EVALUATION OF NEUROPHYSIOLOGICAL PROPERTIES AMONG METHADONE TREATMENT SUBJECTS: EEG AND ERP STUDY

FARID ESMAEILI MOTLAGH

FACULTY OF ENGINEERING
UNIVERSITY OF MALAYA
KUALA LUMPUR

2018

EVALUATION OF NEUROPHYSIOLOGICAL PROPERTIES AMONG METHADONE TREATMENT SUBJECTS: EEG AND ERP STUDY

FARID ESMAEILI MOTLAGH

THESIS SUBMITTED IN FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

FACULTY OF ENGINEERING
UNIVERSITY OF MALAYA
KUALA LUMPUR

2018

UNIVERSITY OF MALAYA

ORIGINAL LITERARY WORK DECLARATION

Name of Candidate: FARID ESMAEILI MOTLAGH

Matric No: KHA130096

Name of Degree: Doctor of Philosophy (Ph.D)

Title of Thesis ("Evaluation of neurophysiological properties among methadone treatment subjects: EEG and ERP study"):

Field of Study: Biosignal processing

I do solemnly and sincerely declare that:

(1) I am the sole author/writer of this Work;
(2) This Work is original;
(3) Any use of any work in which copyright exists was done by way of fair dealing and for permitted purposes and any excerpt or extract from, or reference to or reproduction of any copyright work has been disclosed expressly and sufficiently and the title of the Work and its authorship have been acknowledged in this Work;
(4) I do not have any actual knowledge nor do I ought reasonably to know that the making of this work constitutes an infringement of any copyright work;
(5) I hereby assign all and every right in the copyright to this Work to the University of Malaya ("UM"), who henceforth shall be owner of the copyright in this Work and that any reproduction or use in any form or by any means whatsoever is prohibited without the written consent of UM having been first had and obtained;
(6) I am fully aware that if in the course of making this Work I have infringed any copyright whether intentionally or otherwise, I may be subject to legal action or any other action as may be determined by UM.

Candidate's Signature Date:

Subscribed and solemnly declared before,

Witness's Signature Date:

Name:

Designation:

EVALUATION OF NEUROPHYSIOLOGICAL PROPERTIES AMONG METHADONE TREATMENT SUBJECTS: EEG AND ERP STUDY

ABSTRACT

This thesis presents the investigation of brain electrophysiological attributes of heroin addicts from early stages of withdrawal until three months of methadone maintenance treatment (MMT) with and without electroacupuncture (EA). In this longitudinal study, 96 heroin-dependent subjects had been recruited to MMT program and divided randomly into two groups of MMT+EA and MMT. A comprehensive paradigm was designed to probe the event-related potential (ERP) components and a novel single-trial algorithm was developed to detect and extract the ERP components features. EEG power spectrum and electro-cognitive responses were the main assessments to be evaluated in each phase of the study. The first phase was a cross-sectional study between heroin dependents and healthy control subjects. Study subjects went through three months of treatment, and neurophysiological properties were assessed based on monthly interventions. This study has further confirmed that opioid addiction significantly influences certain brain regions and causes abnormal spectral activity, as well as the decline of brain evoked potentials amplitudes. Monitoring the immediate and short-term effects of treatment revealed the process of cognitive enhancement among the subjects. Furthermore, the results of different phases suggest electro-neurophysiological properties as an index indicate cognitive dysfunction and severity of brain activity dysregulations which can be used for evaluating the treatment effectiveness as well. The results also proved the reliability of single-trial ERP detection algorithm and suggested the employment of this paradigm in the field.

Keywords: Electroencephalogram, Event-related Potential, Wavelet, Heroin Addiction, Methadone treatment.

PENILAIAN SIFAT NEUROFISIOLOGI DI KALANGAN SUBJEK RAWATAN METADONE: KAJIAN EEG DAN ERP

ABSTRAK

Tesis ini membentangkan penyiasatan sifat-sifat electrophysiological otak penagih heroin dari peringkat awal pengeluaran sehingga tiga bulan rawatan penyelenggaraan methadone (MMT) dengan dan tanpa electroacupuncture (EA). Dalam kajian jangka panjang ini, 96 mata pelajaran yang bergantung kepada heroin telah direkrut untuk program MMT dan dibahagikan secara rawak ke dalam dua kumpulan MMT + EA dan MMT. Paradigma yang telah direka untuk meneliti komponen potensi yang berkaitan dengan peristiwa (ERP) dan satu algoritma percubaan baru novel telah dibangunkan untuk mengesan dan mengekstrak ciri-ciri komponen ERP. Spektrum kuasa EEG dan respons elektro-kognitif adalah penilaian utama yang akan dinilai dalam setiap fasa kajian. Fasa pertama adalah kajian keratan rentas antara tanggungan heroin dan subjek kawalan yang sihat. Subjek kajian menjalani tiga bulan rawatan, dan sifat neurofisiologi dinilai berdasarkan intervensi bulanan. Kajian ini seterusnya mengesahkan bahawa ketagihan opioid banyak mempengaruhi kawasan otak tertentu dan menyebabkan aktiviti spektrum yang tidak normal, serta penurunan otak, menimbulkan potensi amplitudo. Memantau kesan segera dan jangka pendek rawatan mendedahkan proses peningkatan kognitif di kalangan mata pelajaran. keputusan fasa yang berbeza menunjukkan sifat-sifat elektro-neurofisiologi sebagai indeks menunjukkan disfungsi kognitif dan keparahan disregulations aktiviti otak yang boleh digunakan untuk menilai kecekapan rawatan juga. Hasilnya juga membuktikan kebolehpercayaan algoritma pengesanan ERP satu percubaan dan mencadangkan penggunaan paradigma ini di lapangan.

Keywords: Electroencephalogram, Berkaitan dengan peristiwa,Wavelet, Ketagihan Heroin, Rawatan metadon.

ACKNOWLEDGEMENTS

To my life heroes, my father Ali Motlagh and my lovely mother Sedigheh Hajibagheri because I owe it all to you. Many Thanks!

Special thanks to Prof. Fatimah and Prof. Hussain my supervisors for their unfailing support and assistance and without their help, support and trust I could not proceed. My forever interested, encouraging and always enthusiastic brother Omid who was always supporting me in all aspects of my life. I miss our interesting and joyful moments. My eldest brother Hamid and his loving wife, Nazi. All my supportive and patient friends Abdi, Sam, Leili, Hossein & Zahra, Amin, Golnosh, Nima & Niusha, Sina, Sara, Vahid, MJ, Karam & Hanna who were like a part of my family and provided me with moral and emotional support in my life.

I am also grateful to my other family members and friends who have supported me along the way. My course mates, basketball and gym buddies! I will miss your screams of joy whenever a significant momentous was reached.

A very special gratitude goes out to all at UMCAS and HIR fund for helping and providing the funding for the work.

With a special mention to Dr. Tahereh, Dr. Rusdi, Ashikin and all members of UMCAS who support me through this important chapter of my life. It was fantastic to have the opportunity to work the majority of my research in your facilities.

And finally, last but by no means least, also to everyone in the CIME it was great sharing laboratory with all of you during last four years.

Thanks for all your encouragement!

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF SYMBOLS AND ABBREVIATIONS

AA	:	Auricular Acupuncture
AC	:	Alternating Current
ANCOVA	:	Analysis of Covariance
ANOVA	:	Analysis of variance
CNS	:	Central Nervous System
CWT	:	Continues Wavelet transform
DC	:	Direct Current
DIPFIT	:	Dipole Fitting
DWT	:	Discrete Wavelet Transform
EA	:	Electro-acupuncture
EEG	:	Electroencephalography
ERP	:	Event related potential
FFT	:	Fast Fourier Transform
FIR	:	Finite Impulse Response
FT	:	Fourier transform
HOC	:	Horizontal Oculogram
IIR	:	Infinite Impulse Response
IC	:	Independent Component
ICA	:	Independent Component Analysis
IQ	:	Intelligence quotient
MATLAB	:	MATrix LABoratory
MEG	:	Magnetoencephalography
MINI	:	Mini-International Neuropsychiatric Interview
MMN	:	Mismatch Negativity

MMT	:	Methadone maintenance treatment
MRI	:	Magnetic Resonance Imaging
NADA	:	National Acupuncture Detoxification Association
NIH	:	National Institute of Health
OCDUS	:	Obsessive Compulsive Drug Use Scale
OOWS	:	Objective Opioid Withdrawal Scale
PCA	:	Principle Component Analysis
PDF	:	Probability Density Function
PET	:	Positron Emission Tomography
PSD	:	Power spectral density
QQ	:	Quantile-Quantile
SOBI	:	Second Order Blind Identification
SOWS	:	Subjective Opioid Withdrawal Scale
SPECT	:	Single Photon Emission Computed Tomography
SVM	:	Support Vector Machine
SPW	:	Slow Positive Wave
TCM	:	Traditional Chinese Medicine
UMMC	:	University Malaya Medical Centre
VOC	:	Vertical Oculogram
WAIS	:	Wechsler Adult Intelligence Scale
WM	:	Working Memory
WHO	:	World Health Organization
WT	:	Wavelet transform

LIST OF APPENDICES

CHAPTER 1: INTRODUCTION

1.1 Overview

Heroin as an opioid drug is converted to morphine in the brain and binds to molecules of opioid receptors, which are especially involved in the perception of pain and controlling automatic processes. Therefore, Opioid addiction as the prevalent cause of physical and psychosocial problems leads to cognitive functioning impairments and a deficit of attentional bias among addicts. In 2011, 4.2 million Americans aged 12 or older had used heroin at least once in their lives. Heroin abuse has been prevalent in Malaysia as well, and opiate was the main drug abused in the early 20th century according to the local statistics from National Anti-Drug Agency in 2010.

According to the World Drug Report 2014, there was estimated 12.8 – 20.2 million people worldwide are engaged with illicit substances such as heroin and opium (Organization, 2014). Methadone treatment has been known to be the standard substitution therapy for heroin addiction, and Methadone Maintenance Treatment (MMT) has been used since the 1960s as the most common substitution therapy for heroin addicts. Methadone is a synthetic half-life opiate developed as a pharmacological treatment that replaces heroin and has been associated with physical reliance; it causes less psychological dependence as compared with other opioids like heroin (Greenwald, 2006).

Since methadone is an opiate itself, safe and low-cost adjunctive therapies like acupuncture were proposed and have been practiced for providing more efficient heroin addiction treatment. Acupuncture denotes one of the most promising cost-effectiveness, and safe therapies for pain control, fibromyalgia, headaches, Parkinson's disease, schizophrenia, and depression (Jordan, 2006b) as well as drug addiction. Acupuncture therapy has been accepted by the National Institutes of Health (NIH) as a suitable complementary procedure alongside Western medicine, particularly for pain relief, and

the World Health Organization (WHO) officially accepted acupuncture as a treatment for drug abuse (Organization, 2002).

Many studies showed that chronic heroin abuse leads to long-lasting impairments in cognitive functioning and alterations in central nervous system (CNS) functioning (London *et al.*, 2000; Polunina *et al.*, 2007). Chronic heroin addiction and its relative neurophysiological effects have been evaluated through various mediums providing information about brain functions in this field. Although functional neuroimaging studies have contributed significantly to the understanding of the human brain, each has its advantages and limitations. However, since imaging techniques are time and cost consuming, over the last few decades, electroencephalographic (EEG) activities have been widely used to study brain cognitive dysfunctions and neurobiological alterations especially among heroin addicts.

Brain electrical activity properties including EEG activities and brain event-related potentials (ERPs) measurements have been used for detailed investigation of brain electrophysiological changes, as well as cognitive biases and information processing related to pre-attentive processing, attentional deficit, and response inhibition among heroin addicts. Previous investigations in the field can be clustered into two main categories namely; exploring the cognitive dysfunctions and abnormal brain activities caused by chronic heroin abuse, and effectiveness of methadone and abstinence from heroin on normalization of cognitive impairments and neuro-electrophysiological abnormalities.

Despite methadone's clinical effectiveness to restore some of the abnormal brain activities among heroin dependents, a number of neuropsychological studies have shown evidence of cognitive function impairments among methadone-treated subjects; including poor performance of attention, impairment of working memory, and deficiencies in

learning and immediate recall tasks (Darke *et al.*, 2000; Gritz *et al.*, 1975; Mintzer & Stitzer, 2002; Papageorgiou *et al.*, 2001; Papageorgiou *et al.*, 2003; Papageorgiou *et al.*, 2004; Prosser *et al.*, 2006; Rapeli *et al.*, 2006; Specka *et al.*, 2000; Wang *et al.*, 2014).

A review of these studies indicated that methodological issues; including intergroup differences, premorbid conditions, and comorbidities have significant direct and indirect effects on neuroelectrophysiological findings of these studies (Wang *et al.*, 2015). Furthermore, opioid withdrawal causes neurophysiological alterations and cognition deficiencies as well (Papageorgiou *et al.*, 2001; Rapeli *et al.*, 2006). Taken together, it remains uncertain whether the observed cognitive impairments among MMT subjects are solely attributable to the pharmacological effects of methadone. In other words, influences of methadone treatment on brain activity and neurocognitive abilities is a matter of debate with no concise conclusion yet.

In addition, aside from the reports that support the postulation of acupuncture therapy as an effective therapy for addiction (Chu *et al.*, 2008b; Cui *et al.*, 2004a; Fallopa *et al.*, 2012a; Hu *et al.*, 2009a; Li *et al.*, 2012; Y. J. Li *et al.*, 2011b; Liang *et al.*, 2006; Overstreet *et al.*, 2008a; Shi *et al.*, 2003a; Shi *et al.*, 2004b; Wang *et al.*, 2000; Wang *et al.*, 2011; Yang *et al.*, 2010a; Zhao *et al.*, 2006a), several clinical trials have shown that acupuncture presented no significant advantage in this field (Gates *et al.*, 2006; Jordan, 2006a; Lin *et al.*, 2012; Rabinstein & Shulman, 2003; Samuels *et al.*, 2008; White *et al.*, 2006; White *et al.*, 2011, 2014). Therefore, investigating the effectiveness of acupuncture as a proper adjunctive therapy is the second main issue in this field.

The next challenging issue in this area is quantifying the ERP characteristics (voltage amplitude and occurrence latency) in assessing an ERP component. Precise detection of amplitude and latency of ERPs is a key challenge in providing accurate conclusions and understanding of information processing and cognitive biases. The most significant

problem in describing an ERP waveform is the small amount of signal to noise ratio of EEG signals and low amplitude of ERPs when compared to baseline EEG (J. Li *et al.*, 2011). Therefore, averaging across a large number of trials is the most common approach in ERP experiments in the field of psychoanalysis. To provide a large number of trials in the conventional method, extended procedures with repetitive tasks of probing ERPs are required in related paradigm to directly affect the participants' attention and awareness which is associated with ERP features. On the other hand, characteristics of ERP components per se vary from trial to trial and cause variability of the waveform in single trials; and moreover, voltage fluctuations of various ERP waveforms overlap each other in time and space, such as auditory MMN and P3.

Taken together, using conventional techniques was a limitation in psychological experiments due to their fundamental goal in the evaluation of human neuro-psychophysiological features on a trial-by-trial basis. To date, there has been little use of advanced single-trial ERP detection techniques to investigate latency and amplitude of ERPs in the addiction field.

Integrating these challenging issues confirmed that there is a lack of a comprehensive evaluation of brain electrophysiological characteristics among heroin dependent subjects during MMT program. To the best knowledge of the authors, there is no study which comprehensively investigated the effect of methadone on EEG power spectrum as well as ERP components. In addition, to date, MMT adjunctive to acupuncture has not been neurophysiologically evaluated as well. Furthermore, no strong consensus opinion on the general efficacy of acupuncture exists, and evaluating acupuncture in cases of substance abuse have shown conflicting results.

1.2 Research Objectives

The main objective of this study is to investigate EEG and ERP attributes of heroin addicts from early stages of withdrawal until three months of MMT with and without electroacupuncture (EA) as an adjunctive treatment. This study also aims to investigate the acute effects of methadone intake as well as acupuncture on brain electrophysiological traits. Furthermore, this study seeks to introduce a reliable single-trial ERP detection algorithm to extract ERP latencies and amplitudes accurately by utilizing a new approach of a comprehensive ERP paradigm in a single experiment. The sub-objectives of this study can be listed as:

i. To design a comprehensive paradigm to probe MMN, P3, P600 components.
ii. To develop a single-trial ERP detection method, which allows the calculation of the latency ratio and the amplitude index of ERP components.
iii. To evaluate and compare the brain electrophysiological properties of chronic heroin addicts with healthy control subjects.
iv. To investigate the immediate and short-term effects of MMT on neuro-electrophysiological properties of drug users and explore any possible electrophysiological effects of EA as an adjunctive therapy to MMT.

1.3 Scope of the Work

The study was approved by the ethics committee of UMMC (Ethics Number: MEC 871.14), and a written consent was obtained from each subject before enrollment. Nicolet EEG diagnostics system (Care Fusion Corporation, 3750 Torrey View Court, San Diego, CA 92130) was used to capture the EEG activities within a frequency band. All the mathematical analysis and plots were performed by using the MATLAB 2013b software.

Male heroin-dependent subjects were recruited from the waiting list in the "Substance Clinic" at the University of Malaya Medical Center (UMMC). This study took place at the clinical engineering laboratory, in the Department of Biomedical Engineering, University of Malaya. The inclusion criteria for this study were at least one year of documented heroin addiction and fulfilled the diagnostic statistical manual IV (DSM-IV) criteria for heroin dependency, syndrome diagnosis, a minimum age of 18 years, and minimum six years of drug abuse. The exclusion criteria from this study were alcohol dependency, the presence of any acute psychiatric or neurological disorder (major head trauma), possessing a lifetime history of a major medical disorder, HIV infection, previous head injury resulting in loss of consciousness, seizures, history of methadone treatment, and record of polysubstance dependence.

1.4 Thesis Organization

This thesis is organized in following chapters:

- Chapter 2 reviews the literature related to acupuncture in addiction treatment as well as utilization of EEG and ERP properties in heroin and methadone studies.
- Chapter 3 describes the methodology and consists of three main sections related to explain the clinical trial, designing the ERP paradigm, and EEG signal processing.
- Chapter 4 presents the results of each phase of the study as well as the related discussion.
- Chapter 5 demonstrates the conclusion and contributions of this study, as well as limitations of this study and recommendations for future research.

CHAPTER 2: LITERATURE REVIEW

2.1 Introduction

This chapter begins with an introduction to heroin dependency, Methadone Maintenance Treatment (MMT) as the substitution therapy for opioid addiction, and acupuncture therapy as a harmless and low-cost adjunctive treatment to methadone which is investigated in this study. There have been several types of neurophysiological measurements suggested as a sophisticated approach providing information about brain functions in this field. However, over the last few decades, electroencephalographic (EEG) activities have been widely used to study brain cognitive dysfunctions and neurobiological alterations, especially among heroin addicts.

Therefore, in this study, electroencephalography (EEG) as an indicator of brain electrical activity is introduced, and a comprehensive review of its related literature in heroin addiction field is provided. For this purpose, a search for relevant documents on this topic was conducted through the ISI Web of Science (all databases) from January 2000 to January 2015, and the current challenges and methodological issues are integrated to form the problem statement of this thesis. A literature review on the efficiency of acupuncture in addiction treatment is provided as well. In the last section of this chapter, a summary of related literature highlighted the main issues of the field.

2.2 Heroin Dependency

Heroin as an opioid drug which occasionally used as a pain medication is synthesized from morphine, a naturally occurring substance extracted from the seedpod of the Asian opium poppy plant. Heroin is converted to morphine in the brain and binds to molecules of opioid receptors (Figure 2.1), which are especially involved in the perception of pain and reward as well as controlling automatic processes (Louria *et al.*, 1967).

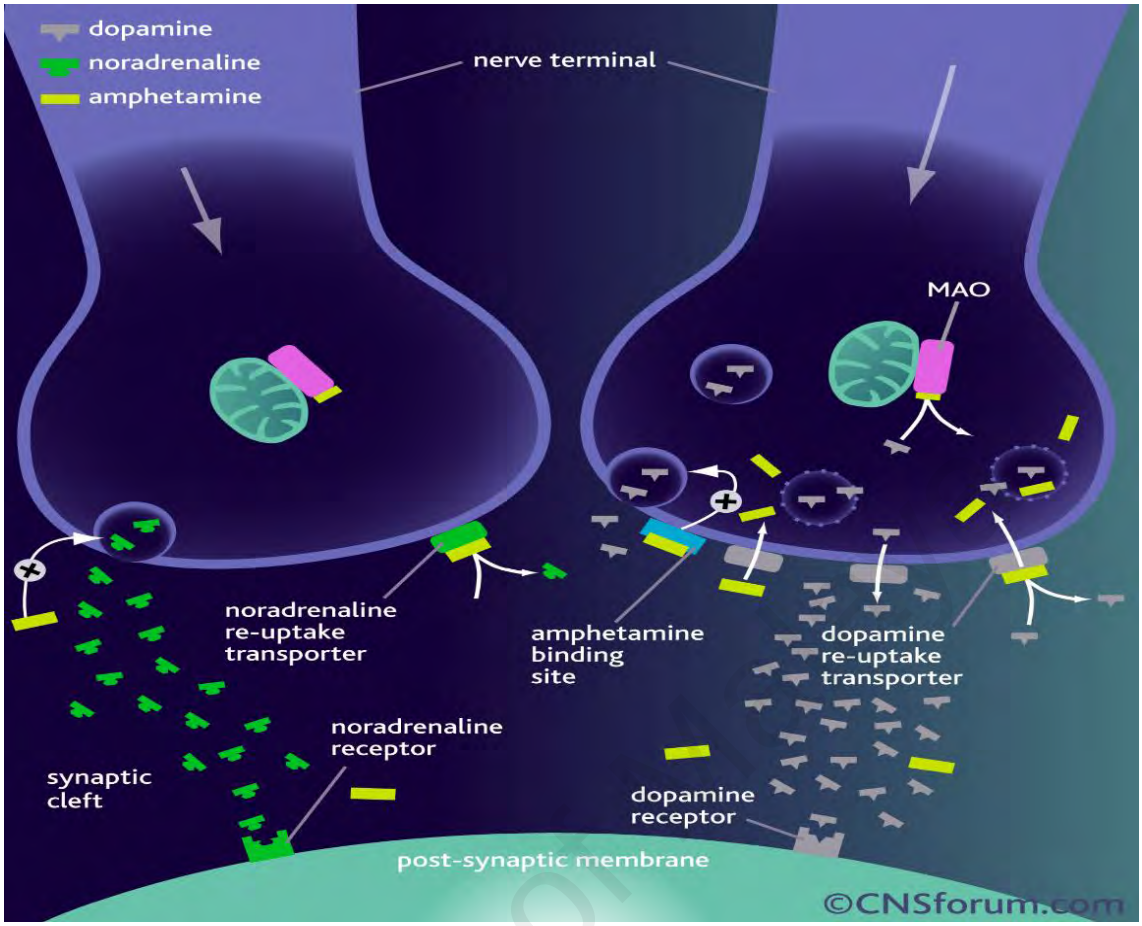
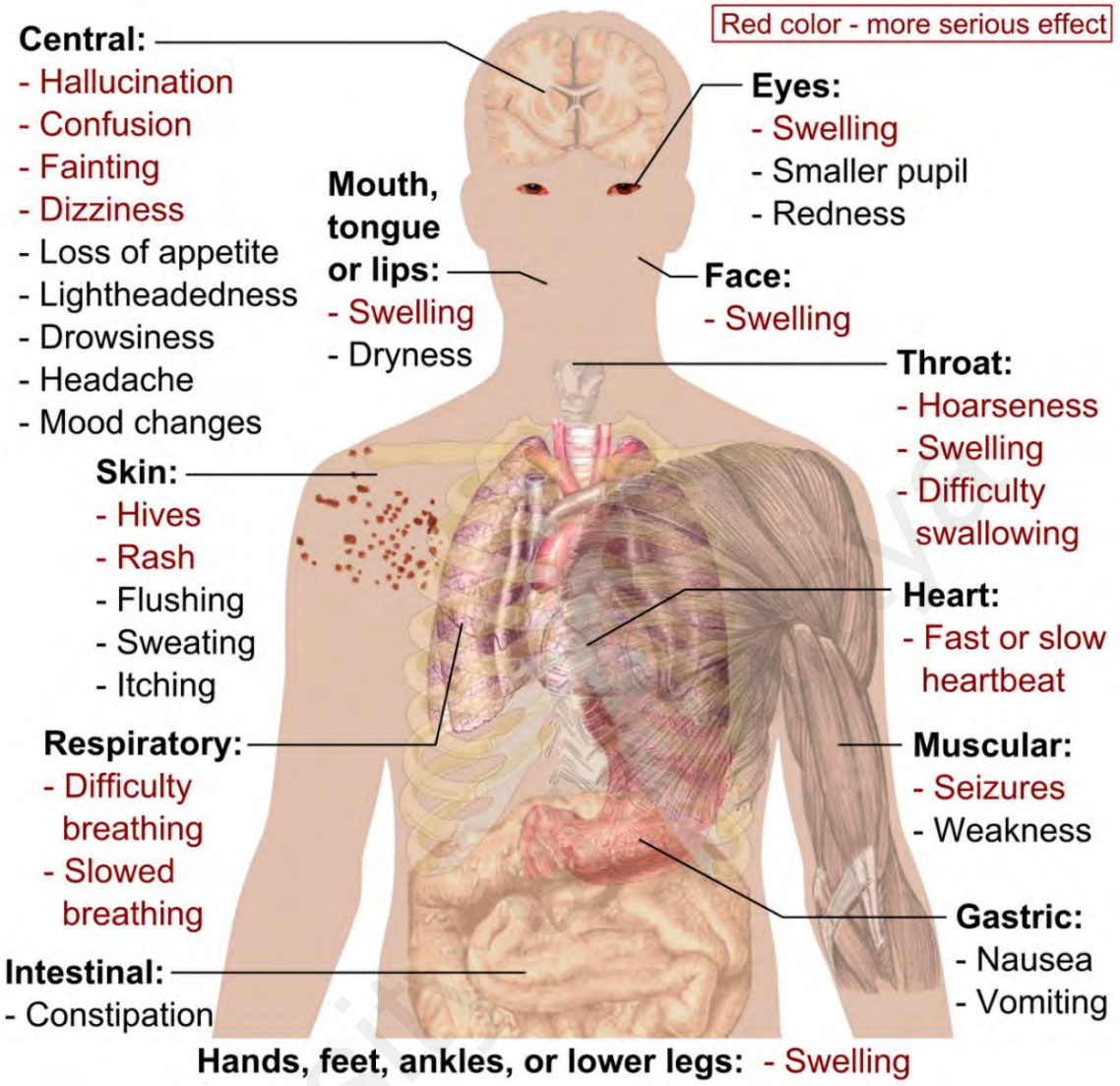


Figure 2.1: Transformation of heroin into morphine and binding to opioid receptors (source: https://www.cnsforum.com).

In 2011, 4.2 million Americans aged 12 or older (or 1.6 percent) had used heroin at least once in their lives. It is estimated that about 23 percent of individuals who use heroin become dependent on it (Clemmey *et al.*, 1997). Heroin abuse has been prevalent in Malaysia as well, and opiate was the main drug abused in the early 20th century according to the local statistics from National Anti-Drug Agency in 2010 (Lua *et al.*, 2013). Besides the psychosocial effects of heroin addiction, a summary of its physiological side effects is depicted in Figure 2.2.



2.2.1 Methadone Maintenance Treatment (MMT)

According to the World Drug Report 2014, there was estimated 12.8 – 20.2 million people worldwide who are engaged with illicit substances such as heroin and opium. However, less than 650,000 people are thought to be receiving substitution treatment globally for opioid dependence, less than 10% of those in need of treatment (Organization, 2014). Methadone treatment has been known to be the standard substitution therapy for heroin addiction. Methadone, also known as Dolophine among other brand names, is a synthetic half-life opiate developed as a pharmacological treatment that replaces heroin (Manfredonia, 2005; Marsch, 1998) to prevent the physical symptoms of withdrawal by affecting the same brain receptors as heroin does (μ-opioid

receptors) (Ball & Ross, 1991; Simpson & Sells, 1982). Methadone is used as a maintenance therapy for people with opioid dependence, in relieves drugs craving and suppresses opioid abstinence syndrome for 24 to 36 hours (Farrell *et al.*, 1994). MMT has been used since the 1960s, and it was the most common substitution therapy for heroin addicts (Marsch, 1998). However, Methadone has been associated with physical reliance; it causes less psychological dependence as compared with other opioids like heroin (Greenwald, 2006).

2.2.2 Heroin and Methadone Effects on Brain

Chronic heroin abuse leads to long-lasting impairments in cognitive functioning and alterations in central nervous system (CNS) functioning (London *et al.*, 2000; Polunina *et al.*, 2007). Neuroimaging findings and clinical implications confirm that chronic heroin abuse affects the prefrontal cortex (Goldstein & Volkow, 2011), temporal insula, and thalamus (Goldstein & Volkow, 2002), as well as the nucleus accumbens (Noel & Gratton, 1995), amygdala (Baxter *et al.*, 2000), and sensorimotor structures (White, 1989). Magnetic resonance imaging (MRI) studies have also confirmed decreases in gray matter density in the prefrontal and temporal cortical regions of chronic heroin addicts (Fu *et al.*, 2008; Haselhorst *et al.*, 2002; Lee *et al.*, 2005; Lyoo *et al.*, 2006). Gray matter contains most of the brain's neuronal cell, and shrinkage of its density can directly affect the muscle control, sensory perception, memory, emotions, speech, and decision-making abilities of the brain. Although these abnormalities have been suggested to be moderate during withdrawal, abstinence, or substitution therapy, there is no concise conclusion on this matter while opioid withdrawal has severe physical and mental symptoms as well.

Previous studies on MMT subjects have shown alterations in cognitive functions, such as changes in cerebral phospholipid metabolite levels (Silveri *et al.*, 2004), poor performance in learning and immediate recall tests (Gritz *et al.*, 1975), increase in serum

leptin levels (Wilczek *et al.*, 2004), higher production of interleukins (IL-6) (Zajocov *et al.*, 2004), and impairment of cognitive functions (Darke *et al.*, 2000; Mintzer & Stitzer, 2002; Prosser *et al.*, 2006; Rapeli *et al.*, 2006; Specka *et al.*, 2000) of methadone-treated subjects compared with healthy subjects. The evidence of MMT limitations has resulted in efforts to explore the potential of safe and effective complementary therapies in addiction treatment field.

2.3 Acupuncture Therapy

The word "acupuncture" is etymologically derived from Latin, meaning "with a needle through the skin" (Kaptchuk, 2002). Acupuncture has been used for more than 3000 years, becoming one of the most popular therapeutic procedures in Traditional Chinese Medicine (TCM). The basic concept of TCM believes in the reality of the flow of Qi ("chi") through the meridian pathways in the body (Jordan, 2006a). The therapeutic effects of acupuncture have been investigated through clinical practices, and several research-works in pain control, fibromyalgia, headaches, Parkinson's disease, Schizophrenia, and depression treatment (Jordan, 2006a). Acupuncture denotes one of the most promising cost-effectiveness and safe therapies for drug addiction based on the core concept of the flow of Qi ("chi") through the meridian pathways in the body (Jordan, 2006a). Acupuncture can be carried out either by manual insertion of specific needles or through a very mild electrical current stimulator called Electro-Acupuncture (EA). New methods of acupuncture involve finger pressure called (acupressure) and Laser therapy (Lin *et al.*, 2012). Acupuncture therapy is often considered cheap, simple, and harmless. To date, increase the levels of enkephalin, epinephrine, endorphin, serotonin, norepinephrine, and dopamine in the CNS and plasma has been described as the most significant effect of acupuncture (Cabioglu *et al.*, 2007).

2.3.1 Acupuncture Therapy in Substance Abuse Treatment

In the 1997 milestone decision by the National Institutes of Health (NIH) consensus, acupuncture therapy has been accepted as a suitable complementary procedure alongside Western medicine, particularly for pain relief. Interests in using acupuncture in substance treatment centers have increased over the last three decades (Cui *et al.*, 2008). Auricular acupuncture (AA) has been proven effective in alcohol and drug abuse treatments, in both Europe and the US (Cowan, 2011). In 1996, the World Health Organization (WHO) officially accepted acupuncture as a treatment for drug abuse (Organization, 2002).

Back in 1972, the pioneering Dr. Wen from Hong Kong reported that acupuncture at six points could relieve opioid withdrawal symptoms (Wen & Cheung, 1973). For this purpose, in 1985, Dr. M. Smith, the head of the National Acupuncture Detoxification Association (NADA) in New York, USA, subsequently finalized a protocol that is currently practiced in over 250 hospitals in the UK and USA (Cui *et al.*, 2008). The latest modification in this treatment was created in 2005 by Dr. Han from Peking University, Beijing, China (reported in (Cui *et al.*, 2008)). Currently, more than 700 addiction treatment centers using acupuncture and clinical trials due to the efficiency of this method per se or as an adjunctive procedure (Black *et al.*, 2011a).

Aside from the reports that support the postulation of acupuncture therapy as an effective treatment, several clinical trials have shown that acupuncture presented no significant advantage for addiction treatment (see: (Gates *et al.*, 2006; Jordan, 2006a; Lin *et al.*, 2012; Rabinstein & Shulman, 2003; Samuels *et al.*, 2008; White *et al.*, 2006; White *et al.*, 2011, 2014)). Existing evidence fails to document the specific benefits and effects of acupuncture in drug addiction treatment.

One of the objectives of this study aims to investigate any possible electrophysiological effects of acupuncture as an adjunctive therapy to MMT. Therefore,

the recent authentic published documents (2000 to September 2014) on acupuncture therapy studies in substance abuse treatments were reviewed systematically to delineate the main controversial issues of the field. The process of this search is explained in details in (Motlagh *et al.*, 2016). Original experimental research articles were reviewed according to the type of substance dependence; the treatment method, subjects, objectives, and assessments of clinical trials for each group were shown in Appendix A. A summary of their findings is categorized and reported in this chapter.

2.3.1.1 Cocaine

Avants and Margolin have evaluated the efficacy of AA for cocaine addiction treatment in four studies on human subjects. Although promising results were reported in their first study on 82 cocaine-dependent subjects (Avants *et al.*, 2000), another study on 83 cocaine-dependent subjects found AA to be effective in reducing cocaine in only one of two trials (Margolin, Avants*, et al.*, 2002). When the original study was repeated with 620 subjects, no effect was found (Margolin, Kleber*, et al.*, 2002). These researchers also conducted a study in 2005 on 40 cocaine abusers who had tested positive for the human immunodeficiency virus and were under methadone maintenance; no difference was found between the standard and reduced NADA protocols for cocaine use (Margolin *et al.*, 2005). Acupoints in NADA protocol are located at (sympathetic: in the deltoid fossa at the junction of the infra-antihelix crus and the medial order of the helix, lung: in the center of the cavum concha, liver: located in the posterior to upper portion of the helix crus, kidney: in the cleft between the upper plateau, and the helix).

Three studies on rats were conducted to explore the effects of bilateral stimulation at the *Shenmen* (HT7) points. Modulation of the central dopaminergic system by acupuncture might be effective in preventing the behavioral effects of cocaine in rats (Lee *et al.*, 2009). By regulating neuronal activation in the nucleus accumbens (NAc) shell,

acupuncture reduced stress-induced relapse (Lee *et al.*, 2012). The effect of acupuncture on the inhibition of cocaine-induced locomotor activity was mediated by A-fiber activation of the ulnar nerve in rats (Lee *et al.*, 2013). Refer to Appendix A for study details.

2.3.1.2 Opioids and opiates

In 2002, Montazeri investigated the efficacy of acupuncture at *Hegu* (LI4), *Neiguan* (PC6), *Shenmen* (HT7), *Taichong* (LR3), *Zusanli* (ST36), *Dazhui* (DU14), and *Baihui* (DU20) in 40 male adult heroin- or opium-addicted patients. The severity of withdrawal symptoms declined when acupuncture was used in rapid opiate detoxification (Montazeri *et al.*, 2002). Liu (2007) used functional magnetic resonance imaging to show that hypothalamus activation associated with manual acupuncture at *Zusanli* (ST36) was more robust in heroin addicts compared with healthy subjects (Liu *et al.*, 2007). EA (2 Hz) at *Zusanli* (ST36) and *Sanyinjiao* (SP6) was effective in reducing active responses elicited by discrete cues in rats (Liu *et al.*, 2012). The same EA treatment showed promise in treating heroin-seeking behaviors when combined with extinction therapy (Hu *et al.*, 2013). EA (2 Hz) at the same points *Zusanli* (ST36) and *Sanyinjiao* (SP6) activated the endogenous opioid-cannabinoid and the dopamine systems in rats (Xia *et al.*, 2011).

An evaluation of the event-related potentials of heroin addicts before and after acupuncture at *Neiguan* (PC6) and *Zusanli* (ST36) suggested that EA might potentially lower relapse rates by inhibiting attention bias to heroin (Jiang *et al.*, 2011). The presentation of heroin cues could induce activation in craving-related brain regions, which are involved in reward, learning and memory, cognition, and emotion. Acupuncture at *Zusanli* (ST36) rapidly suppressed the activation of these specific brain regions related to craving (Cai *et al.*, 2012). Transcutaneous electric acupoint stimulation was a possible adjunctive treatment for pharmacological treatments for heroin

detoxification (Meade *et al.*, 2010). Acupuncture at *Dazhui* (GV14) and *Baihui* (DU20) prevented brain cell apoptosis in heroin-readdicted rats, normalized neuronal ultrastructure in the ventral tegmental area of heroin relapse rate, and protected nerve cells against injury in heroin relapse rate (Hou *et al.*, 2014; Zhang *et al.*, 2014).

Recent studies of acupuncture's effectiveness as an adjunct therapy in methadone maintenance programs have been controversial. In 2009, Bearn demonstrated a lack of effect for adjunctive methadone maintenance treatment with AA upon withdrawal severity or craving (Bearn *et al.*, 2009). In 2013, Pei Lin showed a lack of AA effectiveness on the number of daily consumed cigarettes, relapse rate, and withdrawal symptoms, and examined patients' satisfaction and coping with AA as an adjunct treatment to methadone maintenance treatment among Malaysian subjects (Lua & Talib, 2013; Lua *et al.*, 2013). However, Chan *et al.* (Chan *et al.*, 2014) claimed that two weeks of acupuncture therapy reduced the daily dose of methadone and was also associated with greater improvement in sleep latency. Refer to Appendix A for study details.

2.3.1.3 Nicotine

Acupuncture stimulation at *Zusanli* (ST36) exerted a therapeutic effect on nicotine detoxification (Chae *et al.*, 2004) and acupuncture at *Zusanli* (ST36) or *Shenmen* (HT7) might attenuate anxiety-like behavior following nicotine withdrawal by modulating corticotrophin-releasing factor in the amygdala (Chae *et al.*, 2008). Smoking withdrawal symptoms could be ameliorated by acupuncture treatment (Chae *et al.*, 2010). In one study, acupuncture at *Shenmen* (HT7) attenuated cigarette withdrawal symptoms more than acupuncture at *Shousanli* (LI10) (Chae *et al.*, 2011). Real acupuncture (as opposed to sham acupuncture) at *Shenmen* (HT7) alleviated cue-induced cravings through the regulation of activity in brain regions (medial prefrontal cortex, premotor cortex,

amygdala, hippocampus, and thalamus) related to craving scores in the initial abstinence phase (Kang *et al.*, 2013).

However, one study failed to find any effect of acupuncture on cotinine serum levels, carbon monoxide exhalation, and smoking quit rate in 59 smokers (Yeh *et al.*, 2009). It has been suggested that DRD2 gene TaqI A polymorphism was related to AA response in smoking cessation treatment (Park *et al.*, 2005). Auricular transcutaneous electrical neurostimulation relieved withdrawal symptoms and decreased anxiety and stress levels during the detoxification period in a study of six smokers (Bonnette, 2008). Auricular transcutaneous electrostimulation therapy might be an acceptable alternative therapy for smoking cessation (Thanavaro & Delicath, 2010). Refer to Appendix A for study details.

2.3.1.4 Alcohol

Conflicting results from two large randomized, single-blind, placebo-controlled trials suggested that acupuncture not be effective in reducing alcohol use (Bullock *et al.*, 2002; Karst *et al.*, 2002). However, promising results have been found using acupuncture as an adjunctive treatment to carbamazepine medication to reduce the severity of alcohol withdrawal symptoms (Karst *et al.*, 2002). In one study, AA failed to reduce the duration and severity of alcohol withdrawal symptoms (Kunz *et al.*, 2007); another study found no advantage for laser AA in treating alcohol withdrawal (Trumpler *et al.*, 2003). However, research indicated that laser therapy helps to promote the release of endorphins in the body and decreases discomfort accompanying alcohol withdrawal (Zalewska-Kaszubska & Obzejta, 2004). It might, therefore, be a safe and painless beneficial adjunct treatment for alcoholism (Zalewska-Kaszubska & Obzejta, 2004).

Acupuncture at *Zusanli* (ST36) or *Sanyinjiao* (SP6) modulated postsynaptic neural activation in the striatum and NAc in rats (Zhao *et al.*, 2006b). Acupuncture at *Shenmen* (HT7) normalized dopamine release in the mesolimbic system (Zhao *et al.*, 2006b),

modulated mesolimbic dopamine release, and suppressed the reinforcing effects of ethanol (Yang *et al.*, 2010b). Activation of the endogenous opiate system might be responsible for *Zusanli* (ST36) and *Sanyinjiao* (SP6) stimulation effects on alcohol intake in alcohol-dependent rats (Overstreet *et al.*, 2008b).

EA applied at *Zusanli* (ST36) was more effective than EA at *Shenshu* (BL23) at normalizing alcohol-drinking behavior in rats (Yoshimoto *et al.*, 2001); the activity of serotonergic neurons in the reward system pathway of the brain might be increased and prolonged by acupuncture (Yoshimoto *et al.*, 2006). EA at the combination *Zusanli* (ST36) and *Neiguan* (PC6) (but not at either point alone) prevented sensitization of the mesocorticolimbic pathway induced by ethanol in mice and modulated both the expression of the protein homer1A and glutamatergic plasticity (dos Santos *et al.*, 2009). EA (2 Hz) at *Zusanli* (ST36) could reduce voluntary intake of ethanol, but not sucrose, in rats (J. Li *et al.*, 2011) and 100 Hz EA treatment at *Zusanli* (ST36) effectively reduces preference for ethanol and its consumption in rats (Li *et al.*, 2012). In one study, 2 Hz EA at *Zusanli* (ST36) and *Neiguan* (PC6) or 100 Hz EA at *Dazhui* (DU14) and *Baihui* (DU20) inhibited CB1R upregulation in ethanol-withdrawn mice (Escosteguy-Neto *et al.*, 2012). The behavioral effects of 2 Hz EA at *Dazhui* (DU14) and *Baihui* (DU20), but not 100 Hz EA at *Zusanli* (ST36) and *Neiguan* (PC6), depended on extracellular signal-regulated kinase signaling (Fallopa *et al.*, 2012b). Refer to Appendix A for study details.

2.3.1.5 Morphine

Compared with 100 Hz, 2 Hz peripheral electric stimulation (PES) at *Zusanli* (ST36) and *Sanyinjiao* (SP6) inhibited the expression of morphine-induced conditioned place preference (CPP) (see (Liang *et al.*, 2006) for information on CPP) *via* activation of opioid receptors (Wang *et al.*, 2000). One study found that the release and synthesis of enkephalin in the NAc were accelerated by 2 Hz stimulation of *Zusanli* (ST36) and

Sanyinjiao (SP6) (Liang *et al.*, 2010). In addition, EA suppression of opiate addiction might involve the release of endogenous μ-, δ-, and κ-opioid agonists in the NAc shell (Liang *et al.*, 2006) and might activate the cannabinoid, endogenous opioid, and dopamine systems to induce CPP in rats (Xia *et al.*, 2011). PES (100 Hz) at *Zusanli* (ST36) and *Sanyinjiao* (SP6) activated the suprasegmental δ- and κ-opioid receptors in the central nervous system, which cause the anti-craving effects of PES in rats (Shi *et al.*, 2003b). It was also found that the expression of preproenkephalin and preprodynorphin mRNAs in the NAc was mediated by 2 Hz or 100 Hz PES, with the release of endogenous μ-, δ-, and κ-opioid agonists to suppress morphine-induced CPP (Shi *et al.*, 2004a). Stimulation at *Zusanli* (ST36) and *Sanyinjiao* (SP6) (100 Hz) for 30 min normalized the activity of ventral tegmental area dopamine neurons (Hu *et al.*, 2009b), downregulated p-cAMP response element binding, and accelerated dynorphin synthesis in the spinal cord (Wang *et al.*, 2011).

Some research suggests that 2 Hz EA is a potential complementary therapy for improving immune dysfunction in opiate addicts (Y. J. Li *et al.*, 2011a) and that 2 Hz or 100 Hz EA facilitates the recovery of male sexual behavior in rats during morphine withdrawal (Cui *et al.*, 2004b). Thirty minutes of EA of 2 Hz or 100 Hz at *Zusanli* (ST36) and *Sanyinjiao* (SP6) reversed the morphological alterations induced by chronic morphine administration (Chu *et al.*, 2008a). In addition, by increasing NREM sleep, REM sleep, and total sleep time, EA could be a potential treatment for sleep disturbance during morphine withdrawal (H. Y. Li *et al.*, 2011).

