(162.5)	151.0(144.0)	161.0(147.5)
Subject9	267.5(270.0)	181.5(162.5)	153.0(173.5)	153.0(175.0)	157.5(135.5)	141.0(149.5)
Subject10	230.5(234.5)	127.5(157.5)	157.5(162.5)	159.0(187.5)	160.0(221.0)	277.0(280.5)
Mean	260.0(262.1)	177.7(172.4)	171.2(167.4)	171.9(179.6)	168.7(168.7)	174.6(189.9)
Standard Deviation	40.2(40.0)	34.0(20.8)	13.5(6.5)	15.9(15.5)	31.6(35.2)	45.0(59.4)
Pilot Subject						
Pilot 1	288.0(293.0)	244.5(285.0)	164.5(280.5)	181.5(160.0)	193.5(271.5)	174.5(288.0)
Pilot 2 (500ms ISI)	194.5(207.0)	155.5(160.0)	180.0(178.5)	181.5(183.0)	163.5(155.5)	160.0(171.5)
Pilot 2 (750ms ISI)	221.5(217.0)	161.5(167.0)	169.0(175.5)	164.0(169.0)	188.5(133.5)	174.0(131.5)

Table 5: Comparison of the peak amplitudes of the five mismatch negativity deviants for the raw and filtered data recorded from the electrode Fz

Subject	**Amplitude [Raw Data](μV)**					**Amplitude [Filtered data] (μV)**				
	Duration	Frequency	Intensity	Location	Gap	Duration	Frequency	Intensity	Location	Gap
Subject1	-1.71	-3.88	-2.46	-1.94	-3.49	-0.44	-4.31	-2.62	-1.67	-2.55
Subject2	-2.47	-3.11	-8.15	-3.39	-2.18	-1.39	-1.32	-2.20	-0.84	-1.11
Subject3	-2.68	-4.44	-5.00	-4.05	-2.51	-0.33	-3.78	-4.79	-2.58	-1.61
Subject5	-3.13	-2.00	-3.85	-3.35	-2.88	-3.83	-3.77	-4.56	-4.28	-3.01
Subject6	-2.97	-2.12	-2.92	-2.46	-1.29	-2.74	-2.82	-2.51	-1.23	-0.72
Subject7	-3.48	-5.75	-5.32	-0.12	-7.90	-2.18	-3.40	-4.60	0.43	-1.97
Subject8	-4.01	-1.49	-4.71	-0.75	-4.02	-1.15	-1.96	-3.19	-2.73	-3.26
Subject9	-7.55	-2.38	-4.50	-4.62	-6.13	-2.33	-2.65	-2.30	-1.80	-4.03
Subject10	-4.26	-0.47	-1.41	-1.83	-3.41	-2.19	-1.46	-1.95	-1.10	-1.89
Mean	-3.58	-2.85	-4.26	-2.50	-3.76	-1.84	-2.83	-3.19	-1.76	-2.24
Standard Deviation	1.70	1.60	1.90	1.50	2.10	1.10	1.10	1.10	1.30	1.10
Pilot Subjects										
Pilot 1	-3.80	-1.65	-3.71	-1.32	-2.39	-5.14	-2.43	-3.67	-0.69	-3.61
Pilot 2 (500ms ISI)	-1.83	-1.87	-3.14	-3.38	-1.55	-1.97	-0.72	-3.03	-2.92	-2.11
Pilot 2 (750ms ISI)	-3.15	-4.59	-5.71	-1.37	-1.77	-0.26	-1.91	-3.03	-1.61	-1.52

Table 6: Total number of accepted sweeps in raw and filtered data for P300 in subjects

Subject	Accepted Sweeps for P300 (Raw Data)		Accepted Sweeps for P300 (Filtered Data)	
	Standard P300	Deviant P300	Standard P300	Deviant P300
Subject1	123	29	253	64
Subject2	21	19	139	28
Subject3	210	84	306	114
Subject4	113	28	311	89
Subject5	394	112	423	104
Subject6	389	101	441	110
Subject7	385	102	397	87
Subject8	297	89	378	104
Subject9	189	54	262	86
Subject10	310	116	432	111
Subject11	88	54	191	84
Mean	229	71.63	321.18	89.18
Standard Deviation	133.5	35.98	102.27	25.26
Pilot Subjects				
Pilot 1	217	61	322	94

For determining P300 averages, subject2 and subject11 were excluded from the calculations, as the number of accepted sweeps in the standard P300 was comparatively low.

The P300 amplitude is related to the amount of attention paid to the paradigm. In this study, large amplitudes of the P300 difference wave from the averaged raw data (9.271µV) and the filtered data (4.24 µV) were obtained (Table 9). This highlights the fact that with a longer interstimulus interval, larger amplitude is evident (Duncan et al., 2009). The latency in the P300 difference wave from averaged raw data appears to be delayed to 413.5ms while in the averaged filtered data the latency could be detected much earlier at 328.5 ms (Table 9). Both P300 amplitude and latencies have been associated with performance of neurophysiological cognitive tests (Light & Braff, 2005).

Table 7: Table showing comparison of P300 latency in the standard, deviant and difference wave for the raw and filtered data recorded at the electrode Pz

Subject	**P300 Latency [Raw Data] (ms)**			**P300 Latency [Filtered data](ms)**		
	Standard	Deviant	Difference wave	Standard	Deviant	Difference wave
Subject1	413.5	420.5	419.0	317.5	400.5	394.5
Subject3	377.5	318.5	314.0	331	322.5	319.0
Subject4	369.5	378.5	378.5	281.5	387.0	388.0
Subject5	263.5	359.0	373.5	234.5	358.5	359.0
Subject6	336.5	451.5	451.5	341.0	361.5	362.0
Subject7	375.5	375.0	352.5	385.0	362.0	348.5
Subject8	322.0	427.0	428.0	347.0	390.5	392.0
Subject9	344.5	415.5	398.0	353.5	366.0	409.5
Subject10	340.0	325.5	328.0	294.0	310.0	312.0
Mean	356.4	357.7	382.6	347.7	338.2	364.9
Standard Deviation	32.6	31.6	46.2	30.0	34.9	34.2
Pilot Subjects						
Pilot 1	331.0	397.5	396.5	343.5	366.0	364.0

Table 8: Table showing comparison of P300 peak amplitudes in the standard, deviant and difference wave for the raw and filtered data recorded at the electrode Pz

Subject	**P300 Amplitude [Raw Data] (µV)**			**P300 Amplitude [Filtered Data] (µV)**		
	Standard	Deviant	Difference wave	Standard	Deviant	Difference wave
Subject1	1.43	7.311	9.00	1.12	5.04	3.93
Subject3	1.60	13.15	15.13	0.54	6.52	6.35
Subject4	3.04	12.92	8.57	2.81	7.22	8.37
Subject5	5.01	11.62	14.46	3.24	7.22	9.50
Subject6	2.24	10.58	10.95	0.99	6.01	5.61
Subject7	2.99	2.31	0.94	1.14	7.47	7.37
Subject8	2.07	9.81	9.12	0.20	4.47	5.44
Subject9	5.51	12.55	14.54	1.81	7.53	8.65
Subject10	2.15	26.42	23.62	-0.88	11.94	13.50
Mean	3.57	9.74	11.8	2.71	8.37	7.60
Standard Deviation	2.20	4.50	6.20	1.50	3.80	2.80
Pilot Subjects						
Pilot 1	4.099	20.23	19.40	-0.016	11.51	12.04

Table 9: Grand Averages of the Standard tone and deviants for Mismatch negativity and the standard, deviant and difference wave for P300

Parameters	Raw Data		Filtered Data	
	Latency(ms)	Amplitude(μV)	Latency(ms)	Amplitude(μV)
Standard Tone(Fz)	281.5	-0.847	269.0	-0.176
Duration MMN (Fz)	161.0	-0.911	147.5	-1.459
Frequency MMN (Fz)	173.5	-1.583	169.0	-2.588
Intensity MMN (Fz)	172.5	-2.296	176.0	-2.331
Location MMN (Fz)	157.5	-1.273	150.0	-1.283
Gap MMN (Fz)	157.5	-1.847	143.0	-1.456
P300 Standard (Pz)	350.0	1.431	335.0	-0.012
P300 Deviant(Pz)	404.5	8.565	326.0	5.234
P300 Difference Wave (Pz)	413.5	9.271	328.5	4.240

The filtering of the raw data does not have a large effect on the latency of the peak MMN activity in any of the deviants (table 9). It reduces the amplitude of the gap MMN, increases the amplitude of the duration and frequency MMNs, and has no real effect on the intensity or location MMNs (table 9). The raw data was recorded with a wide bandwidth of DC to 500 Hz. The filtering process removes all low frequency and DC components below 1 Hz, and all high frequency components above 30Hz. The reason that the removal of these components alters the amplitudes in such different ways requires further investigation as it could be linked to underlying differences in how the stimuli are processed, and is another reason for conducting the time-frequency analysis.

There is an obvious N100 response in the filtered standard tone which can be reviewed in figures 4.1 to 4.6 below. This highlights the fact that there is sensory information processing across all the subjects. This effect also indicates the activation of fresh afferent neurons producing an evident N100 sensory response (Näätänen et al., 2007). There is also an N100 type response to the deviant tones, but it has a longer duration which leads to the MMN observed in figures 4.1 to 4.6. The grand averaged duration, location and gap deviants elicit a peak response as early as 143 ms (Figures 4.1, 4.3, 4.5, table 9), while the frequency and intensity responses peak later at 169ms and 176ms respectively. The peak MMN latencies reported in table 9 reflect the latency at which there is maximum difference between the standard and deviant waveforms. The

differences between the waveforms of the deviants are reflected in the peak latency of the MMNs.

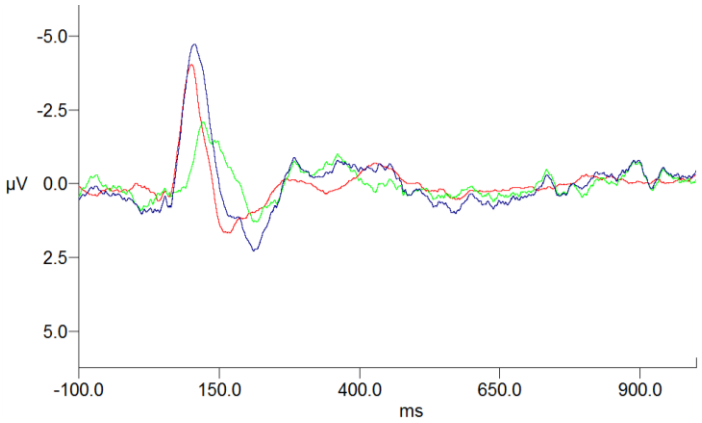

Figure 4.1 : Comparison of Grand average filtered standard tone (red), duration deviant (blue), MMN due to duration deviant (green) at Fz electrode.

In comparison to the other deviants, the frequency deviant (Figure 4.2) elicits a more effective MMN response as peak amplitude of MMN due to frequency deviant is higher than any other deviant (figure 4.8 and table 9). In all the grand averages, the early peak in MMN is followed by a second negative peak of considerable amplitude which might be associated with N200 (N2). N200 is considered to be associated with initial categorization of stimuli based on selective attention. This N200 effect is found to be reduced in the patients of Schizophrenia (Weinberger & Harrison, Schizophrenia, Chapter: 15).

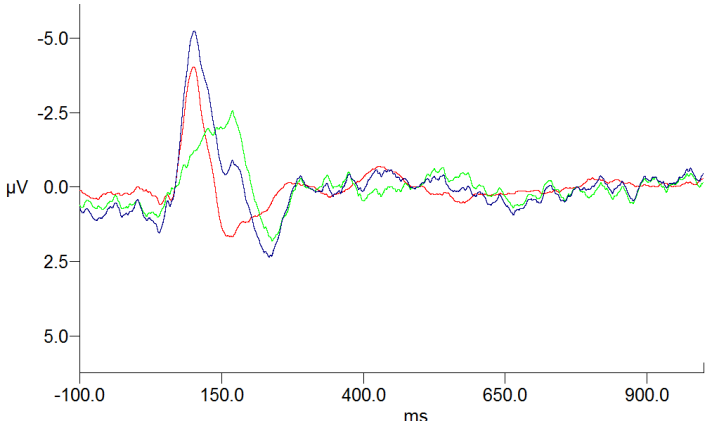

Figure 4.2 : Comparison of Grand average filtered standard tone (red), frequency deviant (blue) and MMN due to frequency deviant (green) at Fz electrode.

MMN due to the filtered intensity deviant shows a peak at 176ms with notable amplitude (Figure 4.3, table 9). The intensity increments are more attention-catching auditory events (Näätänen et al., 2007). This suggests that intensity MMN can also be used to elicit a good MMN response.

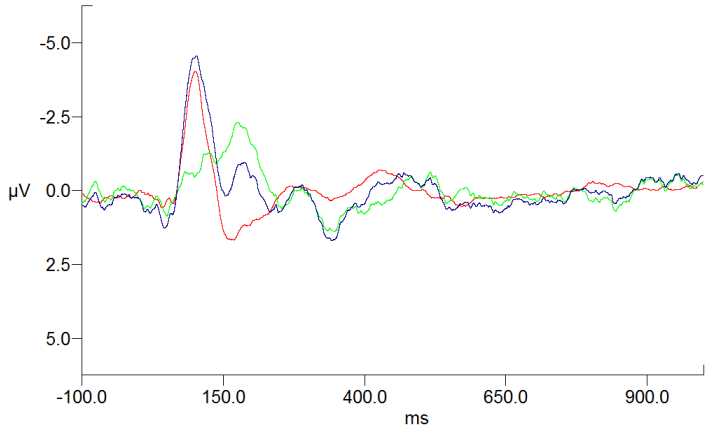

Figure 4.3 : Comparison of Grand average filtered standard tone (red), intensity deviant (blue) and MMN due to intensity deviant (green) at Fz electrode

The MMN to filtered location deviant (Figure 4.4) shows an early peak latency with the smallest amplitude when compared to the other deviants (figure 4.8), but that is still easily detectable.