EA at *Shenshu* (BL23) attenuated the expression of the proto-oncogene c-Fos in the central nucleus of the amygdala (Liu *et al.*, 2005). Acupuncture at *Shenmen* (HT7) inhibited neurochemical and behavioral sensitization to morphine by decreasing dopamine release in the NAc (M. R. Kim *et al.*, 2005). Acupuncture at *Shenmen* (HT7)

significantly suppressed a morphine-induced increase in locomotor activity and Fos expression in the NAc and striatum (Lee *et al.*, 2010). Acupuncture at *Yanggu* (SI5) can reduce the reinstatement of morphine-seeking behaviors by mediating the gamma-aminobutyric acid receptor system (Lee *et al.*, 2013; Lee *et al.*, 2012). Refer to Appendix A for study details.

2.3.1.6 Other substances

Studies of methamphetamine, cannabis, illicit/psychoactive drugs, and polydrug users are shown in Table 2.6. Twelve studies used the NADA 5-point protocol and AA as their treatment method. The findings indicated that people dependent on drugs preferred acupuncture treatment (Ashton *et al.*, 2009), which was associated with a decrease in psychological distress (Bergdahl *et al.*, 2014) and an increase in confidence (Bernstein, 2000), but showed no efficacy for drug consumption and withdrawal symptoms (Ashton *et al.*, 2009; Bergdahl *et al.*, 2014; Berman *et al.*, 2004; Bernstein, 2000). However, the conflicting nature of the research findings remains a controversial issue. Although there was evidence against the effectiveness of acupuncture in drug addiction treatment (Black *et al.*, 2011b; Janssen *et al.*, 2012; Janssen *et al.*, 2005), recent studies have shown an effect for AA (Chang & Sommers, 2014; Chang *et al.*, 2010; Courbasson *et al.*, 2007; Russell *et al.*, 2000; Tian & Krishnan, 2006) and transcutaneous electric acupoint stimulation (Penetar *et al.*, 2012) per se or as adjunct treatments. Issues of safety and placebo effects suggest the need for further research (Courbasson *et al.*, 2007; Janssen *et al.*, 2012; Janssen *et al.*, 2005; Penetar *et al.*, 2012). Refer to Appendix A for study details.

2.4 Brain Electrophysiological Assessments

In this section of thesis electro-neurophysiological evaluations in this field is introduced and reviewed. There have been several types of neurophysiological measurements suggested as a sophisticated approach providing information about brain

functions in this area. Functional neuroimaging studies have utilized positron emission tomography (PET), single photon emission computed tomography (SPECT), and functional magnetic resonance imaging (fMRI). In general, such techniques can measure functional changes in the brain in pathological conditions, in response to pharmacological interventions, and during various activation states. Activation states have included sensory stimulation (visual, auditory, and so forth), motor function and coordination, language, and higher cognitive functions (such as concentration). The changes that can be measured include general physiological processes such as cerebral blood flow and metabolism in addition to many aspects of the neurotransmitter systems as well as serotonin, dopamine, opiate, benzodiazepine, glutamate, and acetylcholine systems. Although functional neuroimaging studies have contributed significantly to the understanding of the human brain, each has its advantages and limitations. These techniques allow for a very accurate determination of the specific areas of the brain that are activated with an excellent temporal resolution, which allows the observation of a brain response that may occur very quickly. However, since imaging techniques are time and cost consuming, over the last few decades, electroencephalographic (EEG) activities have been widely used to study brain cognitive dysfunctions and neurobiological alterations especially among heroin addicts.

2.5 Electroencephalogram (EEG)

Nerve cells consist of axons, dendrites and cell bodies respond to stimuli and transmits information in the brain in the form of electrical activities. EEG, which measures this electrical activity in the brain can be named as one of the most valuable neurophysiological approaches due to its and good temporal resolution and noninvasive technique. Electrical signals of brain show variety of frequencies oscillations with specific spatial distributions which are associated with different states of brain functioning. EEG signals are classified according to their frequency into five major

frequency bands; namely as delta (δ), theta (θ), alpha (α), beta (β) and gamma (γ) Figure 2.3.

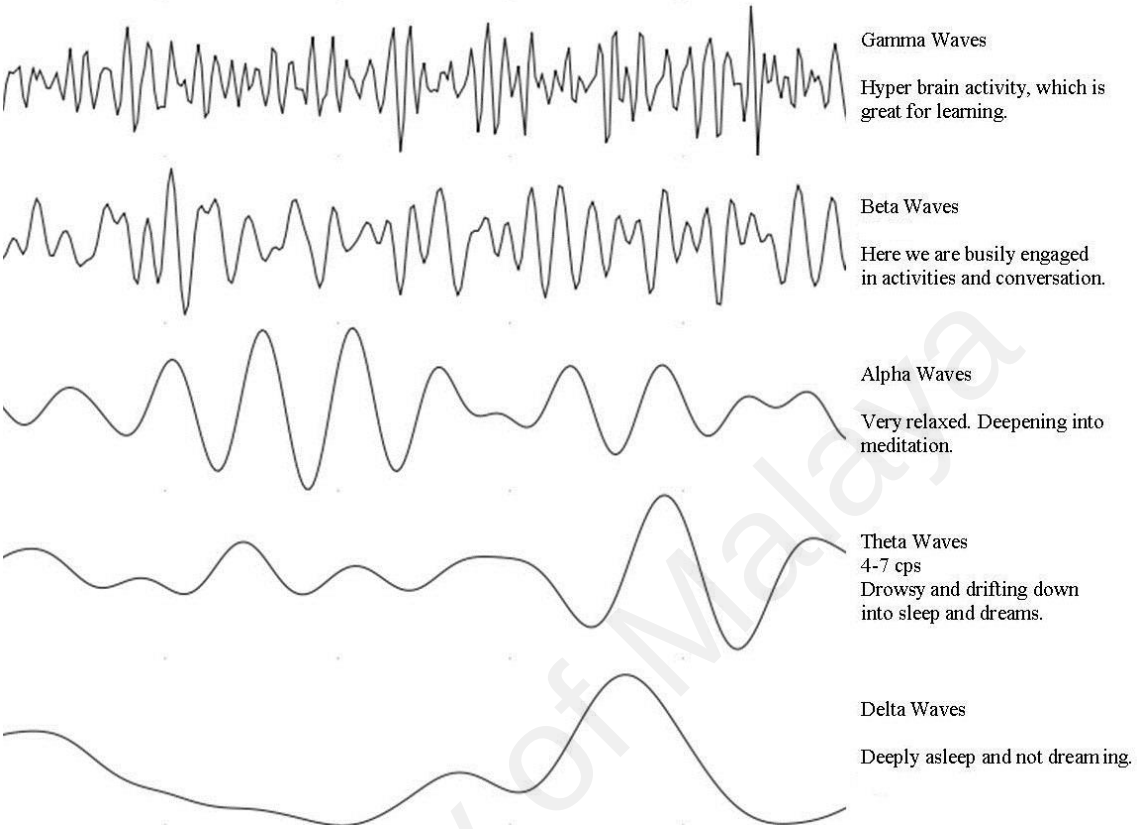


Figure 2.3: EEG Frequency bands from low to high frequency with their description.

Delta waves, slow and loud brainwaves (low frequency and deeply penetrating) lie between 0.5-4 Hz, are generated in deepest meditation and dreamless sleep, suspend external awareness and are the source of empathy. Theta brainwaves are within the range of 4-8 Hz, related to the level of arousal, creative inspiration, and deep meditation acts as our gateway to learning and memory. Alpha band frequency is between 8-12 Hz, and the amplitude of this band is below 50 micro volts (mostly between 30-50 μ volts), which appear in awareness at back of the head and has a stronger amplitude at occipital areas. Beta waves (12-38 Hz) with the amplitude less than 30 μ volt, but the frequency can be up to 50 Hz during the intense mental activity. Beta wave is the normal waking rhythm of the brain for normal adults; which is associated with progressive thinking, active

attention, and focus on the outside world or solving particular problems. Beta waves are larger in amplitude in frontal and central lobes. EEG waves which are more than 30 Hz (mostly till 45 Hz) are called as gamma or fast beta wave. The amplitude of gamma waves are very low, and occurrence of this type of EEG is rare.

2.5.1 Quantitative EEG

Quantitative Electroencephalography (qEEG) shows various frequencies of oscillations with specific spatial distributions associated with different states of brain function, and its coherence measures the functional interactions between brain areas at different frequency bands (Knyazeva & Innocenti, 2001). QEEG is a procedure that processes the recorded EEG activity from a multi-electrode recording using a computer. This multi-channel EEG data is processed with various algorithms (such as the "Fourier" classically, or in more modern applications "Wavelet" analysis). The EEG and the derived qEEG information can be interpreted and used by experts as a clinical tool to evaluate brain function and to track the changes in brain function due to various interventions such as neurofeedback or medication.

In addition to EEG spectral analysis to examine the heroin effects on brain functionality (Davydov & Polunina, 2004; Fingelkurts *et al.*, 2008; Fingelkurts *et al.*, 2006; A. A. Fingelkurts *et al.*, 2007; Fingelkurts *et al.*, 2009; Alexander A. Fingelkurts *et al.*, 2007; I. H. A. Franken *et al.*, 2004; Polunina & Davydov, 2004, 2006), brain event-related potential (ERP) components were evaluated as a reliable approach to study cognitive abilities related to information processing, selective attention, and memory updating of addicts.

2.5.2 Event Related Potential (ERP)

Study the ERP components are one of the most informative and dynamic methods of monitoring the information stream in the living brain as a useful diagnostic tool, in both

psychiatry and neurology. ERPs are the brain signals that measure the electrical response of the brain to sensory, affective, or cognitive events. Typically, peripheral or external stimulations generate ERPs, which is appeared as somatosensory, visual and auditory brain potentials, or as slowly brain activity before voluntary movements and during anticipation of conditional stimulation.

Measuring ERPs provide a reliable approach to study brain-behavior relations (Boutros *et al.*, 1997; Handy, 2005). ERPs as averaging potentials generated by spatially coherent activity within some cortical EEG source areas; consist of time-locked components related to information-processing operations, which can monitor cognitive responses with a high temporal resolution (on the order of milliseconds). ERP properties can reflect cognitive traits related to the processing of sensory information as well as higher level processing that involves selective attention, memory updating, semantic comprehension, and other types of cognitive activity. ERPs provide sufficient temporal precision to distinguish between the rapid perceptual and cognitive processes occurring during stimulus processing (Bernat *et al.*, 2001; Luck *et al.*, 2000). The most popular ERP features are the areas and the peaks of the ERP components (waves) defined by the mean and extremum voltage, respectively, computed in certain windows in the time domain, which is determined by visual inspection of the average ERP waveforms (obtained by averaging across many trials). Compare to the background EEG activity: ERPs are quite small (1-30uV). The ERP waveforms can be quantitatively characterized by amplitude, latency and its distribution in the scalp. The ERPs can be analyzed on their relative latencies. ERPs amplitude presents an index of the extent of activity in neuron and shows how it responds to experimental variables. Meanwhile, the latency presents the activation time, and the scalp distribution provides the voltage gradient pattern of a component over the scalp. The sequence and latencies of ERP components track the time course of processing activity in milliseconds, whereas their amplitudes indicate the extent of

allocation of neural resources to specific cognitive processes. ERPs are sensitive to variables related to information processing (e.g., auditory discrimination acuity, expectancy, semantic processing) and complement traditional and limited performance measures, such as the accuracy and speed of behavioral responses. The brain structures and systems that generate long-latency components are more complex than those underlying shorter latency, sensory components. Nevertheless, as the neural generators of these cognitive components are characterized with greater specificity, abnormalities in their amplitude, latency, or scalp distribution will provide useful diagnostic information. Some of the well-studied ERP components in addiction research are explained in continue.

P100 (P1) and N100 (N1) are the first positive- and negative-going components, respectively, after stimuli onset. P1 and N1 peak at around 80 ms to 130 ms with a maximum amplitude over the lateral occipital region for P1 (Mangun, 1995) and centro-frontal region for N1 (Mangun & Hillyard, 1991). P1 reflects visual-spatial attentional processing, which represents the early stages of visual analysis. N1 is elicited by any unpredictable stimulus in the absence of task demands (Warnke *et al.*, 1994).

P200 (P2) is a positive potential at centro-frontal and the parieto-occipital region, which peaks at about 150-275 ms after the onset of the external stimulus, modulated by attention (Freunberger *et al.*, 2007; Luck & Hillyard, 1994). N200 (N2) is a negative potential that peaks between 200 ms and 350 ms after the start of stimulus (Folstein & Van Petten, 2008). N2 is attributed to novelty or mismatch detection to stimuli. Mismatch negativity (N2a or auditory MMN) exhibits as a negative potential evoked by a deviant stimulus, occurring in a series of frequent standard stimuli, which achieves maximal amplitude at the frontal scalp locations (Naatanen, 1992). N2 and MMN as deviant stimulation-detection mechanisms are attention-independent components associated with

preattentive auditory processing, conscious discrimination ability, and orienting response by activation of the frontal site of the brain (Kraus *et al.*, 1995a, 1995b; Kujala *et al.*, 2007; Naatanen, 1992).

MMN component is thought to reflect an automatic process that detects a difference between an incoming stimulus and the sensory memory trace of preceding stimuli. MMN can be elicited even in the absence of the participant's attention, which makes it useful in the assessment of very young or impaired participants. A common procedure in MMN studies involves the presentation of a series of identical stimuli with occasional mismatching stimuli. The mismatching stimuli can differ on any discriminable auditory dimension such as pitch, duration, intensity, or location. Hence, one of the stimuli (''standard") occurs frequently (e.g., p = 0.80), and the other (''deviant") infrequently occurs (p = 0.20). The two stimuli are usually presented at relatively short inter-stimulus intervals (ISI), such as 500ms to 1s. MMN is typically seen as a centro-frontal negativity occurring in the latency range of 100–250 ms. when the magnitude of the difference increases between the standard and deviant stimuli, the peak latency of MMN becomes progressively shorter and its peak amplitude larger. However, a small MMN can be elicited by even threshold-level differences. MMN can, therefore, be used to evaluate discrimination acuity; it provides what may be the best objective measure available for this purpose. MMN is generated from the auditory cortices, and the biological function represented by this ERP component is to monitor and detect any change in continuous auditory stimulation, irrespective of where attention is directed. The MMN sub-component generated in supra-temporal areas is an auditory-cortex marker of change that occurs automatically (and pre-perceptually). The frontal MMN sub-component is a sign that the frontal cortical mechanisms implicated in the recruitment of attention (involuntary attention switching) have been activated in response to a change in auditory stimulation.

P300 (P3) is a late positive-going ERP generates between 300-500 ms after the onset of relevant stimulation among irrelevant stimuli. The P3 amplitude is associated with the stimuli concerned detection and discrimination, orientation response, selective attention, memory renewal, decision confidence and uncertainty resolution, motivation, task difficulty, stimulus significance, stimulus probability, vigilance (Sommer & Matt, 1990), activation of inhibitory processes over widespread cortical areas (Tomberg & Desmedt, 1998), indexing decisional process of updating working memory (Polich & Herbst, 2000), cognitive closure (Verleger, 1988), fluctuations in the arousal state, and attentional operations (Donchin, 1981; Donchin & Coles, 1988; McEvoy *et al.*, 2001; Polich, 1998). P3 amplitude increases with low probability stimuli, attentional shift, augmented selective attention, emotional stimuli, and increased confidence in the decision (Andreassi, 2000). P3 amplitude has been shown to increase the amount of attentional demands required by the task. Therefore, it has been suggested that P3 amplitude reflects the engagement of attention in updating the contents of working memory.

Slow positive waves (SPWs) reflect the cognitive processes that occur after 300 ms and reflect higher processes such as attention and memory. SPW was suggested as a suitable indicator of motivational relevance and selective cognitive processing, reflecting the activation of a motivational system in the brain (Schupp *et al.*, 2000). The P600 component (500–800 ms after warning stimuli) reflects second-pass parsing processes that are the completion of any synchronized operation immediately following target detection. Psychophysiological research has argued that P600 has much in common with the central executive component of WM (Papageorgiou *et al.*, 2001).

Among various ERP components, measuring amplitude and latency of mismatch negativity (MMN), P3, and P600 components in standard condition have attracted particular attention in ERP evaluation among addicts. These ERPs are associated with

brain discrimination abilities, the orientation of attention, response resolution, and executive working memory. Additionally, examining cognitive responses to drug-related stimuli showed promising results in providing better understanding of brain functional alterations during withdrawal and abstinence periods associated with addiction traits such as craving and anhedonia (Franken *et al.*, 2003; Jiang *et al.*, 2011; Lubman *et al.*, 2007, 2008; Lubman *et al.*, 2009). For this purpose, various types of ERP paradigms have been designed to probe subjects' evoked cognitive responses using deviant, affective, and drug-related stimuli.

MMN has been evaluated among heroin addicts to measure their deficiency in orienting their attention (Kivisaari *et al.*, 2007; Morie *et al.*, 2014; Yang *et al.*, 2009). P300 has been investigated among opioid dependents (Bauer, 2001; Lubman *et al.*, 2007; Lubman *et al.*, 2009; Marques-Teixeira & Barbosa, 2005; Singh *et al.*, 2009). P600 reflects attributes of second-pass parsing processes of information processing in association with working memory (WM) systems (Frisch *et al.*, 2003; Guillem *et al.*, 1999), and suggested to serve as a valuable investigative tool for a more comprehensive understanding of the neurobiological substrate of drug abuse and central executive WM (Papageorgiou *et al.*, 2001).

2.6 Brain Electrophysiological Approaches in Heroin Addiction

The essential goal of neuro-electrophysiological studies in addiction research is to assess the neurophysiological changes among heroin addicts. These studies vary in their approach, experimental conditions, paradigms, and outcomes. However, it is essential to integrate previous findings and experimental methods for a better demonstration of current issues and challenges in designing such studies. In this section, a comprehensive review of human neuro-electrophysiological clinical trials among heroin addicts is provided. Therefore, a search was conducted through the ISI Web of Science (all

databases) from January 2000 to January 2015. The scope of this review is limited to human EEG, and ERP approaches to heroin and methadone studies. The selected studies use EEG and ERP as an evaluation tool for assessing the brain activity differences among addicts who met the standard criteria for heroin dependence [One of the followings: DSM IV axis 1, ICD-10, SCID (I and II), DSM-IIIR (Axis I and II)], and healthy volunteers as well as the brain electrophysiological changes during the abstinence periods. Due to the study objectives, there are differences in exclusion criteria of the studies. However, some of these criteria are fundamental factors for designing an experimental study in this field; including a lifetime history of a major medical disorder (neurological, hepatic, or cardiovascular), HIV infection, hepatitis, a head injury resulting in a loss of consciousness, seizures (including drug-related seizures), schizophrenia, affective disorder, mental retardation, and significant somatic disorders such as Parkinson disease (or symptoms), or any disorder caused cognitive impairments are some of the exclusion criteria. The general condition of subjects and any additional exclusion criteria are reported in the following section where the cognitive assessments methods are compared.

2.6.1 Evaluation of Brain Electrophysiological Properties

Various type of paradigms has been used for measuring neurophysiological traits and probing the attentional biases in these studies. These paradigms are divided into three main groups, namely eye-closed resting time EEG, standard ERP paradigms, and cue reactivity paradigms. Tables 2.1-2.3 summarize the findings, experimental conditions, and subjects of all these studies.

2.6.1.1 Eye-closed resting EEG

EEG shows various frequencies of oscillations with specific spatial distributions associated with different states of brain function, and its coherence measures the functional interactions between brain areas at different frequency bands (Knyazeva &

Innocenti, 2001). Eleven research articles (Table 2.1) recorded the EEG during resting condition with closed eyes for 5-7 minutes (Davydov & Polunina, 2004; Fingelkurts *et al.*, 2008; Fingelkurts *et al.*, 2006; A. A. Fingelkurts *et al.*, 2007; Fingelkurts *et al.*, 2009; Alexander A. Fingelkurts *et al.*, 2007; I. H. A. Franken *et al.*, 2004; Gorricho & Uson, 2008; Polunina & Davydov, 2004, 2006).

Table 2.1: Eye-closed resting state EEG studies among heroin addicts.

Author Year	ABS* Period	Subjects N**	Addiction Period	N channels	Evaluated features	Outcomes***
Franken et al. 2004	2 w	18 addicts (M) (32.4±5.9) 12 control(M) (32.6±9.5)	9 ±6.3 y	21	EEG power & coherence	$\beta 2\uparrow, \gamma\uparrow$ (T)$\beta 2$ & δ coherence ≈ chronic heroin-related thoughts. (F) $\alpha 1$ & (T) δ coherence ≈ chronic heroin craving
Polunina and Davydov 2004	6d-4.5m	33 addicts(M) (24.2±5.6) 13 controls(M) (25.8±5.1)	8.76±3.48m 28.68±5.1m	19	spectral power and mean frequencies shift	$\beta\uparrow, \alpha\downarrow$ Duration of heroin addiction ≈ frequency shifts of (F, C) $\alpha 2$ High dose of drug was ≈low $\alpha 1$ mean frequency at (C, T, O)
Polunina and Davydov 2004	6d-4.5m	33 addicts(M) (24.2±5.6) 12 controls(M) (24.8±4.1)	8.76±3.48m 28.68±5.1m	19	Alpha2 spectral power and mean frequency	$\beta\uparrow, \alpha\downarrow$ $\alpha 2$ frequency in the right hemisphere ≈ chronic heroin intake length.
Polunina and Davydov 2006	6d-4.5m	31 addicts(M) (24.2±5.6) 12 controls(M) (24.8±4.1)	8.76±3.48m 28.68±5.1m	19	spectral power and mean frequencies shift + WAIS IQs	WAIS ≈ EEG mean frequencies and spectral power Long-term memory WAIS ≈ δ mean frequencies, working memory WAIS ≈ $\theta 2$ (T) mean frequency, problem-solving WAIS ≈ α parameters, whereas psychomotor speed ≈ β power.
Fingelkurts et al. 2006	2 w	22 addicts(X) (33±5) 14 healthy(X) (33±5)	11±5 y	20	Local & remote functional cortex connectivity	local functional connectivity↑ and remote functional connectivity↓ (β , α) (T,O) functional connections in the posterior≠ duration of addiction
Fingelkurts et al. 2006	2 w	22 addicts(X) (33±5) 14 healthy(X) (33±5)	11±5 y	20	the composition of brain oscillations	Percentage and periods of (T) $\beta\uparrow$ & $\alpha\uparrow$, $\theta\downarrow$ (OPTF)
Fingelkurts et al. 2007	2 w	13 addicts(X) (32±5) 14 healthy(X) (33±5)	10.7±6.4 y	20	Local & remote functional cortex connectivity	Local and remote cortical functional connectivity increased for α &$\beta\uparrow$. Increase of severity of withdrawal symptoms ≈ the percentage of θ-β and β
Fingelkurts et al. 2008	2 w	13 addicts(X) (33±5) 14 healthy(X) (33±5)	10±5 y	20	the composition of brain oscillations	Increased the percentage of α 2↑, $\beta\uparrow$ and decrease the percentage of $\delta\downarrow$, $\theta\downarrow$and $\alpha 1\downarrow$
Fingelkurts et al. 2007	2w (22) 6mMMT(6)	22 addicts(X) 14 healthy(X) (33±5)	11±6 y	20	the composition of brain oscillations	A lower percentage of $\alpha 1\downarrow$, $\alpha 2\downarrow$, and $\beta 1\downarrow \delta-\theta 1\uparrow$, θ, and $\theta 2$–$\alpha 2\uparrow$
Fingelkurts et al. 2009	11±6 m MMT	6 addicts(X) (33±5) 14 healthy(X) (33±5)	11±7 y	20	Local & remote functional composition of brain oscillations	Amplitude for both $\alpha\uparrow$ and $\beta\uparrow$ (F,T) percentage of ($\alpha\downarrow$, $\beta\downarrow$ bands), right parietal ($\beta\downarrow$), and midline occipital ($\alpha\downarrow$ bend) cortex.
Gorricho et al. 2008	59±33 m MMT	15 addicts (X) (22.7±5.3)	Not available	20	spectral power	$\alpha\downarrow$, $\delta\uparrow$,$\theta\uparrow$ Acute effects of methadone

* Abstinence period (w=week, m=month, y=year)
** Number of subjects (M=male, X=male & female)
*** T=Temporal, F=Frontal, C=Central, O=Occipital, P= Parietal, ≈= correlation,≠ is not correlated, arrows show the increase or decrease of each component, controls are healthy volunteers

Franken et al. evaluated EEG power and coherence of male heroin addicts after two weeks of abstinence and free of withdrawal symptoms compared to healthy controls (I. H. A. Franken *et al.*, 2004) to examine the heroin effects on brain functionality. Sixteen out of 18 heroin dependent subjects had a history of additional cocaine use, and ten subjects tried MMT in the past, and their chronic craving (using Obsessive Compulsive Drug Use Scale), and drug use severity (using addiction severity index) were assessed as well.

Polunina and Davydov investigated heroin abuse history (i.e., duration and dosage of daily heroin abuse and abstinence length) in association with EEG spectral power and mean frequencies among male heroin dependents (Davydov & Polunina, 2004; Polunina & Davydov, 2004, 2006). Abstinence length of subjects vary from 6 days to 4.5 months, and almost all patients reported the occasional use of cannabis, ecstasy, and pervitin-ephedrone while most of them reported excessive use of barbiturates, benzodiazepines, and tramadol (five of them also used alcohol excessively).

Fingelkurts et al. explored the role of local and remote functional cortex connectivity and the composition of brain oscillations in current heroin addicts going for withdrawal and methadone maintenance treatment (MMT) in a longitudinal study (Fingelkurts *et al.*, 2008; Fingelkurts *et al.*, 2006; A. A. Fingelkurts *et al.*, 2007; Fingelkurts *et al.*, 2009; Alexander A. Fingelkurts *et al.*, 2007). They evaluated 22 subjects (male and female) with 4–26 years heroin addiction, during short-term withdrawal period (lofexidine was used three times a day to moderate the withdrawal symptoms), as well as long-term abstinent during MMT, compared to 14 age-matched control subjects. Almost all patients reported the irregular use of benzodiazepines, amphetamine, cannabis, and alcohol; some of them even met DSM-IV criteria for benzodiazepine dependence and personality

disorders (antisocial, obsessive–compulsive, paranoid, borderline, narcissistic, schizoid, passive–aggressive, dependent, and depressive personality disorders).

Gorricho et al. studied the acute effect of methadone usage among 15 subjects (male and female who were under MMT for 59±33 months) (Gorricho & Uson, 2008). However, addiction period was not stated in their research report, and several subjects reported the use of cannabis, cocaine, alcohol, benzodiazepines, and other psychoactive substances.

2.6.1.2 Standard ERP paradigms

The standard ERP investigations include three types of paradigms (Table 2.2): (a) Oddball paradigms (standard and target stimuli) during resting condition (Bauer, 2001; Kivisaari *et al.*, 2007; Muller *et al.*, 2007; Singh *et al.*, 2009) or during the exposure of various emotional targeting slides (Marques-Teixeira & Barbosa, 2005). (b) Visual Go-NoGo paradigms using neutral or emotional conditions (Morie *et al.*, 2014; Yang *et al.*, 2009), and (c) Standard Digit Span Wechsler Test paradigm (Kalatzis *et al.*, 2005; Papageorgiou *et al.*, 2001; Papageorgiou *et al.*, 2003; Papageorgiou *et al.*, 2004).

Standard oddball paradigms have been the most common paradigms in study cognitive biases and information processing. Bauer examined the process of CNS recovery from opioids by evaluation of P300 amplitude among 29 opioid addicts (1-5 months of abstinence) compared to 14 controls (Bauer, 2001). Subjects abstinence period was verified by urine toxicology and breath alcohol screens performed at frequent intervals. In this study, childhood conduct disorder, conduct disorder problems, paternal alcohol or drug dependence, depression score, and state of anxiety were also assessed, and duration of education and IQ was controlled. Kivisaari et al., employed simultaneous EEG and magnetoencephalography (MEG) techniques to evaluate cognitive processing (P1,N1, Mismatch negativity, and P3) in current opioid (heroin and buprenorphine) dependents

who also had antisocial personality disorder (Kivisaari *et al.*, 2007). Muller investigated the effect of nicotine on P3 component among MMT subjects whom most of them used non-opioid drugs as well (benzodiazepines and cannabis) (Muller *et al.*, 2007).

Table 2.2: ERP studies among heroin addicts using standard paradigm.

Author Year	ABS* Period	Subjects N**	Addiction Period	N Channels	Evaluated Features***	Outcomes****
Bauer 2001	1-5 m	29 addicts(X) (33.6±6.6) 14 control(X) (34.2±5.9)	9.9 years (C,A)	15	(a1) P300 amplitude	P300(A) ↓ P300(A) ≈ duration of abstinence
Papageorgiou et al. 2001	6 m	20 addicts (X) (31.05±5.3) 20 control(X) (30.7±4.82)	9±6.29	15	(C) P600	P600 (L) ↑ At right hemisphere, specifically at Fp2.
Papageorgiou et al. 2003	6 m	20 addicts (X) (31.05±5.3) 20 control(X) (30.7±4.82)	9±6.29	15	(C) P300	P300(A) ↓ (L) ↑ P300 (A) (C, F) ↓ and at the left O ↑
Papageorgiou et al. 2004	Current addicts and 6 m	20 addicts(X) (31.05±5.3) 18 addicts(X) (29.57±6.23) 20 control(X) (30.7±4.82)	9.25±6.29 4.74±2.9	15	(C) P300	P300(A)(C,F) ↓ (L) ↑, P300 (A) abstinent < addicts < healthy
Kalatzis et al. 2005	1m	16 addicts(X) (NA) 20 control(X) (NA)	Not available	15	(C) P600	P600 (L) ↑ Highest accuracies were achieved at (F)(91.7%) and left temporal-central (86.1%). Highest single-lead precision (86.1%) was found at P3, C5 and F3 leads.
Marques et al. 2005	2 w	15 addicts (M) (31.47±5.48) 11 controls (M) (26.5±4.23)	12±4.11 years (C, O)	19	(a2) P300 amplitude, latency	P300(A) ↓ Less spread of P300 activation on brain of addicts and ERP modulation ≈ relapse
Kivisaari et al. 2007	0	21 opioid dependents(X) (32.2±6.5) 15 controls(X) (31.6±7.4)	10±6 years (Opioids)	15	(a2) P1, N1, MMN and P3a	MMN (L) ↑
Müller et al. 2007	MMT 19.2±17.2 m	47 addicts (X) (29.3±5.6) 65 control(X) (29.4±6.1)	9.5±5.8 years (Polydrug)	Fz, Cz, Pz	(a1) P3 amplitude and latency	P300(A) ↓ P300(A) controls with nicotine <MMT subjects< controls without nicotine
Yang et al. 2009	4.67±6.44 m	14 addicts(M) (41±7.11) 14 control(M) (41±10.5)	13.54±5.71	Fz, Fcz, Cz, Cpz, Pz	(b) N2 and P3 amplitudes and latencies	Go-N2(A) ↑
Singh et al. 2009	1 w	20 addicts (M) (24.4) their brothers (24.45) 20 controls (M) (24.7)	Not available	Cz and Pz	(a) P300 (A,L)	P300(A) ↓ (L) ↑, P300 (A) addicts <brothers<control P300 (L) addicts> brothers> control
Morie et al. 2014	15±26 m	20 addicts(X) (39±10) 21 controls(X) (41±10)	124±100 months	72	(b) N2, P3	nogo N2 (A) ↓or nogo P3(A) ↓ nogo N2 (A) or nogo P3(A) negative or positive conditions ≠ abstinence duration, duration of drug use. nogo P3 amplitude in the neutral condition ≈ abstinence duration

* Abstinence period (w=week, m=month, y=year)
** Number of subjects (M=male, X=male & female)
*** a1= standard Oddball paradigms a2= Oddball paradigms of various emotional, b = Visual Go-NoGo paradigms using neutral or emotional conditions, c = Standard Digit Span Wechsler Test paradigm
****A= amplitude, L= Latency, T=Temporal, F=Frontal, C=Central, O=Occipital, P= Parietal ≈= correlation,≠ is not correlated. Arrows show the increase or decrease of each component

For the first time Singh et al., compared auditory P3 and cognitive functions in opioid-dependent, their brothers, and normal controls to explore any shared genetic factors in the development of opioid dependence (Singh *et al.*, 2009). In this study, Hamilton Rating Scale for Anxiety, short opiate withdrawal scale score, and severity of addiction score was assessed and neurophysiological functioning of subjects evaluated by Wisconsin card sorting test (speed of attention, sequencing, mental flexibility, visual search and motor functioning, memory Scale, concentration and immediate memory). Marques et al., also analyzed the amplitude and latency of P3 among heroin addicts (also dependent on cocaine and other drugs) after two weeks of abstinence (using trazodone tranquilizers during the abstinence), confronting various emotional conditions (Marques-Teixeira & Barbosa, 2005).

Morie et al., evaluated inhibitory deficit and ERP modulation to emotionally valenced inputs in associations with relapse in emotional contexts outside the treatment center among heroin addicts who were different in their abstinence period (15±26 months) (Morie *et al.*, 2014). In this study, subjects were polydrug users and had high rates of comorbidity of alcohol and drug abuse. Life history of aggression, Buss-Perry Aggression Questionnaire, and Barratt's impulsiveness scale was assessed in this research. In a study by Yang et al., ERP proprieties were evaluated to explore the response inhabitation time among heroin addicts who categorized as moderate and severe addicts with different length of abstinence (Yang *et al.*, 2009). Beck anxiety test and opioid addiction severity inventory were used for clustering the addicts based on their addiction severity.

Papageorgiou et al., evaluated the P600 and P3 components of addicts after six months of heroin abstinence during a working memory test (Papageorgiou *et al.*, 2001; Papageorgiou *et al.*, 2003; Papageorgiou *et al.*, 2004). He also in collaboration with Kalatzis developed a multi-probabilistic neural network (multi-PNN) classification

structure for distinguishing one-month abstinent heroin addicts from normal controls through the P600 component to facilitate computer-aided analysis of ERPs (Kalatzis *et al.*, 2005).

2.6.1.3 ERP Studies in Cue-reactivity Conditions

The third group of studies analyze the brain responses to drug-related stimuli (cue-reactivity condition) compared with neutral and arousal stimuli, to elaborate the brain electrical activity properties associated with heroin addiction properties (Franken *et al.*, 2003; Jiang *et al.*, 2011; Lubman *et al.*, 2007, 2008; Lubman *et al.*, 2009). ERP responses of heroin abusers in cue-reactivity experiments during the early stages of withdrawal and abstinence can provide neurophysiological evidence regarding craving, anhedonia, and relapse based on the brain responses to drug stimuli (Table 2.3).

Table 2.3: ERP studies among heroin addicts during cue-reactivity conditions.

Author Year	ABS* Period	Subjects N**	Addiction Period	N Channels	Evaluated Features	Outcomes***
Franken et al. 2003	2 w	19 addicts (M) (33.5±7.7) 14 control (M) (33.7±9)	9±6.7	21	SPW amplitudes	SPW (A) ↑ for heroin-related pictures SPW(A) ≈ Drug cues
Lubman et al. 2009	2 m pharmacotherapy	33 addicts(X) (31.6±7.6) 19control(X) (30±6.8)	10±5.8	Fz, Cz, Pz.	P300	P300 (A) ↓ for pleasant pictures and P300 (A)↑ for drug-related ones. P300(A) ≈ Drug cues & affective stimuli
Lubman et al. 2007	3 m of MMT	14 MMT subjects(M) (29.6±7.4) 14 controls(M) (28.5±5.1)	NA	9	P300	P300 (A)↑ for opiate related stimuli. P300 (A) ≈ Drug cues
Lubman et al. 2008	1-3 m (methadone,cyclomorphine/dihydrocodeine)	16 addicts (M) (29.6±7.4) 12 controls(M) (28.5±5.1)	11.6±7	Fz, Cz, Pz	P300	P300 (A)↑ for drug-related stimuli P300(A) ≈ Craving
Jiang et al. 2011	70.2±20.1 d	10 addicts (M) (29.6±4.1) 10 controls(M) (26.3±5.2)	8.2±3.4	Fz, Cz, Pz before and after EA	P200	Before EA, P200 (A)↑ was higher for Heroin cue. After EA, the P200 (A) ↓ P200(A) ≈ Drug cue and Acupuncture affect P200

* Abstinence period (w=week, m=month, y=year)
** Number of subjects (M=male, X=male & female)
*** A= amplitude, ≈= correlation, Arrows show the increase or decrease of each component

Lubman et al., explored the impact of drug cues and affective stimuli on central attentional processing (P3 amplitude) and examined the motivational significance of drug cues among heroin dependents compared with healthy subjects (Lubman *et al.*, 2007,

2008). He aimed to explore the enhancement of P3 in cue-reactivity conditions as a neurophysiological marker in correlation with craving and heroin abuse (Lubman *et al.*, 2007; Lubman *et al.*, 2009). In these studies, Beck depression inventory and state–trait anxiety inventory, the severity of dependence scale, and short opiate withdrawal scale were also assessed.

Franken et al., investigated Slow positive waves (SPW) among male heroin addicts (2 weeks of abstinence) free of withdrawal symptoms confronting heroin-related visual stimuli (Franken *et al.*, 2003). Conditions of subjects were same as their previously discussed (I. H. A. Franken *et al.*, 2004), and drug use severity index, drug desire questionnaire and valence and arousal properties of the pictures were assessed. Jiang et al., also studied attentional bias to heroin-related stimuli of heroin addicts (Jiang *et al.*, 2011).

2.7 EEG and ERP Findings in Opioid Addiction

The results of original investigations, clustered into three main groups. Studies that investigate: 1) cognitive dysfunctions and abnormal brain activities caused by chronic heroin abuse, 2) brain electrophysiological alteration of heroin addicts in early stages of withdrawal, and cognitive alterations among short-term abstinent subjects (3-6 months), 3) effectiveness of methadone and long-term abstinence from heroin on normalization of cognitive impairments and neuro-electrophysiological abnormalities.

2.7.1 Chronic Heroin Addiction

This type of studies aimed to elaborate the brain activity abnormalities caused by chronic heroin abuse during the addiction period, as well as the first two weeks of withdrawal (Davydov & Polunina, 2004; Fingelkurts *et al.*, 2008; Fingelkurts *et al.*, 2006; A. A. Fingelkurts *et al.*, 2007; Alexander A. Fingelkurts *et al.*, 2007; I. H. A. Franken *et al.*, 2004; Polunina & Davydov, 2004, 2006).

It was shown that chronic heroin abuse alters brain functioning and cause EEG power, coherence, and information processing ability abnormalities. Franken et al., found that beta2 power and gamma coherence increased for heroin-dependent subjects compared with healthy subjects (I. H. A. Franken *et al.*, 2004). It was also found that SPW amplitudes were increased for heroin addicts observing heroin-related pictures compared to neutral pictures, while there was no difference on the SPW among healthy subjects (Franken *et al.*, 2003).

Polunina and Davydov investigated heroin abuse history (i.e., duration and dosage of daily heroin abuse and abstinence length) in association with EEG spectral power and mean frequencies (Davydov & Polunina, 2004; Polunina & Davydov, 2004, 2006). Based on their findings, the cognitive dysfunctions of heroin abusers are correlated with alpha2 mean frequency shifts at the central regions. The duration of heroin addiction is correlated with frequency shifts of alpha2 at frontal and central locations, especially in the right hemisphere, and heroin dosage is associated with lower alpha1 mean frequency at central, temporal, and occipital sites (Davydov & Polunina, 2004; Polunina & Davydov, 2004).

Polunina and Davydov also investigated the associations between Wechsler adult intelligence scale (WAIS) performance and resting EEG power spectral properties among heroin addicts with different length of heroin addiction compared with healthy volunteers. Slower mean frequencies at right lateral frontal/temporal locations were shown to be associated with WAIS scores. Performance on subtests of long-term memory was associated with Delta, working/short-term memory subtests were related to temporal theta2 mean frequency, problem-solving abilities related to alpha, and psychomotor speed is related to beta band properties (Polunina & Davydov, 2006).

Fingelkurts et al., explored local and remote functional cortex connectivity and the composition of brain oscillations in current opioid addicts (Fingelkurts *et al.*, 2006). It

was found that chronic opioid abuse increases local cortical functional connectivity and temporal stabilization for alpha and beta bands. It also decreases remote functional connectivity of alpha and beta bands and shorter periods of theta activity (Fingelkurts *et al.*, 2006). This alteration was reported to be widely distributed across the cortex with the maximum magnitude in the occipital, right parietal, temporal, and frontal areas. In addition, the functional connectivity in the posterior site is negatively correlated to opioid dependency duration (Fingelkurts *et al.*, 2006).

Kivisaari employed simultaneous EEG and magnetoencephalography (MEG) techniques to evaluate cognitive processing in current opioid (heroin and buprenorphine) dependents. Although latencies and amplitudes of P1, N1, MMN, and P3a evoked by deviant stimuli were not significantly different between the groups. Delayed preattentive auditory processing (MMN latency) to novel stimuli was found in opioid dependents (Kivisaari *et al.*, 2007). N2 and P3 amplitudes and latencies evaluation during a visual Go/Nogo task by Yang et al., confirmed the impairment of frontocentral areas of heroin addicts during the inhibition process. The results suggested an abnormal conflict monitoring process and inhibitory control dysfunction in heroin addicts (Yang *et al.*, 2009).

Marques evaluated the amplitude and latency of P3 among heroin addicts confronting neutral, pleasant, and unpleasant emotional conditions. Based on the results, addicts showed no differences between all three emotional conditions. Smaller P3 amplitudes and less spread in the brain areas for unpleasant and neutral emotional conditions among heroin addicts suggested that addicts have deficiencies in extracting relevant information from sensory stimuli under unpleasant and neutral stimulation, as well as in directing attentional resources to environmental stimuli of positive emotional valence (Marques-Teixeira & Barbosa, 2005). Based on the findings of P3 among heroin addicts and the

hypothesis of ERP as putative endophenotypes for opioid addiction, Singh et al. designed a study to evaluate this cognitive component among drug abusers, their brothers, and healthy control subjects. The results confirmed diminished P3 amplitude and longer latency of P3 in heroin addicts and their brothers and suggested a common genetic substrate as a component of the addiction etiology (Singh *et al.*, 2009).

2.7.2 Withdrawal and Short-term Abstinence

Polunina and Davydov argued that during the first two weeks of abstinence, alpha power significantly decreases whereas beta power significantly increases. As the abstinence period lengthens, theta2 and alpha1 powers, as well as the mean frequency of theta, increase, whereas the beta1 mean frequency and beta power significantly decrease (Davydov & Polunina, 2004; Polunina & Davydov, 2004).

Researchers found that during early stages of withdrawal, significant reorganization of EEG composition was found equally in all locations (Fingelkurts *et al.*, 2008; Fingelkurts *et al.*, 2006) and brain local and remote cortical functional connectivity disruption for alpha and beta were enhanced significantly (A. A. Fingelkurts *et al.*, 2007). Bauer also evaluated the P3 amplitude among opioid addicts and examined the process of CNS recovery from opiates (Bauer, 2001). The results showed a significant decline of P3 amplitude among heroin addicts compared to controls.

2.7.3 MMT and long-term Abstinence

It has been demonstrated that six months of methadone treatment moderates distribution of functional connectivity of alpha and beta, normalizes the composition of EEG activities and restores the temporal structure of brain oscillations (A. A. Fingelkurts *et al.*, 2007; Fingelkurts *et al.*, 2009; Alexander A. Fingelkurts *et al.*, 2007; Gorricho & Uson, 2008). The average amplitude of alpha and beta increase in left frontal and right temporal cortical areas, and a decrease in the right parietal location. The average length

of EEG segments was shorter in right central (for both alpha and beta), right parietal (beta), and midline occipital (alpha) cortex. (Fingelkurts *et al.*, 2009). Also, it was seen that the percentage of alpha1, alpha2, and beta1 rhythmic segments decreased, while the percentage of delta–theta1, theta, and theta2–alpha2 rhythmic segments increased (Alexander A. Fingelkurts *et al.*, 2007).

Papageorgiou et al. evaluated the P600 and P3 components after six months of heroin abstinence during a working memory test. Results showed that although P600 amplitude recovered for abstinent heroin addicts (Papageorgiou *et al.*, 2001). Moreover, although P3 latency was normalized, P3 amplitude after abstinence is not completely comparable to healthy subjects, and no correlation between P3 and duration of heroin abuse exists (Papageorgiou *et al.*, 2001; Papageorgiou *et al.*, 2004).

N2 and P3 were evaluated among heroin-abstinent (average of 15 months) during a Go/No-Go task consisting of both neutral and emotionally valenced stimuli (Morie *et al.*, 2014). Although results could not confirm any relationship between the amplitudes of No-Go P3 and N2 and duration of abstinence or addiction, a marginal relationship was found between abstinence length and No-Go P3 amplitude in the neutral condition (Morie *et al.*, 2014).