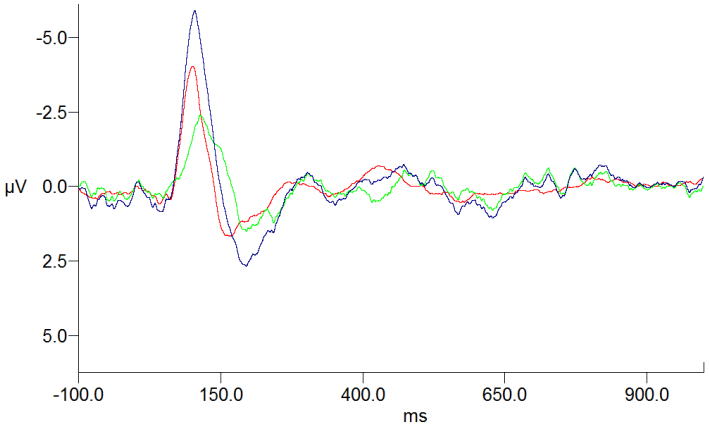

Figure 4.4 : Comparison of Grand average filtered standard tone (red), location deviant (blue) and MMN due to location deviant (green) at Fz electrode

It is clear from the figure 4.5 that the grand average of the filtered gap deviant has earlier peak latency when compared to the other deviants (figure 4.7) and is followed by a distinct negative peak around 300ms.

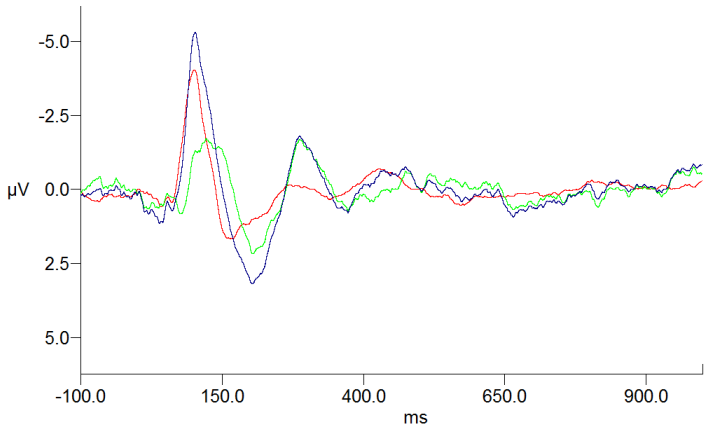

Figure 4.5 : Comparison of Grand average filtered standard tone (red), gap deviant (blue) and MMN due to gap deviant at Fz electrode

Figure 4.6 shows the comparison between the filtered grand averages of P300 standard and deviant which clearly portrays the effect of the deviant in the waveform. There is a large broad P300 observed at 328.5ms at the Pz electrode. As discussed earlier in section 2.6, the P300 amplitude is proportional to the amount of attention allocated to the given task. So, here the average amplitude represents the cumulative performance of the subjects in the task of responding to the deviants from the standards by pressing buttons. The effects of filtering decrease the peak latency and amplitude of both the standard and P300 waveforms, that is probably linked to the fact that the P300 is a high amplitude, low frequency waveform that will be effected by the 1 Hz filter, the time-frequency analysis should reveal more.

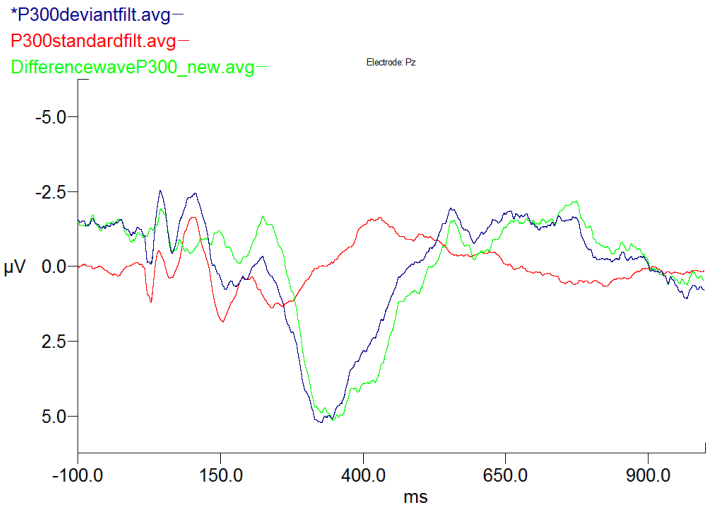

Figure 4.6 : Comparison between Grand average filtered P300 standard tone (red), P300 Deviant tone (blue) and their difference wave (green) at Pz electrode.

Figures 4.7 and 4.8 show a comparison of the latencies and amplitudes between the averaged raw data and the filtered data. As previously mentioned, there is no difference between the latencies of the raw and filtered data for each deviant, and there is not a very large difference in the latencies across all deviants (figure 4.7). There is a greater difference between the amplitudes of the raw and filtered data, but all deviants are well below 0 (figure 4.8).

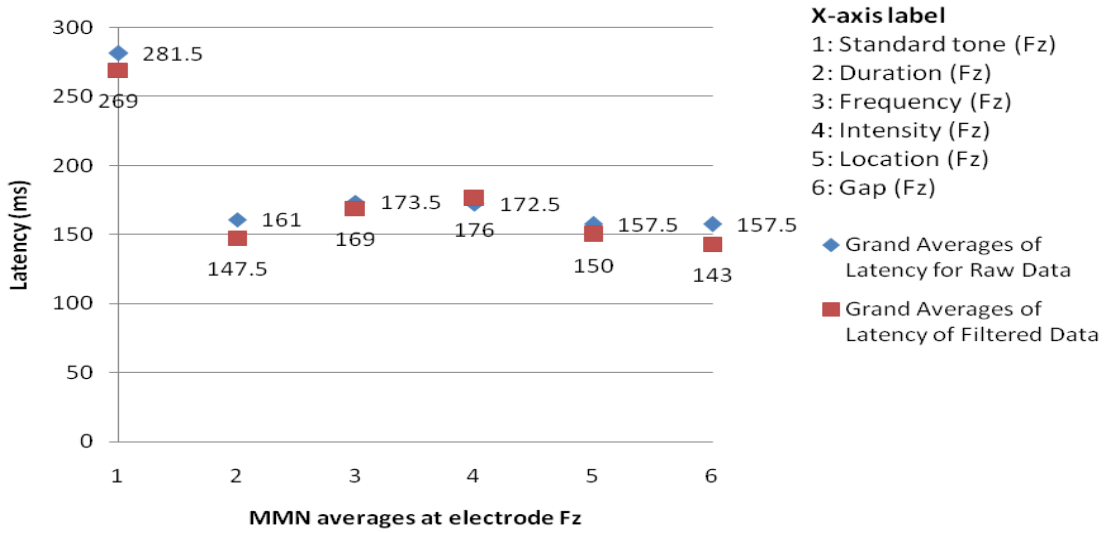

Figure 4.7 : Chart comparing the Latencies of averaged Raw and Filtered Data for Mismatch Negativity