Lubman et al. explored the impact of drug cues and affective stimuli on central attentional processing by evaluating the P3 amplitude and examined the motivational significance of drug cues among heroin dependents compared with healthy subjects. Results showed that MMT subjects and heroin dependents with stabilized opiate-substitution exhibit lower P3 amplitude for pleasant pictures and greater for drug-related cues compared with healthy subjects (Lubman *et al.*, 2007; Lubman *et al.*, 2009). This result was repeated for drug cues compared to affective and neutral stimuli, and the degree

of P3 facilitation to the drug-related stimuli was seen to be related to the craving rate (Lubman *et al.*, 2008).

2.8 Summary

Brain electrophysiological approaches in heroin addiction studies provide an excellent medium to understand neurobiological dysregulation and identify the location of cognitive responses in the brain. EEG and ERP provide accurate information regarding brain functionality and attentional biases, which offer a more direct measure to evaluate information processing and cognition. Since the year 2000, twenty-seven research works have been published in ISI journals in this field, and although their methodological approaches and subjects conditions were varied; they all aimed to explore brain electrophysiological properties of heroin addicts.

Based on the reviewed literature, EEG studies showed abnormal EEG patterns and dysfunctions in cognitive abilities were observed among heroin-dependent subjects. ERP findings show that event potentials changes caused by heroin addiction include declines in P3 amplitude, increased latency of P3, MMN.

This chapter introduces the MMT as the most common substitution therapy for heroin addiction. However, since studies showed controversial findings of the methadone effect on the brain, the adjunctive therapy is used in heroin addiction treatment. This chapter also intends to classify the experimental trials on the consequences of acupuncture in drug dependence treatment. Based on the discussed literature, while many types of research failed to prove any effect of acupuncture in addiction treatment, acupuncture at HT7, ST36, and SP6 acupoints have been shown to be effective in drug-induced activities and reported as a potential therapy for the treatment of opiate addiction. However, no strong consensus opinion on the general efficacy of acupuncture exists, and evaluating acupuncture in cases of substance abuse have shown conflicting results.

In summary, although various studies aimed to investigate the efficiency of methadone as well as exploring its neurophysiology effects. There is a small number of brain electrophysiological research in this field which lack of some methodological issues. Subjects' addiction status, premorbid conditions, and comorbidities have significant direct and indirect effect on neuroelectrophysiological findings of these studies. Intergroup differences and multiple substance issues are two main methodological challenges in heroin addiction studies. In addition, some of these studies lack of controlling confounding factors such as pharmacological and medical comorbidities (e.g., hypertension, diabetes, HIV, hepatitis, etc.), comorbid psychiatric conditions (e.g., antisocial personality disorder, depressive disorder, etc.), premorbid personality and genetic differences, medication use and other premorbid brain dysfunctions (e.g., risky lifestyle with high prevalence of non-fatal opiate overdoses, strokes, head traumas, and chronic infections) .

Various types of ERP paradigms have been designed for evaluation of addicts' evoked cognitive responses from deviant, affective, and drug-related stimuli. However, there is a lack of a comprehensive paradigm to record major ERPs like MMN, P3, and P6 in a single experiment. Evaluating these three components using a uniform paradigm helps to a better understanding of brain electrophysiological alterations among heroin addicts. The designed paradigm should be based on the combination of modified well-known paradigms in heroin addiction research. Therefore, it requires a longitudinal study to monitor MMT subjects from the early stages of withdrawal till long-term abstinence with respect to all confounding factors and a multi-method approach for EEG and ERP evaluation to elaborate the exact effects of methadone therapy. It is imperative to note that investigation of cognitive traits associated with striking features of addiction in a single study can help advance the understanding of neurological features of addiction. Investigating qEEG, MMN, P3, and P600 properties can lead to a better understanding of

brain neurobiological features alteration among heroin addicts and offers more insight for a reliable analysis of brain-behavior relations related to pre-attentive processing, attentional deficit and response inhibition associated with the development and maintenance of addictive behaviors. Therefore a well-designed double-blind, sham controlled, and pragmatic randomized study is required to assess the therapeutic effects of acupuncture from both acute and long-term perspectives.

CHAPTER 3: METHODOLOGY

3.1 Introduction

This longitudinal study has multifold objectives to evaluate brain electrophysiological characteristics of heroin addicts during three months of methadone therapy adjunctive to EA. The flowchart of this study with a comprehensive protocol for the clinical trial is depicted in Figure 3.1. The first objective of the study is to design a comprehensive paradigm to obtain the EEG and ERP components which explain in detail in section 3.2. The applied EEG signal processing in this study as well as developing the single-trial ERP detection method as the second objective of this study is explained in section 3.4.

The first phase of the clinical trial is a cross-sectional observation of various neurophysiological properties among heroin dependents and healthy control subjects with no history of heroin or other substance abuse. This phase aims to investigate and compare the significant differences in brain electrophysiological characteristics between these two groups which may be associated with chronic heroin abuse. Heroin addict subjects were then randomly divided into two treatment groups to be treated with methadone or methadone adjunctive to electro-acupuncture. Brain electrophysiological properties including quantitative EEG (power spectral activity), and ERPs (MMN, P3, P600) were monthly measured as well as clinical psychiatric assessments related to opioid withdrawal scales.

In this study, amplitudes, and latencies of MMN, P3, and P600 associated with brain discrimination abilities, the orientation of attention, response resolution, and working memory were evaluated. Monitoring cognitive EEG changes during the treatment period aims to discover the effects of these treatment methods on EEG and ERP characteristics. Furthermore, to explore the acute effects of methadone intake, the EEG and ERP characteristics of addicts were measured before and after their methadone intake at the

third interval (second month) when the subjects methadone dosage is stabilized, and the withdrawal symptoms can be neglected.

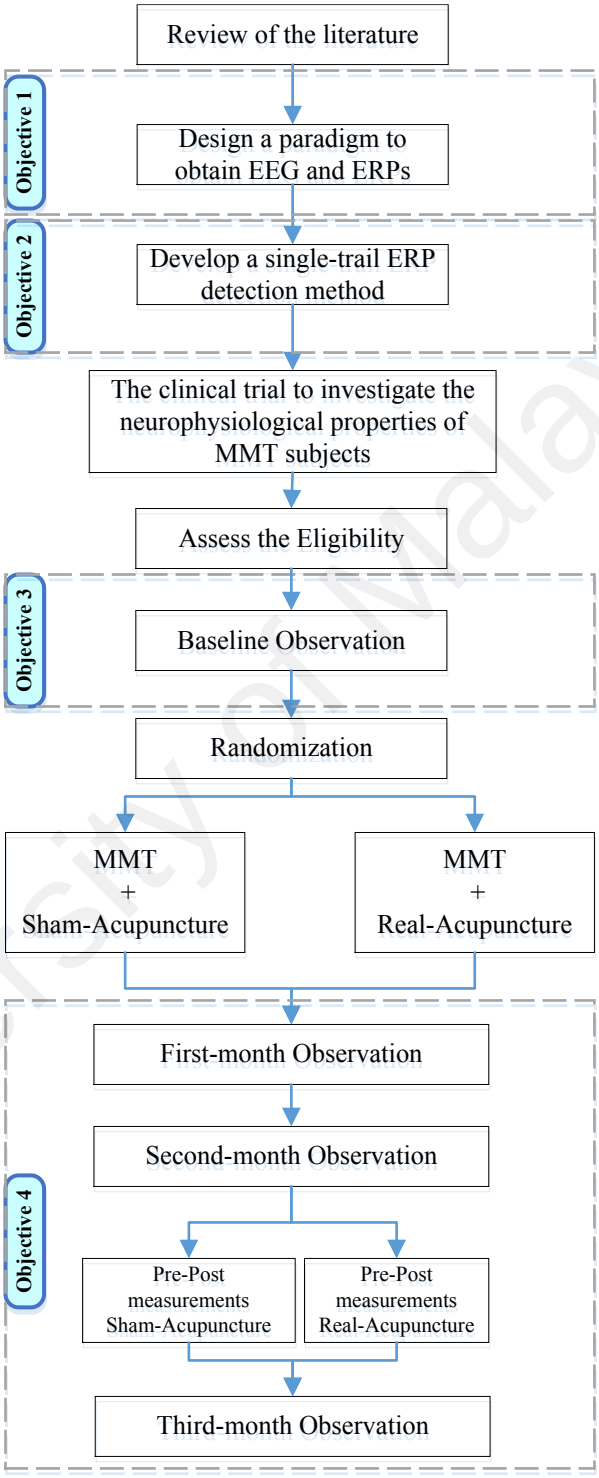


Figure 3.1: The flowchart of the study and protocol of the clinical trial.

3.2 Designing the EEG Paradigm

It is imperative to note that investigation of cognitive traits associated with striking features of addiction in a single study can help advance the understanding of neurological features of addiction. However, lack of a comprehensive evaluation of electrophysiological variables, as a sensitive measure of impaired cognitive control, in a single experiment is a major deficiency in the field (Buzzell *et al.*, 2014). Investigating qEEG, MMN, P3, and P600 properties can lead to a better understanding of brain neurobiological features alteration among heroin addicts and offers more insight for a reliable analysis of brain behavior relations related to pre-attentive processing, attentional deficit and response inhibition associated with the development and maintenance of addictive behaviors. This study aims to introduce a new approach consisting of a comprehensive paradigm to evaluate EEG power spectral density (PSD) and properties of MMN, P300, P600 components simultaneously.

A fusion of well-known paradigms in addiction studies was used in this study to probe N2, P3, and P600 in a single distinct experiment (Figure 3.2). The paradigm consists of three sub-paradigms including a modified version of the computerized auditory digit span Wechsler Test and the two 3-stimulus "oddball" paradigms as standard auditory and heroin-related visual stimuli.

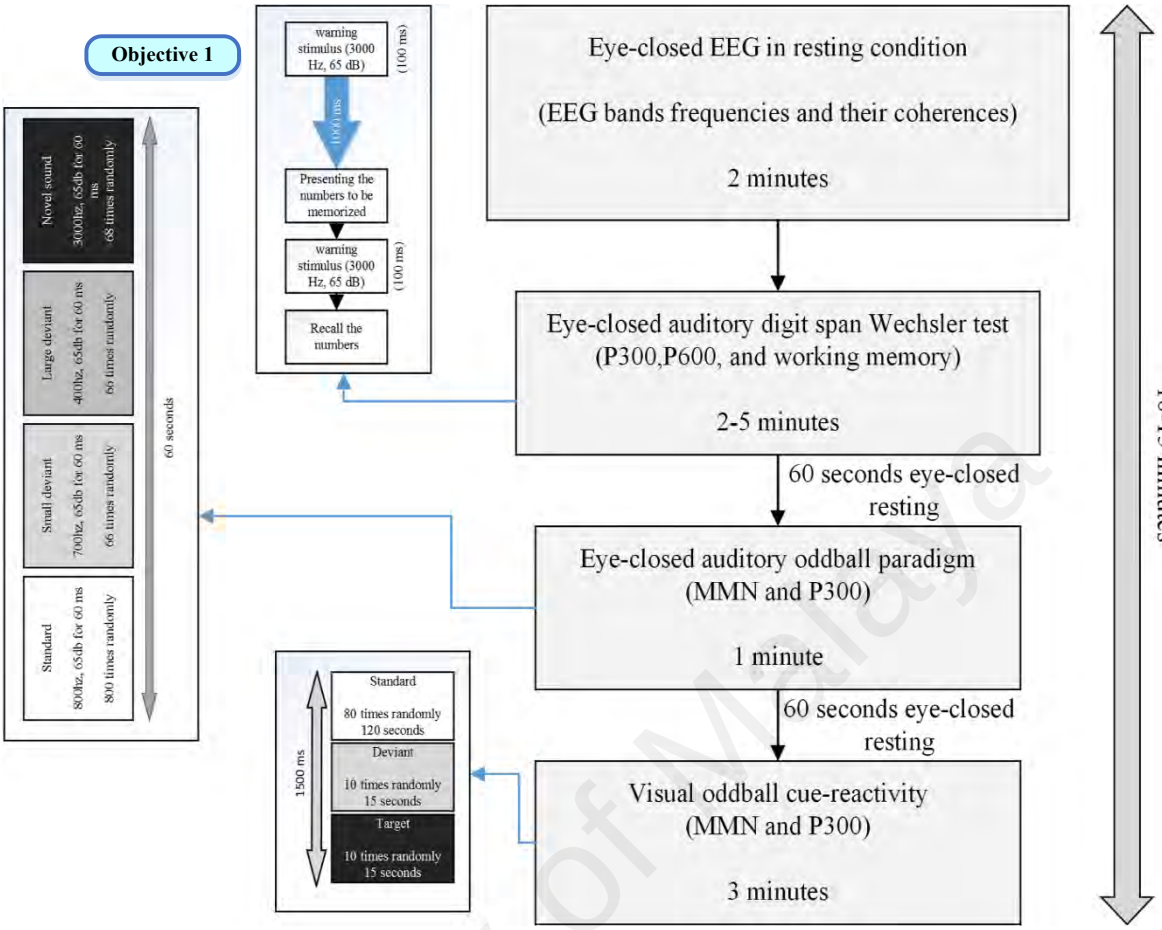


Figure 3.2: ERP paradigm for evaluation of MMN, P3, and P600 at standard and cue-reactivity conditions. Duration of the paradigm is 10-15 minutes including the resting time between each task (Objective 1).

3.2.1 Auditory Digit Span Wechsler Test

This task of paradigm was designed for probing the P3 and P600 components to assess working memory based on a computerized modified version of the digit span Wechsler Test (Wechsler, 2000) (Figure 3.3). Subjects were required to sit in an armchair in a comfortable position, and follow the instructions presented binaurally via earphones at an intensity of 65 dB sound pressure level and asked close his/her eyes during the test to minimize eye movements and blinks. Subjects were requested to memorize a series of numbers in a certain order (2-9 digits, started from two digits and it will be increased after each successful performance) after a warning tone, and then repeated in the same order after the subjects had heard the warning tone for the second time. A single resonance of high frequency (3000 Hz) was presented to the subject with duration of 100 ms as the

warning stimulus. An interval of total silence was given for one second, and this was followed by the warning tone. The numbers to be memorized were recited trough the earphones. The recital of the numbers was not timed as the number of digits were read out at a normal pace. At the end of the number sequence presentation, the same warning stimulus was repeated for 100 ms, and the subjects were asked to recall the given numbers as quickly as possible (this procedure was repeated till the first mistake happens).

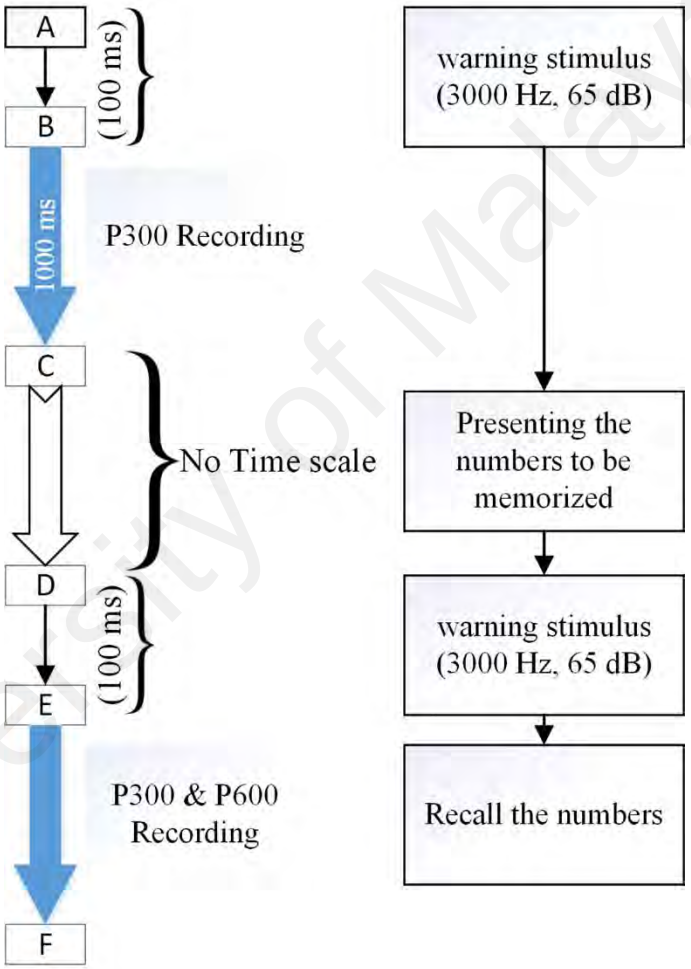


Figure 3.3: Process of modified version of digit span Wechsler test including the precise timing and the process of ERP recording.

3.2.2 Auditory Oddball

This task was designed for the evaluation of MMN and novelty P3 using four classes of auditory stimuli. MMN is calculated by subtraction of the N2 elicited by the standard stimulus from those elicited by the deviant stimulus when both of these stimuli are un-

attendant or ignored. Therefore, in this paradigm, four stimuli were represented as probable standard, improbable target, and high/low improbable deviant stimuli. This paradigm was presented in the same criteria of the auditory digit span Wechsler Test described in 3.2.1 (Figure 3.4). Subjects were asked to press a key upon hearing a single high-frequency tone. 1000 stimuli were binaurally delivered through earphones in a random order with following characteristics:

- Standard stimuli 80% (800hz, 65db, 50 ms + 10 ms for rise and fall)
- Small deviant stimuli 6.6% probability (700hz, 65db, 50 ms + 10 ms for rise and fall)
- Large deviant stimuli 6.6% probability (400hz, 65db, 50 ms + 10 ms for rise and fall)
- Novel sound stimuli 6.8% probability (3000hz, 65db, 50 ms + 10 ms for rise and fall)

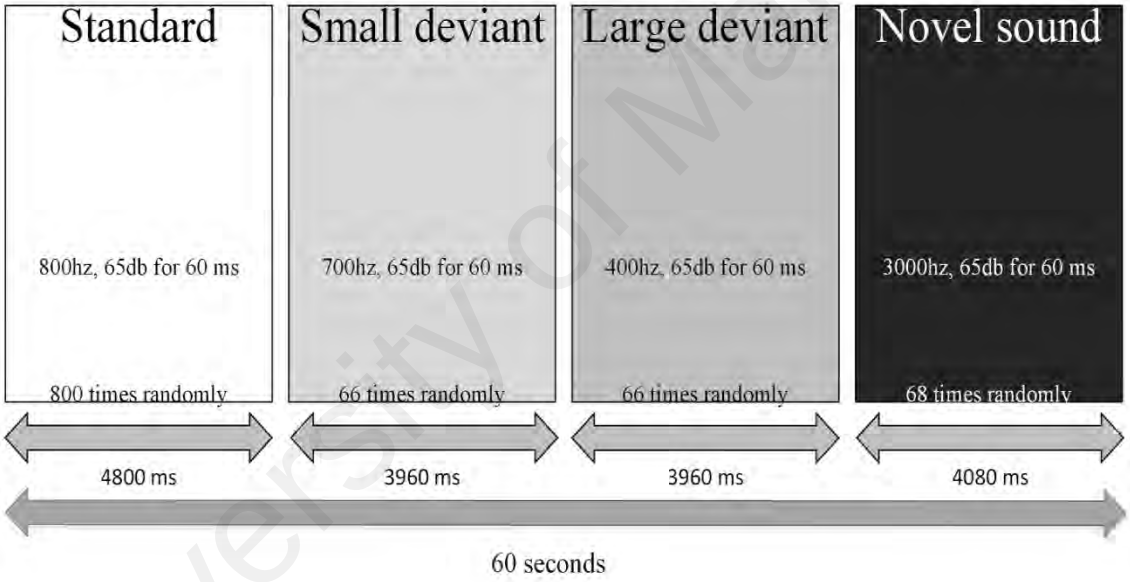


Figure 3.4: Auditory oddball paradigm for evaluation of P3 and MMN with duration of 1 minute including 1000 stimuli presented randomly.

3.2.3 Visual Oddball Cue-reactivity

The 3-stimulus "oddball" paradigm is appropriate for processing the focus of attention, or novelty processing for investigation of P3 when each deviant stimuli is unique and includes probable standard stimuli, improbable target stimuli, and equally improbable deviant stimuli. In this paradigm, three types of stimuli were represented as probable standard, improbable target, and equally improbable deviant stimuli. This paradigm was

presented while the subject was looking at the computer screen and he/she was asked to press a key once a heroin-related image was presented. At the beginning of the paradigm, the screen shows a white fixation cross to cue subject attention to the particular region of the monitor. There were three types of images displayed with the same size for 500 ms in the middle of the screen followed by 1000 ms of a "crosshair" before the next image was presented. There was a total of 100 stimuli (standard pictures included neutral pictures from households, neutral l pictures of trees and landscapes along with deviant stimuli and color pictures of heroin use and heroin paraphernalia) presented randomly as described in Figure 3.5.

- Standard stimuli 80% probability (neural pictures 500ms + 1000 ms crosshair)
- Deviant stimuli 10% probability (nature pictures 500ms + 1000 ms crosshair)
- Target stimuli 10% probability (heroin related pictures 500ms + 1000 ms crosshair)

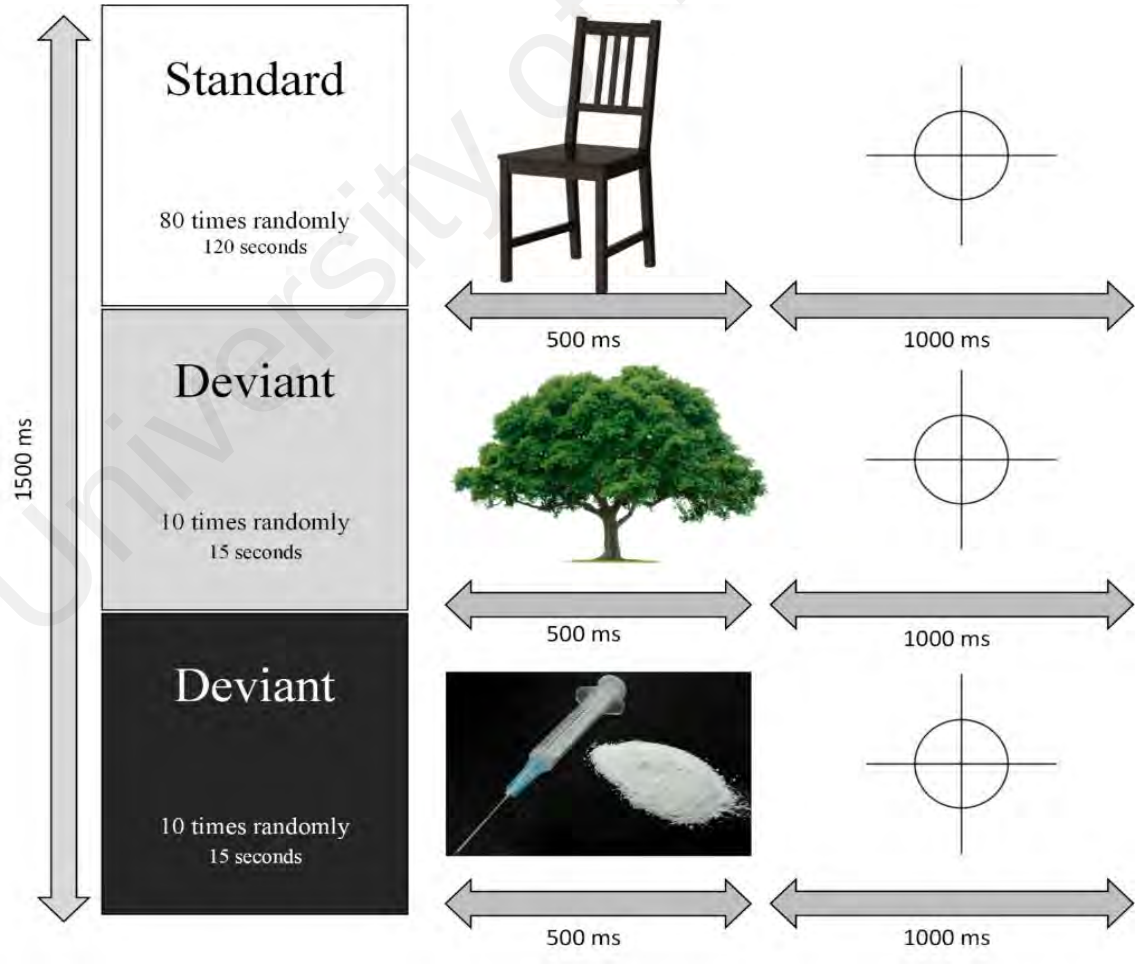


Figure 3.5: Visual cue-reactivity oddball paradigm for evaluation of P300 with duration of 150 seconds including 100 stimuli presented randomly.

3.3 Trial Design and Subjects

In this study, spectral analysis of EEG signals during the relaxing time as well as ERP components (MMN, P3, and P600) amplitude and latency of subjects were monitored using this paradigm. Male heroin dependent subjects were recruited from the waiting list in the "Substance Clinic" at the University of Malaya Medical Center (UMMC). This study took place at the clinical engineering laboratory, in the Department of Biomedical Engineering, University of Malaya. The inclusion criteria for this study were at least one year of documented heroin addiction and fulfilled the diagnostic statistical manual IV (DSM-IV) criteria for heroin dependency, syndrome diagnosis, a minimum age of 18 years, and minimum six years of drug abuse. Also, non-smoking volunteers were included as healthy control subjects and were recruited from the university's portal email broadcast. The exclusion criteria for this study were alcohol dependency, the presence of any acute psychiatric or neurological disorder (major head trauma), possessing a lifetime history of a major medical disorder, HIV infection, previous head injury resulting in loss of consciousness, seizures, history of methadone treatment, and record of polysubstance dependence. All the subjects were informed that their last dose of heroin should be taken at least six hours before attending the assessment session. The study was approved by the ethics committee of UMCC (Ethics Number: MEC 871.14), and a written consent was obtained from each subject before enrollment.

Each subject underwent routine clinical assessments by psychiatrists in the substance clinic of UMMC. The alcohol breath screening test, baseline blood investigations for HIV, viral hepatitis B/C screening and a rapid urine drug test for illicit drugs use were carried out as objective measures to verify the absence of alcohol and other substance abuse prior to the EEG procedures in addition to verbal information collected from the subjects. The *Fagerstrom* test was used to assess the intensity of physical addiction to nicotine among addicts. The *Fagerstrom* test evaluates the quantity of cigarette

consumption, the compulsion to use, and dependence. Also, Mini-International Neuropsychiatric Interview (MINI) for DSM-IV for controlling the premorbid psychiatric effects was performed for both groups (Sheehan *et al.*, 1998), as well as the objective and subjective withdrawal scales (OOWS, SOWS).

After the initial assessment in the substance clinic and signing the written consent form, clinic staff brought the subjects immediately to the EEG recording room. Once the instruments were calibrated and the electrodes were placed on the scalp, the subjects were lulled into a comfortable situation in a dimmed and an electromagnetically shielded recording room. Subjects were instructed to be relaxed and comfortable without any movement. These criteria reduced artifact effects that might be derived from muscle tensions during the EEG recording. Subjects underwent 30 minutes of EEG recording from 19 channels during the performance of the paradigm.

Subjects recruited in this study were given the methadone dosage (20-125 mg/ml). The starting dosage of methadone is 20-30 mg per day, and it increased by the psychiatrists directly. All the patients were randomly assigned into two groups which are treatment and sham group. The treatment group was given methadone plus (electro-acupuncture) EA stimulation at 1.1+80Hz while sham group received methadone plus sham EA without actually receiving any electrical stimulation. Both groups received the EA treatments 30 minutes per session. The treatment protocol applied in this study was based on the Biophysical Solutions^{TM} methods. The acupuncture protocol for the first month of treatment was three times a week, and it reduced to one session a week for the rest of the treatment period. Four acupuncture points were stimulated for the treatment include Jerome point (ear lobe at the end of the helix), St36 (four finger breadth above the internal malleolus), Sp6 (four finger breadth below the tibial creast) and meridian point (spleen meridian: bilateral big toe).

3.3.1 EEG Recording

Nicolet EEG diagnostics system (Care Fusion Corporation, 3750 Torrey View Court, San Diego, CA 92130) was used to capture the EEG activities within a frequency band of 0.5-70Hz (with a sampling rate of 256 Hz). Considering the technical limitations and reducing the complexity of the analysis, EEG signals from 19 electrodes - ($Fp_{1/2}$, $F_{7/8}$, Fz, $F_{3/4}$, $T_{3/4}$, $C_{3/4}$, Cz, $T_{5/6}$, $P_{3/4}$, Pz, $O_{1/2}$) according to the 10-20 standard of electrode placement with $A_{1/2}$ (earlobes) as the reference were recorded. These electrodes were widely distributed across the scalp, and their installation was based on the standard protocols explained in (Light *et al.*, 2010). Before data collection, the impedances of all the electrodes were monitored for each subject, to verify its value to be under 5 kΩ. Then, the paradigm timing system and the EEG recorder were synchronized followed by 30 minutes of signal recording during the performance of the described paradigm.

3.4 EEG Signal Processing

The signal processing flowchart of this study for extracting the PSD of resting-state EEG and ERP components features is depicted in Figure 3.6. EEG recording is highly susceptible to various forms and sources of noise, which present significant difficulties and challenges in the analysis and interpretation of the EEG data. Different signal processing techniques were applied for qEEG and ERP analysis; noisy epochs can be rejected from the recorded EEG for qEEG analysis; while artifacts should be detected and removed from EEG for ERP analysis. It is critical to detect artifact contamination of evoked-potential EEG data for several reasons. First, artifactual signals usually have high amplitudes. Thus, even if their distribution in the recorded EEG is sparse, they can bias the evoked potential averages constructed from the data and, as a consequence, bias the results of an experiment. Therefore, pre-processing and analysis of EEG spectrum and ERPs are explained in separate sections.

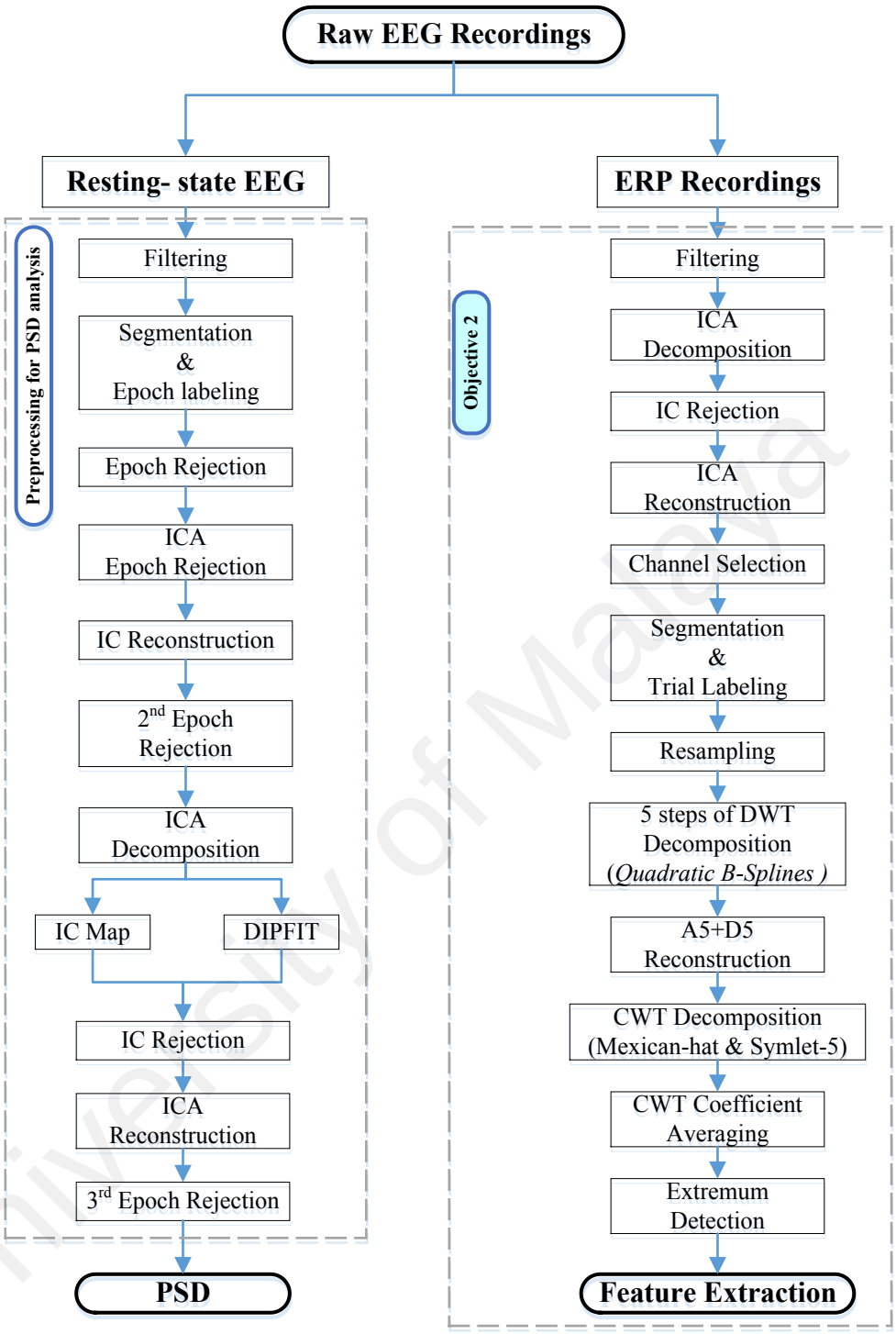


Figure 3.6: The signal processing flowchart of this study to extract the PSD and ERP features.

3.4.1 EEG Power Spectrum

To measure the resting-state EEG power spectrum, only resting state eye-closed EEG recordings were used at this stage to analyze the spectral activity of the EEG signals for each frequency band. Data was recorded within 0.5-70 Hz, and evaluation of power

spectrum comparison was defined to be within 0.5-30 Hz for Delta (0.5-4 Hz), theta (4-8 Hz), alpha (8-13 Hz), and beta (13-30 Hz). However, the artifactual and noisy parts of recorded signals should be rejected for providing reliable PSD values.

3.4.1.1 Pre-processing of resting-state EEG

To have a uniform and standard procedure for rejecting the EEG artifacts; an automated algorithm was followed for all recording signals (Figure 3.7). In this study, unlike the common practice in psychiatric research (experts screening the signals to reject the contaminated epochs), an automated artifact rejection method was performed using the *EEGLAB* toolbox (Delorme & Makeig, 2004) in *MATLAB*. In this approach, first, the continuous data were filtered from 0.1-70 Hz using a combination of a low-pass and high-pass two-way infinite impulse response (IIR) elliptic filters. Although IIR filtering introduce different phase delays at various frequencies, it can be compensated by applying the filter forward and then backward. This applies the filter forward and then again backward, to ensure that phase delays introduced by the filter are nullified. Since filtering the continuous data minimizes the introduction of filtering artifacts at epoch boundaries, after that, all signals were divided into epochs with a duration of two seconds. A uniform algorithm for identifying clean EEG epochs was applied to all recorded signals. All epochs that went through the same algorithms were subsequently labeled as clean or contaminated EEG epochs. Software routines for performing the artifact detection methods described above are available within the EEGLAB toolbox (Delorme & Makeig, 2004). In the first step, epochs were rejected based on four criteria:

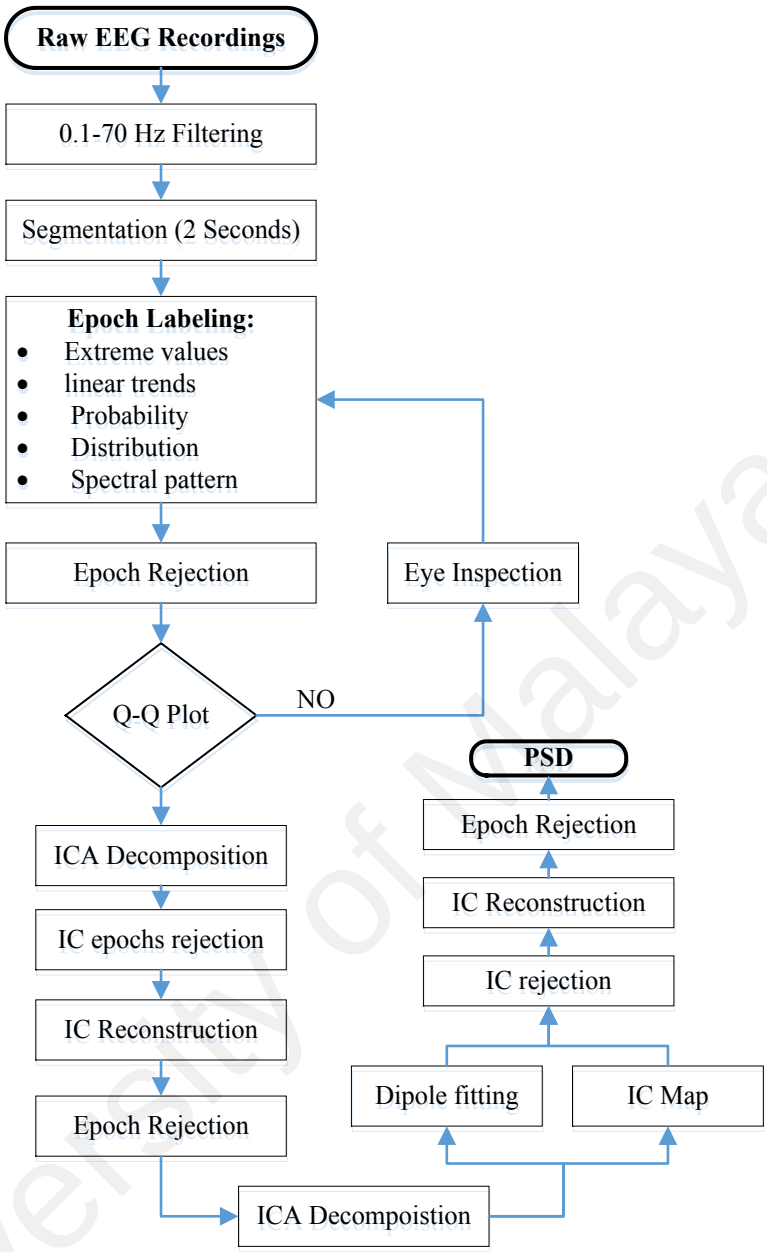


Figure 3.7: The flowchart of PSD computation of EEG signals during resting time.

(a) ***Extreme values***

Extreme values can be named as the most widely used method for detecting contaminated EEG trials by gross eye blinks and eye movements. This method known as threshold rejection consists of defining the standard threshold of ±75 μv for potential values based on the amplitude of EEG signals (Nolan *et al.*, 2010). If the absolute value of any data points in the epoch exceeds this range, the epochs are labeled as artifactual.

(b) ***Abnormal linear trends***

Since during acquisition of EEG transient, recording-induced current drifts and electrode movements causes linear drifts in some channels, in this method data points of each epoch fit to a straight line and if the slope of the calculated line is more than 60 μv (0 μv for the beginning of epoch and 60 μv at the end of 2-second epoch), it is deemed as an artifactual epoch (Nolan *et al.*, 2010). In this method, a standard R-square measure is used to determine the minimal fit between the epoch data-points and a line of the minimal slope.

(c) ***Improbable data***

In this study, data were recorded during the resting time of the subjects, and it is expected that the majority of EEG epochs have same time courses. Since some of the artifacts are caused by the occurrence of sudden movements or improbable noises, in this step transient odd or unexpected value points in each epoch are identified by the comparison of the epoch values and normal statistical values recorded during the whole trial using probability density function of all data-points for each electrode. If the probability value of an epoch is low, then it considers as "oddness". In this method, Probability Density Function (PDF) of all data points of each electrode (e) has been calculated. Then, the joint log probability was computed for every epoch value at each electrode using the Equation 1, while "e" indicates the electrode, "i" is the epoch number," A_i" is the activity values and "$P_{De}(x)$" is the probability of observing the value "x" in the probability distribution "D_e" of activity at channel "e". Lograitmic join probability was used for better graphical presentation of low probability values.

$$J_e(i) = -Log(\prod_{x \in A_i} P_{De}(x)) \tag{3.1}$$

Probability measure is applied both to each channel as well as all electrodes and standardized the measures into mean 0 and standard deviation of 1. Then, improbable

epochs are labeled based on the threshold of 5×standard deviations of the average probability distribution. Although the data supposed to be normally distributed (5×SD provides 99.99% of data), Chebyshev's inequality ensures that, for all distributions for which the standard deviation is defined, the amount of data within a number of standard deviations of the mean is at least as equal to 96% for 5×SD (Hazewinkel, 2001).

(d) ***Abnormal distributed data***

Another method of detecting artifactual epochs is based on the kurtosis of the activity values to locate the unusual peaky distribution of values which occurs due to strong transient muscle activity and flat distribution caused by AC/DC artifacts. Therefore, this method is used to find high kurtosis values which indicate abnormal peaky and flat activity distribution in an epoch. Similar to improbable data detection, it is calculated for both single- and all-channels with the same threshold from mean kurtosis value.

(e) ***Abnormal spectral pattern***

This method has been suggested as the most efficient method for rejecting the artefactual epochs (Delorme *et al.*, 2007). Some EEG artifacts have a specific activity and scalp topographies that are more easily identifiable in the frequency domain. To detect these artifacts, the *Slepian* multitaper spectrum is computed for all epochs and channels, using *MATLAB* "*pmtm*" function defaults (four orthogonal tapers; FFT length of 500 data points for each data epoch). The main advantage of using multitaper over standard spectral methods is that, for rhythmic activity in the data; the signal/noise ratio is lower. Subsequently, the average power spectrum of the whole trial over each channel is subtracted from the calculated epoch spectrum. If the subtraction value exceeds a value of ± 50 dB in the range of 0.5-2 Hz and +25 or -100 dB for 20-45 Hz, then, the epoch is labeled as a contaminated epoch and is selected for rejection. The purpose of using a 0.5-2 Hz and 20-30 Hz is to help single out eye movements and muscle activities.

(f) ***Validation***

In the next step, all marked epochs were rejected from the trials and for evaluating the success of each channel artifact rejection process; the signal mean, standard deviation, skewness, kurtosis, and median were calculated, and *kolmogrov-Smirnov* test was applied for the estimation of distribution subsequently on raw data and processed signals after the artifact rejection (Delorme *et al.*, 2007). The result of this test at a significance level of $p \leq 0.05$ will indicate whether the data distribution of the signal was Gaussian or not. In this step, empirical quantile-quantile plot (*QQ-plot*) was used to depict the quantiles of the data versus the quantiles of standard normal (Gaussian) distribution. The result of this step was used for validation of artifact rejection as a pre-condition evaluation to apply ICA for artifact removal in the next steps. Manual eye-inspection was applied to the specific channel signals if the explained examinations did not classify the artifact-free signals as a Gaussian distribution.

(g) ***ICA decomposition***

The epoch rejection steps can only partially remove some types of artifacts (e.g., line noise or muscle artifact). In addition, the epoch rejection is developed based on statistical properties of single electrodes while EEG signals recorded from different channels are highly correlated which contains redundant information. Therefore, as an alternative approach, the EEG data are separated into active cortical and artifact sources based on the physiology and statistically adequate assumption of the ICA (Ball & Ross, 1991; Delorme & Makeig, 2004; Delorme *et al.*, 2007). Decomposing data by ICA (or any linear decomposition method, including PCA and its derivatives) involves a linear change of basis from data collected at single scalp channels to a spatially transformed "virtual channel" basis. ICA separated the channel signals into independent signals (on the basis of orthogonality utilizing singular value decomposition algorithm) as a common method

to remove artifacts and source localization of EEG signals (this method is explained in (Li *et al.*, 2006; Schlögl *et al.*, 2007)).

ICA has been shown to be more efficient for this purpose than other algorithms such as principal component analysis (PCA). PCA utilizes the first and second dimensions of the measured data and hence relying heavily on Gaussian features, while ICA exploits inherently non-Gaussian features of the data and employs higher dimensions. Furthermore, EEG signals seem to fulfill the major assumptions of ICA (i.e. ICA component projections sum linearly on the electrodes, source independence, and the non-gaussianity of the sources) (Makeig *et al.*, 1996). The main two assumptions of ICA about linearly summed components and independence of component is fulfilled by considering that in EEG recording both active and passive electrodes are equally receptive to cortical and artifact sources while they are related to brain activity and muscle contractions, that is triggered by activity in the motor cortex and the time courses of the resulting artifacts and the triggering of brain events should typically be distinctive across all or some trials, which make the cortical and artifactual source signals nearly independent (Figure 3.8). The third assumption concerns the non-gaussianity of the source activity distributions, which is quite plausible for artifacts, which are usually sparsely active and thus far from Gaussian in value distribution.

The concept of ICA as a subclass of "blind source separation" was first developed around 1990 and aims to separate individual source signals from multidimensional data in which they are mixed (Lee, 1998). Technically, ICA finds a set of fixed spatial filters that together constitute the most distinct (i.e., temporally near-independent) signals available in the input data. The main goal of using ICA is brain source localization; by making a single Independent Component (IC) from joining all the information content of the EEG signals from different electrodes which constitute one particular EEG source

(Figure 3.9). This component includes the projection of those activities to all the scalp electrodes, whereas the unrelated EEG source activities will be eliminated from this IC and contributed to other ICs (Delorme *et al.*, 2007).

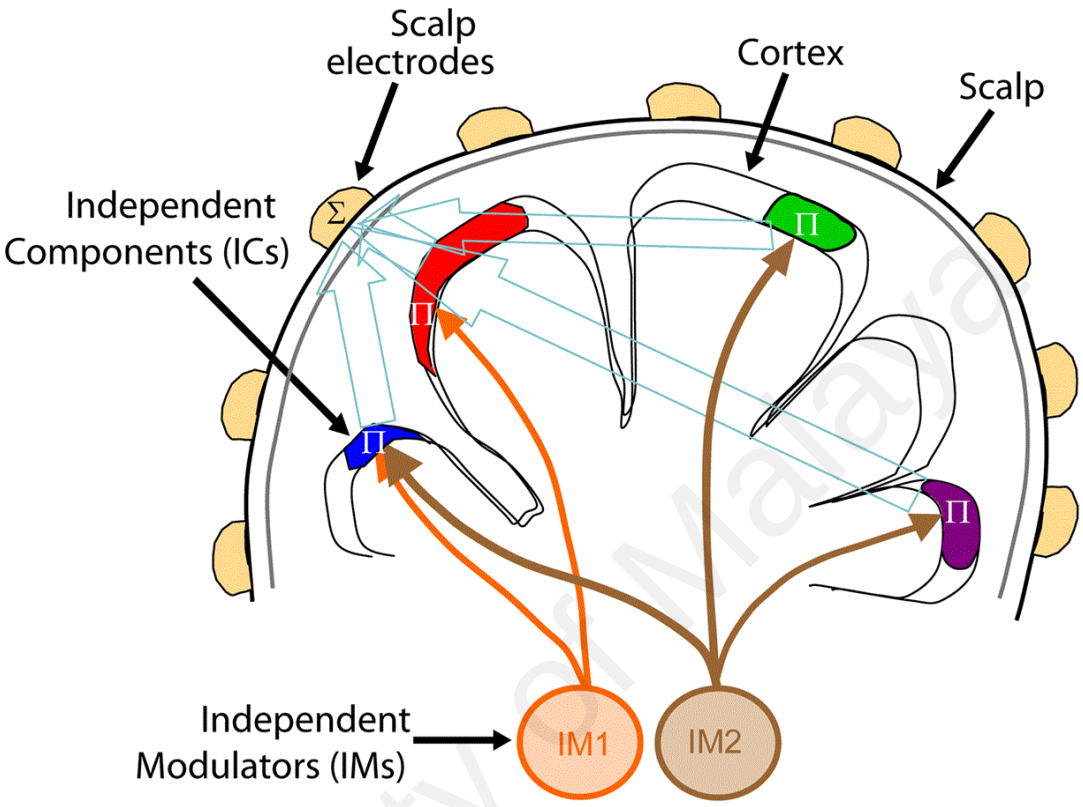


Figure 3.8: A schematic of EEG independence sources and their combination to form the scalp-EEG recording (Onton & Makeig, 2009).

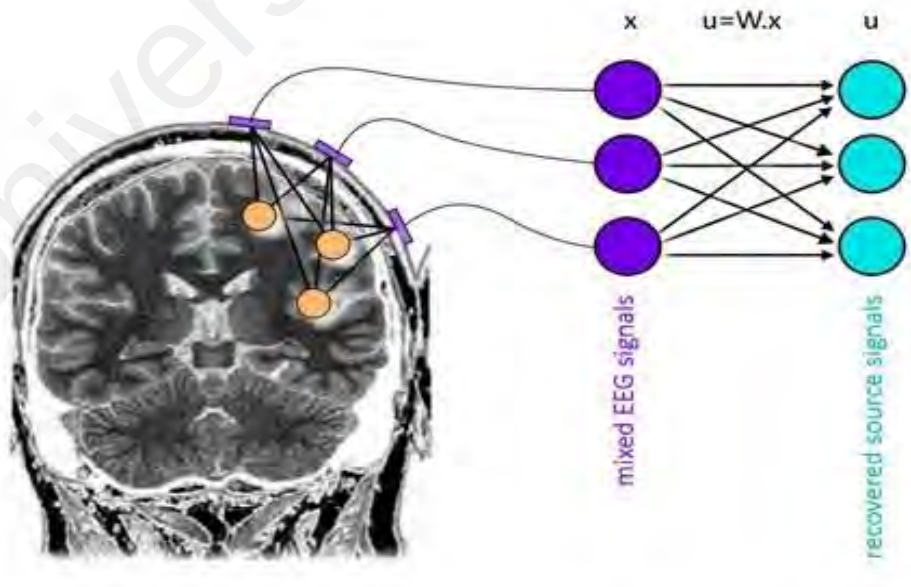


Figure 3.9: ICA decomposition aims to recover the source signals from mixed scalp-EEG recorded signals.

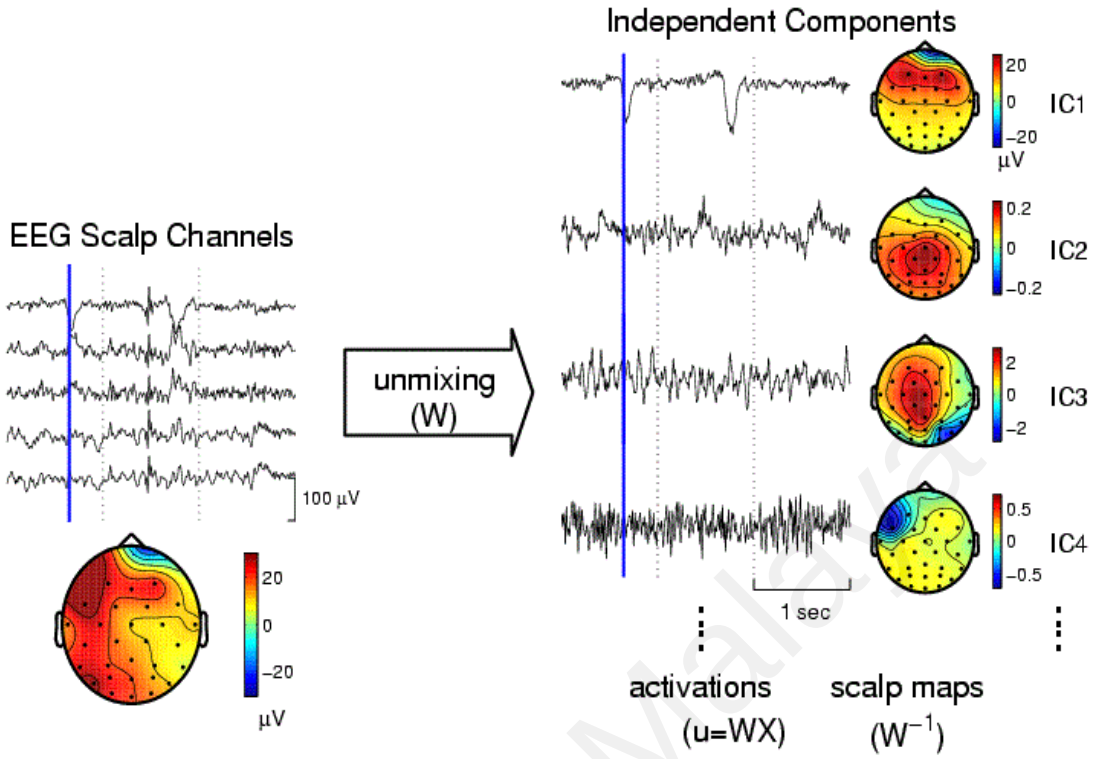


Figure 3.10: ICA decomposition of matrix X (scalp-EEG recording) into Independent Components (U).

Based on the assumptions for applying ICA, the scalp-EEG recording from each channel x_j is the linear summation of cortical and artifactual sources $s_{1\text{-}n}$ (Equation 3.2) (Hyvärinen *et al.*, 2004). The EEG recordings from all channels form a matrix of $n \times t$ (n is the number of channels and t is all data-points of recording) regardless of channel locations. The source locations of the ICs are presumed to be stationary for the duration of the recording (spatially fixed). ICA performs a blind separation of the data matrix (X) and finds a matrix (W) that when multiplied by the original data (X), yields the matrix (U) based only on the criterion that resulting source time courses (U) are maximally independent (Equation 3.3) (Figure 3.10).

$$x_j = a_{j1}s_1 + a_{j2}s_2 + a_{j3}s_3 + \cdots + a_{jn}s_n \rightarrow x_j = \sum_{i=1}^{n} a_{ji}s_i \quad (3.2)$$

$$U_{n\times t} = W_{n\times n}X_{n\times t} \rightarrow X = W^{-1}U \quad (3.3)$$

After ICA decomposition, The ICs (U-matrix) may represent the synchronous or partially synchronous activity of cortical sources as well as activity from non-cortical sources (e.g., potentials induced by eyeball movements or produced by single muscle activity, line noise, etc.). Figure 3.11 depicts a sample of ICA decomposition of high-density EEG recording from (Onton & Makeig, 2006), and it illustrates remarkable ability of ICA to separate out activity sources typically contained in EEG.

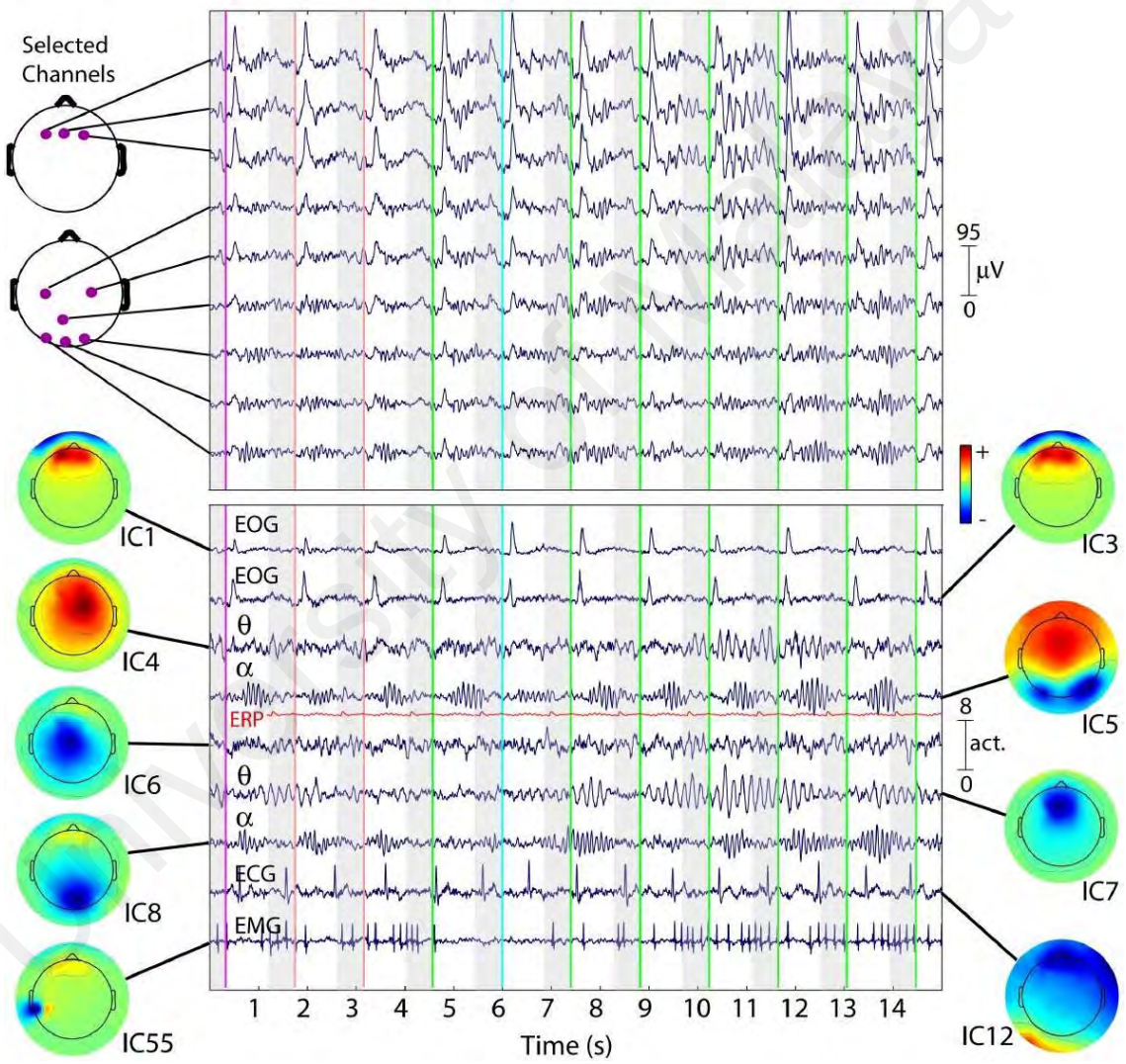


Figure 3.11: Fifteen seconds of EEG data at 9/100 channels (top panel) and ICs at 9/100 (bottom panel).

Since *Infomax* algorithm was suggested to perform better than the other two ICA algorithms, SOBI and *FastICA*, *Runica* from *EEGLAB* was used in this study (Delorme & Makeig, 2004). The *Runica infomax* algorithm can only be applied for channel's

components with a super-gaussian activity distribution. Non-stereotyped artifacts may confound and affect the ICA decompositions. Therefore, it is important to identify and discard non-stereotyped noise epochs from the data before running the ICA, as was performed in this study, by using the described inspection methods. Applying the explained methods for rejection of contaminated epochs is indispensable for fulfilling the preconditions of applying ICA on trials of each experiment. However for relatively low dimensional data set of this study *runica* algorithm recommended providing stable decompositions (assuming enough training data). *Runica* suggested giving stable decompositions for up to hundreds of channels (Delorme & Makeig, 2004; Onton & Makeig, 2006). For having N stable components (from N-channel data) typically requires more than $kN^{\wedge 2}$ data sample points (at each channel), where $N^{\wedge 2}$ is the number of weights in the W matrix that ICA is trying to learn and k is a multiplier which increases as the number of channels increases. It was recommended by (Delorme & Makeig, 2004; Onton & Makeig, 2006) to choose $k > 25$ to produce more regular and dipolar component maps. In this study a minimum period of two minutes of 19 recording channels provides 30000 data points, giving $30000/19^{\wedge 2} = 83$ pts/weight points which are enough for 19 components.

(h) IC statistics for epoch rejection

In the next step of the pre-processing analysis, probability and kurtosis as explained earlier were used to define and reject the improbable activity or epochs with unusual peaky distribution. By cleaning the ICs' epochs using probability and kurtosis criteria, the ICs were used to reconstruct the EEG signal channels. In general, it is important to give ICA as many similar and mostly clean data as possible for successful training. Therefore, using the reconstructed EEG signals, ICA decomposition was performed for the second time. The results of ICA as this stage were used to form the dipoles of brain source distributions provided by *EEGLAB* (Delorme & Makeig, 2004).

(i) Equivalent dipole source localization of independent components

It is worth mentioning that in this study, the source localization of EEG activities was only used for a better visualization of ICs and removing the noisy components. The ICs were assumed to reflect the electrical activity of independent sources which can be utilized for finding the location of a single equivalent dipole. DIPFIT2 plug-in of EEGLAB employs the calculated ICs for localizing equivalent dipole locations of independent component scalp maps by fitting an equivalent current dipole model using a nonlinear optimization technique (Scherg, 1990) in a 4-shell spherical model including sagittal, coronal, and top viewing angles. Each dipole is associated with a particular orientation that determines the pattern of its scalp distribution. It is notable that some bilaterally symmetrical ICs are modeled by two symmetrically located dipoles with different dipole orientations. Using this apt tool, single equivalent dipoles models can be found for IC maps with percentage indication of residual variance which specifies the percent difference between the scalp projection of the dipole model and the actual IC scalp map. Figure 3.12 demonstrates an example of using DIPFIT2 to find dipole scalp projection with less than 15% residual variance with IC scalp map.

(j) Removing ICA components

As it mentioned earlier, in this study ICA decomposition and source localization of EEG signals was used for pre-processing purposes to remove the stereotyped artifacts from the data. In this step of preprocessing, ICA components from each subject were investigated for eye movement, muscle activity or any other signal sources not related to brain activity. The process of determining whether an IC accounts for an artifact or a cortical activity is simple for a specialist in the field with the trained eye, but typically the main criteria to determine the source and type of an IC are the dipoles of the head model, ICs' scalp map, activity spectra, and time courses (Figure 3.13).

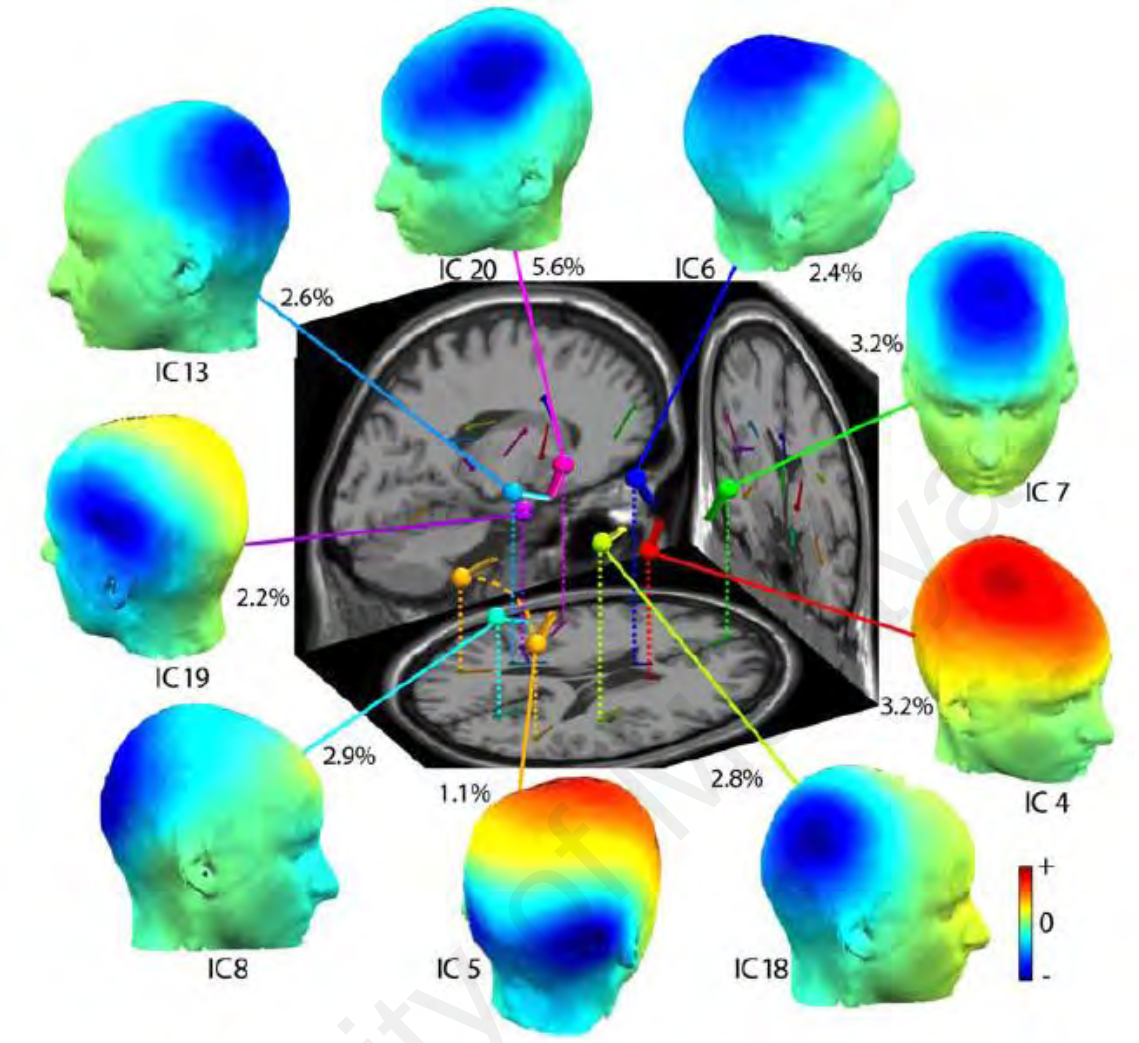


Figure 3.12: A combination of ICs scalp map and their model as single dipolar sources with a low variance between them in a conventional 3-D (Talairach) brain space.

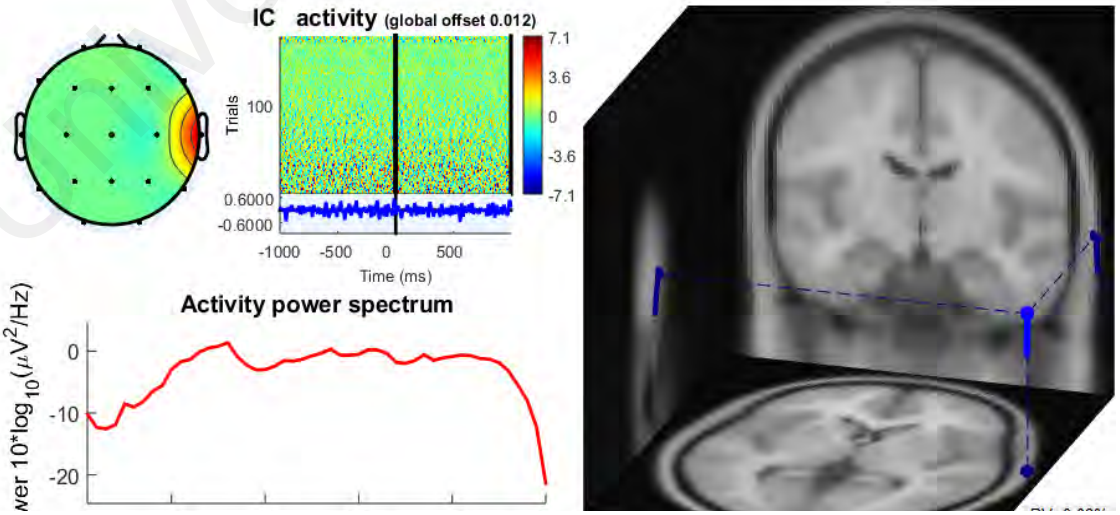


Figure 3.13: IC related to muscle activity affecting temporal channels with a high frequency and fluctuations related to its inconsistency.

For instance, a typical spectrum of eye blink activity, consisting of relatively high power at low frequencies and no spectral peaks with a smoothly decreasing spectrum is and relative projection at frontal sites. Muscle component spectra (EMG) activity typically have the highest power at frequencies above 20 Hz, while other frequencies may be present. On the other hand, from the dipole fitting mode, ICs with more than 15% residual variance overall scalp electrodes from the IC scalp map, or ICs with an equivalent dipole located well outside the model brain volume considered to be omitted from further analysis (Oostenveld & Praamstra, 2001).

3.4.1.2 Power spectral density

By identifying and removing the artefactual ICs (replacing the entire k^{th} row of the component activation matrix *U* with zeros), the remaining ICA components can use to reconstruct the EEG trials for all channels. Although, this resulted in an artifact-free scalp-EEG trails, steps (a) to (e) was performed again to reject any abnormal epochs and providing clean EEG signals for spectral analysis.

Calculation of power spectrum density (PSD) within 0.5-30 Hz frequency range was done for Delta (0.5-4 Hz), theta (4-8 Hz), alpha (8-13 Hz), and beta (13-30 Hz) bands. PSD of each trial was computed using the *Pwelch* function of *MATLAB* with 0.5 Hz resolution without overlapping. *Pwelch* function applied four orthogonal tapers of 500 data points (2 seconds) for each trial using the *Hanning* window (Percival & Walden, 1993). The estimation of the PSD values of each trial consisted of 59 values for 0.5-30 Hz with the resolution of 0.5 Hz. The PSD modality was evaluated for each group based on 2×SD of PSD (as the selection boundary), and the mean and standard deviation were recalculated after eliminating the outliers; then data were scaled to reduce the skewness. Finally, the absolute values, mean value, and the relative PSD were stored.

3.4.2 Single-trial ERP Detection

In this study, EEG recordings during the performance of ERP paradigms were separated from resting-time EEG-recording to extract the ERP features including amplitude index and latency ratio. EEG recording is highly susceptible to various forms and sources of noise, which present significant difficulties and challenges in the analysis and interpretation of the EEG data. The predominant method for reducing the complexity of ERP collected in sensory and cognitive paradigms is to form ERP averages of the trial to generate the same or essentially similar brain responses {Buckner, 1996 #264}. Meantime, a novel multi-dimensional approach was used in this study for single-trial detection of ERP components using wavelet transform technique. In addition, the efficiency of utilized single-trial method was evaluated by comparing to the traditional averaging technique.

3.4.2.1 Pre-processing for ERP recordings

Epoch rejection procedure (explained in section 3.4.1.1) cannot be used for cleaning the ERP recording due to the importance of recording time relative to task performance. Therefore, different steps of signal processing techniques were applied for pre-processing the ERP recordings.

A uniform semi-automated pre-processing method based on the artifact removal (instead of rejection) concept was adopted in this study using *EEGLAB* toolbox (Delorme & Makeig, 2004) in *MATLAB*. This was necessary due to the significance of timing relative to the paradigm and recorded signals. Every channel signal was band-pass filtered between 0.1 and 45 Hz utilizing *Slepian* multitaper spectrum (*MATLAB* "pmtm" function) by implementing four orthogonal tapers, (a combination of high pass and low pass filters), to eliminate power lines, high-frequency noises, and DC biases. To use ICA for artifact removal, recordings of all 19 channels were used to apply *Runica infomax* from *EEGLAB*.

The certain artifactual ICs (i.e. eye movements, blinking, muscle movements, and external noises) were identified -as explained earlier- and removed from the recordings, and the ICs that remained were utilized for reconstruction of the channel signals. After the artifact removal, the constructed EEG of four active channels (Cz, Fz, Pz, Fcz) was used for extracting the ERP features based on the guidelines of recording ERPs in clinical research (Duncan *et al.*, 2009).

In the next step, one second (1000 ms) of EEG signal from the onset of each stimulus was extracted to be called as a "trial". Four types of trials were identified for each subject as N2, P3, and P6 (for all the P6 trials a separate trial was stored as the P3 as well) which labeled as "Target trial" and trials extracted by the onset of neutral stimuli were labeled as "Non-target trial".

3.4.2.2 Single-trial ERP detection

Due to the importance of getting a reliable value representing the amplitude and latency of ERP components, and in addition to the lack of Fourier Transform in analyzing the time transient signals, a newly established method for ERP detection was used in this study. A fusion of continuous and discrete WT was used for feature extraction from trials. WT can be seen as an extension of Fourier transform (Figure 3.14), which provides a time-frequency representation of each trial and is defined as the convolution between the signal $f(t)$ and the wavelet functions $\psi(t)$ which can be scaled and shifted (a,b) (Equation 3.4, 3.5). Detailed information on time-frequency localization and signal analysis using WT are explained in (Daubechies, 1990). Wavelet analysis is capable of revealing aspects of data that other signal analysis techniques miss, aspects like trends, breakdown points, discontinuities in higher derivatives, and self-similarity. Further, because it affords a different view of data than those presented by traditional techniques, wavelet analysis can often compress or de-noise a signal without appreciable degradation.

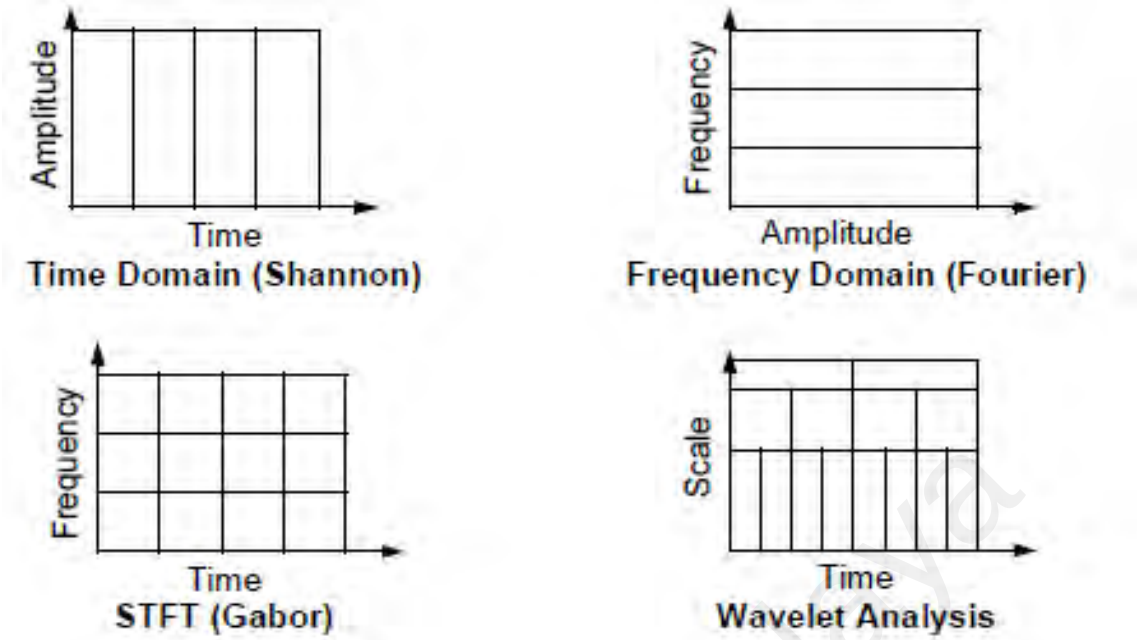


Figure 3.14: A comparison between the dimensionality of four main domains in signal processing. Wavelet allows the use of long time intervals for more precise low-frequency information and shorter regions for high-frequency information.

$$C(\text{scale,position}) = \int_{-\infty}^{\infty} f(t)\psi(\text{scale}, \text{position}, t)dt \quad (3.4)$$

$$\psi_{a,b} = |a|^{-\frac{1}{2}} \psi\left(\frac{t-b}{a}\right) \quad (3.5)$$

Wavelet Transform (WT) can be seen as an extension on Fourier Transform which components with specified shape replace sinusoid components. On the other hand, brain activity signals have specific shape with a defined frequency specification. Thanks to WT properties, it was suggested as an optimal convolution for non-stationary signals like EEG (Bostanov & Kotchoubey, 2006; Motlagh *et al.*, 2013; Quiroga & Garcia, 2003; Xu *et al.*, 2004). A wavelet is a waveform of effectively limited duration that has an average value of zero with a particular shape and having activity in the low-frequency range. There are various types of wavelets with different characteristics which are more or less appropriate to be compared with the signal depends on the desired application. Indeed, the possibility of selecting these wavelets (mother-waves) for comparison with the signal is one of the major advantages of wavelets.

Wavelet analysis is the breaking up of a signal into shifted and scaled versions of the original (mother) wavelet. Wavelet transform can be in forms of discrete or continuous (DWT and CWT). CWT consists of two main procedures, scaling and shifting. Scaling a wavelet in a simple language means stretching (or compressing) it and shifting means moving the wavelet through the length of the main signal in time domain. Extracted features from WT are conversation coefficients indicate the similarity of the signal with components corresponding to its coefficients.

Applying CWT includes five consecutive steps (Lilly & Olhede, 2012):

i. In the first phase, a wavelet (mother-wave) should be chosen to be compared with the particular section of the original signal.
ii. In the comparison of wavelet and the special part of the signal, their correlation value (a number between 0-1) is obtained as the correlation coefficient (C).
iii. The chosen wavelet should be shifted along the length of the original signal and first and second steps repeat until the whole signal covered.
iv. By increasing the scale (stretch) of the wavelet, transform procedure repeats from step 1 to 3.
v. In the last step, all previous steps should be repeated for all scales.

Small scales of wavelet can be compressed into properties which are following rapid changing details in high frequency. Logically, for large scales stretched wavelets can be matched by slowly changing coarse features of low frequency. Contracted scales of the wavelet are matched with the high-frequency components of the original signal, and the dilated scales of the mother-wave are matched with the low-frequency oscillations. Therefore, by analyzing the original signal with the wavelet from different scales, all the details can be obtained at different scales.

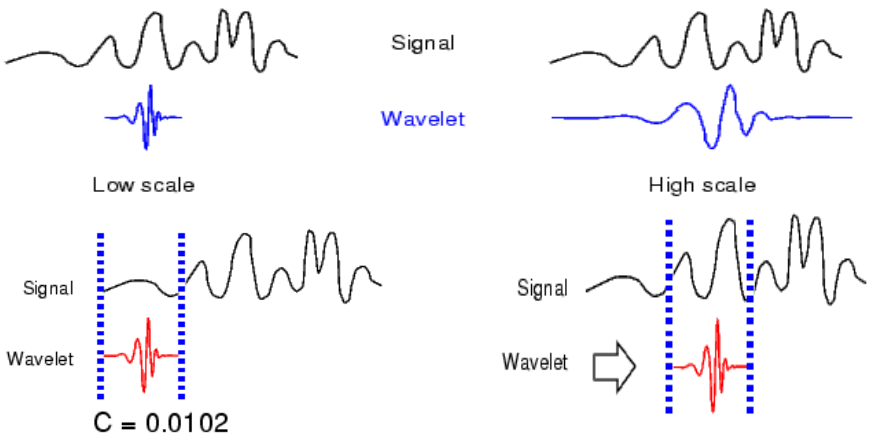


Figure 3.15: Representing the steps of CWT, shifting, scale, and wavelet coefficient.

On the other hand, DWT divides the signal into "Approximations" (A) and "Details" (D). The filtering process, at its most basic level displays in Figure 3.16 the original signal "S" passes through two paired filters and emerges as two signals. In the next step of the multiresolution decomposition, approximation signal (A) will be filtered into detail and approximation. By performing this operation on a real digital signal, the number of samples for each A and D is equal to S. Therefore, a down-sampling procedure should be applied to A and D by keeping only one point out of two in each of the two signals samples for getting the complete information, while an up-sampling happens when A and D are used to reconstruct S. Figure 3.17 depicts the schematic of DWT recursive implementation of the multiresolution decomposition and reconstruction. H and G are the low and high-pass filters, respectively, H′ and G′ are the Inverse filters used for the reconstruction. Downward arrows mean decimation by 2 and upward arrows mean up-sampling.

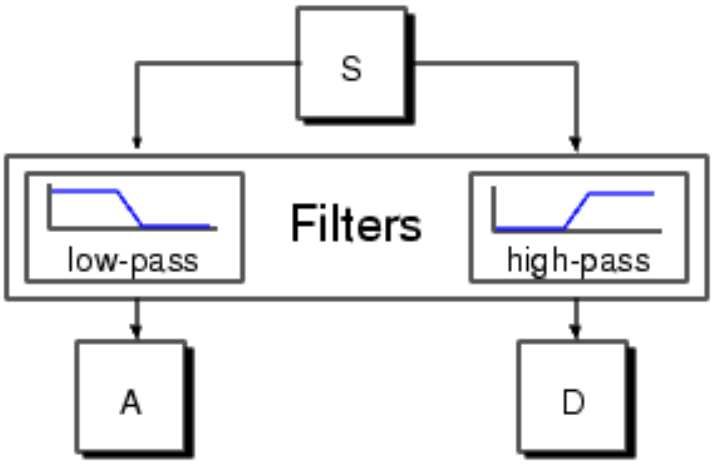


Figure 3.16: Decomposition of the signal into approximation and detail signals.

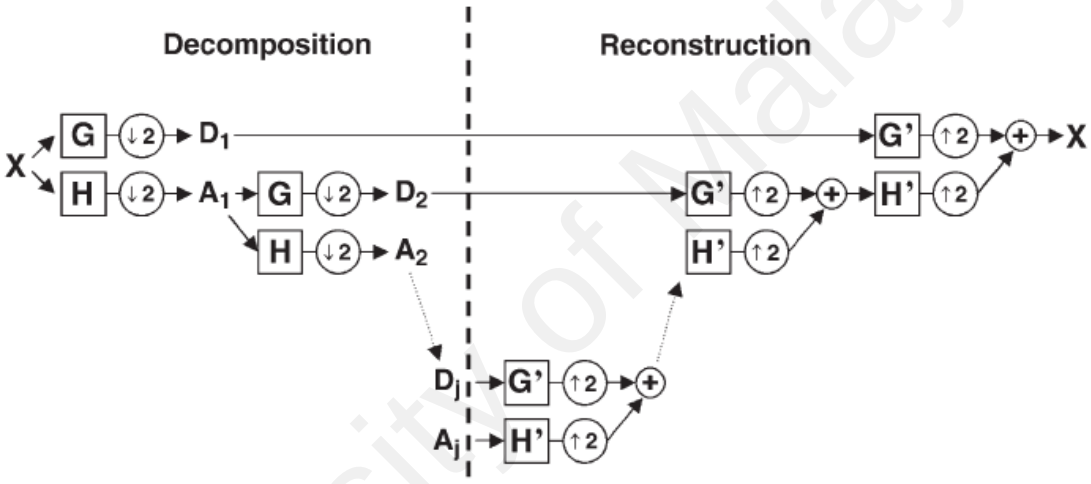


Figure 3.17: Schematic of DWT for decomposition and reconstruction of signal X as well as showing the down and up samplings.

(a) ***Discrete Wavelet Transform (DWT)***

In the first phase of single-trial detection of ERP components, the artifact-free EEG signals from four (Cz, Fcz, Fz, Pz) channels – outputs of preprocessing step explained in section 3.6.2.1 – were resampled from 240 Hz into 256 Hz to be suitable for DWT filters. DWT was used for decomposition of a signal into 'details' and 'approximation' (high frequency and low frequency) components. It is believed that *Quadratic B-Splines* functions have high similarity with ERP waveforms and have optimal time-frequency resolution (Quiroga & Garcia, 2003). Therefore, these functions were selected as the mother-wave for DWT in this study. The first level of DWT decomposition transformed the trials into approximation (A) and detail (D) components; subsequently, another four

levels of DWT decomposition of each level approximations provided six decompositions of D1-5 and A5 (Figure 3.18). DWT time-frequency decompositions were relative to 64–128 Hz (D1), 32–64 Hz (D2), 16–32 Hz (D3), 8–16 Hz (D4), 4–8 Hz (D5) and 0.1–4 Hz (A5), as depicted in Figure 3.18. Thanks to low-frequency characteristics of evoked potentials, A5 and D5 decomposition (representative of delta and theta band frequencies) were used for reconstructing the trials.

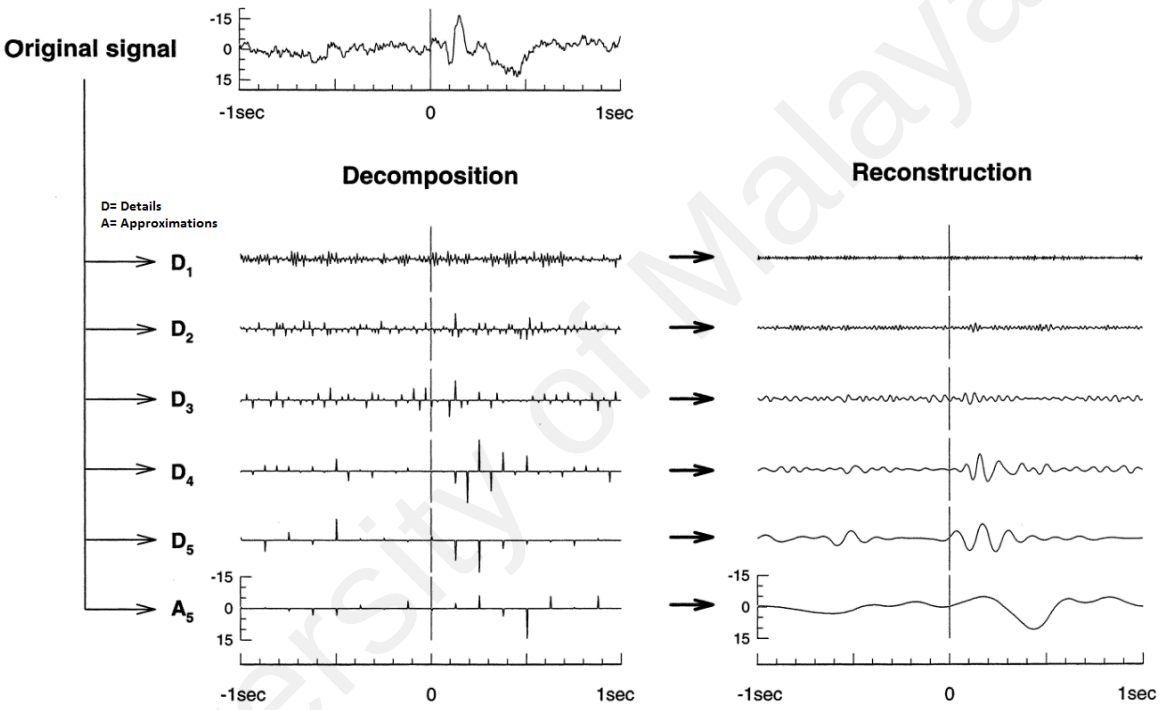


Figure 3.18: EEG waveforms decompose into five-octaves by using the discrete wavelet transform. Six sets of coefficients (including residual scale) within the following frequency bands obtain; 0–4 Hz, 4–8 Hz, 8–16 Hz, 16–32 Hz, 32–62 Hz and 62–128 Hz.

In the next step, CWT was applied on the reconstructed trials (A5+D5 levels) to extract the ERP components' features based on the correlation of trials and wavelets, which are similar to ERP components. Selecting similar wavelet functions to ERP components as a valuable advantage of WT analysis provides a variety of functions to be chosen from. In this study, *Symlet-5* as a modified version of *Daubechies* wavelets with increased symmetry was used for N2 trials analysis and *Mexican-hat* (Figure 3.19) was used for analysis of P3 and P600 trials.

Moreover, for increasing the efficiency of CWT calculation, based on the pseudo frequency of wavelet functions' scales and center frequency of ERP components, scales of 80-100 for *Symlet-5* and 30-100 for *Mexican-hat* were selected. Different scales of wavelet swept each trial, and their correlation (wavelet function and signal) was calculated for each time-scale as similarity coefficient.

The considering wavelet used is the Mexican-hat has defined as:

$$\varphi(s,p,t) = \frac{2}{\sqrt{3}}\pi^{-\frac{1}{4}}(1-\alpha^2)e^{-\frac{1}{2}\alpha^2} \tag{3.6}$$

$$\text{where } \alpha = \frac{t-p}{s}$$

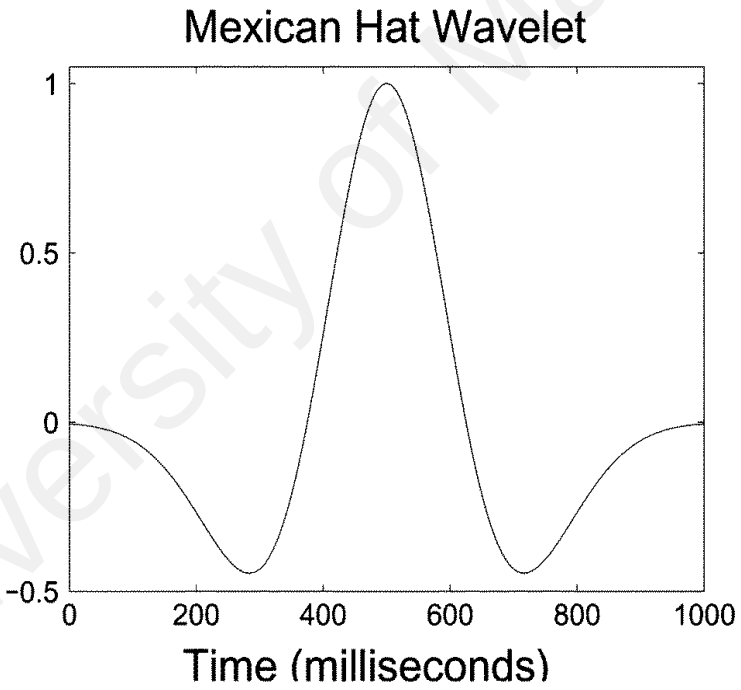


Figure 3.19: Mexican-hat wavelet.