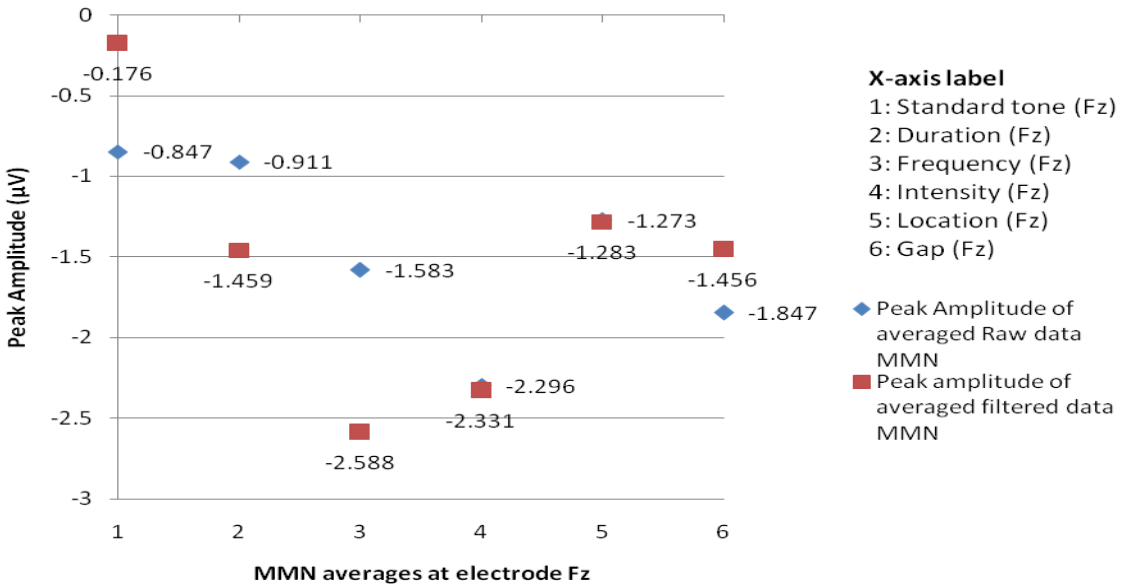

Figure 4.8 : Chart comparing the peak amplitudes of averaged Raw and Filtered Data for Mismatch Negativity

4.2 Pilot Study Results

A study with different interstimulus intervals of 500ms, 750ms and 1000ms was conducted on a pilot subject to determine any specific changes in the peak latency window and amplitude in mismatch negativity to the five deviants due to the different ISIs. In figure 4.9, filtered duration deviants of the three epoch lengths have been shown from -100 to 500ms. The peak latency and amplitude of the duration deviant MMN for 500ms, 750ms and 1000ms epoch lengths were found to be very similar at 160ms, -1.996µV; 167ms, -0.26µV and 157.5ms, -2.190µV respectively. The peak latencies are broadly similar in raw and filtered data. This observation is consistent with the other 4 MMN deviants for the filtered data (Table 4). The comparison of the latency and peak amplitudes is shown in table 4 and 5 respectively. As the interstimulus interval (the timing between the successive stimuli) is reduced the stimulus are presented at a faster rate (Javitt et al., 1997). With an increase in ISI, the chance of being initially exposed to more standard tones helps in the formation of a better memory representation of the standard tones before the deviants are introduced (Javitt et al., 1997).

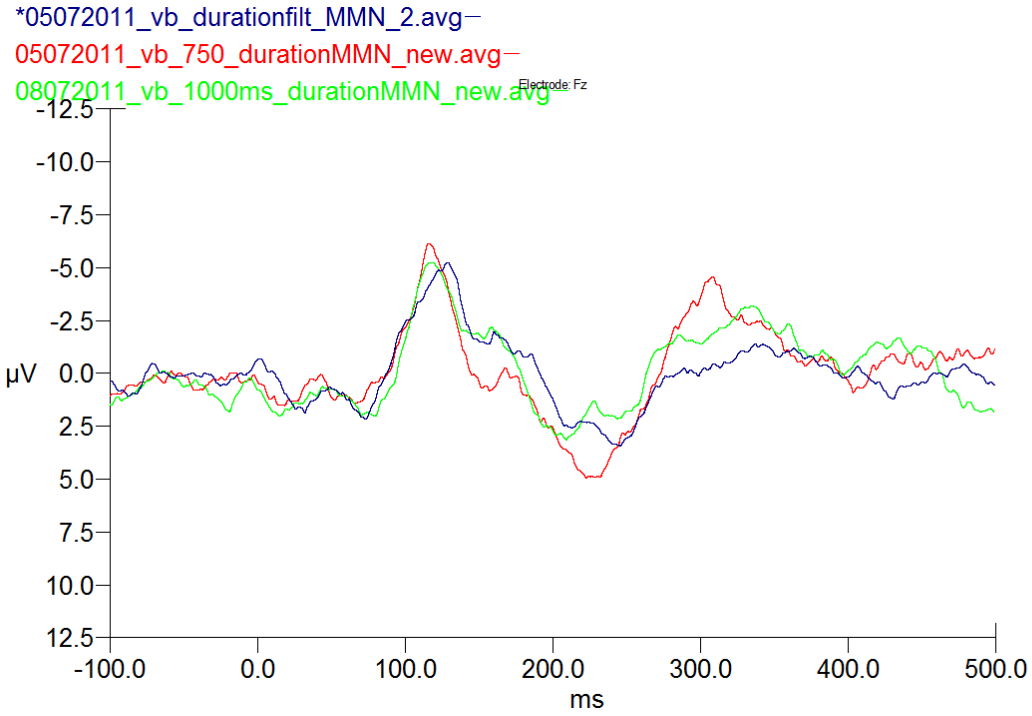

Figure 4.9: Comparison of the filtered Duration MMN data with 500ms, 750ms and 1000ms epochs at electrode Fz in the pilot subject.

4.3 Time-frequency Analysis

Time-frequency analysis is believed to reveal the correlation of the spectral properties of the ongoing EEG to various behavioral changes like cognitive processing (Delmore and Makeig 2004).

Single-trial EEG data time-locked to a particular event consists of an average ERP whose time course and polarity is fixed across the trial. As the time-frequency analysis was performed using the grand averages across the subject 7, there are certain limitations to the analysis. It is likely that event-related dynamics might be poorly represented across the average response or may not be presented at all. Also, the average response might ignore the ongoing EEG processes which are partially time and phase-locked when the auditory stimuli were presented (Delmore and Makeig, 2004). However, in this analysis distinctive increase in power has been noted across all the plots more or less around the time period where the peak latencies occur in the time-domain.

In the plot (figure 4.10) of averaged raw data representing the standard tone for the mismatch negativity, there is a significant increase in power around 100ms across all the frequency bands. In the theta, delta and alpha bands the energy is as high as 27dB relative to the pre-stimulus baseline. This indicates the presence of the N100 effect in the standard epoch which is evident even in the time-frequency plot. There is an increase in power in the frequency range of 0-16Hz around 200-300ms and a decrease in power, -3dB to -21dB, across the same time period is noticed in the higher frequency range from 35-45Hz.