Wavelet coefficients of a signal *x(t)* at time point p are defined as:

$$\int_0^t x(t)\varphi(s,p,t)dt \tag{3.7}$$

Where *s* is the wavelet scale, *t* is trial data-points, and φ is the wavelet function. Calculated CWT coefficients (Figure 3.20) averaged over different scales resulted in a function of *s(t)* which projects data points in the time domain to averaged correlation

values (Figure 3.21). Averaged CWT coefficients (*s(t)*) have local extremums with maximums related to the time points with the highest similarity between signal *x(t)* and the wavelet function. Based on the label of each trial, a local maximum was selected based on the time-locked property of ERPs: 100-300 ms for N2 trials, 300-700 ms for P3 trials, and 600-800 ms for P600 trials. It was assumed that the maximum of *s(t)* in a predefined period would have the amplitude of A_0 that happens at time T_0. The goal was to find two local minimums, one just after A_0 and another just before A_0 with amplitudes of B1 and B2 respectively. Summation of value differences of A_0 with B1 and B2 (Equation 8) provides value (A) relative to the amplitude of the ERP. T_0 is the latency of the ERP, and it is assumed to be near 100 ms for N2, and 300 ms for P3, and 600 for P600 components. Therefore, in Equation 9 the ideal latency of each ERP (shown as ERP latency) was subtracted from the real latency of ERP, then it was scaled by the perfect latency to provide a similar latency ratio for each trial. The process of computing A and T is presented in Figure 3.22 for a P3 target trial collected from the Cz channel.

$$A = (A_0 - B_1) + (A_0 - B_2) \tag{3.8}$$

$$T = \frac{|T_0 - ERP.Latency|}{ERP.Latency} \tag{3.9}$$

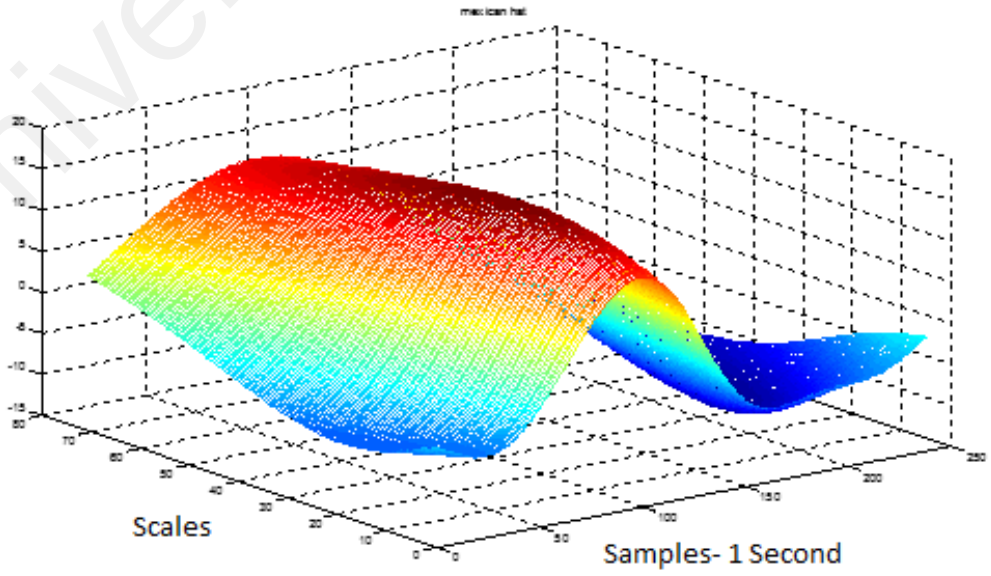


Figure 3.20: CWT coefficients for one second of ongoing Target signal from Cz channel using Mexican-hat for scales of 30-100.

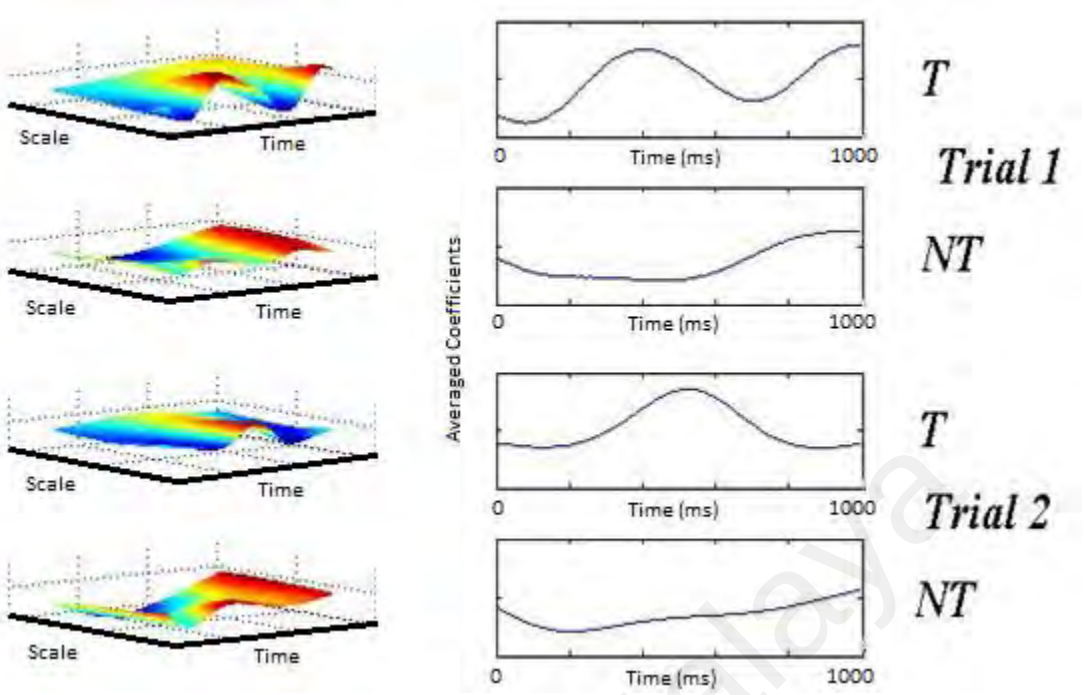


Figure 3.21: Averaged CWT coefficients for a sample of target and non-target trials.

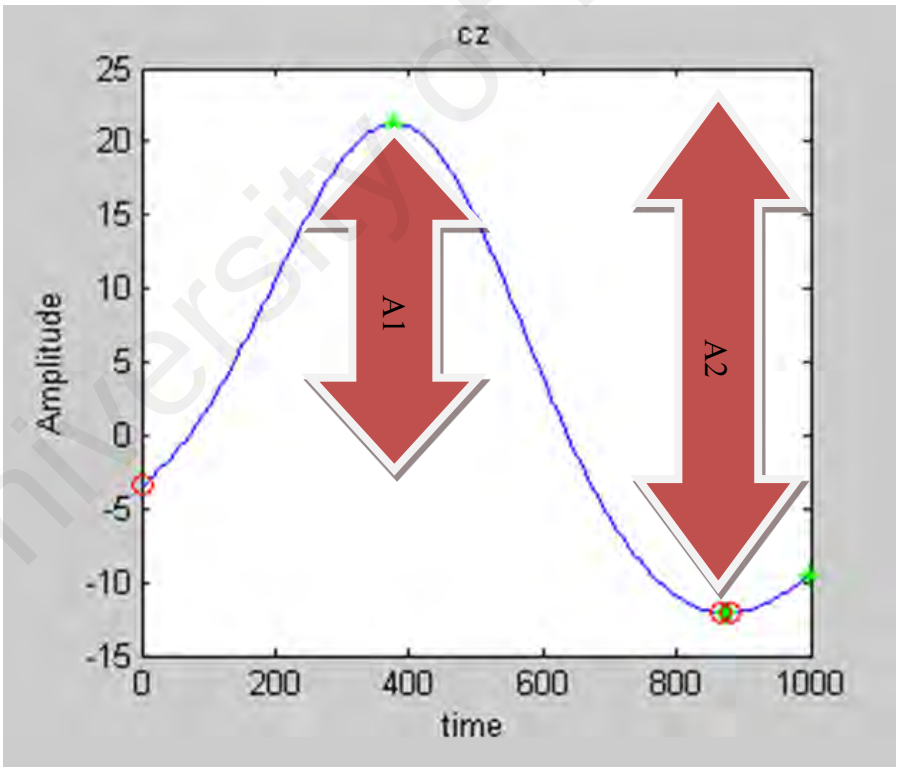


Figure 3.22: Amplitude and latency of P300 based on averaged coefficients.

(b) V***alidation***

In this section, we aim to validate the accuracy of feature extraction algorithm in the single-trial evaluation of an ERP. Classification of Target and Non-target signals based on supervised learning algorithms considers different validation stages (Foster *et al.*, 2014). Support vector machines (SVM) were employed as a well-known supervised classifier (Fletcher, 2009). SVM is a binary linear classifier, which is trained by a set of training data related to two groups and it decide for the test data which groups it is associated with. SVM classifier has been widely used as an optimized classifier in various machine learning applications (Foster *et al.*, 2014).

Due to the existence of N2 in neutral stimuli (Non-target trials), the classification was applied to P3 Target trials and Non-target trials. Each category of P3 Targets (50×50 trials) and Non-targets (50×800 trials) was randomly divided into 60% training (predefined label for each trial as Target or Non-target), and 40% test datasets. Based on the relative value of A and T to P3 amplitude and latency, it is assumed that for detecting Target trials, the amplitude feature (A) should have a "large" value and the time difference feature (T) should be as "small" as possible (zero is considered ideal). For each trial, these eight (A and T of four channels) features were extracted from all four channels using the described feature extraction techniques. The class of each trial was defined by 1 for Target and 0 for Non-target trials $Y \in [1,0]$.

Using *MATLAB* built-in SVM as a large margin classifier defines a decision boundary with different choices of kernel functions. In this study, two different types of kernels (linear, Gaussian) were used to train the SVM. Values of “T” and “A” of four channels were used as eight identified features of each trial. It is noteworthy that the nature of classes in this study was skewed due to a large number of Non-target trials. Therefore, for a better numerical evaluation of classifier accuracy, F-score was calculated based on

precision and recall values of the SVM classifier (Equation 3.10). Precision was calculated based on the number of true-positive trials divided by all positive predicted trials (true-positive and false-positive), and recall value was equal to the number of true-positive trials divided by the number of actual positive trials (true-positive and false-negative).

$$F - score = \frac{2 \times Precision \times Recall}{Precision + Recall} \quad (3.10)$$

3.4.2.3 ERP Averaging

Averaging of trials to define the shape of ERP components is a typical approach for visualizing the ERP waveform in psychiatric studies. With this method, all trials of the same category (standard, deviant, and novel) are averaged together in a time-space domain. In the next step, the resulting waveforms of standard stimuli were subtracted from the deviant and novel stimuli waveforms. The maximum value of this subtraction is considered as the amplitude of the ERP component. Subsequently, the projection of this maximum value in time domain was indicated as the ERP latency. To compare the variability of extracted ERP features extracted from averaged and single-trial methods, the differences between the methods were plotted versus their mean value. This method of comparison was suggested by J. Martin Bland and Douglas G. Altman (Altman & Bland, 1983), which allows the investigation of any possible relationship between the measurement error and the actual value.

3.5 Statistical Analysis

This longitudinal study includes four main phases of statistical analysis. The Initial group comparisons for demographic and clinical characteristics were performed using one-way ANOVA. *Lilliefors* test (two-sided goodness-of-fit test) was used to check the normality assumption of the demographic features and electrophysiological properties of groups. EEG measurements were used as the primary outcomes to define the

electrophysiological differences between addicts and healthy subjects. Relative and mean PSD values of each frequency band were used to assess the EEG spectral changes. In order to reduce multiple testing and evaluating the statistical differences of PSD among the controls and addicts, as a common approach (Brickman *et al.*, 2005; Gatt *et al.*, 2008; Liddell *et al.*, 2007; Wang *et al.*, 2015; Whitford *et al.*, 2007), averaged PSD of channels over certain brain regions [frontal ($Fp_{1/2}$, $F_{3/4}$, $F_{7/8}$, and Fz), central ($C_{3/4}$, and Cz), temporal ($T_{3/4}$, and $T_{5/6}$), and occipital-parietal ($P_{3/4}$, Pz, $O_{1/2}$)] were used for further analysis.

Group comparisons for spectral values and ERP features were performed using repeated measure ANOVA while the group of subjects was chosen as a between subject factor. For fitting the data into a repeated measure model, time intervals, frequency band (delta/theta/alpha/beta) and the brain region (frontal/central/temporal/occipital-parietal) were within-subjects factors. Furthermore, to explore the differences in cognitive response features, each ERP component data were fitted into a separated repeated measure ANOVA model to evaluate if the model terms were significant in their effect on the response by measuring how they contribute to the overall covariance. The multivariate responses for each subject were a vector of repeated measures while the task and the type of the features were used as within-subjects factors.

For accurate *p*-value calculations in the repeated measure ANOVA models, the *Mauchly's* test was used for sphericity assessment, and if the compound symmetry assumption was not satisfied, the *p*-value was adjusted using three different correction methods (Greenhouse-Geisser/Huynh-Feldt/Lower bound adjustment). Based on the results of repeated measure mix models, significant main effects were followed up with post hoc pairwise analysis of variances using the *Tukey-Kramer* correction method. The default significant level was chosen for p-value<0.5.

It is worth mentioning that repeated measure ANOVA model was used to answer the question of whether the mean changes of the outcome from one measurement interval to the other one is differed in two groups. Therefore ANOVA compares the groups on differences between post-test and pre-test. However, if the ANOVA model results show any significant differences in pre-measurement properties, data were analyzed using an ANCOVA model (pre- measurements as covariates) as well. The ANCOVA with the condition as the independent variable and the pre-measure as the covariate and post measure as the dependent variable answers the question of whether the post-test means, adjusted for pre-test scores, differ between the two groups. The adjustment for the pre-test score in ANCOVA has two benefits. One is to make sure that any post-test differences truly result from the treatment, and aren't some left-over effect of (usually random) pre-test differences between the groups. The other is to account for variation around the post-test means that comes from the variation in where the patients started at pre-test. Furthermore, Even though subjects are assigned to treatment at random, there may be some concern that any difference in the post-test measurements might be due a failure in the randomization. Perhaps the groups differed in their pre-test measurements.

The analysis of covariance is a combination of an ANOVA and a regression analysis. In basic terms, the ANCOVA examines the influence of an independent variable on a dependent variable while removing the effect of the covariate factor. ANCOVA first conducts a regression of the independent variable (i.e., the covariate) on the dependent variable. The residuals (the unexplained variance in the regression model) are then subject to an ANOVA. Thus the ANCOVA tests whether the independent variable still influences the dependent variable after the effect of the covariate(s) has been removed.

Furthermore, *Pearson's r* and *Spearman's rho* correlations between the EEG and ERPs is calculated. It is notwithstanding that due to the mismatch between the statistical test

outcomes, and interpretation of data distributions suggested by (Rousselet *et al.*, 2016), scatter plots and strip-charts of repeated measurements as well as quantities were used for interpretation of results in this study.

CHAPTER 4: RESULTS AND DISCUSSION

4.1 Introduction

This chapter presents the results and related discussions of this study. The results (power spectrum of resting-state EEG activity and ERP components features of chronic heroin addicts through 3 months of MMT in the form of two groups of with and without EA as adjunctive therapy) are presented in separate sections, and a concise summary of findings and its related discussion is provided by the end of this chapter. In addition, the results of signal-processing steps are provided for a better elaboration of enhanced techniques used in this study.

4.2 EEG Paradigm

In this study, an integration of paradigms was introduced to probe MMN, P300, and P600 components during standard and cue-reactivity conditions. This paradigm was designed based on a modification of well-known paradigms in electro-neurophysiological studies of the addiction field and follows the methodological recommendation of ERP eliciting and recording in clinical trials (Duncan *et al.*, 2009). The paradigm consists of various tasks related to salient brain electrophysiological features of addiction studies. Closed-eye qEEG and ERPs' (MMN, P3, and P600) associated with brain discrimination abilities, the orientation of attention, response resolution, and working memory were assessed among chronic heroin addicts and healthy control subjects.

This study showed that using this paradigm can provide comprehensive information regarding the electrophysiological features in this field. This comprehensive investigation of cognitive traits associated with striking features of addiction could effectively contribute to improving the understanding of neurological features of addiction.

4.3 Resting-time EEG Pre-processing

EEG signals were recorded from 19 electrodes - ($Fp_{1/2}$, $F_{7/8}$, Fz, $F_{3/4}$, $T_{3/4}$, $C_{3/4}$, Cz, $T_{5/6}$, $P_{3/4}$, Pz, $O_{1/2}$) according to the 10-20 standard of electrode placement and the impedance of all the electrodes was under 5 kΩ. Since the raw EEG recording is highly susceptible to various forms and sources of noise, which present significant difficulties and challenges in the analysis and interpretation of the EEG data (Figure 4.1). Therefore a semi-automated algorithm (as explained in 3.4.1.1) was applied for preprocessing to define and eliminated the noisy parts of the signal.

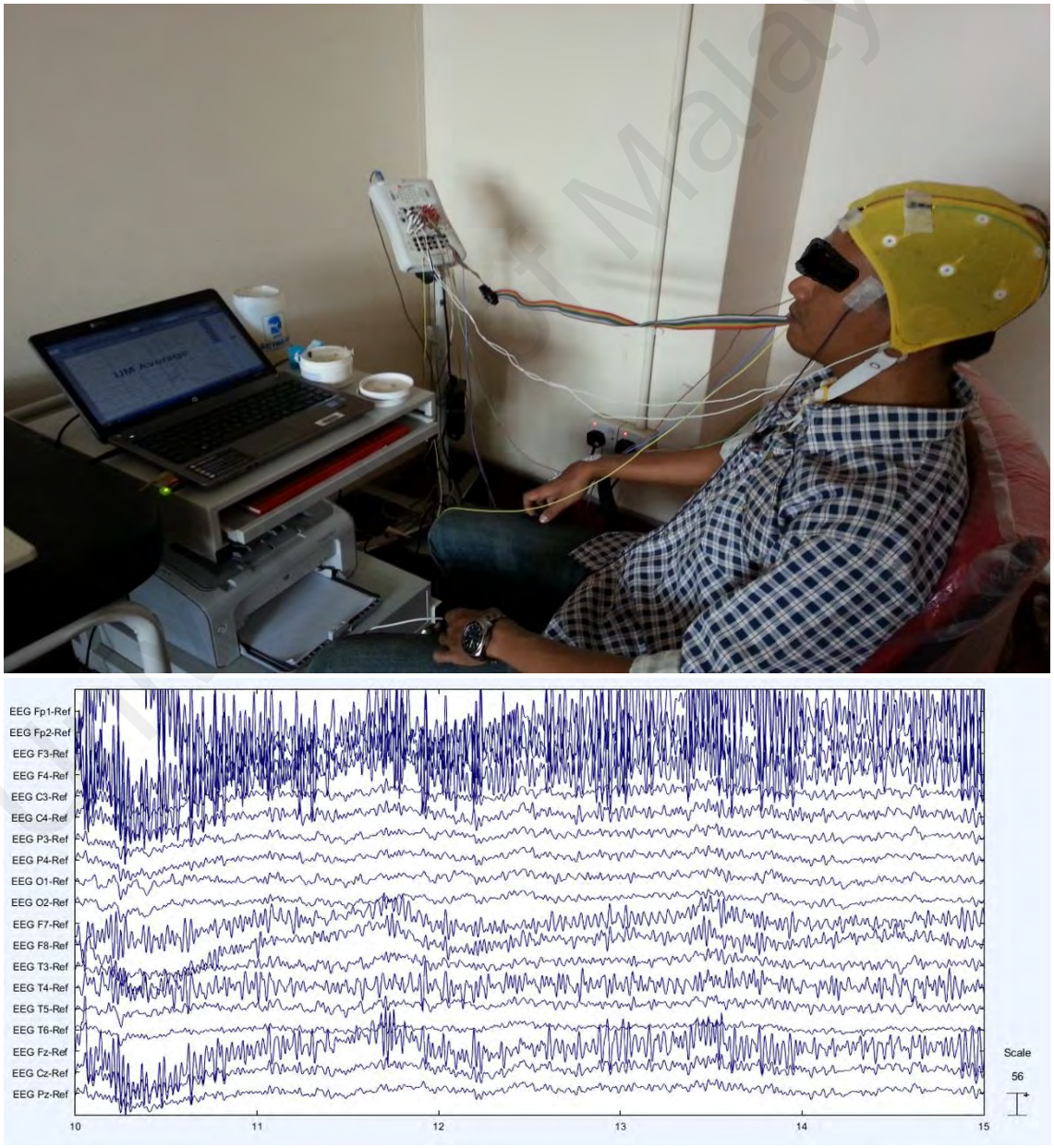


Figure 4.1: A sample of raw EEG recorded during the resting-time.

A uniform procedure was applied to the raw signal including the epoch segmentation, noise removal and epoch rejection to provide an artifact-free resting state EEG. This approach as elaborated in Chapter 3 was performed using the *EEGLAB* toolbox (Delorme & Makeig, 2004) in *MATLAB*. The 2-seconds epochs extracted from the filtered signals went through explained algorithms and subsequently labeled as clean or contaminated EEG epochs. Extreme values, abnormal linear trends, improbable data, abnormal distributed data, and abnormal spectral patterns as the main epoch rejection criteria respectively helps to reject the contaminated EEG epochs with high amplitudes (due to gross eye blinks and eye movements), linear drifts (caused by current drifts and electrode movements), sudden movements or improbable noises, unusual peaky distribution of values (due to strong transient muscle activity and flat distribution caused by AC/DC artifacts), and epochs with abnormal and improbable spectral activities (due to any physiologic and extra-physiologic artifacts). Figure 4.2 shows the EEG recording after epoch rejection based on five criteria (extreme values, abnormal linear trends, improbable data, abnormally distributed data, and abnormal spectral patterns).

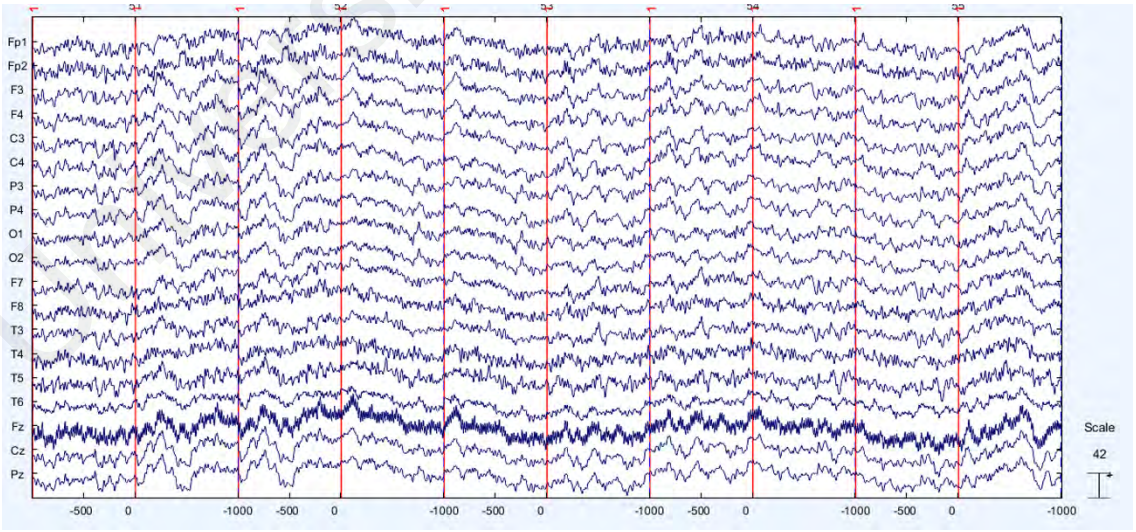


Figure 4.2: EEG recording of resting time after rejecting the noisy epochs.

After elimination of noisy epochs, distribution of EEG signals of each channel was assessed using *kolmogrov-Smirnov* test with the significance level of $p \leq 0.05$ to indicate whether the data distribution of the signal was Gaussian or not. It depicts the quantiles of

the data versus the quantiles of standard normal (Gaussian) distribution using the QQ-plot (Figure 4.3). The result of this step was used for validation of artifact rejection as a pre-condition evaluation to apply ICA for artifact removal in the next steps.

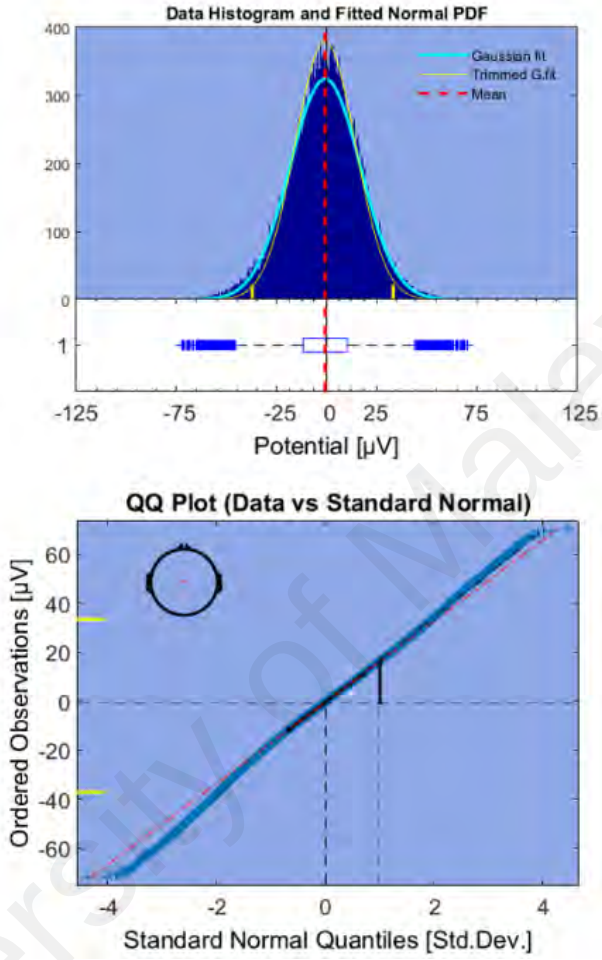


Figure 4.3: The quantiles of the data versus the quantiles of standard normal (Gaussian) distribution using the QQ-plot.

The epoch rejection steps can only partially remove some types of artifacts, therefore, as an alternative approach, the EEG data are separated into active cortical and artifact sources based on the physiology and statistically acceptable assumption of the ICA. The main two assumptions of ICA about linearly summed components and independence of component is fulfilled by considering that in EEG recording both active and passive electrodes are equally receptive to cortical and artifact sources while they are related to brain activity and muscle contractions. The third assumption concerns the non-

gaussianity of the source activity distributions, which is quite plausible for artifacts, which are usually sparsely active and thus far from Gaussian in value distribution.

Non-stereotyped artifacts may confound and affect the ICA decompositions (Figure 4.4). Albeit the rejection of noisy epochs in the first step, it is important to identify and discard non-stereotyped noise epochs from the data before removing the noise-related ICs. Therefore, before discarding any ICs, probability, and kurtosis were used to define and reject the improbable activity or epochs with unusual peaky distribution using ICs epochs.

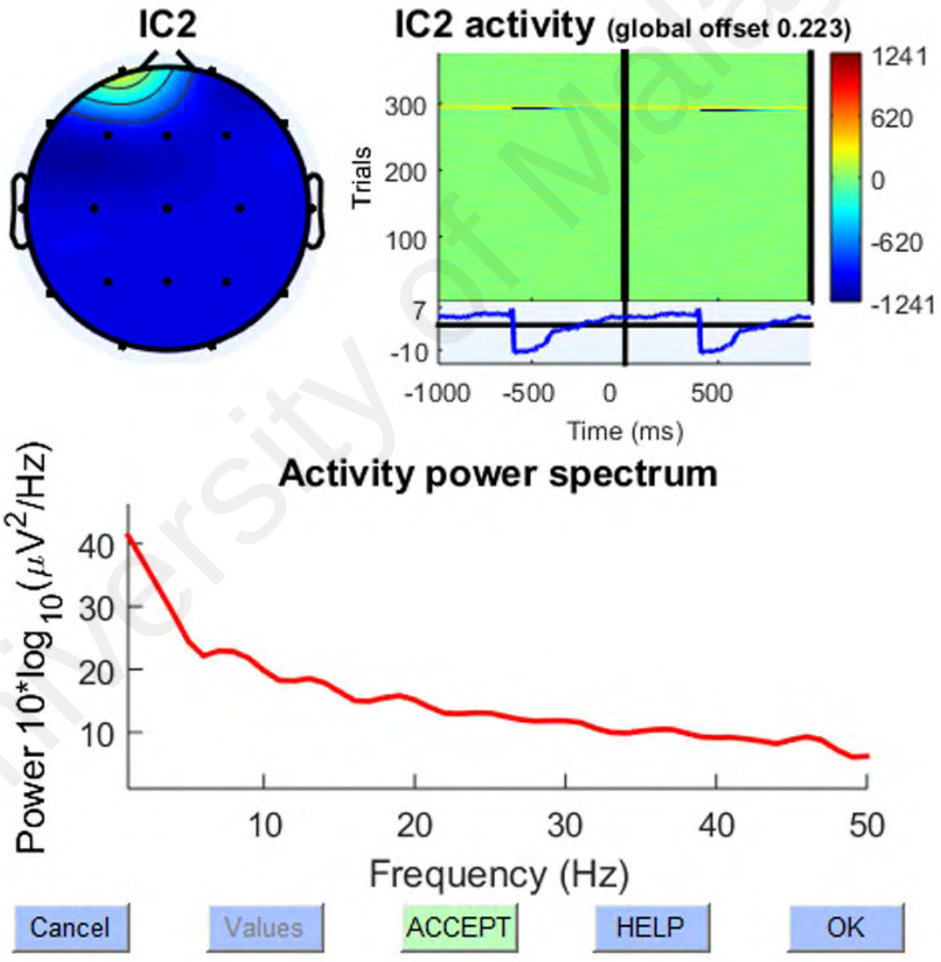


Figure 4.4: Sample of a non-stereotyped noise which can be visualized with ICA decomposition and related epoch can be marked for rejection.

By cleaning the ICs' epochs using probability and kurtosis criteria, the ICs were used to reconstruct the EEG signal channels. This step was utilized due to increasing the ICA decomposition by providing clean data. Therefore, the second ICA supposed to perform

accurately and the results were used to form the dipoles of brain source distributions. In this study, source localization of EEG activities was only used for a better visualization of ICs and selecting the artifact-related ICs. Figure 4.5 represents the equivalent dipoles of ICs for the sample IC at Figure 4.4 in a 4-shell spherical model including sagittal, coronal, and top viewing angles.

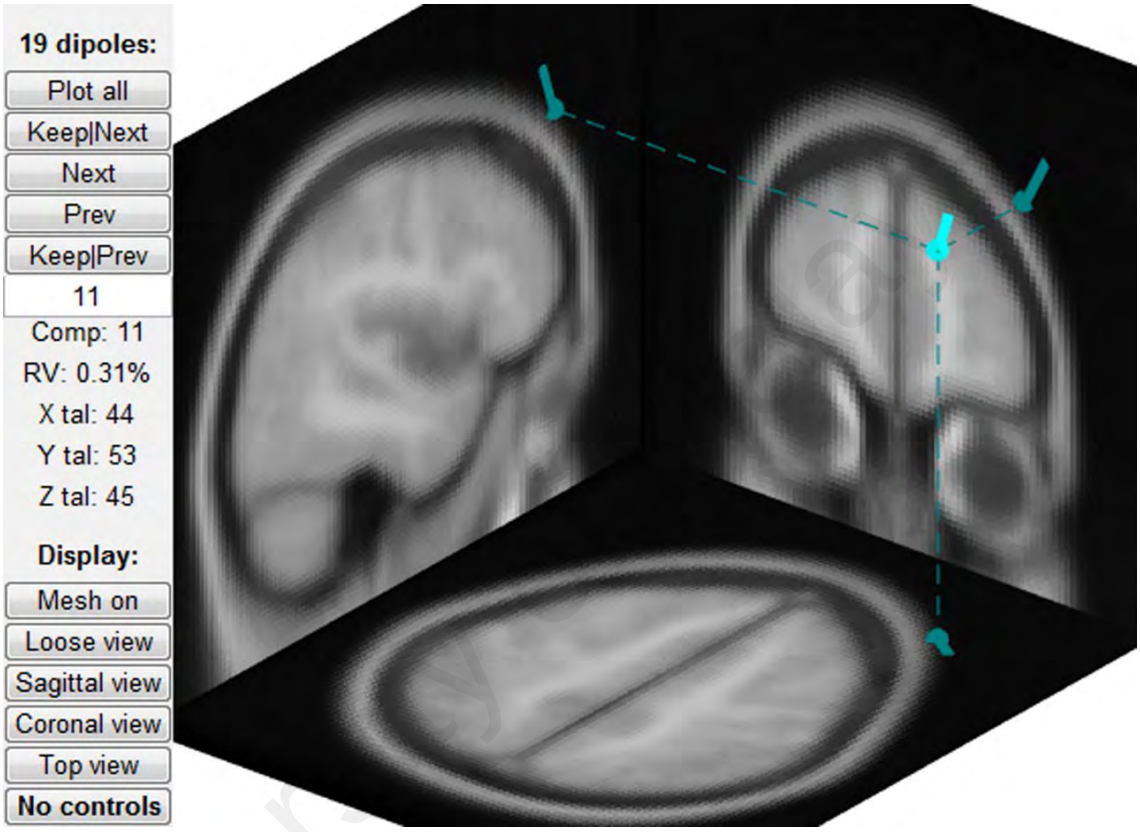


Figure 4.5: The equivalent dipoles of a noisy IC in a 4-shell spherical model including sagittal, coronal, and top viewing angles.

ICA components related to artifacts were determined based on the dipoles locations, ICs' scalp map, activity spectra, and time courses. The ICs related to artifacts including muscle activity (with a high frequency and fluctuations related to its inconsistency), eye activity (consisting of relatively large power at low frequencies and no spectral peaks with a smoothly decreasing spectrum is and relative projection at frontal sites), ICs with an equivalent dipole located well outside the model brain was marked and removed (Figure 4.6 and Figure 4.7 are two samples of noisy ICs). By identifying and removing the artifactual ICs, the remaining ICA components were used to reconstruct an artifact-free EEG trial for all channels.

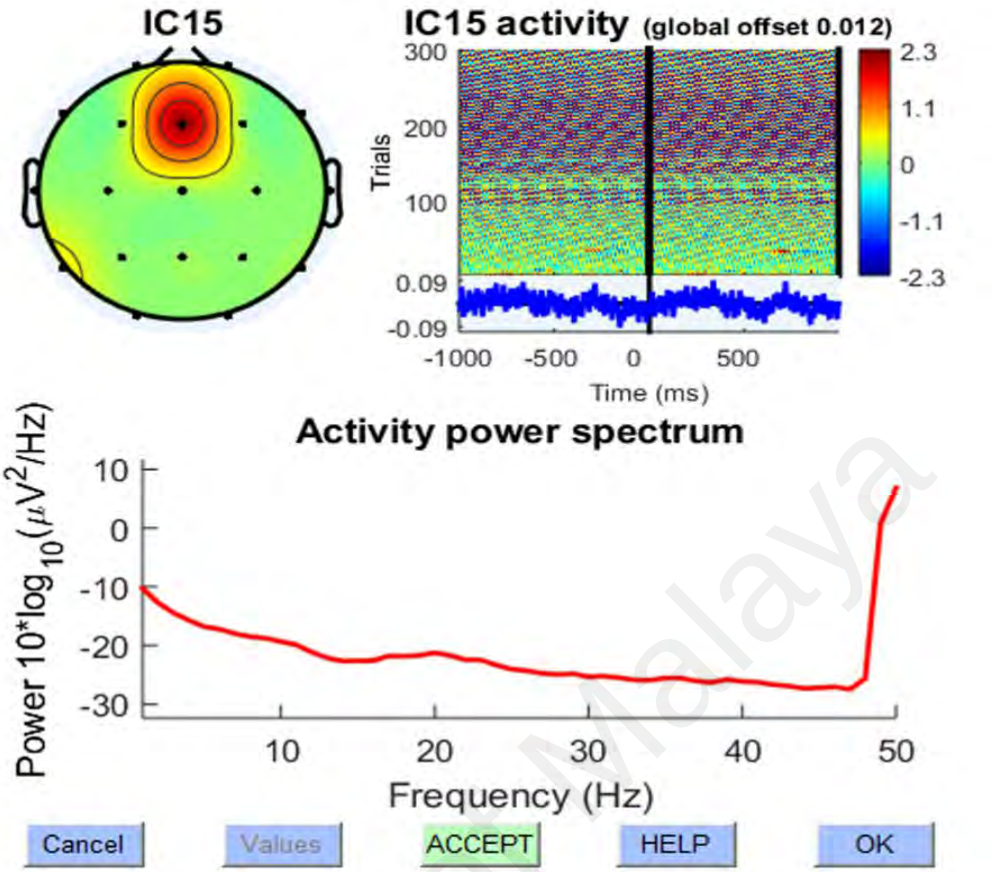


Figure 4.6: A sample of a noisy IC with high-frequency noise at frontal channel.

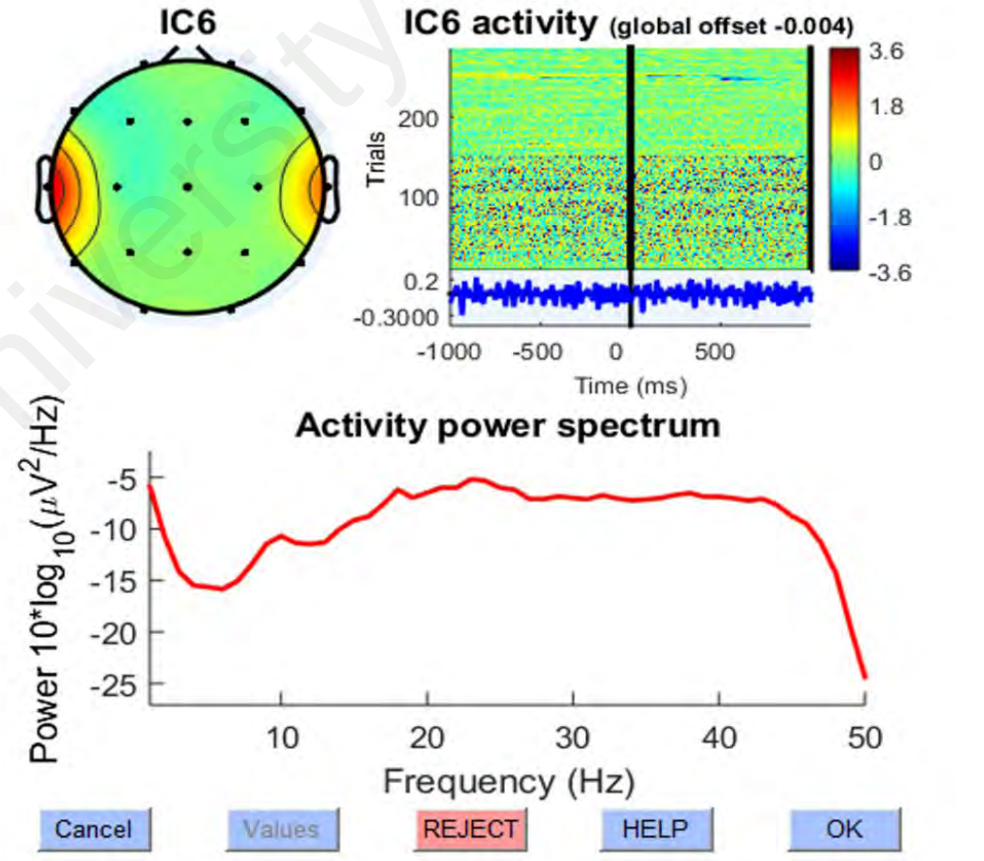


Figure 4.7: A sample of a noisy IC showing the muscle activity at temporal channels.

4.4 Single-trial Detection of ERP Features

In this study, a combination of continuous and discrete WT was used for feature extraction from trials. DWT time-frequency decompositions were relative to 64–128 Hz (D1), 32–64 Hz (D2), 16–32 Hz (D3), 8–16 Hz (D4), 4–8 Hz (D5) and 0.1–4 Hz (A5), and this process is depicted in Figure 4.8. In this study, *Symlet-5* as a modified version of *Daubechies* wavelets with increased symmetry was used for N2 trials analysis and *Mexican-hat* was used for analysis of P3 and P600 trials. The mean of CWT results provides a curve (Figure 4.9) which as explained earlier is used to extract the amplitude index and latency ratio of ERP components. Figure 4.10 and Figure 4.11 show the comparison of two target and non-target trials for a P3 collected from the Cz channel.

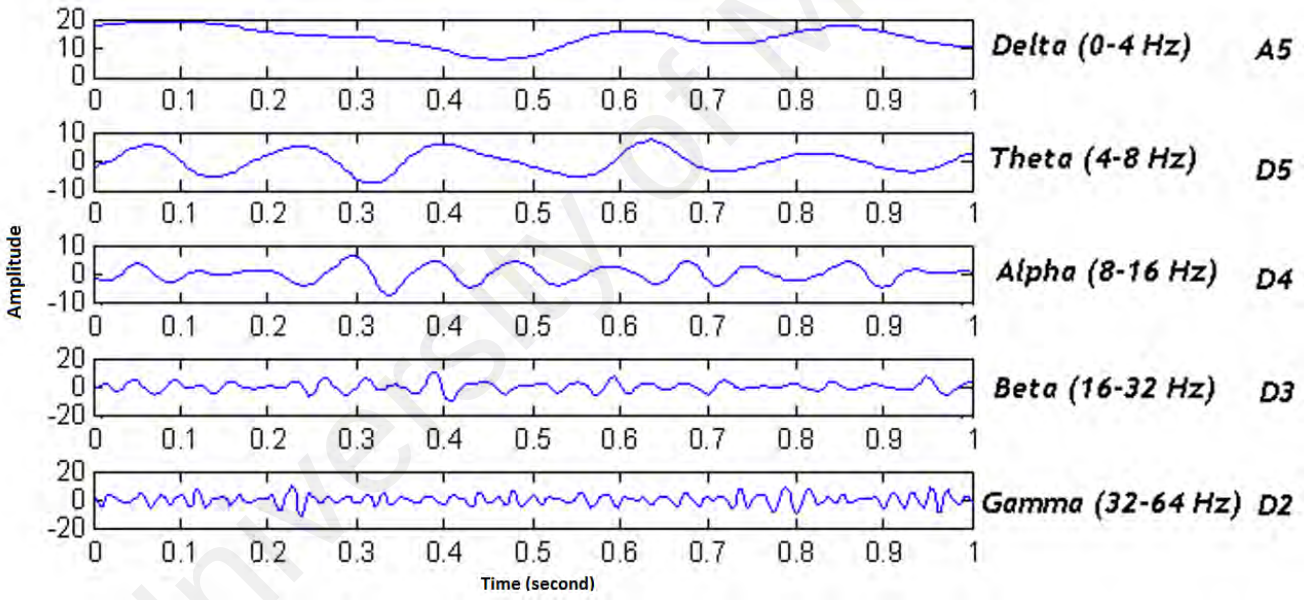


Figure 4.8: DWT decomposition of a P3 Target signal into five sub-bands.

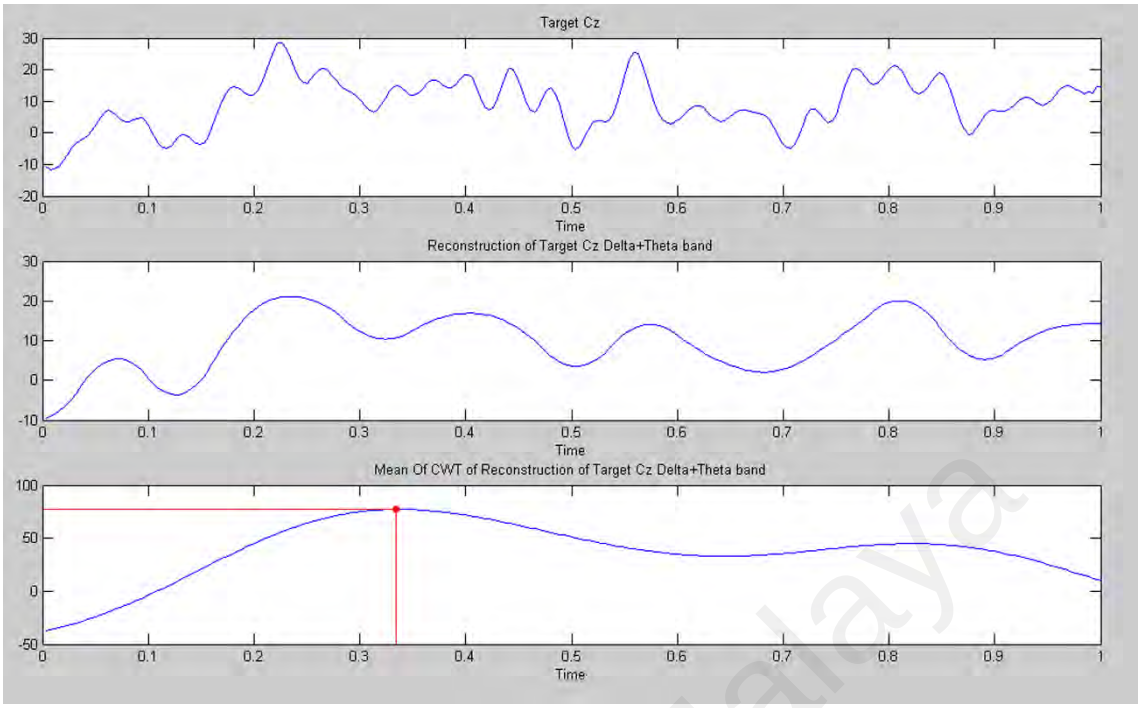


Figure 4.9: A sample of P3 target trial recorded at Cz channel filtered by DWT and features extracted based on averaged CWT coefficients.

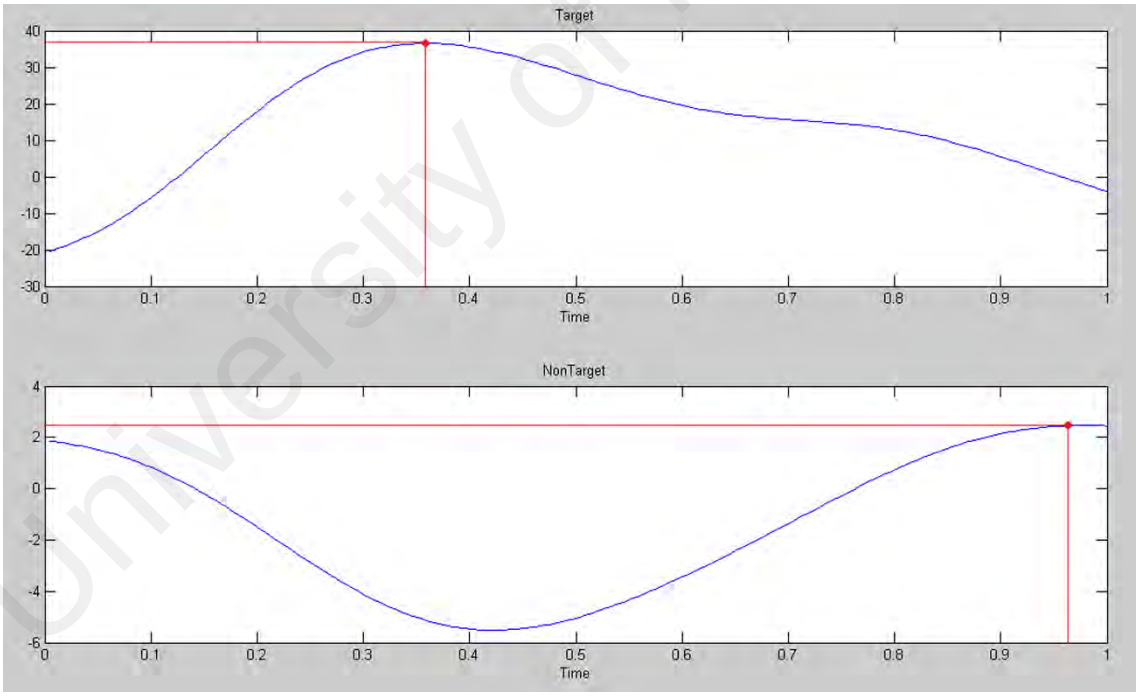


Figure 4.10: Averaged CWT coefficients over scales for a target and Non-target P3 signals.

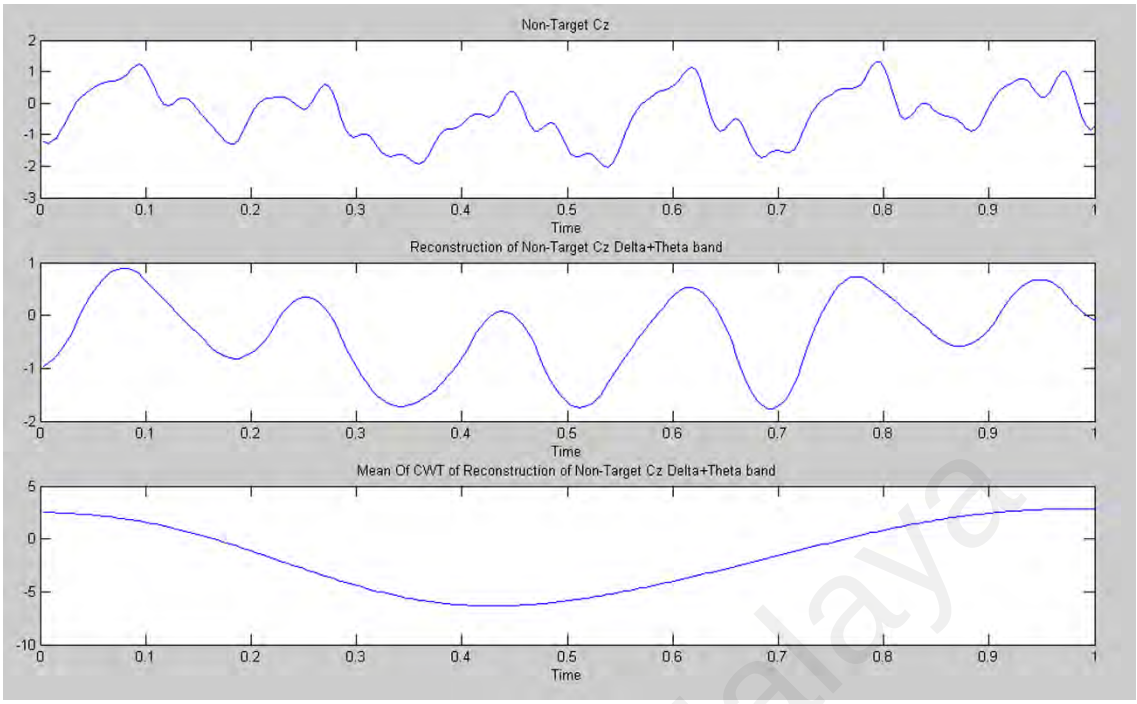


Figure 4.11: A sample of P3 Non-target trial recorded at Cz channel filtered by DWT and features extracted based on averaged CWT coefficients.

4.4.1 Validation

Since this technique is a semi-automated method, we aim to assess the validity of single-trial feature extraction algorithm in the evaluation of an ERP. Therefore, the classification accuracy of Target and Non-target signals based on features extracted from a single-trial method using SVM. Using *MATLAB* built-in SVM as a large margin classifier defines a decision boundary with several choices of kernel functions. In this study, two different types of kernels (linear, Gaussian) were used to train the SVM. Values of "T" and "A" of four channels were used as eight identified features of each trial. Also, for providing a more sophisticated classification method, the average of "A" and "T" values of four channels as well as first two principle components (PC1 and PC2) of these eight features were extracted using built-in PCA algorithm of MATLAB and used as inputs for the SVM machine.

It is noteworthy that the nature of classes in this study was skewed due to the large number of Non-target trials. Therefore, for a better statistical evaluation of classifier

accuracy, F-score was calculated based on precision and recall values of the SVM classifier. The results of test dataset validation for linear kernel (precision=0.75, recall=0.96, F-score=0.85) and Gaussian kernel (precision=0.76, recall=0.97, F-score=0.85) show the high accuracy of the applied algorithm for single-trial ERP detection in this study.

Table 4.1: Results of single-trial P3 detection using SVM classifier.

Classifier	SVM											
Number of input features	8-inputs				2-averaged inputs				2-PCA inputs			
Kernel function	Linear Kernel		Gaussian Kernel		Linear Kernel		Gaussian Kernel		Linear Kernel		Gaussian Kernel	
Dataset	T	CV	T	CV	T	CV	CV	T	T	CV	T	CV
precision	0.75	0.74	0.76	0.74	0.75	0.72	0.75	0.73	0.76	0.74	0.76	0.74
Recall	0.95	0.92	0.97	0.90	0.92	0.84	0.94	0.86	0.96	0.89	0.96	0.91
F-score	0.84	0.82	0.85	0.81	0.83	0.78	0.84	0.79	0.85	0.81	0.85	0.82
Accuracy	0.96	0.96	0.97	0.96	0.96	0.96	0.96	0.96	0.96	0.96	0.96	0.96

4.5 Comparing the Results of ERP Detection Algorithms

The ERP recordings were pre-processed to remove the artifacts without rejecting epochs, and both conventional method (averaging) and the single-trial method (multi-dimensional approach) was used to detect ERP components in this study. Then, the efficiency of utilized single-trial method was evaluated by comparing its results with the traditional averaging technique. Therefore, the characteristics of ERP components of 31 male heroin-dependent subjects [age (mean=36.8 and SD=6.5), with the period of addiction (mean=16.8 and SD=5.8)] at baseline phase and 19 healthy volunteer control subjects (age: mean=34.3 and SD =7.5) were extracted using both single-trial and averaging approaches. Due to the various number of factors in this comparison namely; group, location, ERP component, and ERP characteristics (amplitude and latency), only the auditory MMN and P3 components which extracted from task II were used in this investigation.

Table 4.2 provides a summary of subject characteristics; indicating the mean (SD) and one-way ANOVA results for age, and ERP features of each group using two methods (averaged and single-trial). *Lilliefors* test (two-sided goodness-of-fit test) was used to

check the normality assumption of the demographic characteristics and electrophysiological properties of groups. It was shown that no significant association was found in all demographic characteristics for both groups (p<0.05).

Table 4.2: Demographic and electrophysiological data of subjects indicate the mean±SD as well as the ERP features differences using one-way ANOVA.

Effect		Heroin Addicts (n=31)		Healthy Controls (n=19)		$F_{(1,48)}$		P-value	
Age (years)		36.8±6.5		34.3±7.5		-		-	
		Averaged	Single-Trial	Averaged	Single-Trial	Averaged	Single-Trial	Averaged	Single-Trial
MMN Amplitude	Fz	1.54±0.55	1.55±0.34	1.57±0.58	2.48±0.43	0.06	70.54	0.81	<0.001
	Fcz	1.52±0.50	1.63±0.36	1.27±0.52	2.59±0.41	2.8	75.38	0.10	<0.001
	Cz	1.48±0.39	2.05±0.35	2.19±0.39	3.06±0.31	39.31	105.9	<0.001	≈0.000
	Pz	1.28±0.39	1.76±0.38	1.99±0.49	2.69±0.43	34.17	63.79	<0.001	<0.001
MMN Latency	Fz	0.47±0.03	0.45±0.02	0.42±0.02	0.38±0.02	33.08	121.4	<0.001	≈0.000
	Fcz	0.47±0.03	0.46±0.02	0.44±0.02	0.39±0.02	7.94	121.8	<0.01	≈0.000
	Cz	0.47±0.03	0.47±0.02	0.44±0.03	0.40±0.02	11.13	118.3	<0.01	≈0.000
	Pz	0.46±0.03	0.48±0.02	0.43±0.04	0.42±0.02	7.98	114.3	<0.01	≈0.000
P300 Amplitude	Fz	3.65±1.36	11.20±1.23	5.93±1.48	14.96±1.81	30.87	76.23	<0.001	<0.001
	Fcz	3.49±1.59	11.06±1.21	5.47±1.92	14.83±1.96	15.58	70.83	<0.001	<0.001
	Cz	3.72±1.47	11.47±1.38	5.20±1.72	15.29±1.70	10.45	75.26	<0.01	<0.001
	Pz	4.02±1.47	11.96±1.18	5.91±1.37	15.87±1.42	20.5	110.6	<0.001	≈0.000
P300 Latency	Fz	0.54±0.08	0.48±0.06	0.34±0.05	0.25±0.04	94.47	251.8	<0.001	≈0.000
	Fcz	0.56±0.07	0.49±0.06	0.35±0.09	0.26±0.03	92.72	252.9	<0.001	≈0.000
	Cz	0.56±0.07	0.50±0.06	0.34±0.09	0.27±0.04	105.46	251.3	<0.001	≈0.000
	Pz	0.54±0.08	0.52±0.05	0.33±0.07	0.28±0.03	90.63	301.4	<0.001	≈0.000

In averaging method, all trials of the same category (standard, deviant, and novel) are averaged together in a time-space domain. In the next step, the resulting waveforms of standard stimuli were subtracted from the deviant and novel stimuli waveforms (Figure 4.13). Figure 4.14 depicts the average of the Target and Non-target trials for MMN and P3 waveforms recorded at Cz channel. The maximum value of mean-voltage polarity of each curve was considered as the amplitude of the ERP component. Subsequently the projection of this maximum value in time domain was indicated as the ERP latency.

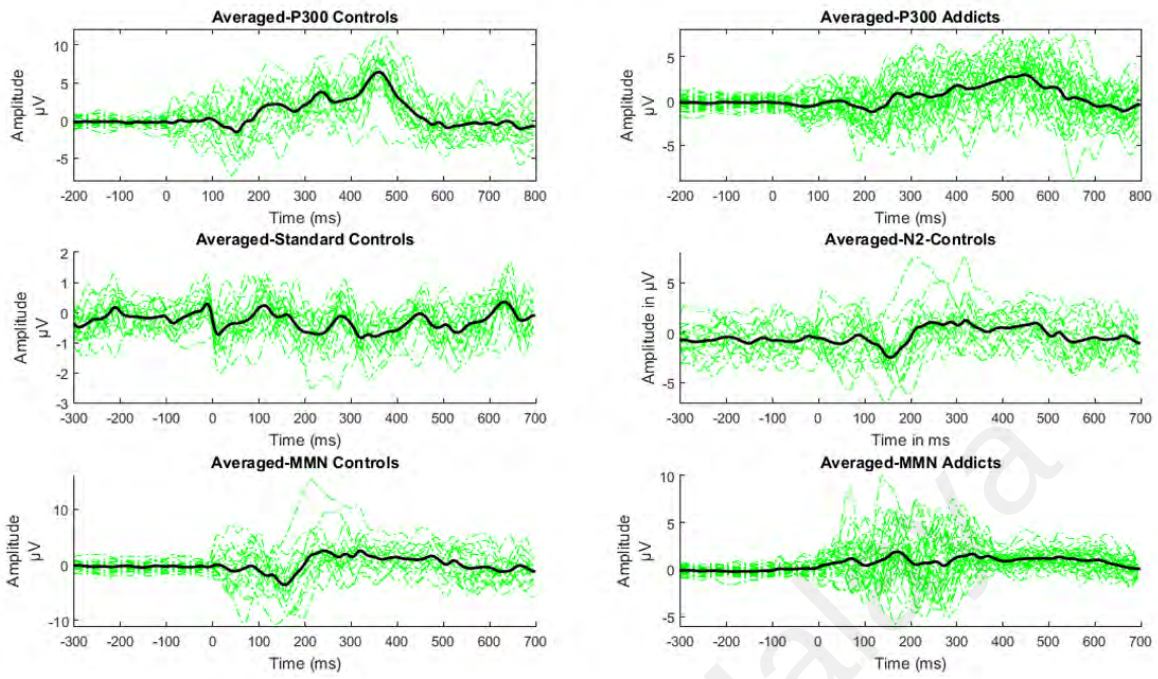


Figure 4.12: Detecting the MMN and P3 of study groups using averaging approach.

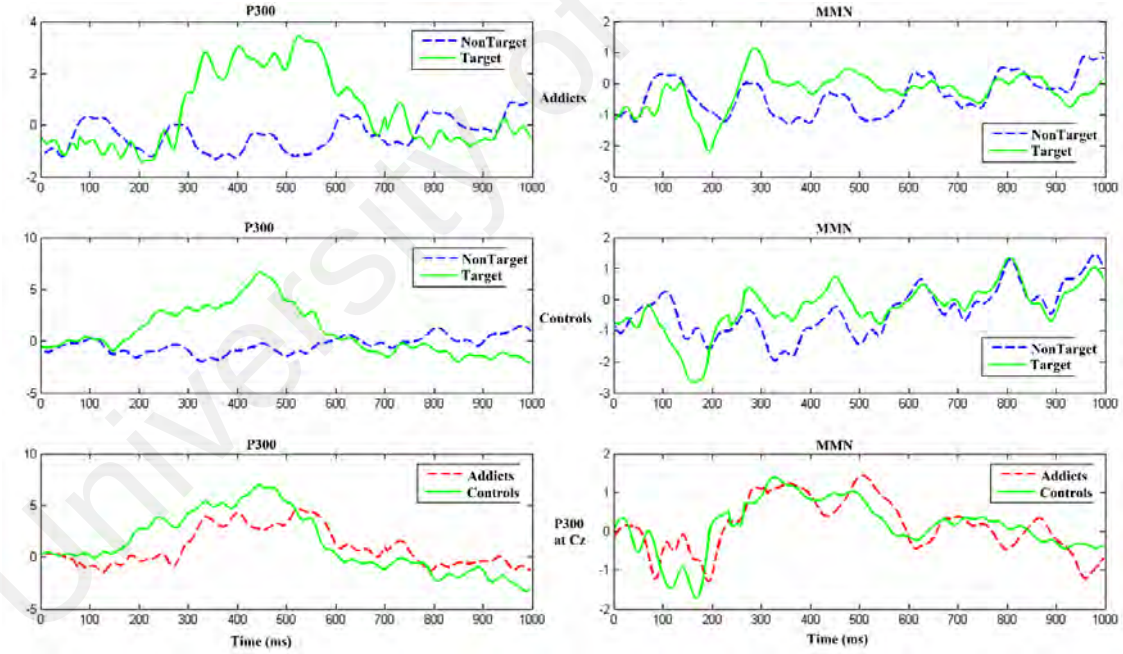


Figure 4.13: Target and Non-Target ERPs trials is averaged for both groups to define the MMN and P3 components' amplitude and latency at Cz channel. The comparison of these components between addicts and controls in the lower window was based on subtracting the Non-target from the Target.

Group comparisons for ERP features were studied using mixed models of between and within subject factors. To explore the differences in cognitive response features among the addicts and controls, data were fitted into a repeated measure ANOVA model to

evaluate if the model terms were significant in their effect on the response by measuring how they contributed to the overall covariance. The multivariate response for each subject was a vector of repeated measures, and *Lilliefors* test (two-sided goodness-of-fit test) was used to check the normality assumption of each variable. For an accurate *p*-value calculation in the repeated measure models, the *p*-value was adjusted using three different correction methods (*Greenhouse-Geisser/Huynh-Feldt/Lower bound* adjustment). Based on the results of repeated measure mix models, significant main effects were followed up with *post hoc* pairwise analysis of variances using the *Tukey-Kramer* correction method. These analyses were done separately for both approaches of feature extraction, and their statistical results were compared.

The *Bland-Altman* plots quantifies the bias and a range of agreements, which shows wide limits of agreement of both methods. (Figure 4.14 and Table 4.3) confirm the lower interquartile range of values for each feature using the single-trial method of feature extraction.

In addition, ERP features of both methods were fitted into a repeated measure ANOVA model (Table 4.3) to investigate the effects of the method on comparative analysis. The results of this model showed the significant effect of the method on ERP feature measurements of all subjects. In addition, follow-up analysis of each ERP feature further confirmed that method has a significant effect on the ERP latency ratio; intercept×method interaction had significant effect on analysis of MMN ($F_{(1,48)}$=35.99, *p*-value<0.001) and P3 ($F_{(1,48)}$=41.45, *p*-value<0.001), and group×method had significant effect only on MMN latency ($F_{(1,48)}$=19.68, *p*-value<0.001). It should be noted that MMN and P3

amplitude ratio was defined differently in each method, which contributed to the significant effect of the method on ERP amplitudes comparisons.

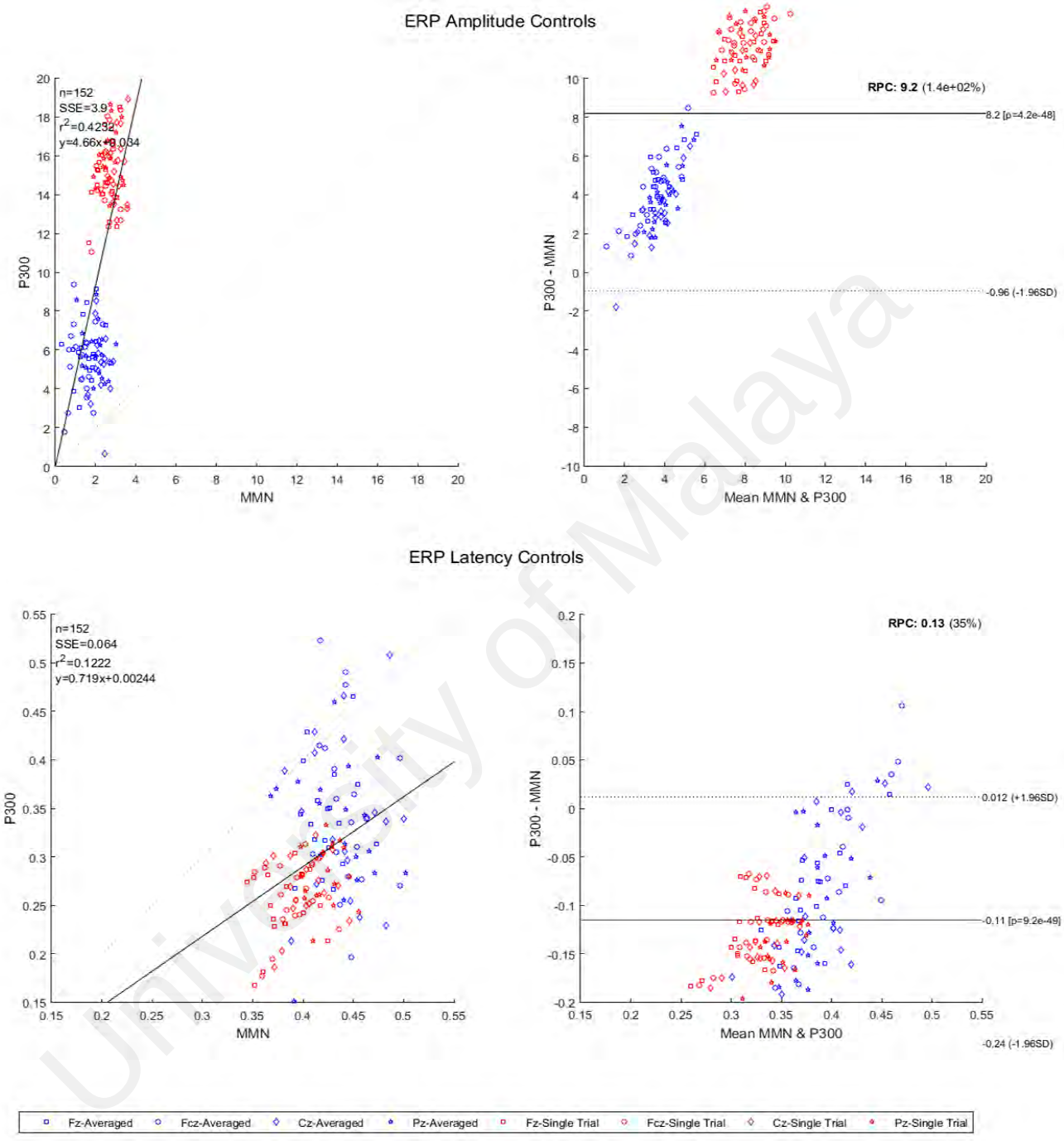


Figure 4.14: Bland-Altman plot of latency ratio and amplitude index extracted from both averaging and single-trial methods. The figure shows a higher variance of data for averaged values compared to single-trials. RPC is the reproducibility coefficient (1.96*SD) and % of mean values.

Table 4.3: Method comparison of auditory MMN and P3 measurements using repeated measure ANOVA model.

Effect	Statistics	P_value*	P_GG	P_HF	P_LB
Intercept/Method	$F_{(1,48)}$=2131	≈0.000			
Group/Method	$F_{(1,48)}$=39.33	<0.001			
Intercept/Method/Location	$F_{(3,144)}$=3.277	<0.05	<0.05	<0.05	<0.08
Group/Method/Location	$F_{(3,144)}$=0.503	<0.7	<0.7	<0.7	<0.7
Intercept/Method/Feature	$F_{(1,48)}$=2158	≈0.000			
Group/Method/Feature	$F_{(1,48)}$=41.96	<0.001			
Intercept/Method/ERP	$F_{(1,48)}$=1601	≈0.000			
Group/Method/ERP	$F_{(1,48)}$=10.01	<0.003			
Intercept/Method/Location/Feature	$F_{(3,144)}$=2.573	<0.06	<0.07	<0.07	<0.2
Group/Method/Location/Feature	$F_{(3,144)}$=0.527	<0.7	<0.7	<0.7	<0.7
Intercept/Method/Location/ERP	$F_{(3,144)}$=1.544	<0.3	<0.3	<0.3	<0.3
Group/Method/Location/ERP	$F_{(3,144)}$=2.687	<0.05	<0.06	<0.06	<0.2
Intercept/Method/Feature/ERP	$F_{(1,48)}$=1588	≈0.000			
Group/Method/Feature/ERP	$F_{(1,48)}$=9.564	<0.004			
Intercept/Method/Location/Feature /ERP	$F_{(3,144)}$=1.427	<0.3	<0.3	<0.3	<0.3
Group/Method/Location/Feature /ERP	$F_{(3,144)}$=2.639	<0.06	<0.07	<0.06	<0.2
Amplitude MMN					
Intercept/Method	$F_{(1,48)}$=121.2	≈0.000			
Group/Method	$F_{(1,48)}$=33.02	<0.001			
Intercept/Method/Location	$F_{(3,144)}$=2.806	<0.06	<0.06	<0.06	<0.2
Group/Method/Location	$F_{(3,144)}$=10.74	<0.001	<0.001	<0.001	<0.01
Amplitude P300					
Intercept/Method	$F_{(1,48)}$=2023	≈0.000			
Group/Method	$F_{(1,48)}$=24.54	<0.001			
Intercept/Method/Location	$F_{(3,144)}$=2.128	<0.1	<0.2	<0.2	<0.2
Group/Method/Location	$F_{(3,144)}$=0.654	<0.6	<0.6	<0.6	<0.5
Latency MMN					
Intercept/Method	$F_{(1,48)}$=35.99	<0.001			
Group/Method	$F_{(1,48)}$=19.68	<0.001			
Intercept/Method/Location	$F_{(3,144)}$=10.82	<0.001	<0.001	<0.001	<0.002
Group/Method/Location	$F_{(3,144)}$=0.885	<0.5	<0.5	<0.5	<0.4
Latency P300					
Intercept/Method	$F_{(1,48)}$=41.45	<0.001			
Group/Method	$F_{(1,48)}$=1.709	<0.2			
Intercept/Method/Location	$F_{(3,144)}$=3.483	<0.02	<0.02	<0.02	<0.07
Group/Method/Location	$F_{(3,144)}$=0.112	<0.96	<0.96	<0.96	<0.8

* Repeated ANOVA computes three *p*-values using *Greenhouse-Geisser, Huynh-Feldt,* and *Lower-bound* corrections when the factors are more than one.

Quantifying the ERP characteristics (voltage amplitude and occurrence latency) is the main goal in assessing an ERP component, and precise detection of amplitude and latency of ERPs is a key challenge in providing accurate conclusions and understanding of information processing and cognitive biases. The most significant problem in describing an ERP waveform is the very low amount of signal to noise ratio of EEG signals and low amplitude of ERPs when compared to baseline EEG (J. Li *et al.*, 2011). Therefore, averaging across a large number of trials is the most common approach in ERP experiments in the field of psychoanalysis. For instance, P3 as the most robust ERP component requires 35-60 trials, and MMN quantification needs at least 150 trials, per

condition from each subject (Duncan *et al.*, 2009; Woodman, 2010). To provide a large number of trials in the conventional method, extended procedures with repetitive tasks of probing ERPs are required in related paradigm to directly affect the participants' attention and awareness which is associated with ERP features. On the other hand, characteristics of ERP components per se vary from trial to trial and cause variability of the waveform in single trials; and moreover, voltage fluctuations of various ERP waveforms overlap each other in time and space, such as auditory MMN and P3 (Woodman, 2010). Taken together, these facts lead to the conclusion that restriction of ERP analysis to the average ERP hides potential violations and provides wide deflections in the time domain which affect the exact identification of ERP latencies and result in poor estimation of the amplitude and latency of ERPs (Picton, 1992; Quiroga & Garcia, 2003).

Although it was shown that single-trial detection of ERP components provides more accurate values compared to the conventional method, the averaging technique has been practiced in neuropsychiatric clinical studies. Therefore, to define the effects of measurement accuracy on outcome result, factorial analysis of this observational study was done using both measurement values. Figure 4.15 presents the ERP visualization using averaged method for all four locations, and Figure 4.16 shows the mean and standard error of ERPs characteristics extracted from both measurement techniques. To compare the amplitude and latency ratio of recorded ERP components, subjects group were selected as three within-subjects factors to fit the data into was chosen as a between-subjects factor, and ERP type, features, and locations were two repeated measure models using two described methods' values (Table 4.4).

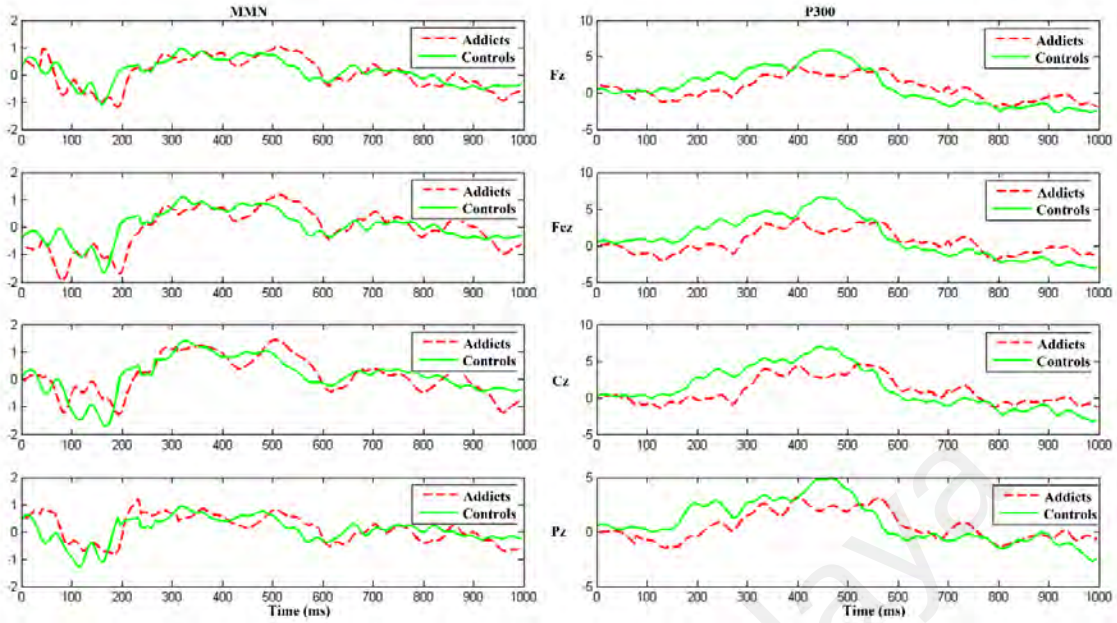


Figure 4.15: Grand average of MMN and P3 components of both control and addict groups over four selected channels.

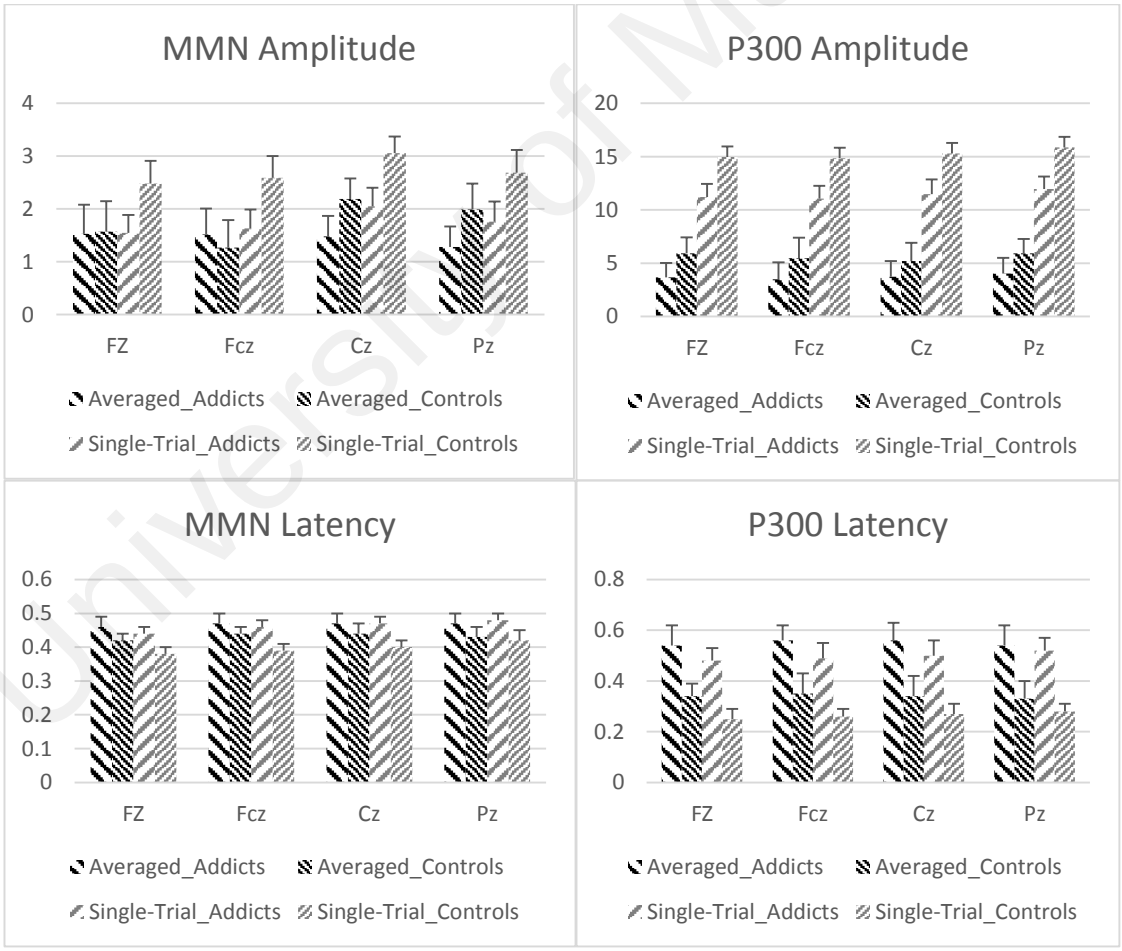


Figure 4.16: Group comparison of mean and standard-error of ERP amplitude and latency ratio over channels using both methods.

The results of repeated measure ANOVA models showed that location factor had significant effect on all interactions of single trial method ($F_{(3,144)}$=89.88, $p_{\text{-valueGG}}$<0.001, $p_{\text{-valueHF}}$<0.001, $p_{\text{-valueLB}}$ <0.001), while it was not a significant factor in averaged method interactions ($F_{(3,144)}$=1.54, $p_{\text{-valueGG}}$<0.3, $p_{\text{-valueHF}}$<0.3, $p_{\text{-valueLB}}$ <0.3). Table 4.4 provides the results of the related factorial analysis for a better comparison of both methods. Hence, the follow-up analysis for each ERP feature was presented in separated sections and the analysis results of both methods compared.

The results of both models confirmed that heroin addicts group had lower MMN amplitude relative to the controls. However, *post hoc* analysis of each model showed that in the single-trial method, diminished amplitude of MMN was statistically significant in all channels, while in the averaged method it was only significant at Cz and Pz channels. In addition, the results showed the significant effect of location interactions on amplitude of MMN in all subjects [averaged method ($F_{(3,144)}$=6.62, $p_{\text{-valueGG}}$<0.001, $p_{\text{-valueHF}}$<0.001, $p_{\text{-valueLB}}$ <0.05) and single-trial method ($F_{(3,144)}$=231, $p_{\text{-valueGG}}$<0.0001, $p_{\text{-valueHF}}$<0.0001, $p_{\text{-valueLB}}$ <0.001)]. Results indicate that in addition to the greater value of MMN amplitude at Cz channel in both groups, the variability of this component amplitude for both groups at Cz channel is higher compared to other channels.

Table 4.4: Factorial analysis for comparison of ERP properties using two measurement techniques (averaging and single-trial).

	Averaging Method					Single-trial Method				
Effect	**Statistics**	**P_value***	**P-GG**	**P-HF**	**P-LB**	**Statistics**	**P_value**	**P-GG**	**P-HF**	**P-LB**
Intercept	$F_{(1,48)}$=3524	≈0.000				$F_{(1,48)}$=5582	≈0.000			
Group	$F_{(1,48)}$=66.77	<0.0001				$F_{(1,48)}$=104.2	≈0.000			
Intercept /Location	$F_{(3,144)}$=1.538	0.207	0.212	0.209	0.221	$F_{(3,144)}$=89.88	≈0.000	≈0.000	<0.001	<0.001
Group / Location	$F_{(3,144)}$=0.613	0.607	0.587	0.597	0.437	$F_{(3,144)}$=0.423	0.74	0.67	0.68	0.52
Intercept /Feature	$F_{(1,48)}$=1986	≈0.000				$F_{(1,48)}$=4395	≈0.000			
Group / Feature	$F_{(1,48)}$=103.3	≈0.000				$F_{(1,48)}$=129.1	≈0.000			
Intercept /ERP	$F_{(1,48)}$=694.5	≈0.0000				$F_{(1,48)}$=2972	≈0.000			
Group /ERP	$F_{(1,48)}$=37.59	<0.0001				$F_{(1,48)}$=43.96	<0.001			
Intercept / Location / Feature	$F_{(3,144)}$=1.752	0.1591	0.166	0.162	0.192	$F_{(3,144)}$=74.36	<0.001	<0.001	<0.001	<0.001
Group / Location / Feature	$F_{(3,144)}$=0.625	0.599	0.581	0.591	0.433	$F_{(3,144)}$=0.37	0.78	0.71	0.72	0.55
Intercept / Location / ERP	$F_{(3,144)}$=1.804	0.149	0.159	0.157	0.185	$F_{(3,144)}$=47.92	<0.001	<0.001	<0.001	<0.001
Group / Location / ERP	$F_{(3,144)}$=2.612	0.0536	0.065	0.062	0.113	$F_{(3,144)}$=0.41	0.75	0.69	0.7	0.52
Intercept / Feature / ERP	$F_{(1,48)}$=668.4	≈0.000				$F_{(1,48)}$=2950	≈0.000			
Group / Feature / ERP	$F_{(1,48)}$=55.88	<0.0001				$F_{(1,48)}$=54.5	<0.001			
Intercept / Location / Feature / ERP	$F_{(3,144)}$=1.804	0.149	0.159	0.156	0.185	$F_{(3,144)}$=48.44	<0.001	<0.001	<0.001	<0.001
Group / Location / Feature / ERP	$F_{(3,144)}$=2.531	0.0595	0.071	0.067	0.118	$F_{(3,144)}$=0.53	0.66	0.61	0.62	0.47
Amplitude MMN										
Intercept	$F_{(1,48)}$=2480	≈0.000				$F_{(1,48)}$=1781	≈0.000			
Group	$F_{(1,48)}$=22.72	<0.001				$F_{(1,48)}$=81.9	<0.001			
Intercept /Location	$F_{(3,144)}$=6.622	<0.001	<0.01	<0.001	0.013	$F_{(3,144)}$=231.6	≈0.000	≈0.000	≈0.000	≈0.000
Group / Location	$F_{(3,144)}$=11.90	<0.0001	<0.01	<0.001	0.001	$F_{(3,144)}$=1.53	0.21	0.21	0.21	0.22
Amplitude P300										
Intercept	$F_{(1,48)}$=1663	≈0.000				$F_{(1,48)}$=4196	≈0.000			
Group	$F_{(1,48)}$=69.34	<0.0001				$F_{(1,48)}$=85.93	<0.001			
Intercept /Location	$F_{(3,144)}$=1.229	0.301	0.301	0.301	0.273	$F_{(3,144)}$=54.49	<0.001	<0.001	<0.001	<0.001
Group / Location	$F_{(3,144)}$=0.554	0.646	0.619	0.63	0.460	$F_{(3,144)}$=0.35	0.79	0.72	0.73	0.55
MMN Latencies										
Intercept	$F_{(1,48)}$=33805	≈0.0000				$F_{(1,48)}$=24220	≈0.0000			
Group	$F_{(1,48)}$=41.34	<0.0001				$F_{(1,48)}$=127.5	≈0.0000			
Intercept /Location	$F_{(3,144)}$=2.008	0.11	0.120	0.115	0.1629	$F_{(3,144)}$=292.7	≈0.0000	≈0.000	≈0.000	≈0.000
Group / Location	$F_{(3,144)}$=0.889	0.45	0.443	0.448	0.353	$F_{(3,144)}$=7.44	<0.001	<0.008	<0.008	<0.008
P300 latency										
Intercept	$F_{(1,48)}$=6550	≈0.0000				$F_{(1,48)}$=28.46	<0.001			
Group	$F_{(1,48)}$=359.8	≈0.0000				$F_{(1,48)}$=266.5	≈0.000			
Intercept /Location	$F_{(3,144)}$=0.783	0.505	0.503	0.505	0.381	$F_{(3,144)}$=207.7	≈0.000	≈0.000	≈0.000	≈0.000
Group / Location	$F_{(3,144)}$=0.124	0.946	0.944	0.946	0.726	$F_{(3,144)}$=0.11	<0.98	<0.8	<0.8	<0.8

* Repeated ANOVA computes three *p*-values using *Greenhouse-Geisser, Huynh-Feldt,* and *Lower-bound* corrections when the factors are more than one.

The analysis of MMN latency ratio extracted from both methods confirmed the prolonged latency of MMN occurrence among addicts compared to controls. The *post hoc* analysis showed that averaged method values indicated prolonged latency in all channels with no statistically significant effect of location ($F_{(3,411)}$=42.3, $p_{\text{-valueGG}}$<0.1, $p_{\text{-valueHF}}$<0.1, $p_{\text{-valueLB}}$ <0.3). On the other hand, latency ratio values extracted from single-trial method indicated that Fz channel has the shortest latency among all four recorded channels ($F_{(3,144)}$=292, $p_{\text{-valueGG}}$<0.0001, $p_{\text{-valueHF}}$<0.0001, $p_{\text{-valueLB}}$ <0.0001) for all participants. Also, single-trial values for MMN latency showed a significant effect of group×location interaction ($F_{(3,144)}$=7.44, $p_{\text{-valueGG}}$<0.01, $p_{\text{-valueHF}}$<0.01, $p_{\text{-valueLB}}$ <0.01), which indicated Cz and Fcz with the maximum differences of MMN latency ratio between groups.

The follow-up analysis for P3 component, as depicted in the figure, shows lower amplitude and prolonged latency ratio of addicts compared to healthy controls in both methods. Comparison of *post hoc* analysis of both approaches showed that there was no significant effect of location in the averaging method for both amplitude ($F_{(3,144)}$=1.23, $p_{\text{-value}}$<0.4) and latency ratio ($F_{(3,144)}$=0.78, $p_{\text{-value}}$<0.6). Conversely, analysis of single-trial method values showed that although there is no significant effect of group×location ($F_{(3,144)}$=0.35, $p_{\text{-valueGG}}$<0.8, $p_{\text{-valueHF}}$<0.8, $p_{\text{-valueLB}}$ <0.6), there was significant effect of intercept×location interaction for P3 amplitude ($F_{(3,144)}$=54.5, $p_{\text{-valueGG}}$<0.001, $p_{\text{-valueHF}}$<0.001, $p_{\text{-valueLB}}$ <0.001); which showed higher amplitude of P3 in Pz channel compared to the other three channels. Similarly, for the P3 latency ratio measured by single-trial method; group×location interaction ($F_{(3,144)}$=0.11, $p_{\text{-valueGG}}$<0.98, $p_{\text{-valueHF}}$<0.9, $p_{\text{-valueLB}}$ <0.9) was not significant, but there was a significant effect of intercept×location interaction ($F_{(3,144)}$=207, $p_{\text{-valueGG}}$<0.0001, $p_{\text{-valueHF}}$<0.0001, $p_{\text{-valueLB}}$ <0.0001), which indicated lower latency ratio of P3 component at Fz channel.

The results of this section confirmed that method of measurement has a significant effect on the measurement of ERP latency ratio and amplitude index. This significant effect of measurement method especially for the interaction of method×location shows the increase of variability of amplitude index and latency ratio over different locations for all participants as well as its effect on group differences. It should be pointed out that single-trial method provides features with lower interquartile value for each group, which results in higher variability of ERP characteristics between groups over channel location. It is notable that brainstem-based arousal 'neuromodulatory' systems based in brainstem areas project to widespread cortical sites, which causes spatial variability of ERP characteristics. Therefore, single-trial analysis can be a beneficial tool to investigate this variability of ERP features relative to their spatial source domains.

The results of separate ANOVA models of each method showed that location factor had a significant effect on all interactions of the single-trial method, while it was not an important factor in averaged method interactions. This indicates that single-trial detection of ERP components provides more accurate and reliable values compared to the averaging method. This is related to the main drawback of averaging method, which disregards inconsistency of ERP occurrence features, including their spatial and temporal trial-to-trial variability, which leads to wide deflection of polarity after averaging the trials. In contrast, single-trial detection of ERPs can avoid problems by defining the occurrence of ERP more accurately which potentially reveals much richer information about the investigation of event-related brain dynamics. Although the amplitude index of ERP components was measured differently in these approaches and the variability of this feature could not be addressed precisely; results showed significant accuracy of single-trial approach in detecting latency ratio which is argued to be more important than amplitude polarity in quantifying the ERP traits (Luck, 2005; Woodman, 2010). In fact, the main limitation of the averaging method is related to the measurement of ERP

component latencies, caused by overlapping cognitive responses especially earlier offset of the N2 and earlier onset of the P3 component (Luck, 2014). This problem also results in difficulty of measuring voltage amplitude using the peak of an ERP component in averaging technique.