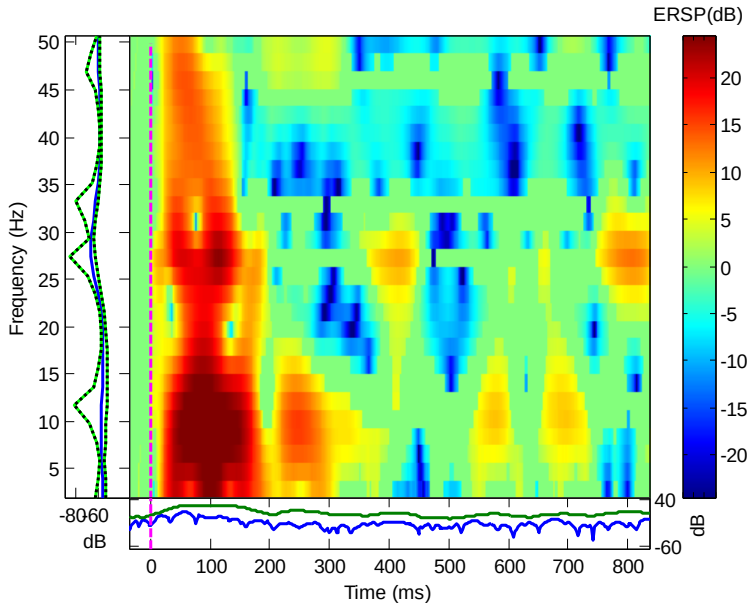

Figure 4.10: Time-Frequency plot of raw standard wave data for MMN averaged at electrode Fz.

In the time-frequency plot of averaged MMN due to duration deviant (figure 4.11), an increase in power (3-16dB) is noticed from 79-200ms in the frequency range of 0-18Hz. In the time-domain, the peak latency for the duration MMN is noticed around 175ms. In the spectral plot, this activity is highlighted in the lower frequency bands. A uniform increase in power (ranging from 7-13dB) is noticed across the entire frequency band from 298-394ms. However, a decrease in power (-5dB to -25dB) is observed in the frequency ranges 0-20Hz and 33-47Hz about 276-278ms and 182-250ms respectively. These two features are not present in the standard wave ERSP. Filtering data above 30 Hz would remove some activity in the 45 to 50Hz range, that could increase the amplitude of the ERP in the time domain, as observed in table 9.

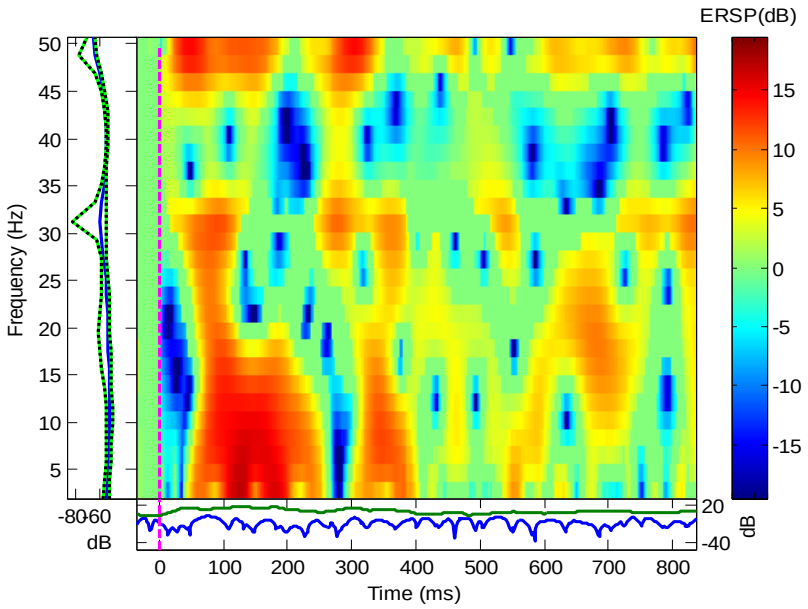

Figure 4.11: Time-Frequency plot of raw Duration deviant MMN data averaged at electrode Fz.

In the plot of the mismatch negativity due to frequency deviant (figure 4.12), there is increase in power (5dB-19dB) in the lower frequency band in the range 0-28Hz in the period 55-330ms. There is an evident increase in power of about 16dB at 18Hz in the range 162-178ms, approximately the same time when the frequency MMN peaks in the time-domain. In the higher frequency band 29-50Hz, there is a decrease in power (-5dB to -35dB) from 23-272ms. The concentration of power in frequencies below 30 is very different to the standard data ERSP, and may also explain the increase in amplitude seen in the time domain ERP when the filter was applied to the data (table 9).

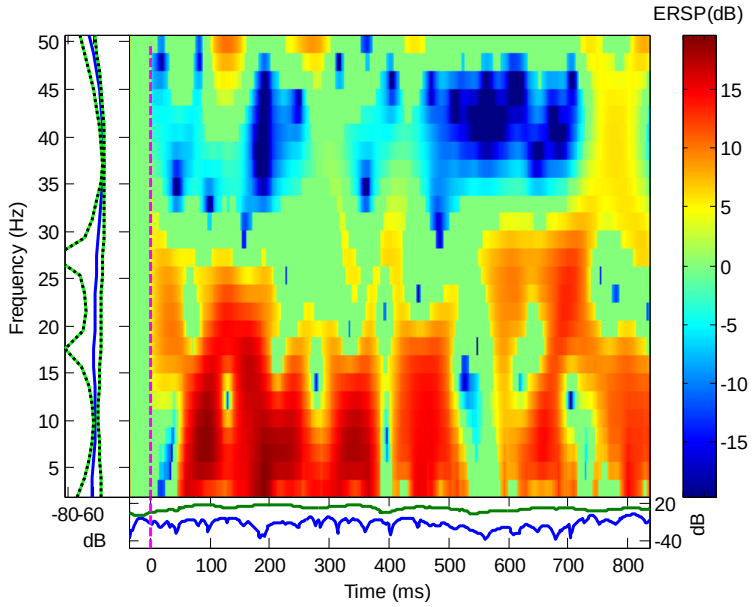

Figure 4.12: Time-Frequency plot of raw Frequency deviant MMN data averaged at electrode Fz.

In the plots of intensity deviant (figure 4.13) and location deviant (figure 4.14), an increase in power is seen in the frequency range of 0-18Hz from 50-425ms and in the frequency range of 18-40Hz from 75-340ms respectively. A decrease in power (-5dB to -18dB) is evident in the frequency range of 18-33Hz from 260-395ms in the plot for intensity deviant while in the location plot a decrease in power (-3dB to -30dB) is seen higher frequency, 39-56Hz from 33-250ms. The intensity and gap deviant ERSP are very different from each other, being almost inverted over the 1 to 35Hz range, and are also very different from the standard data ERSP.