The effectiveness of the location factor in the analysis of factor interaction effects in single-trial method indicates the higher precision in ERP features which leads to comparability of these features over the location factor. Analysis of ERP traits using single-trial method indicated the diminished amplitude of MMN in all channels, which suggested a correlation with the degree of difference between standard and deviant stimuli (Stefanics *et al.*, 2014), and its lower amplitude among chronic heroin addicts might be related to their pre-attentive processing deficits. The analysis of averaged method results for MMN latency ratio showed prolonged latency in all channels with no statistically significant effect of location, while latency ratios extracted from single-trial detection method indicated that Fz channel had the shortest latency among all four recorded channels for all participants. This result is in line with the arguments regarding frontal lobe involvement in MMN generation (Alho, 1995). In addition to the greater value of MMN amplitude at Cz in both groups, the results indicated that difference of MMN latency of both groups is more significant at Cz and Fcz channels. The results of single-trial method analysis also showed the higher amplitude of P3 in Pz channel, and a lower latency ratio at Fz channel and have further confirmed lower amplitude and prolonged latency ratio of P3 component among addicts compared to healthy controls. Therefore, it can be concluded that assessing MMN and P3 components at these channels by utilizing the proposed single-trial technique could provide rigorous indexes regarding the pre-attentive and selective attention processing to be used for investigating the severity of addiction and monitoring the treatment of addicts.

4.6 Subjects Demographic

116 of 200 participants who were enrolled in UMMC met the inclusion criteria of this study as heroin dependent group while 21 healthy volunteers were selected based on the inclusion and exclusion criteria shown in Figure 3.1. The psychiatrist interviewed subjects at UMCAS, and blood investigations, as well as electrophysiological measurements, were assessed one day after the enrollment. Based on the exclusion criteria of this study, the electrophysiological data of 96 heroin addict subjects and 21 healthy volunteers were included in the first phase of this study (baseline phase). Then 96 heroin addicts of baseline stage were randomly divided into two groups of MMT+sham EA (n=48) called as Sham group, and MMT+real EA (n=48) called as EA group. By the end of the first month of the treatment regime, 25 number of subjects quit the program and 11 number of them were eliminated from the analysis based on the exclusion criteria. 28 subjects of sham group and 32 of EA group remained, and their measurements were used to investigate the short-term effects of the treatment regime. After three months of treatment, only 44 subjects remained (23 sham group and 21 EA group). Also, acute effects of treatments were evaluated for the subjects with no record of withdrawal syndrome between the second and third month of the treatment (28 sham group and 19 EA group).

The demographic and clinical characteristics of the subjects included in each phase of the study are shown in Table 4.5. The healthy control subjects had higher education (years) compared to the addicts. The digit-span Wechsler score for subjects revealed that healthy controls achieved higher scores in this test compared to the addicts.

Table 4.5: Demographic of subjects indicate the mean (SD) as well as ERP features differences using one-way ANOVA.

Effect	Addicts Baseline (n=96)	Healthy Controls (n=21)	Sham First-month (n=28)	EA First-month (n=32)	Sham Acute (n=28)	EA Acute (n=19)	Sham Third-month (n=23)	EA Third-month (n=21)
Age (years)	43.2±9.6	39.3±7.5	42.8±9.9	43.4±10.3	42.8±9.9	43.1±10.1	42.7±9.8	43.2±10.1
Education (years)	3.4±1.0	5.8±3.4	3.3±0.8	3.2±0.9	3.3±0.8	3.1±0.9	3.2±0.8	3.2±0.9
Duration of opioid addiction (Years)	21.7±9.4	-	22.9±9.5	23.2±1.0	22.9±9.5	23.1±0.9	22.8±9.5	23.0±0.9
Smoking Index FSTROM	2.1±0.8	-	-	-	-	-	-	-
OOWS	2.3±1.7	-	1.9±1.2	2.0±1.1	-	-	-	-
SOWS	5.5±5.7	-	5.1±5.2	5.2±5.1	2.1±1.0	1.9±1.1	1.8±0.7	1.7±0.6
DSWT	5.4±0.8	6.1±0.6	5.3±0.8	5.2±0.9	5.3±0.8	5.1±0.9	5.2±0.8	5.2±0.9

To investigate the intergroup differences of addicts who participate in this study, age, education, duration of addiction, OOWS, SOWS, nicotine dependence, and opioid treatment index was examined in a mixed model. This evaluation can benefit further analysis of results to address the significant effective factors. The results of mix-model analysis are shown in Appendix C for all the electrophysiological measurements. The analysis shows that age has significant effect only on mean PSD values of delta ($F_{(1,88)}$=4.87, p<0.05), and alpha ($F_{(1,88)}$=4.09, p<0.05), while its interaction with location is significant for relative beta power ($F_{(3,264)}$=3.15, p<0.05). SOWS has significant effect on mean of PSD at theta ($F_{(1,88)}$=3.80, p<0.05) and alpha ($F_{(1,88)}$=7.28, p<0.05), as well as relative PSD of delta band ($F_{(1,88)}$=6.75, p<0.05), P3 amplitude ($F_{(1,77)}$=5.26, p<0.05), and P600 ($F_{(1,77)}$=6.56, p<0.05). Therefore, it can be suggested that age and withdrawal syndromes have a significant effect on brain electrophysiological activities of subjects in this study. ANOVA analysis (Appendix C) shows no significant effect of other factors especially for education and duration of addiction. Furthermore, *Pearson's r* and *Spearman's rho* correlations showed no significant correlation ($p < 0.05$) between the EEG and ERPs measurements and duration of addiction. The results of this longitudinal study are reported in 4 different sections namely; 1) baseline, 2) short-term, 3) three-months, 4) acute effects.

4.7 Baseline Observation

In the first phase of this study, baseline obsevations of 96 heroin addicts were compared to 21 healthy control subjects to describe the brain electrophysiological differences between these two groups. Since brain electrophysiological features were extracted during the performance of different tasks, the results are shown in separate sections including the power spectral density of resting EEG, digit-span P600, auditory MMN, and P3.

4.7.1 PSD of Resting-state EEG

Tables 4.6 and 4.7 show the ANOVA analysis of the mean and relative value of PSD during the resting time EEG recordings. The absolute values of PSD are reported in Appendix B. Group comparisons for PSD of resting-state EEG recording showed significant group differences as a between-subjects factor for both mean ($F_{(1,115)}$=96.48, p<0.001), and relative PSD values ($F_{(1,115)}$=15.23, p<0.01). Results show a significant effect of interactions between the within-subjects and between-subjects factors (Table 4.6 and Table 4.7). The post hoc analysis of mean PSD measurements showed lower value for delta, theta, and alpha bands in all locations while mean value of beta band was higher compared to controls (Table 4.6). Figure 4.17 and Figure 4.18 illustrate the mean and relative PSD comparison (mean and standard-error) of each frequency band of each region for both groups. The post hoc analysis of relative PSD (Table 4.7), as it is shown in the error-bar plot of relative PSD values (Figure 4.18), shows lower activity for delta (at frontal and temporal), theta (central, frontal, and temporal), and alpha (all locations) among addicts compared to controls. However, beta band activity among addicts has higher values compared to controls while these differences are the most significant differences between these two groups.

Table 4.6: Results of the factorial ANOVA for the mean value of resting EEG PSD measurements. There are two groups of subjects, four regions, and four frequency bands.

QEEG Mean	F_statistics	P_value*	Location	Diff**	StdErr	P_value	Lower	Upper
Group	$F_{(1,115)}$= 96.48	p< 0.001						
Group/Band	$F_{(3,345)}$=87.08	p< 0.001 p_{gg}<0.001 P_{hf}<0.001 P_{lb}<0.001						
Group/Band/Location	$F_{(9,1035)}$=11.16	p< 0.001 p_{gg}<0.001 P_{hf}<0.001 P_{lb}<0.01						
DELTA								
Group	$F_{(1,115)}$=41.31	p< 0.001						
Group/Location	$F_{(3,345)}$=1.31	p= 0.27 pgg= 0.27 Phf= 0.27 Plb= 0.25	'C'	-0.98	0.21	p< 0.01	-1.40	-0.56
			'F'	-1.31	0.18	p< 0.01	-1.67	-0.96
			'P'	-1.01	0.24	p< 0.01	-1.47	-0.54
			'T'	-1.06	0.19	p< 0.01	-1.43	-0.68
THETA								
Group	$F_{(1,115)}$=49.63	p< 0.001						
Group/Location	$F_{(3,345)}$=2.66	p< 0.05 Pgg=0.07 Phf=0.07 Plb=0.11	'C'	-1.61	0.23	p< 0.01	-2.06	-1.16
			'F'	-1.66	0.23	p< 0.01	-2.12	-1.21
			'P'	-1.31	0.23	p< 0.01	-1.78	-0.85
			'T'	-1.36	0.23	p< 0.01	-1.82	-0.90
ALPHA								
Group	$F_{(1,115)}$=138.45	p< 0.001						
Group/Location	$F_{(3,345)}$=14.56	p< 0.001 pgg<0.001 Phf<0.001 Plb<0.001	'C'	-2.97	0.29	p< 0.01	-3.54	-2.40
			'F'	-2.69	0.27	p< 0.01	-3.24	-2.15
			'P'	-4.04	0.34	p< 0.01	-4.71	-3.38
			'T'	-3.06	0.31	p< 0.01	-3.66	-2.45
BETA								
Group	$F_{(1,115)}$=44.69	p< 0.001						
Group/Location	$F_{(3,345)}$=0.63	p=0.59 pgg=0.57 Phf=0.58 Plb=0.43	'C'	0.29	0.05	p< 0.01	0.18	0.39
			'F'	0.27	0.05	p< 0.01	0.17	0.36
			'P'	0.22	0.05	p< 0.01	0.12	0.33
			'T'	0.22	0.05	p< 0.01	0.12	0.33

* RANOVA computes three p-values using Greenhouse-Geisser, Huynh-Feldt, and Lower-bound corrections.
**Estimated difference between the means of addicts and controls.

Table 4.7: Results of the factorial ANOVA for the relative value of resting EEG PSD measurements. There are two groups of subjects, four regions, and four frequency bands.

QEEG Relative	F_statistics	P_value*	Location	Diff**	StdErr	P_value	Lower	Upper
Group	$F_{(1,115)}$= 15.23	p< 0.001						
Group/Band	$F_{(3,345)}$=25.80	p< 0.001 p_{gg}<0.001 P_{hf}<0.001 P_{lb}<0.001						
Group/Band/Location	$F_{(9,1035)}$=10.91	p< 0.001 p_{gg}<0.001 P_{hf}<0.001 P_{lb}<0.01						
DELTA								
Group	$F_{(1,115)}$=8.52	p< 0.01						
Group/Location	$F_{(3,345)}$=11.21	p< 0.001 p_{gg}<0.001 P_{hf}<0.001 P_{lb}<0.001	'C'	-0.02	0.02	p< 0.30	-0.07	0.02
			'F'	-0.13	0.03	p< 0.01	-0.19	-0.07
			'P'	-0.01	0.02	p< 0.70	-0.06	0.04
			'T'	-0.07	0.02	p< 0.01	-0.12	-0.03
THETA								
Group	$F_{(1,115)}$=6.88	p< 0.02						
Group/Location	$F_{(3,345)}$=1.89	p< 0.2 Pgg=0.13 Phf=0.13 Plb=0.17	'C'	-0.06	0.02	p< 0.01	-0.09	0.02
			'F'	-0.05	0.02	p< 0.01	-0.08	0.01
			'P'	-0.03	0.02	p< 0.08	-0.07	0.00
			'T'	-0.04	0.02	p< 0.04	-0.07	0.00
ALPHA								
Group	$F_{(1,115)}$=30.18	p< 0.001						
Group/Location	$F_{(3,345)}$=16.84	p< 0.001 pgg<0.001 Phf<0.001 Plb<0.001	'C'	-0.11	0.03	p< 0.01	-0.17	-0.06
			'F'	-0.07	0.02	p< 0.01	-0.12	-0.03
			'P'	-0.19	0.02	p< 0.01	-0.24	-0.14
			'T'	-0.09	0.02	p< 0.01	-0.14	-0.05
BETA								
Group	$F_{(1,115)}$=57.63	p< 0.001						
Group/Location	$F_{(3,345)}$=1.90	p=0.13 pgg=0.13 Phf=0.13 Plb=0.17	'C'	0.10	0.02	p< 0.01	0.06	0.15
			'F'	0.15	0.02	p< 0.01	0.11	0.19
			'P'	0.13	0.02	p< 0.01	0.09	0.17
			'T'	0.13	0.02	p< 0.01	0.09	0.16

* RANOVA computes three p-values using Greenhouse-Geisser, Huynh-Feldt, and Lower-bound corrections.
**Estimated difference between the means of addicts and control.

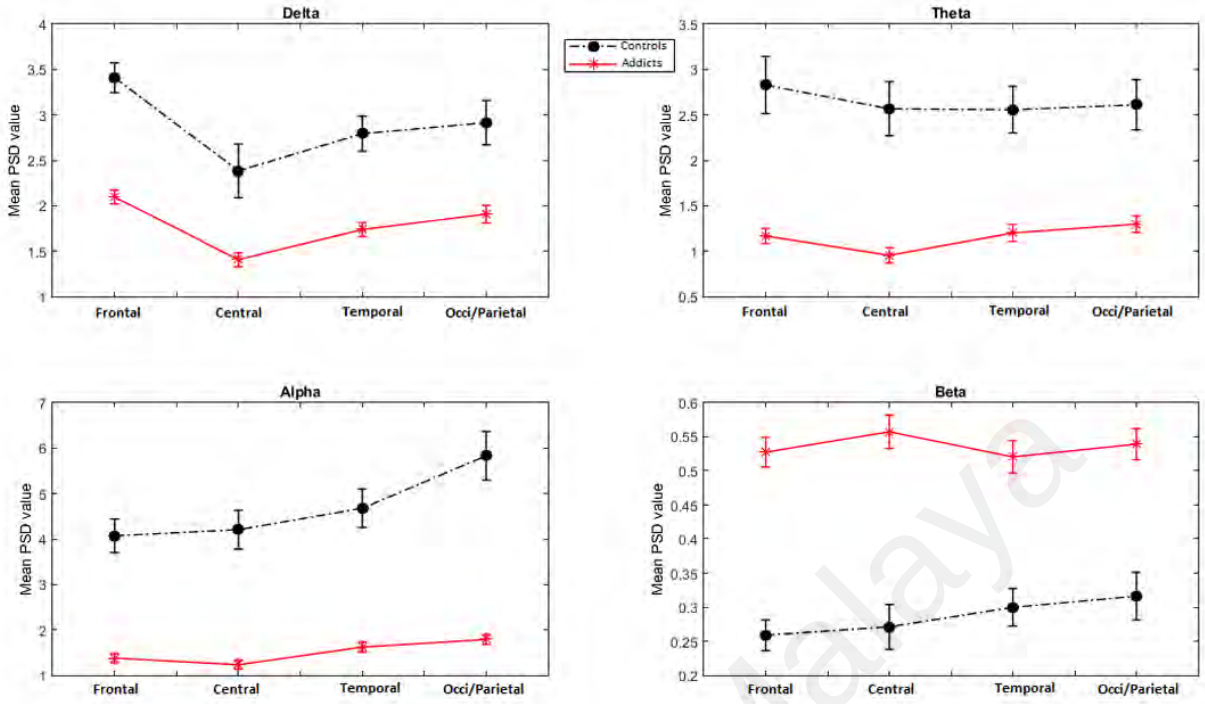


Figure 4.17: Error bars represents the differences of mean PSD values between two groups (Controls and Addicts) for all four locations.

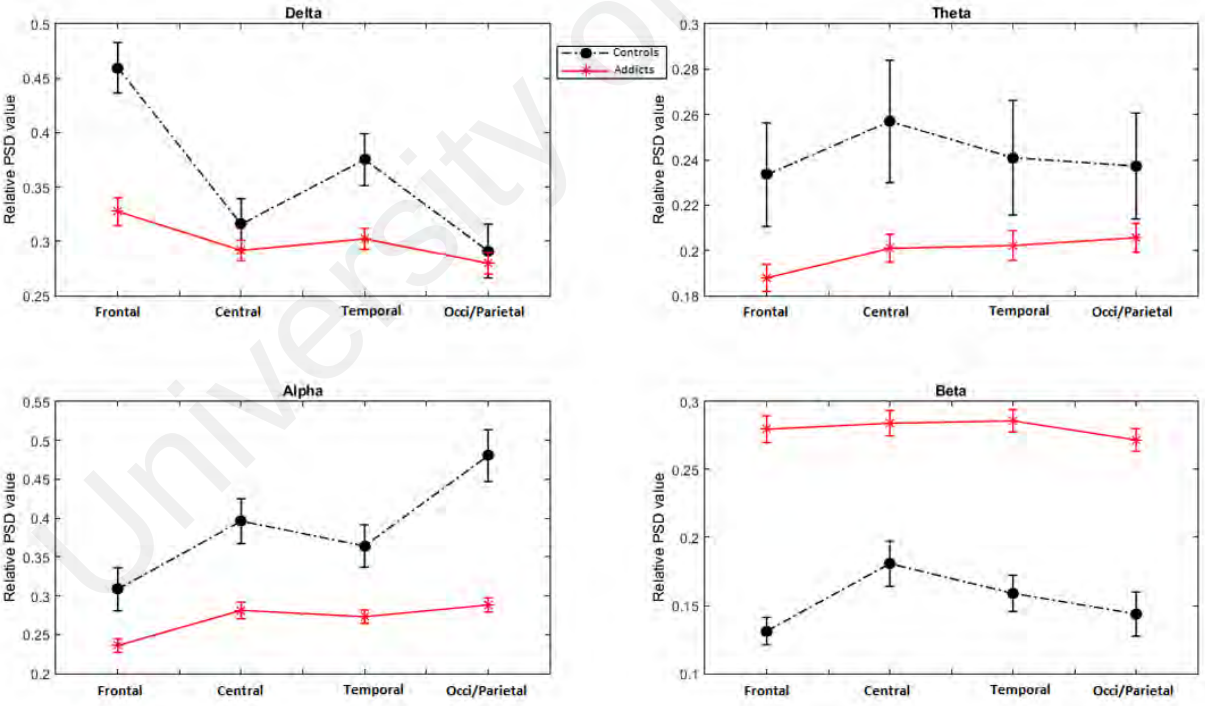


Figure 4.18: Error bars represents the differences of relative PSD values between two groups (Controls and Addicts) for all four locations.

4.7.2 P300

To analyze the P3 features, data were fitted into a repeated measure model while tasks were chosen as a within-subjects factor (Table 4.8). Results reveal that P3 amplitude of

addicts is lower compared to the healthy controls ($F_{(1,102)}$=417, p<0.001), while their latency is longer ($F_{(1,102)}$=1384, p<0.001). Results indicate that task type has a significant effect on between-group differences in P3 amplitude, and the post hoc analysis as it can be confirmed by the error bars in Figure 4.19, shows diminished P3 amplitude among the addicts. Also, the correlation was observed between the P3 features recorded from all tasks (r=0.53, p=0.0006). The latency of P3 (Figure 4.20) is prolonged among addicts compared to controls, and this latency is linearly correlated with the recording channels for all subjects.

Table 4.7: ANOVA model of baseline phase for P3 features. There are two groups of subjects and four tasks.

P300	F_statistics	P_value*	Task	Diff**	StdErr	P_value	Lower	Upper
AMPLITUDE								
Group	$F_{(1,102)}$=417.02	p< 0.001						
Group/Task	$F_{(3,306)}$=3.92	p< 0.01 Pgg< 0.01 Phf< 0.01 Plb< 0.06	Attention	-3.79	0.35	0.000	-4.48	-3.09
			Auditory	-2.82	0.36	0.000	-3.54	-2.10
			Recall	-3.48	0.33	0.000	-4.14	-2.82
			Visual	-2.26	0.28	0.000	-2.83	-1.69
LATENCY								
Group	$F_{(1,102)}$=1384	p< 0.001						
Group/Task	$F_{(3,306)}$=1.90	p< 0.2 Pgg< 0.2 Phf< 0.2 Plb< 0.2	Attention	0.221	0.012	0.000	0.198	0.244
			Auditory	0.233	0.012	0.000	0.209	0.258
			Recall	0.257	0.012	0.000	0.233	0.282
			Visual	0.224	0.012	0.000	0.200	0.248

* RANOVA computes three p-values using Greenhouse-Geisser, Huynh-Feldt, and Lower-bound corrections.
**Estimated difference between the means of addicts and control.

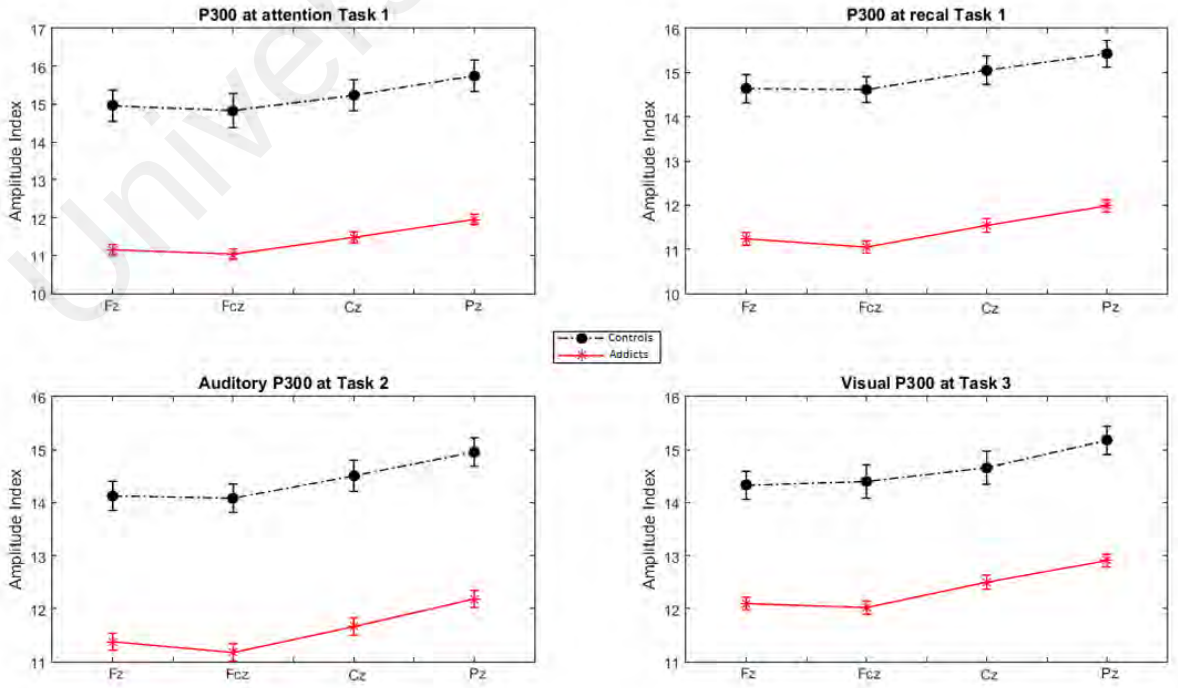


Figure 4.19: Comparison of error bars for P3 amplitude index of heroin addicts and healthy controls.

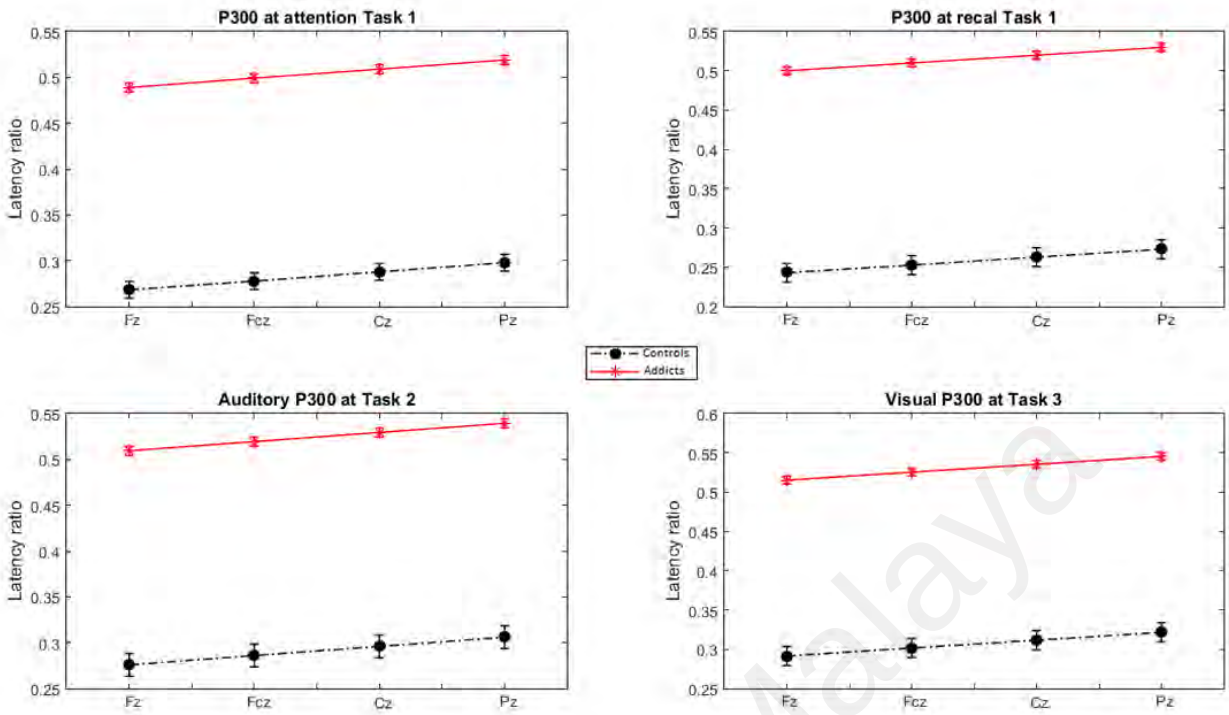


Figure 4.20: Comparison of error bars for P3 latency ratio of heroin addicts and healthy controls. Controls show lower latency for all locations and tasks.

4.7.3 Auditory MMN

MMN amplitude and latency for small and large deviant auditory stimuli were fitted into a repeated measure ANOVA model with subjects group as a between-subjects factor and stimuli types were chosen as a within-subjects factor (Table 4.9). Results show that there was a significant difference in between-subjects factor ($F_{(1,102)}$=76.21, p<0.001), and MMN amplitude was lower among addicts for both small and large deviant stimuli compared to the healthy subjects. The latency ratio of MMN components was also longer among addicts compared to the controls (p<0.0001). There was also a significant correlation between both MMN amplitudes (r=0.52, p=0.0007) and novel P3 amplitude extracted from this task (r=0.40, p=0.012).

Table 4.8: ANOVA model of baseline phase for MMN features. There are two groups of subjects, and two types of MMN.

MMN	F_statistics	P_value	Type	Diff	StdErr*	P_value	Lower	Upper
AMPLITUDE								
Group	$F_{(1,102)}$=76.21	p< 0.001						
Group/Task	$F_{(1,102)}$=6.62	p< 0.02	Large	-0.92	0.12	0.000	-1.156	-0.692
			Small	-0.51	0.11	0.000	-0.737	-0.292
LATENCY								
Group	$F_{(1,102)}$=987	p< 0.001						
Group/Task	$F_{(1,102)}$=3.74	p< 0.06	Large	0.10	0.005	0.000	0.094	0.112
			Small	0.09	0.005	0.000	0.079	0.098

*Estimated difference between the means of addicts and control.

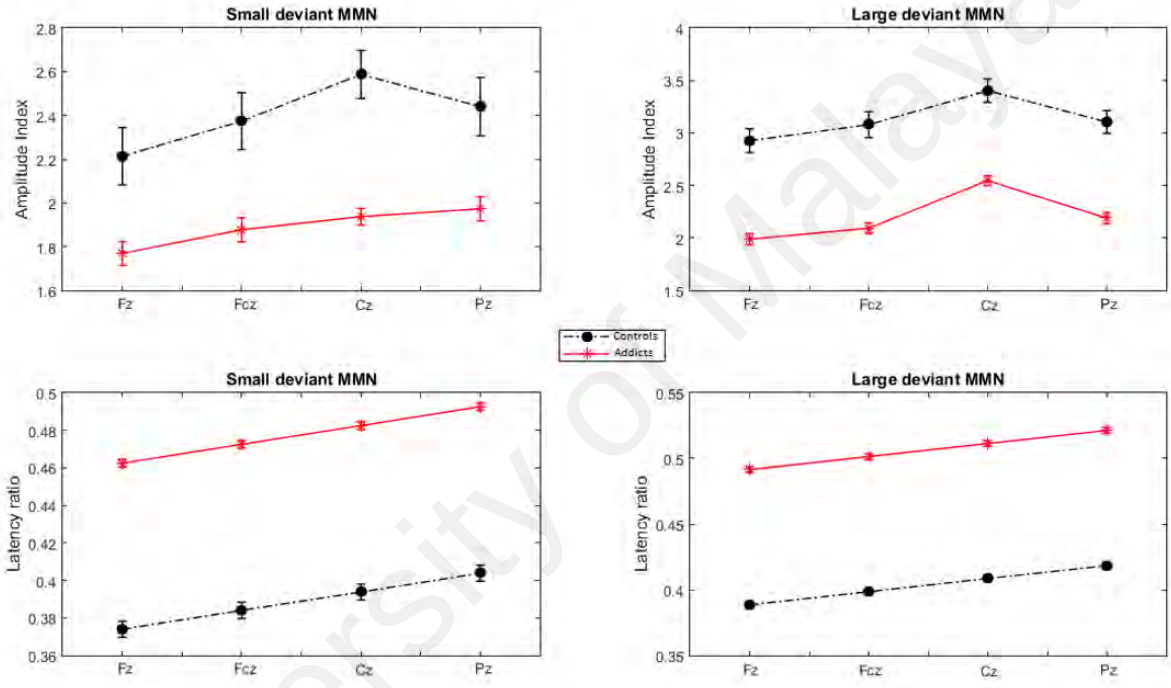


Figure 4.21: Comparison of error bars for MMN features of heroin addicts and healthy controls.

4.7.4 P600

ANOVA analysis of the P600 features (Table 4.10) shows significant differences between two groups ($F_{(1,102)}$=138, p<0.001), and follow up analysis of within factor shows higher amplitude and lower latency of the P600 component among the healthy controls compared to the addicts (Figure 4.22). There was also a significant correlation between the digit-span score of subjects and P600 amplitude (r=0.47, p=0.002), as well as its latency ratio (r=-0.66, p<0.0001). Moreover, a significant correlation was observed

between the P600 and the P3 recorded at both attention (r=0.65, p<0.0001), and recall phases (r=0.66, p<0.0001).

Table 4.9: ANOVA model of baseline phase for P600 features. There are two groups of subjects, and two types of features.

P600	F_statistics	P_value	Feature	Diff	StdErr*	P_value	Lower	Upper
Group	$F_{(1,102)}$=138	p< 0.001	0.000	0.000	0.000	0.000		
Group/Task	$F_{(1,102)}$=176	p< 0.001	Amplitude	-3.479	0.278	0.000	-4.030	-2.929
			Latency	0.204	0.009	0.000	0.185	0.222

*Estimated difference between the means of addicts and control.

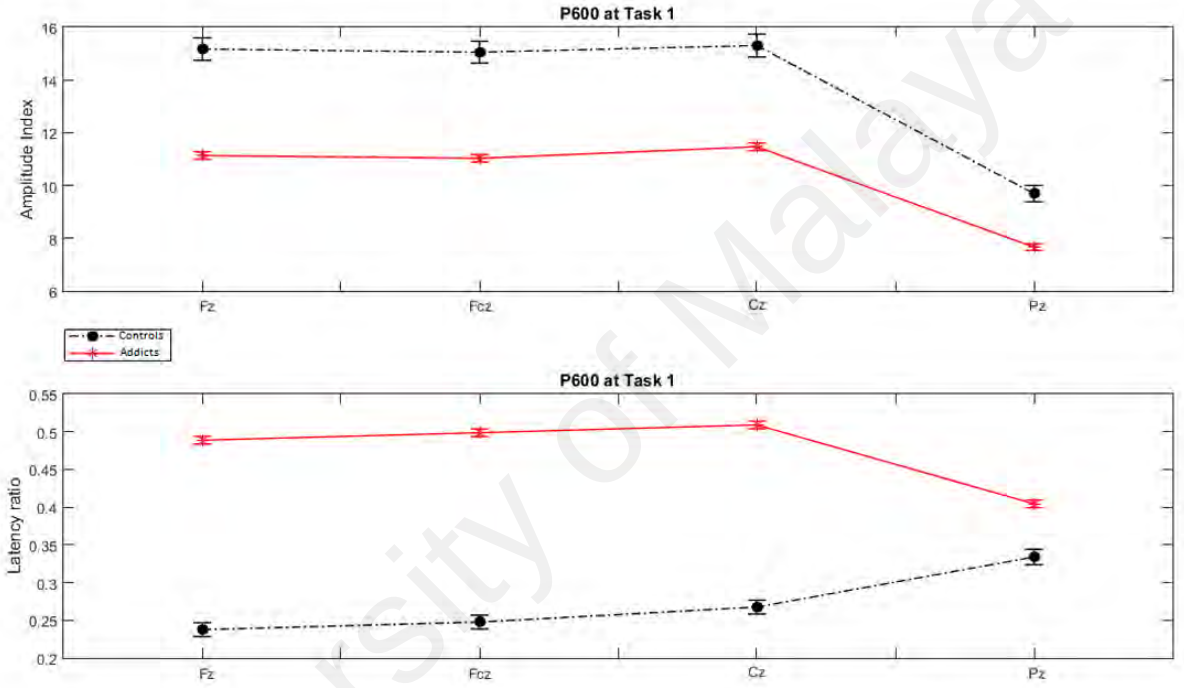


Figure 4.22: Comparison of error bars for P600 features of heroin addicts and healthy controls.

4.8 First Month Interval

As it explained earlier, 96 heroin addicts of baseline stage were randomly divided into two groups of sham (n=48) and EA (n=48) groups. By the end of the first month of their treatment regime, 25 number of subjects quite the program and 11 number of them were eliminated from the analysis based on the exclusion criteria. 28 subjects of sham group and 32 of EA group remained, and their electrophysiological measurements were used to investigate the short-term effects of the treatment regime. In addition, for the better elaboration of results investigation, the intergroup differences of heroin addicts were

analyzed as well. Based on the baseline analysis age of subjects used as a factor in repeated measurements of EEG properties at different intervals. The results of the detailed statistical analysis are depicted in Appendix D.

4.8.1 PSD of Resting-state EEG

To explore the brain electro-neurophysiological alterations during the four weeks of MMT with real and sham EA, data were fitted into a factorial repeated measure ANOVA model (Table 4.11 and Table 4.12). For delta band, there was no significant effect of factors for both mean and relative PSD values. However, the post hoc analysis (Table 4.11) shows the significant increase of mean PSD value at the frontal location for both treatment groups. Also, relative PSD of delta band was increased significantly for both groups at all head locations.

For theta band, the analysis did not reveal any significant effect of factors or their interaction while the post hoc analysis shows a significant increase of theta activity for both groups at all locations. For the alpha band, the effect of group×time ($F_{(1,56)}$=10.29, p-value<0.01) was significant for mean PSD values, and post hoc analysis showed that increase of alpha activity is significantly more for EA group compared to sham group. However, the increase of mean PSD of alpha was more significant at central, frontal and occi-parietal locations for both groups. Furthermore, relative PSD value of alpha band increased significantly at all head locations regardless of treatment group. For beta band, while the mean PSD were significantly decreased for both groups at all sites, group×time ($F_{(1,56)}$=5.62, p-value<0.05) had a significant effect on PSD changes. It is noteworthy that age interaction with group and time was also significant for both alpha and beta bands activity.

Table 4.10: Repeated measure ANOVA for mean PSD values during resting time.The post hoc analysis is computed for both group and location factors of each frequency bands, and estimated difference is calculated between the means of first and second intervals.

Mean PSD	F_statistics	P_value	P_GG	P_HF	P_LB	Post hoc Factor	Diff	StdErr	P_value	Lower	Upper
DELTA											
Intercept/Time	$F_{(1,56)}$=0.00	0.947									
Group/Time	$F_{(1,56)}$=0.71	0.403				Acupuncture	-0.16	0.13	0.214	-0.43	0.10
Age/Time	$F_{(1,56)}$=0.10	0.753				Sham	-0.19	0.14	0.195	-0.47	0.10
Group/Age/Time	$F_{(1,56)}$=0.76	0.386									
Intercept/Time/Location	$F_{(3,168)}$=0.34	0.798	0.725	0.733	0.563	Central	-0.14	0.10	0.139	-0.33	0.05
Group/Time/Location	$F_{(3,168)}$=0.75	0.521	0.479	0.484	0.389	Frontal	-0.31	0.15	0.042	-0.60	-0.01
Age/Time/Location	$F_{(3,168)}$=0.30	0.828	0.755	0.764	0.588	Occi-parietal	-0.12	0.17	0.499	-0.46	0.23
Group/Age/Time/Location	$F_{(3,168)}$=0.54	0.656	0.594	0.601	0.466	Temporal	-0.14	0.12	0.273	-0.38	0.11
THETA											
Intercept/Time	$F_{(1,56)}$=0.67	0.416									
Group/Time	$F_{(1,56)}$=2.37	0.129				Acupuncture	-0.49	0.15	0.002	-0.79	-0.19
Age/Time	$F_{(1,56)}$=0.00	0.999				Sham	-0.37	0.16	0.026	-0.70	-0.05
Group/Age/Time	$F_{(1,56)}$=2.06	0.157									
Intercept/Time/Location	$F_{(3,168)}$=0.45	0.717	0.655	0.663	0.505	Central	-0.40	0.11	0.001	-0.63	-0.17
Group/Time/Location	$F_{(3,168)}$=0.14	0.935	0.884	0.891	0.708	Frontal	-0.52	0.12	0.000	-0.77	-0.28
Age/Time/Location	$F_{(3,168)}$=0.79	0.501	0.465	0.470	0.378	Occi-parietal	-0.45	0.13	0.001	-0.72	-0.19
Group/Age/Time/Location	$F_{(3,168)}$=0.33	0.802	0.735	0.744	0.566	Temporal	-0.36	0.12	0.004	-0.60	-0.12
ALPHA											
Intercept/Time	$F_{(1,56)}$=1.80	0.186									
Group/Time	$F_{(1,56)}$=10.29	0.002				Acupuncture	-0.49	0.17	0.005	-0.83	-0.16
Age/Time	$F_{(1,56)}$=0.58	0.451				Sham	-0.21	0.18	0.265	-0.57	0.16
Group/Age/Time	$F_{(1,56)}$=8.94	0.004									
Intercept/Time/Location	$F_{(3,168)}$=0.06	0.983	0.966	0.970	0.815	Central	-0.38	0.14	0.007	-0.65	-0.11
Group/Time/Location	$F_{(3,168)}$=0.11	0.952	0.922	0.929	0.737	Frontal	-0.45	0.15	0.004	-0.76	-0.15
Age/Time/Location	$F_{(3,168)}$=0.05	0.983	0.966	0.971	0.816	Occi-parietal	-0.34	0.15	0.032	-0.64	-0.03
Group/Age/Time/Location	$F_{(3,168)}$=0.02	0.997	0.991	0.993	0.896	Temporal	-0.23	0.14	0.107	-0.51	0.05
BETA											
Intercept/Time	$F_{(1,56)}$=3.46	0.068									
Group/Time	$F_{(1,56)}$=5.64	0.021				Acupuncture	0.19	0.05	0.001	0.08	0.29
Age/Time	$F_{(1,56)}$=0.29	0.590				Sham	0.28	0.05	0.000	0.18	0.39
Group/Age/Time	$F_{(1,56)}$=4.47	0.039									
Intercept/Time/Location	$F_{(3,168)}$=0.26	0.857	0.805	0.814	0.614	Central	0.28	0.04	0.000	0.19	0.37
Group/Time/Location	$F_{(3,168)}$=0.91	0.439	0.419	0.422	0.345	Frontal	0.20	0.04	0.000	0.12	0.29
Age/Time/Location	$F_{(3,168)}$=0.44	0.727	0.676	0.685	0.511	Occi-parietal	0.24	0.05	0.000	0.15	0.34
Group/Age/Time/Location	$F_{(3,168)}$=0.64	0.591	0.552	0.558	0.428	Temporal	0.21	0.05	0.000	0.11	0.31

Table 4.11: Repeated measure ANOVA for relative PSD values during resting time.The post hoc analysis is computed for both group and location factors of each frequency bands, and estimated difference is calculated between the means of first and second intervals.

Relative PSD	F_statistics	P_value	P_GG	P_HF	P_LB	Post hoc Factor	Diff	StdErr	P_value	Lower	Upper
DELTA											
Intercept/Time	$F_{(1,56)}$=0.11	0.738									
Group/Time	$F_{(1,56)}$=2.27	0.138				Acupuncture	-0.05	0.02	0.022	-0.09	-0.01
Age/Time	$F_{(1,56)}$=0.10	0.752				Sham	-0.05	0.02	0.044	-0.09	0.00
Group/Age/Time	$F_{(1,56)}$=2.26	0.139									
Intercept/Time/Location	$F_{(3,168)}$=0.81	0.492	0.468	0.473	0.373	Central	-0.05	0.02	0.009	-0.08	-0.01
Group/Time/Location	$F_{(3,168)}$=1.15	0.331	0.326	0.327	0.288	Frontal	-0.05	0.02	0.022	-0.10	-0.01
Age/Time/Location	$F_{(3,168)}$=0.79	0.502	0.477	0.482	0.378	Occi-parietal	-0.04	0.02	0.016	-0.07	-0.01
Group/Age/Time/Location	$F_{(3,168)}$=1.00	0.396	0.384	0.387	0.323	Temporal	-0.05	0.02	0.008	-0.09	-0.01
THETA											
	F_statistics	**P_value**	**P_GG**	**P_HF**	**P_LB**	**Post hoc Factor**	**Diff**	**StdErr**	**P_value**	**Lower**	**Upper**
Intercept/Time	$F_{(1,56)}$=0.01	0.941									
Group/Time	$F_{(1,56)}$=0.19	0.662				Acupuncture	-0.02	0.01	0.062	-0.04	0.00
Age/Time	$F_{(1,56)}$=0.60	0.442				Sham	-0.04	0.01	0.000	-0.06	-0.02
Group/Age/Time	$F_{(1,56)}$=0.56	0.457									
Intercept/Time/Location	$F_{(3,168)}$=0.22	0.879	0.848	0.858	0.638	Central	-0.03	0.01	0.002	-0.05	-0.01
Group/Time/Location	$F_{(3,168)}$=0.66	0.576	0.552	0.559	0.419	Frontal	-0.03	0.01	0.000	-0.05	-0.02
Age/Time/Location	$F_{(3,168)}$=0.27	0.846	0.814	0.824	0.605	Occi-parietal	-0.04	0.01	0.000	-0.06	-0.02
Group/Age/Time/Location	$F_{(3,168)}$=0.92	0.434	0.422	0.426	0.343	Temporal	-0.02	0.01	0.014	-0.04	0.00
ALPHA											
	F_statistics	**P_value**	**P_GG**	**P_HF**	**P_LB**	**Post hoc Factor**	**Diff**	**StdErr**	**P_value**	**Lower**	**Upper**
Intercept/Time	$F_{(1,56)}$=0.00	0.972									
Group/Time	$F_{(1,56)}$=3.07	0.085				Acupuncture	-0.04	0.02	0.025	-0.07	-0.01
Age/Time	$F_{(1,56)}$=0.49	0.485				Sham	-0.04	0.02	0.033	-0.08	0.00
Group/Age/Time	$F_{(1,56)}$=3.16	0.081									
Intercept/Time/Location	$F_{(3,168)}$=3.76	0.012	0.015	0.013	0.058	Central	-0.03	0.01	0.040	-0.06	0.00
Group/Time/Location	$F_{(3,168)}$=1.07	0.364	0.360	0.362	0.305	Frontal	-0.04	0.02	0.006	-0.07	-0.01
Age/Time/Location	$F_{(3,168)}$=3.75	0.012	0.015	0.013	0.058	Occi-parietal	-0.05	0.02	0.005	-0.08	-0.01
Group/Age/Time/Location	$F_{(3,168)}$=1.19	0.316	0.315	0.316	0.281	Temporal	-0.04	0.01	0.003	-0.07	-0.01
BETA											
	F_statistics	**P_value**	**P_GG**	**P_HF**	**P_LB**	**Post hoc Factor**	**Diff**	**StdErr**	**P_value**	**Lower**	**Upper**
Intercept/Time	$F_{(1,56)}$=1.76	0.190									
Group/Time	$F_{(1,56)}$=4.08	0.048				Acupuncture	0.08	0.01	0.000	0.05	0.10
Age/Time	$F_{(1,56)}$=0.57	0.452				Sham	0.09	0.01	0.000	0.06	0.11
Group/Age/Time	$F_{(1,56)}$=4.83	0.032									
Intercept/Time/Location	$F_{(3,168)}$=2.95	0.034	0.038	0.035	0.091	Central	0.08	0.01	0.000	0.05	0.10
Group/Time/Location	$F_{(3,168)}$=0.06	0.982	0.978	0.981	0.813	Frontal	0.10	0.01	0.000	0.08	0.13
Age/Time/Location	$F_{(3,168)}$=4.16	0.007	0.009	0.007	0.046	Occi-parietal	0.07	0.01	0.000	0.05	0.09
Group/Age/Time/Location	$F_{(3,168)}$=0.22	0.880	0.867	0.877	0.638	Temporal	0.07	0.01	0.000	0.05	0.09

4.8.2 P300

Statistical analysis of P3 amplitude revealed that interaction of factors with time was only significant for intercept×time ($F_{(1,56)}$=13.60, p-value<0.0001). The post hoc analysis (Table 4.13) and Figure 4.23 show that the P3 amplitude was enhanced for all the subjects of both groups for all tasks without any significant differences between treatment groups. Moreover, analysis of P3 latency ratio shows that there are no major differences within one month of therapy for either of groups.