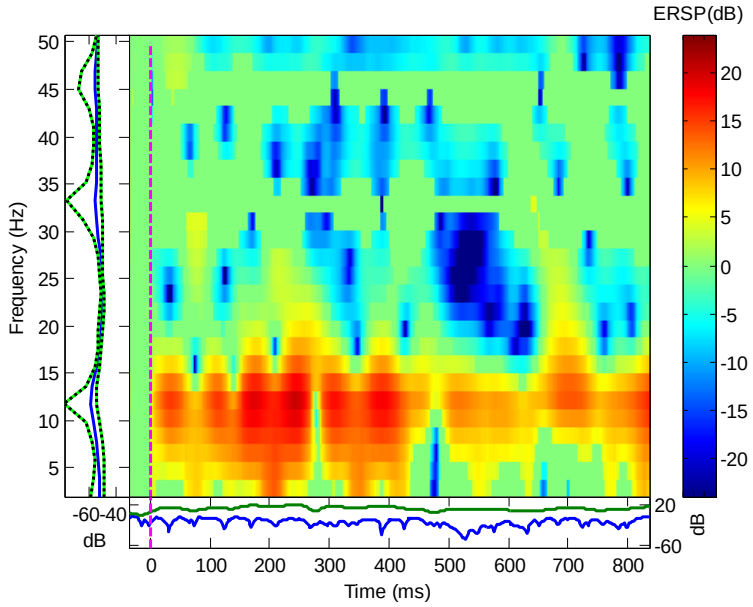

Figure 4.13: Time-Frequency plot of raw Intensity deviant MMN data averaged at electrode Fz.

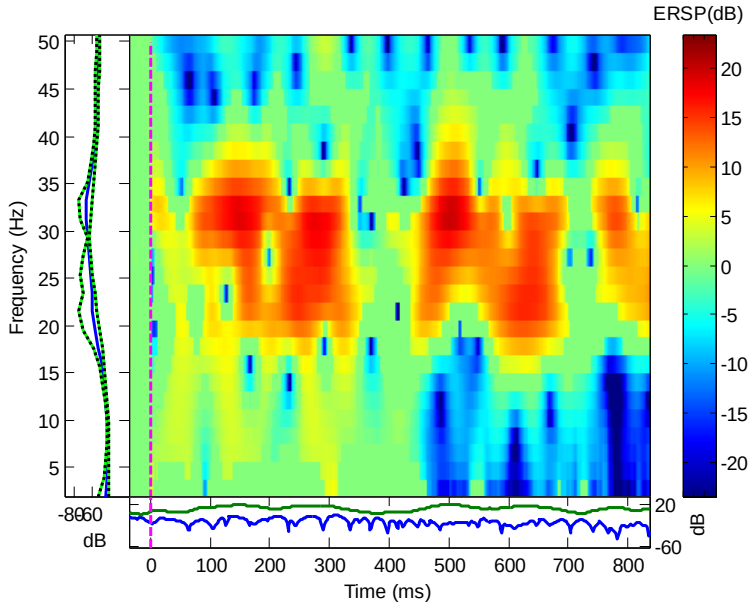

Figure 4.14: Time-Frequency plot of raw Location deviant MMN data averaged at electrode Fz.

In the plot of raw averaged mismatch negativity due to gap deviant (figure 4.15), there is increase in power in the frequency range of 0-20Hz from 127-259ms. There is also a decrease in power (-4dB to -25dB) from 97-340 ms, seen across higher frequency band

of 31-48Hz. There is a general decrease in power across the whole epoch, and the increase in power is confined to the time window during which the peak latency of the ERP occurred. This ERSP is the most different from the standard wave ERSP, and the other deviants.

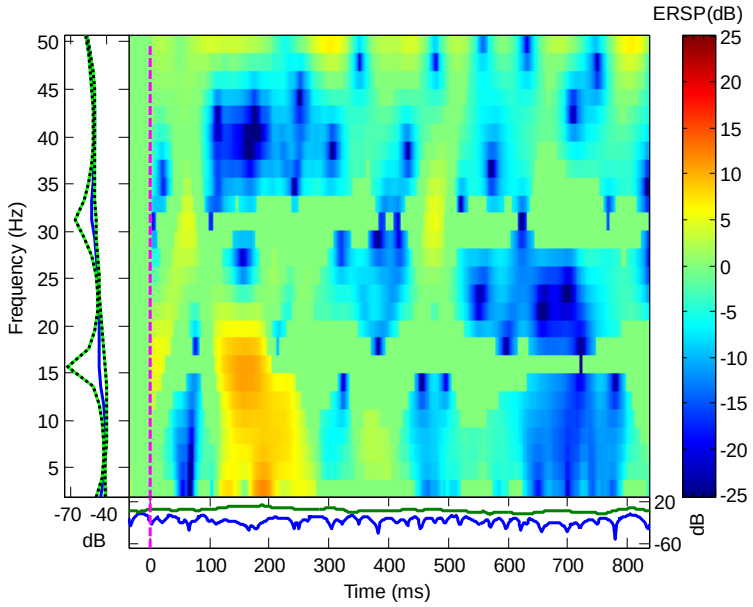

Figure 4.15: Time-Frequency plot of raw Gap deviant MMN data averaged at electrode Fz.

There is an increase in the power of frequency bands below 30Hz noticed across all the mismatch negativity plots due to the various deviants, but each deviant is strikingly different from the other deviants, and also from the standard data ERSP. An increase in the total power in the lower frequency bands (delta, theta bands) suggests the existence of a phase-locked event related potential (Roach & Mathalon, 2008). According to a recent study, subjects who have strong frontal midline slow wave oscillations (5-7Hz) tend to have higher cognitive ability (Weinberger & Harrison, Schizophrenia, Chapter: 15). All the participants in this study were involved in post-graduate education, suggesting a high cognitive ability.

The P300 standard wave (figure 4.16) displays increase in energy (as high as 18dB) in all the frequency bands between 50 and 175ms, especially across 33-55Hz. A similar increase in power (4-10dB) is also noticed in the frequency ranges of 0-23Hz about 260-444ms. The P300 deviant wave average (figure 4.17) shows a significant increase in

power around 170-395ms in the frequency range of 0-28Hz, indicating the presence of the large broad P300 effect in the time-domain that is not present in the standard data ERSP. There is also an increase in power at ~22Hz at a number of time points in the deviant ERSP compared to the standard. The P300 difference wave (figure 4.18) displays energies in the range of 3-13dB and 6-12dB in the frequency band of 0-27 Hz at 174-355ms and 40-130ms respectively. However, a decrease in power, -9dB to -20dB, is noticeable in the gamma frequency band of 45-50Hz from 92-189ms.

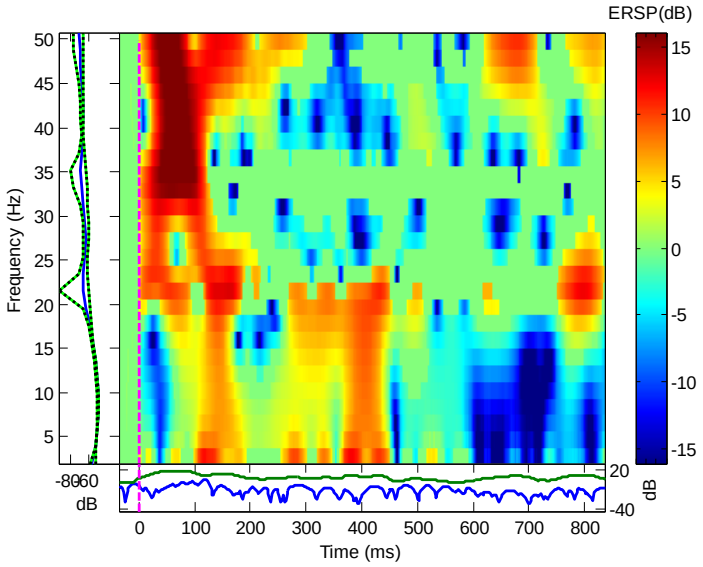

Figure 4.16: Time-Frequency plot of P300 raw standard wave averaged at electrode Pz.

However, in the time-frequency plot of P300 standard, deviant and the P300 difference wave, there is a considerable increase in energy of 4-18dB evident in the frequency range of 30-50 Hz about the interval from 23-200ms, 33-121ms and 20-106ms, 248-460ms respectively. The presence of activity over 30 Hz may also explain the reduced amplitude of the P300 ERP when the data was filtered instead of low frequency filter effects. The frequency band from 31-100 Hz represents gamma frequency band oscillations. Gamma-band oscillations reflect the synchronous activity of activated neurons in the different regions of cortex which primarily include auditory, parietal, somatosensory and visual regions as well as the hippocampus. Gamma activity relates to cognitive processing, sensory stimulation, attentional selection, working memory and

perceptual integration usually seen about 200 ms after the stimulus onset (Weinberger & Harrison, Schizophrenia, Chapter: 15; Roach & Mathalon, 2008). In the present analysis, it is evident that subject7 shows a significant presence of gamma-band oscillations in P300, but also some beta band activity that is associated with the deviant tone, and the largest power increase in power associated with the peak latency of the P300 occurs below 15 Hz. This is similar to the automated stimulus processing of the MMN, suggesting that the feature detection of the stimulus and the cognitive aspects of responding to the deviant tones might be controlled by separate cortical systems.

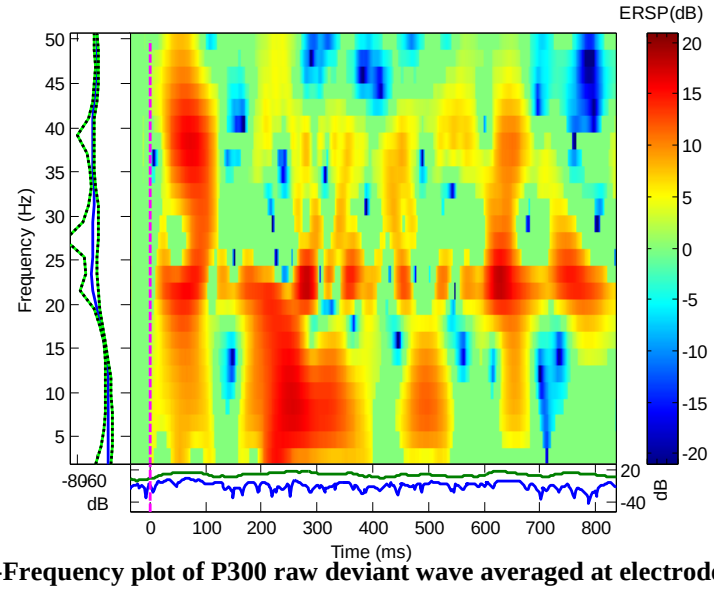

Figure 4.17: Time-Frequency plot of P300 raw deviant wave averaged at electrode Pz.

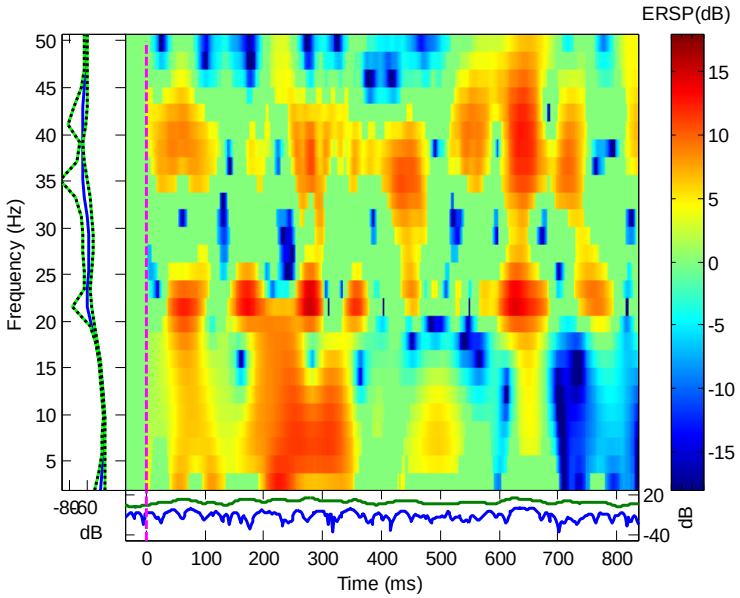

Figure 4.18: Time-Frequency plot of raw P300 difference wave data averaged at electrode Pz.

Chapter 5: DISCUSSION

Mismatch negativity is a short-duration cognitive ERP component which is elicited in response to stimulus deviance (Javitt et al., 1997). It is elicited by any detectable change in auditory stimulus and does not require the attention of the participant which makes it easier to be used in a patient group. As MMN is sensitive to small stimulus changes which correspond to behavioural discrimination, it can be used in both the healthy group and the patients of Schizophrenia. Also, it can be used as an index of discrimination accuracy in evaluating the auditory central processing in both control and patient groups (Näätänen et al., 2008). Deficits in mismatch negativity are relatively specific in the patients of Schizophrenia in contrast to the other psychiatric disorders and exist in the clinically unaffected family members of the patients. This strongly suggests that MMN is not always associated with functional impairments (Light & Braff 2005). Mismatch negativity shows pre-attentive disturbances which are closely associated with cognitive and psychosocial deficits and appear as disrupted activity in the fronto-central cortical region that are evident in the patient group. The fronto-central region is associated with the development of the negative symptoms. As the disease progresses, the positive symptoms such as auditory hallucinations become more prominent and are correlated to the disrupted activity in the temporal region (Hermens et al., 2010).

P300 amplitude is a measure for cognitive tasks that require maintenance of working memory. The P300 component size represents the degree to which the information is processed, establishing it as a measure of the activity in the central nervous system corresponding to the stimulus memory representation generation (Jeon & Polich, 2003).

Though anti-psychotic medications do not affect either MMN or P300 amplitudes, the age of onset and the duration of illness do have an effect on the amplitude of P300 (Jeon & Polich, 2003). However, the MMN deficits due to duration deviants are seen earlier in the course of illness when compared to the deficits due to other deviants (Javitt et al., 1997). Both these biomarkers can be studied together to research the underlying genetic

structure that affects the patients of Schizophrenia using P300 and the various behavioral and cognitive states using mismatch negativity.

The present study was conducted to assess a test protocol which would help determine deficits in the patients at a later stage. The time-frequency analysis of the raw data displayed a significant gamma activity in the grand averages across all the MMNs to the five deviants as well as in the P300 difference wave suggesting its critical presence among the healthy controls. Gamma activity represents the interaction between the pyramidal neurons in the cortex and the GABAergic inhibitory neurons which produce high frequency spikes and contain calcium binding protein called parvalbumin. This activity is found to be abnormal in the patients of Schizophrenia (Weinberger & Harrison, Schizophrenia, and Chapter: 15).

In the present study, the MMN responses have been found to be preceded by an obvious and large N1 event-related potential which reflects a strong sensory response. According to the study by Umbricht et al., 2003, as the difference between the standard and the deviant increases, the MMN to duration deviant stimulus becomes associated with N1 response. In the grand averages across all subjects, a large N1 to the standard tone has been noted in the present group of healthy males. It is believed that N1 to the standard is smaller in the patients of Schizophrenia. In the patient group the N1 effect is found to be masked by the MMN deficits indicating impairment in the temporal processing of information among the patient group and highlighting the presence of deficits in the MMN generation in them (Umbricht et al.,2003).

With a relatively small group of healthy controls (N=9), the study demonstrated that the latencies of MMNs due to various deviants were found to be well within the expected window however, a comparatively diminished value of amplitude has been found both in raw and filtered averaged data.

There are several shortcomings in the present study. The timing of the ERP peaks across the latency ranges varies with many factors for an individual practically. It depends on his mental status, attentional demands, processing resources and susceptibility to

distraction by irrelevant stimuli (Light & Braff, 2010). These factors were not specifically controlled during the study. In addition to this, there are two types of neurons which are involved in the MMN process, namely computational and amplifying neurons. The amplifying neurons might be modulated by attention (Näätänen et al., 2008). These could be the contributing factors for the reduced amplitudes that were observed in the grand averages of mismatch negativity due to the various deviants. Although a nose reference was used, the MMN components did not show any inverted polarity over the mastoids, even when the data was re-referenced to the averaged mastoid channels. This may be due to the exact location of the nose reference and mastoid channels, and could be further investigated.

In time-frequency analysis, by increasing the time window to estimate the data at a given time point, we increase the frequency resolution while making a compromise on the temporal resolution (Roach & Mathalon, 2008). In the study, the use of 1000 ms epochs to elicit MMNs and P300 in the control group allows the assessment of frequency components as low as 1Hz while a shorter ISI would not show the activities in the theta and delta bands. A significant amount of activity has been found in the time-frequency plots of the subject7 in these bands, as well as at higher frequency bands that are commonly removed by filtering. The longer ISI and increased signal bandwidth allows the frequency analysis to investigate activity in low delta to high gamma frequency bands that would not be possible using short ISIs and typical filter settings. A single power spectrum determines the average magnitude of oscillations for individual frequency bands over the entire epoch length of 1s. As in this case, the power spectra is calculated over just one epoch from the averaged data for one subject, the frequency resolution is enhanced with the increase in the total number of the time points in the time window (Roach & Mathalon, 2008). This implies that in time-domain we are able to obtain and assess a good MMN response while also being able to assess the power spectrum with a good frequency resolution.

Having demonstrated the presence of interesting features in the time-frequency analysis, the next stage would be to calculate the ERSP based upon the individual epochs and not

just the grand averaged data. This should significantly increase the sensitivity of the analysis and increase the signal to noise ratio. Widening the analysis to other electrodes could also reveal interesting features that are of importance in understanding MMN and P300 processing. Features common to each subject could then be investigated to help define what frequency features are common to the conscious and unconscious processing of deviant stimuli across the normal population. This will be vital when the test is applied to patients with Schizophrenia.

With a long interstimulus interval the MMN amplitude is found to be more attenuated in the older male age group in comparison to younger males. Traces of relatively short duration are required to elicit MMN. In younger healthy subjects, this trace lasts for about 5-10s and gets shorter with the increase in age (Näätänen et al., 2007). Therefore, an age-matched study with varying interstimulus interval could be carried out in future with a larger sample size to obtain significant results with enhanced signal-to-noise ratio for the deviant waveforms. Filtering the data increased the number of accepted sweeps while there was no significant difference observed in the peak latency and amplitude in both MMN and P300 event-related potentials. However, the usage of the filter smoothes the data out and helps in determining the peak latency and amplitude better. A time-frequency analysis was carried out on the raw data for one of the subjects with the cleanest data. Time-frequency analysis could be run on individual subjects to match the various findings in time-domain. Raw data was used to obtain the power spectra because the bandwidth of the applied bandpass filter [1-30 Hz] would not show any gamma activity in the gamma bands. Applying different filters to the raw data and seeing their effect could provide a better insight on the time-frequency analysis.

A majority of the participants in the study were restless after a certain period of time while performing the tasks, as it took a while for the preparation of the test, applying the gels to the electrodes and checking for the levels of impedance so that better data could be obtained. The length of the experiment and the method might pose a problem for the patients with Schizophrenia. The patients might get very anxious and frustrated with the whole set up and the attachment of electrodes from the scalp might trigger delusional

ideas and thoughts such as the recording being a way through which signals are being transmitted to/ from his brains. If the patient has any motor disorders, then it might interfere with obtaining good data if the recording is conducted for a very short period.

Chapter 6: CONCLUSION

This study tested three protocols in healthy male controls. First, it tested the optimal paradigm for mismatch negativity using a fixed epoch length of 1s. Second, the P300 paradigm was tested and the attention-dependent factor associated with this ERP component was assessed. Third, auditory stimuli with three different epoch lengths of 500ms, 750ms and 1000ms were presented in a pilot subject and its effect on the MMN was observed. The present study was a single trial with 11 healthy controls and 2 pilot subjects. The latencies in mismatch negativity due to various deviants occur about the same time window as has been recorded among healthy controls using other standard paradigms. P300 averaged data reveals a great deal of information about the attention parameter devoted by each subject to the task which is evident with their recorded amplitudes. However, to strengthen the relevance of the findings it would be better to recruit more subjects. The relationship between interstimulus interval and frequency resolution in the time-frequency analysis is an interesting area to be looked at, as there is an obvious trade-off between the two factors.

Both mismatch negativity and P300 appear to be potential biomarkers which can be employed for detection of Schizophrenia while MMN can be preferred over P300 for early detection of the disease in the patients. A few factors have to be taken into consideration in designing the paradigm for MMN namely epoch length, the interstimulus interval, the number of deviants used, the complexity of the paradigm, the location of the reference electrode (nose or mastoids), the video task and the duration of the test.

The successful demonstration of features in the time-frequency analysis that are unique to each deviant also add to the potential for use as biomarkers and require a more detailed and comprehensive analysis.

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