Table 4.12: Repeated measure ANOVA for P3 amplitude and latency.The post hoc analysis is done for both group and tasks, while estimated difference is calculated between the means of first and second intervals.

P300	F_statistics	P_value	P_GG	P_HF	P_LB	Post hoc Factor	Diff	StdErr	P_value	Lower	Upper
AMPLITUDE											
Intercept/Time	$F_{(1,56)}$=302.68	0.000				Acupuncture	-0.59	0.01	0.000	-0.60	-0.57
Group/Time	$F_{(1,56)}$=1.22	0.275				Sham	-0.52	0.01	0.000	-0.54	-0.50
Age/Time	$F_{(1,56)}$=0.06	0.814									
Group/Age/Time	$F_{(1,56)}$=0.00	0.985									
Intercept/Time/Task	$F_{(3,168)}$=0.11	0.955	0.948	0.954	0.743	Attention	-0.58	0.02	0.000	-0.61	-0.54
Group/Time/Task	$F_{(3,168)}$=0.69	0.560	0.551	0.559	0.410	Auditory	-0.53	0.01	0.000	-0.56	-0.51
Age/Time/Task	$F_{(3,168)}$=0.06	0.981	0.977	0.980	0.808	Recall	-0.55	0.01	0.000	-0.58	-0.52
Group/Age/Time/Task	$F_{(3,168)}$=0.71	0.549	0.540	0.548	0.404	Visual	-0.54	0.01	0.000	-0.56	-0.52
LATENCY											
Intercept/Time	$F_{(1,56)}$=2.59	0.113				Acupuncture	0.00	0.00	0.718	0.00	0.00
Group/Time	$F_{(1,56)}$=0.19	0.667				Sham	0.00	0.00	0.161	0.00	0.00
Age/Time	$F_{(1,56)}$=1.82	0.182									
Group/Age/Time	$F_{(1,56)}$=0.37	0.548									
Intercept/Time/Task	$F_{(3,168)}$=0.36	0.784	0.764	0.775	0.553	Attention	0.00	0.00	0.750	0.00	0.00
Group/Time/Task	$F_{(3,168)}$=0.56	0.642	0.625	0.634	0.457	Auditory	0.00	0.00	0.677	0.00	0.00
Age/Time/Task	$F_{(3,168)}$=0.44	0.725	0.705	0.716	0.510	Recall	0.00	0.00	0.426	0.00	0.00
Group/Age/Time/Task	$F_{(3,168)}$=0.56	0.643	0.625	0.635	0.458	Visual	0.00	0.00	0.206	0.00	0.00

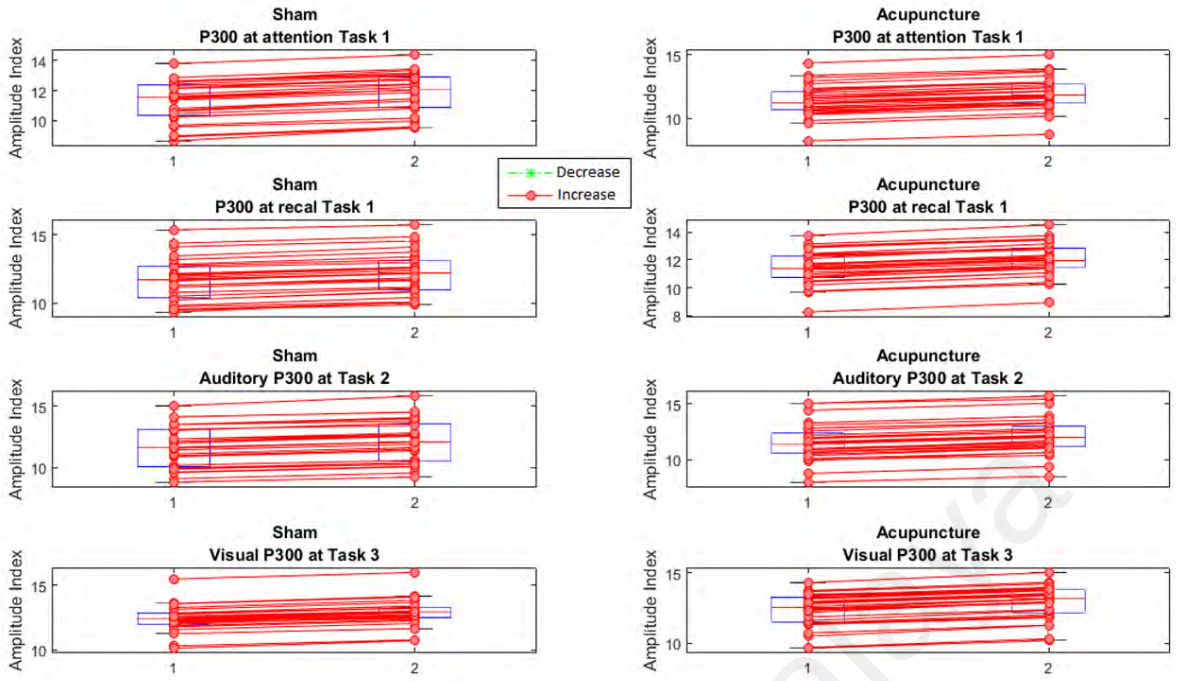


Figure 4.23: Strip-charts shows the paired observation of P3 amplitude index changes for all tasks separately for both study groups.

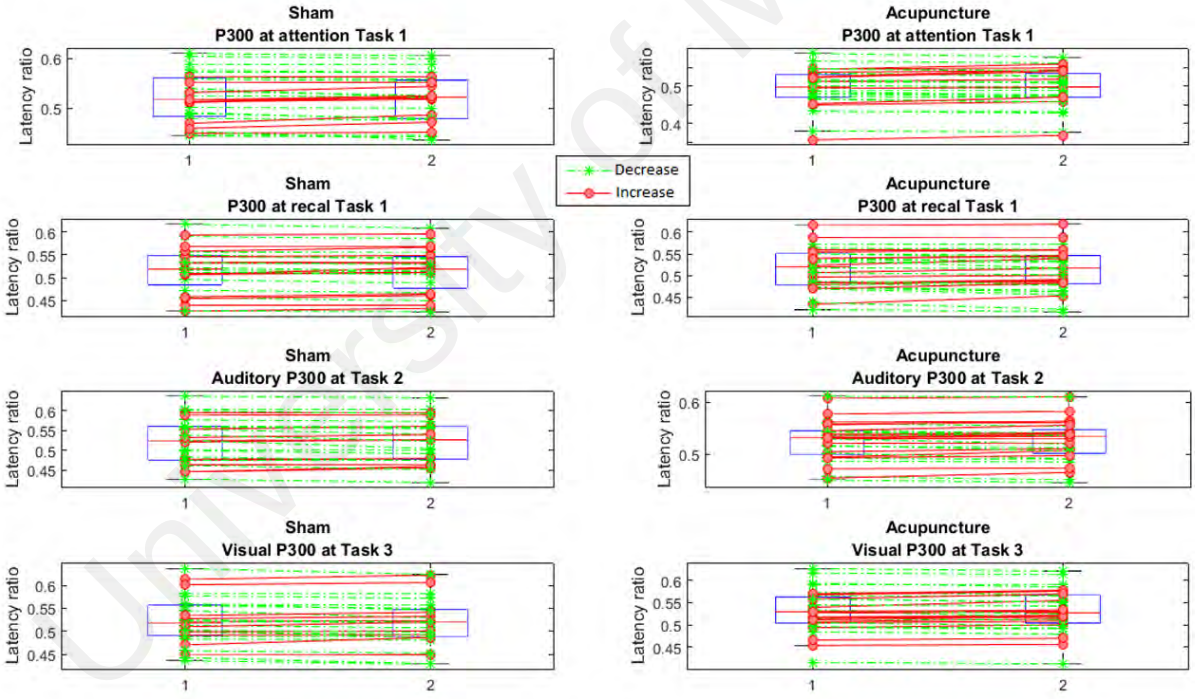


Figure 4.24: Strip-charts shows the paired observation of P3 latency ratio changes for all tasks separately for both study groups.

4.8.3 Auditory MMN

For the alteration of MMN properties, the results of repeated measure ANOVA is depicted in Table 4.14, and the strip-charts of changes for all subjects of each group is presented in below figures. The results show the significant effect of intercept×time factor

($F_{(1,56)}$=477, $p_{\text{-value}}$<0.0001) for MMN amplitude which further analysis, as well as the Figure 4.25, shows the enhancement of MMN amplitude for both small and large deviant stimuli regardless of treatment group. Although no interaction of factors with time have found to be significant for the latency ratio, strip-charts of Figure 4.26 shows that most of the subjects have an enhancement in their MMN latency ratio for the small deviant stimuli.

Table 4.13: Repeated measure ANOVA for MMN amplitude and latency.The post hoc analysis is done for both groups, while estimated difference is calculated between the means of first and second intervals.

MMN	F_statistics	P_value	Post hoc Factor	Diff	StdErr	P_value	Lower	Upper
AMPLITUDE								
Intercept/Time	$F_{(1,56)}$=477.69	0.000	Acupuncture	-0.10	0.00	0.000	-0.10	-0.10
Group/Time	$F_{(1,56)}$=0.09	0.760	Sham	-0.10	0.00	0.000	-0.11	-0.10
Age/Time	$F_{(1,56)}$=0.06	0.811						
Group/Age/Time	$F_{(1,56)}$=0.45	0.504						
Intercept/Time/Task	$F_{(1,56)}$=3.97	0.051						
Group/Time/Task	$F_{(1,56)}$=0.01	0.914						
Age/Time/Task	$F_{(1,56)}$=0.48	0.490	Large	-0.11	0.00	0.000	-0.11	-0.11
Group/Age/Time/Task	$F_{(1,56)}$=0.08	0.778	Small	-0.09	0.00	0.000	-0.09	-0.09
LATENCY								
Intercept/Time	$F_{(1,56)}$=1.93	0.170	Acupuncture	0.00	0.00	0.271	0.00	0.00
Group/Time	$F_{(1,56)}$=0.03	0.872	Sham	0.00	0.00	0.006	0.00	0.00
Age/Time	$F_{(1,56)}$=1.24	0.270						
Group/Age/Time	$F_{(1,56)}$=0.59	0.447						
Intercept/Time/Task	$F_{(1,56)}$=2.74	0.103						
Group/Time/Task	$F_{(1,56)}$=1.21	0.277						
Age/Time/Task	$F_{(1,56)}$=2.41	0.126	Large	0.00	0.00	0.666	0.00	0.00
Group/Age/Time/Task	$F_{(1,56)}$=1.94	0.169	Small	0.00	0.00	0.166	0.00	0.00

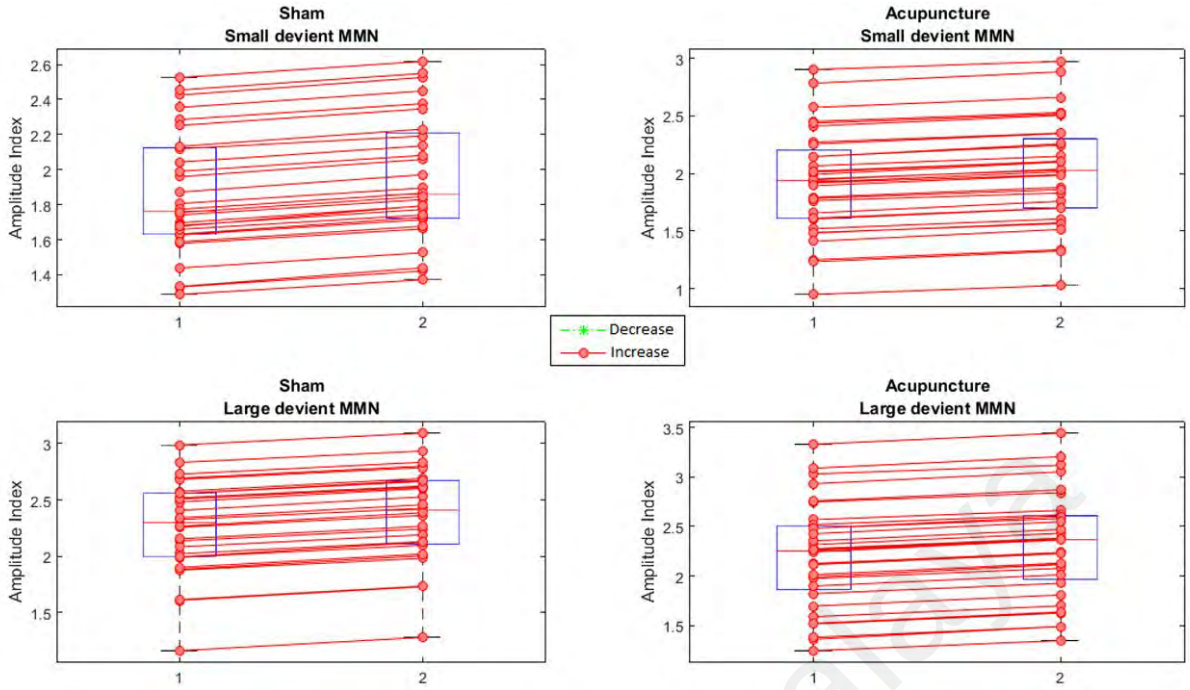


Figure 4.25: Strip-charts shows the paired observation of MMN amplitude index changes for small and large deviant stimuli.

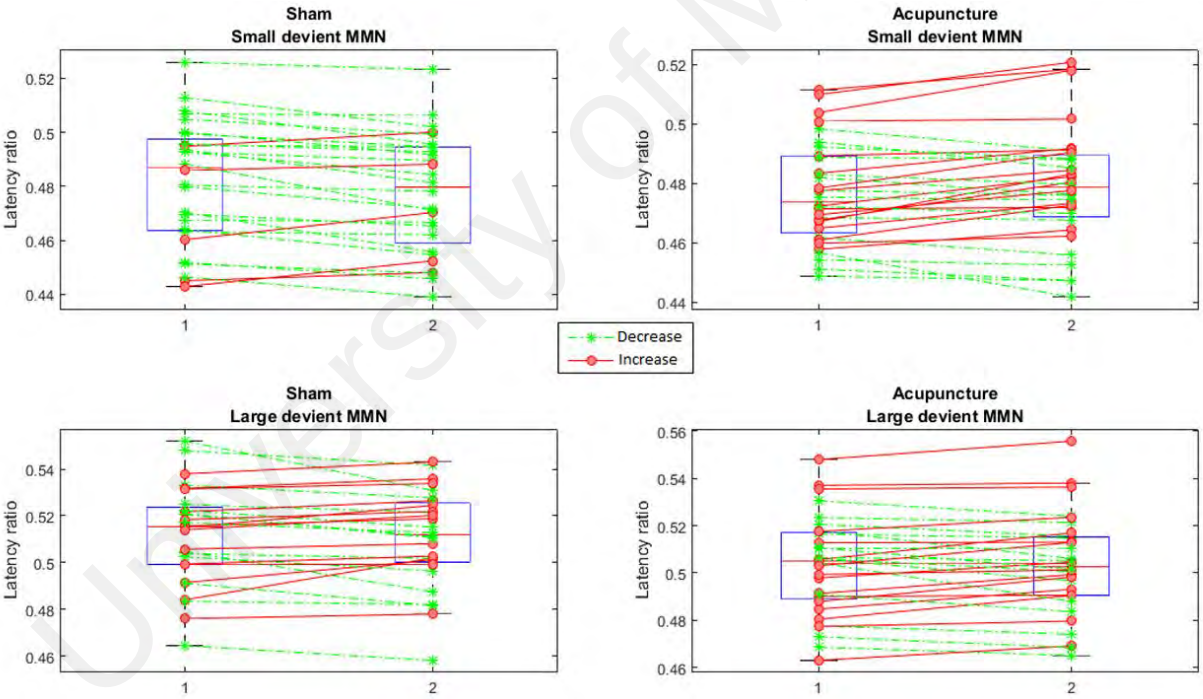


Figure 4.26: Strip-charts shows the paired observation of MMN Latency ratio changes for small and large deviant stimuli.

4.8.4 P600

P600 features were fitted into a repeated measure ANOVA model (Table 4.15), and the strip-charts of paired observations are shown for all participants of both treatment

groups (Figure 4.27 and Figure 4.28). The results of the analysis in line with the strip-chart of paired observation of the subjects shows the enhancement of P600 amplitude for both groups. Although as it shown in Figure 4.28, latency ratio of more subjects in sham group was found to be decreased compared to EA group, statistical analysis shows no significant changes in latency ratio of both groups.

Table 4.14: Repeated measure ANOVA for P600amplitide and latency.The post hoc analysis is done for both groups, while estimated difference is calculated between the means of first and second intervals.

P600	F_statistics	P_value	Post hoc Factor	Diff	StdErr	P_value	Lower	Upper
Intercept/Time	$F_{(1,56)}$=60.83	0.000	Acupuncture	-0.30	0.01	0.000	-0.32	-0.28
Group/Time	$F_{(1,56)}$=1.55	0.218	Sham	-0.23	0.01	0.000	-0.25	-0.21
Age/Time	$F_{(1,56)}$=0.11	0.741						
Group/Age/Time	$F_{(1,56)}$=0.02	0.882						
Intercept/Time/Type	$F_{(1,56)}$=68.52	0.000						
Group/Time/ Type	$F_{(1,56)}$=1.23	0.273						
Age/Time/ Type	$F_{(1,56)}$=0.00	1.000	Latency	0.00	0.00	0.927	0.00	0.00
Group/Age/Time/ Type	$F_{(1,56)}$=0.00	0.986	Amplitude	-0.53	0.01	0.000	-0.56	-0.50

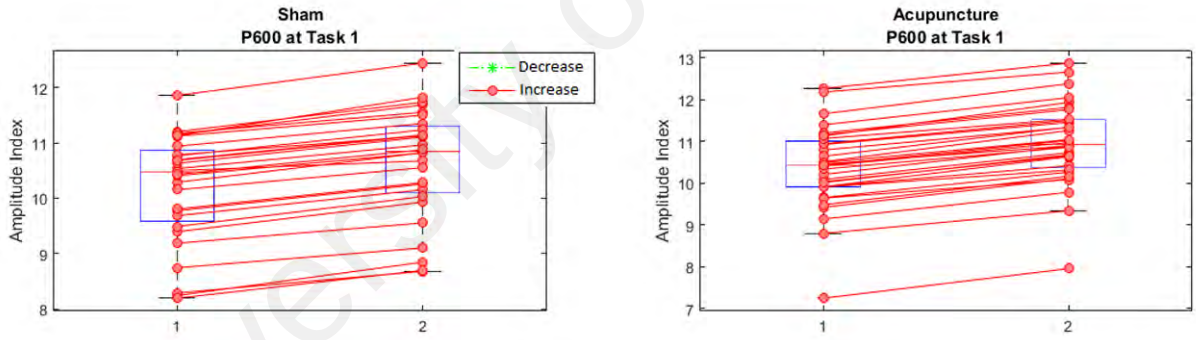


Figure 4.27: Strip-charts shows the paired observation of P600 amplitude index changes for both groups.

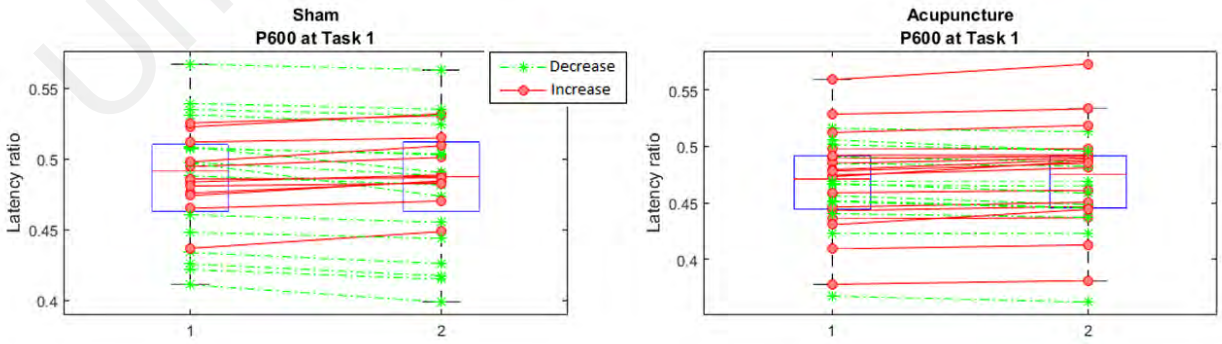


Figure 4.28: Strip-charts shows the paired observation of P600 latency ratio changes for both groups.

4.9 Three months intervals

By the end of the third month of treatment, 23 subjects of sham group and 21 of EA group remained, and their monthly measurements were used to investigate the effects of treatment regime on EEG spectrum and ERP components. However, since the remaining subjects' age was not significantly different from the intergroup factor (Table 4.5) the measurement of each EEG property was fitted into a repeated measure ANOVA model, and the results are presented in following sections. In addition, the detailed statistical analysis results are shown in Appendix E as well as the strip-charts of observations for all intervals.

4.9.1 PSD of Resting-state EEG

4.9.1.1 Delta

For delta band, time factor has a significant effect for both mean ($F_{(3,126)}$=6.28, p<0.01), and relative ($F_{(3,126)}$=5.54, p<0.05) PSD values among all subjects with no significant group differences. Therefore, the post hoc analysis was performed based on time and location factors (Table 4.16). It is worth mentioning that only the significant differences are reflected in Table 4.16. The post hoc analysis and paired observations are shown in Appendix E. In addition, the post hoc analysis for location factor shows that increase of PSD values from baseline is more significant at frontal and temporal locations.

Table 4.15: Repeated measure ANOVA for delta mean and relative PSD values.

Mean PSD							Relative PSD				
Delta		**F_statistics**	**P_value**	**P_GG**	**P_HF**	**P_LB**	**F_statistics**	**P_value**	**P_GG**	**P_HF**	**P_LB**
Intercept/Time		$F_{(3,126)}$=6.28	0.001	0.001	0.001	0.016	$F_{(3,126)}$=5.54	0.001	0.002	0.002	0.023
Group/Time		$F_{(3,126)}$=0.79	0.499	0.487	0.494	0.378	$F_{(3,126)}$=1.05	0.373	0.367	0.370	0.312
Intercept/Location/Time		$F_{(9,378)}$=0.90	0.525	0.503	0.517	0.348	$F_{(9,378)}$=3.11	0.001	0.008	0.005	0.085
Group/Location/Time		$F_{(9,378)}$=1.21	0.287	0.298	0.292	0.278	$F_{(9,378)}$=0.67	0.735	0.656	0.679	0.417
Location	**Interval A**	**Interval B**	**Diff**	**StdEr**	**P_value**	**Location**	**Interval A**	**Interval B**	**Diff**	**StdErr**	**P_value**
Frontal	Baseline	Second	-0.58	0.16	0.00	Frontal	Baseline	First	-0.08	0.03	0.02
Frontal	Baseline	Third	-0.54	0.16	0.01	Frontal	Baseline	Third	-0.12	0.03	0.00
Temporal	Baseline	Second	-0.40	0.13	0.02	Temporal	Baseline	Third	-0.07	0.02	0.00
Temporal	Baseline	Third	-0.45	0.13	0.01						

4.9.1.2 Theta

The repeated measure analysis for theta band, shows a significant effect of time for both mean ($F_{(3,126)}$=11.55, p<0.001), and relative ($F_{(3,126)}$=9.88, p<0.001) PSD values with no significant difference for group factor. Therefore, the post hoc analysis was performed based on time and location factors (Table 4.17). It is worth mentioning that only the significant differences are reflected in Table 4.17 for the post hoc analysis and the detailed results of the statistical analysis are shown in Appendix E. In addition, the post hoc analysis for location factor shows that increase of PSD values from baseline is significant at all locations.

Table 4.16: Table shows the repeated measure ANOVA results for mean and relative PSD values of theta band activity.

Mean PSD							Relative PSD				
Theta		**F_statistics**	**P_value**	**P_GG**	**P_HF**	**P_LB**	**F_statistics**	**P_value**	**P_GG**	**P_HF**	**P_LB**
Intercept/Time		$F_{(3,126)}$=11.55	0.000	0.000	0.000	0.001	$F_{(3,126)}$=9.88	0.000	0.000	0.000	0.003
Group/Time		$F_{(3,126)}$=0.90	0.442	0.436	0.442	0.348	$F_{(3,126)}$=1.08	0.362	0.357	0.359	0.306
Intercept/Location/Time		$F_{(9,378)}$=2.09	0.030	0.066	0.056	0.156	$F_{(9,378)}$=2.09	0.030	0.066	0.056	0.156
Group/Location/Time		$F_{(9,378)}$=1.02	0.420	0.406	0.410	0.317	$F_{(9,378)}$=1.02	0.420	0.406	0.410	0.317
Location	**Interval A**	**Interval B**	**Diff**	**StdErr**	**P_value**	**Location**	**Interval A**	**Interval B**	**Diff**	**StdErr**	**P_value**
Central	Baseline	Second	-0.74	0.12	0.00	Central	Baseline	First	-0.03	0.01	0.01
Central	Baseline	Third	-0.77	0.16	0.00	Central	Baseline	Third	-0.06	0.01	0.00
Central	First	Second	-0.50	0.15	0.01	Frontal	Baseline	First	-0.03	0.01	0.00
Central	First	Third	-0.53	0.18	0.02	Frontal	Baseline	Third	-0.07	0.01	0.00
Frontal	Baseline	First	-0.34	0.11	0.02	Frontal	First	Third	-0.03	0.01	0.04
Frontal	Baseline	Second	-0.53	0.14	0.00	Frontal	Second	Third	-0.04	0.01	0.05
Frontal	Baseline	Third	-0.71	0.16	0.00	Occi-Par	Baseline	First	-0.04	0.01	0.01
Frontal	First	Third	-0.37	0.14	0.05	Occi-Par	Baseline	Third	-0.05	0.01	0.00
Occi-Par	Baseline	Second	-0.41	0.13	0.01	Tempora	Baseline	Third	-0.05	0.01	0.00
Occi-Par	Baseline	Third	-0.63	0.16	0.00						
Temporal	Baseline	Second	-0.39	0.14	0.03						
Temporal	Baseline	Third	-0.60	0.12	0.00						
Temporal	First	Third	-0.34	0.12	0.03						

4.9.1.3 Alpha

For alpha band also mean ($F_{(3,126)}$=10.21, p<0.001) and relative ($F_{(3,126)}$=11.65, p<0.001) PSD changes were significant for time factor while group factor was not significant. Post hoc analysis of location factor (Table 4.18) shows that PSD increases from baseline stage significantly at all locations for both groups.

Table 4.17: Table shows the repeated measure ANOVA results for mean and relative PSD values of alpha band activity.

Mean PSD							Relative PSD				
Alpha		**F_statistics**	**P_value**	**P_GG**	**P_HF**	**P_LB**	**F_statistics**	**P_value**	**P_GG**	**P_HF**	**P_LB**
Intercept/Time		$F_{(3,126)}$=10.21	0.000	0.000	0.000	0.003	$F_{(3,126)}$=11.65	0.000	0.000	0.000	0.001
Group/Time		$F_{(3,126)}$=1.19	0.317	0.314	0.315	0.282	$F_{(3,126)}$=0.35	0.789	0.773	0.788	0.557
Intercept/Location/Time		$F_{(9,378)}$=4.00	0.000	0.001	0.001	0.052	$F_{(9,378)}$=2.77	0.004	0.013	0.008	0.103
Group/Location/Time		$F_{(9,378)}$=1.98	0.040	0.077	0.066	0.167	$F_{(9,378)}$=0.92	0.507	0.480	0.491	0.343
Location	**Interval A**	**Interval B**	**Diff**	**StdErr**	**P_value**	**Location**	**Interval A**	**Interval B**	**Diff**	**StdEr**	**P_value**
Central	Baseline	Second	-0.83	0.20	0.00	Central	Baseline	Second	-0.07	0.02	0.00
Central	Baseline	Third	-0.84	0.25	0.01	Central	Baseline	Third	-0.07	0.02	0.00
Central	First	Second	-0.63	0.21	0.02	Central	First	Second	-0.05	0.02	0.04
Frontal	Baseline	Second	-0.93	0.22	0.00	Central	First	Third	-0.05	0.02	0.02
Frontal	Baseline	Third	-0.70	0.20	0.01	Frontal	Baseline	Second	-0.07	0.01	0.00
Frontal	First	Second	-0.65	0.22	0.03	Frontal	Baseline	Third	-0.07	0.02	0.00
Occi-parietal	Baseline	Second	-1.16	0.29	0.00	Occi-par	Baseline	Second	-0.09	0.02	0.00
Occi-parietal	Baseline	Third	-1.50	0.38	0.00	Occi-par	Baseline	Third	-0.11	0.03	0.00
Occi-parietal	First	Second	-0.92	0.29	0.01	Occi-par	First	Second	-0.06	0.02	0.01
Occi-parietal	First	Third	-1.26	0.36	0.01	Occi-par	First	Third	-0.08	0.02	0.00
Temporal	Baseline	Second	-0.84	0.23	0.00	Tempora	Baseline	Second	-0.07	0.01	0.00
Temporal	Baseline	Third	-0.76	0.26	0.03	Tempora	Baseline	Third	-0.07	0.02	0.00
Temporal	First	Second	-0.74	0.21	0.01						

4.9.1.4 Beta

For the beta band, both mean ($F_{(3,126)}$=37.9, p<0.001) and relative ($F_{(3,126)}$=73.39, p<0.001) PSD values were decreased significantly in all locations for both groups compared to the baseline. The results of repeated ANOVA analysis and post hoc results (Table 4.19) can be visualized in Figure 4.29 and Figure 4.30 which depict the decline of beta activity among the subjects regardless of their treatment group. Comparing the result of post hoc analysis of beta band with other frequency bands confirms that changes in this frequency band are more significant compared to other bands.

Table 4.18: Table shows the repeated measure ANOVA results for mean and relative PSD values of beta band activity.

Mean PSD							Relative PSD				
Beta		**F_statistics**	**P_value**	**P_GG**	**P_HF**	**P_LB**	**F_statistics**	**P_value**	**P_GG**	**P_HF**	**P_LB**
Intercept/Time		$F_{(3,126)}$=37.09	0.000	0.000	0.000	0.000	$F_{(3,126)}$=73.39	0.000	0.000	0.000	0.000
Group/Time		$F_{(3,126)}$=0.66	0.580	0.534	0.542	0.422	$F_{(3,126)}$=0.49	0.687	0.679	0.687	0.486
Intercept/Location/Time		$F_{(9,378)}$=0.81	0.606	0.531	0.544	0.373	$F_{(9,378)}$=3.54	0.000	0.003	0.001	0.067
Group/Location/Time		$F_{(9,378)}$=1.34	0.216	0.254	0.249	0.254	$F_{(9,378)}$=1.36	0.205	0.234	0.225	0.250
Location	**Interval A**	**Interval B**	**Diff**	**StdErr**	**P_value**	**Location**	**Interval A**	**Interval B**	**Diff**	**StdEr**	**P_value**
Central	Baseline	First	0.30	0.04	0.00	Central	Baseline	First	0.08	0.01	0.00
Central	Baseline	Second	0.28	0.04	0.00	Central	Baseline	Second	0.09	0.01	0.00
Central	Baseline	Third	0.31	0.04	0.00	Central	Baseline	Third	0.10	0.01	0.00
Frontal	Baseline	First	0.26	0.05	0.00	Frontal	Baseline	First	0.12	0.01	0.00
Frontal	Baseline	Second	0.28	0.04	0.00	Frontal	Baseline	Second	0.13	0.01	0.00
Frontal	Baseline	Third	0.31	0.05	0.00	Frontal	Baseline	Third	0.15	0.01	0.00
Occi-Parietal	Baseline	First	0.28	0.05	0.00	Frontal	First	Third	0.03	0.01	0.00
Occi-Parietal	Baseline	Second	0.28	0.05	0.00	Occi-Par	Baseline	First	0.09	0.01	0.00
Occi-Parietal	Baseline	Third	0.30	0.05	0.00	Occi-Par	Baseline	Second	0.11	0.01	0.00
Temporal	Baseline	First	0.22	0.05	0.00	Occi-Par	Baseline	Third	0.13	0.01	0.00
Temporal	Baseline	Second	0.25	0.06	0.00	Occi-Par	First	Third	0.04	0.01	0.01
Temporal	Baseline	Third	0.25	0.05	0.00	Tempora	Baseline	First	0.08	0.01	0.00
						Tempora	Baseline	Second	0.10	0.01	0.00
						Tempora	Baseline	Third	0.11	0.01	0.00

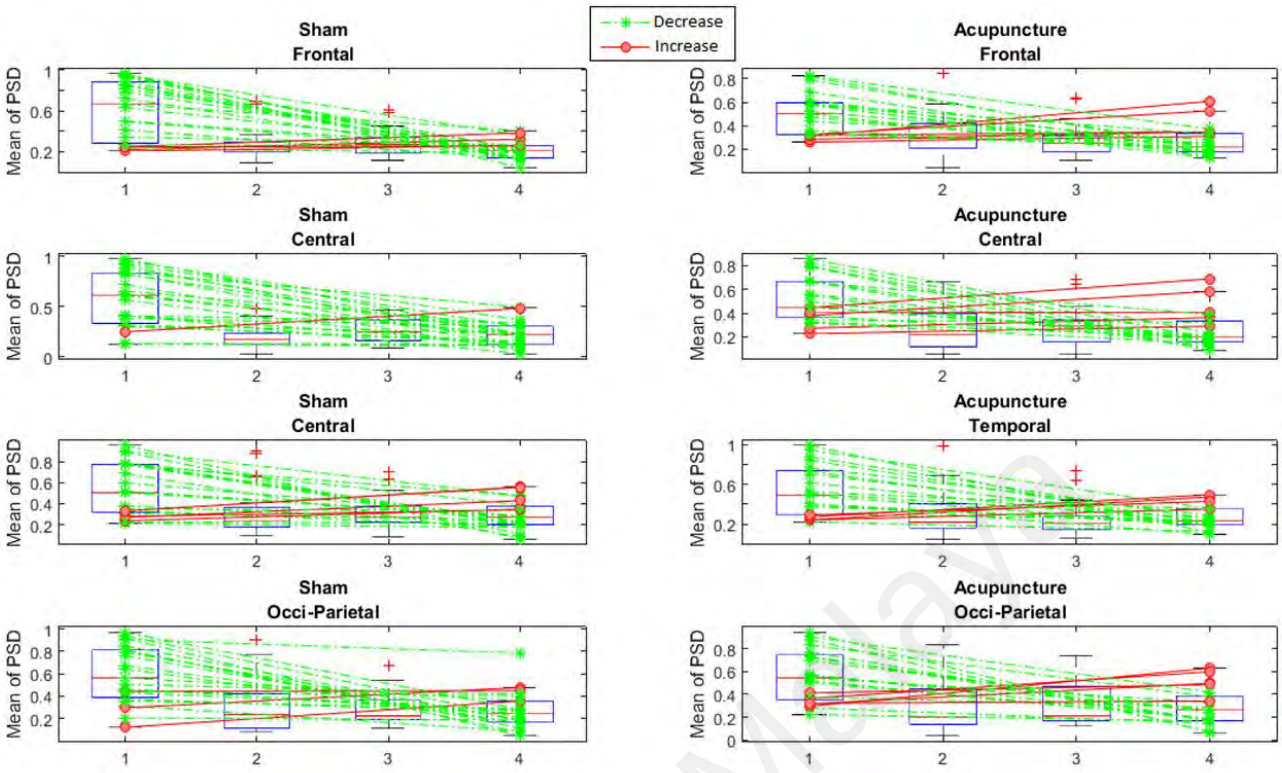


Figure 4.29: Mean PSD changes of subjects for both treatment groups for all locations.

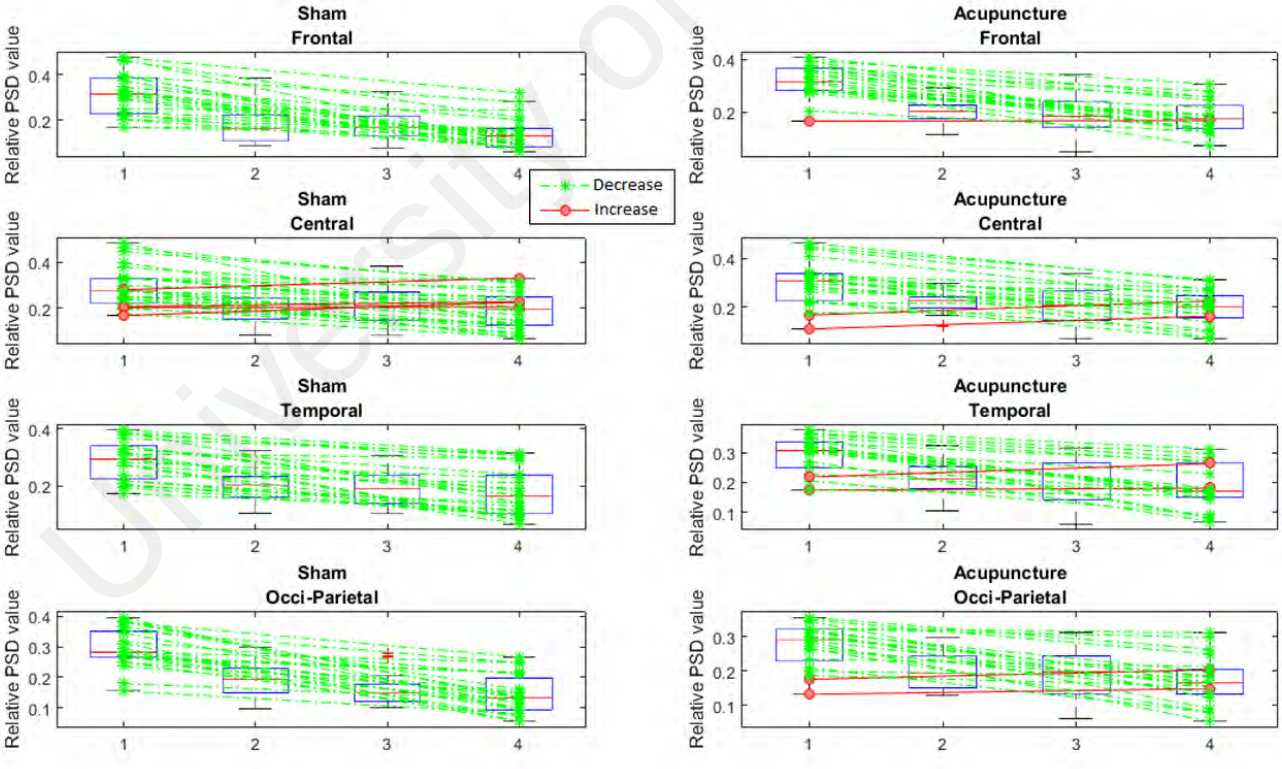


Figure 4.30: Relative PSD changes of subjects for both treatment groups for all locations.

4.9.2 P300

Statistical analysis (Table 4.20) revealed that interaction of factors with time was only significant for intercept×time for both amplitude index ($F_{(3,126)}$=29.75, p-value<0.001) and latency ratio ($F_{(3,126)}$=22.25, p-value<0.001). The post hoc analysis (Table 4.20) show significant enhancement of both amplitude and latency of P3 after three months of treatment regardless of the treatment group. However, changes were seen to be more significant on P3 amplitude compared to the enhancement of the latency.

Table 4.19: Table shows the repeated measure ANOVA results for P3 features (amplitude and latency). The post hoc analysis for p3 features of both groups at different tasks. The effect shows the main study factor and "A" and "B" are the first and second set of variables to be compared.

		Amplitude						Latency				
P300		**F_statistics**	**P_value**	**P_GG**	**P_HF**	**P_LB**	**F_statistics**	**P_value**	**P_GG**	**P_HF**	**P_LB**
Intercept/Time		$F_{(3,126)}$=29.75	0.000	0.000	0.000	0.000	$F_{(3,126)}$=21.25	0.000	0.000	0.000	0.000
Group/Time		$F_{(3,126)}$=0.231	0.874	0.858	0.872	0.633	$F_{(3,126)}$=1.08	0.360	0.360	0.360	0.305
Intercept/Task/Time		$F_{(9,378)}$=0.963	0.471	0.457	0.467	0.332	$F_{(9,378)}$=1.31	0.23	0.243	0.23	0.259
Group/ Task /Time		$F_{(9,378)}$=1.181	0.306	0.314	0.309	0.283	$F_{(9,378)}$=2.24	0.02	0.029	0.02	0.142
Task	**Interval A**	**Interval B**	**Diff**	**StdErr**	**P_value**	**Task**	**Interval A**	**Interval B**	**Diff**	**StdErr**	**P_value**
'ATT'	Baseline	Second	-1.16	0.20	0.000	'ATT'	Baseline	First	0.04	0.01	0.002
'ATT'	First	Second	-1.13	0.22	0.000	'ATT'	Baseline	Second	0.05	0.01	0.000
'ATT'	Second	Third	0.65	0.22	0.022	'ATT'	Baseline	Third	0.04	0.01	0.001
'AUD'	Baseline	Second	-1.02	0.26	0.002	'AUD'	Baseline	First	0.03	0.01	0.035
'AUD'	Baseline	Third	-1.09	0.34	0.013	'AUD'	Baseline	Second	0.05	0.01	0.000
'AUD'	First	Third	-0.96	0.33	0.028	'AUD'	Baseline	Third	0.04	0.01	0.004
'REC'	Baseline	Second	-0.97	0.25	0.002	'REC'	Baseline	Second	0.05	0.01	0.000
'REC'	Baseline	Third	-0.73	0.26	0.038	'REC'	Baseline	Third	0.03	0.01	0.004
'REC'	First	Second	-0.75	0.21	0.004	'VIS'	First	Second	0.03	0.01	0.033
'VIS'	Baseline	Second	-0.60	0.17	0.005						
'VIS'	First	Second	-0.83	0.17	0.000						
'VIS'	First	Third	-0.63	0.21	0.021						

4.9.3 Auditory MMN

For the alteration of MMN properties, the results of repeated measure ANOVA is depicted in Table 4.21, and the strip-charts of changes for all subjects of each group is presented in below figures. The results shows the significant effect of intercept×time factor for both amplitude index ($F_{(3,126)}$=5.74, p-value<0.001) and latency ratio ($F_{(3,126)}$=29.28, p-value<0.001). Although the post hoc analysis shows the enhancement of

both amplitude and latency ratio for the subjects of both groups, Figure 4.31 shows that latency ratio of large deviant stimuli was enhanced for more number of subjects after three-month of treatment compared to small deviant stimuli.

Table 4.20: Table shows the repeated measure ANOVA results for MMN features (amplitude and latency). The post hoc analysis for MMN features of both groups at different tasks. The effect shows the main study factor and "A" and "B" are the first and second set of variables to be compared.

Amplitude							Latency				
P300		**F_statistics**	**P_value**	**P_GG**	**P_HF**	**P_LB**	**F_statistics**	**P_value**	**P_GG**	**P_HF**	**P_LB**
Intercept/Time		$F_{(3,126)}$=5.743	0.001	0.001	0.001	0.021	$F_{(3,126)}$=29.28	0.000	0.000	0.000	0.000
Group/Time		$F_{(3,126)}$=0.723	0.540	0.529	0.538	0.400	$F_{(3,126)}$=2.16	0.096	0.100	0.096	0.149
Intercept/Task/Time		$F_{(3,126)}$=0.525	0.666	0.630	0.642	0.473	$F_{(3,126)}$=2.30	0.081	0.085	0.081	0.137
Group/ Task /Time		$F_{(3,126)}$=2.633	0.053	0.065	0.061	0.112	$F_{(3,126)}$=2.41	0.070	0.074	0.070	0.128
Type	**Interval A**	**Interval B**	**Diff**	**StdErr**	**P_value**	**Type**	**Interval A**	**Interval B**	**Diff**	**StdErr**	**P_value**
Large	Baseline	First	0.02	0.00	0.00	Large	Baseline	Second	-0.22	0.08	0.03
Large	Baseline	Second	0.03	0.00	0.00	Large	Baseline	Third	-0.26	0.10	0.05
Large	Baseline	Third	0.03	0.00	0.00	Small	Baseline	Second	-0.21	0.08	0.04
Small	Baseline	First	0.01	0.00	0.01						
Small	Baseline	Second	0.02	0.00	0.00						
Small	Baseline	Third	0.01	0.00	0.01						

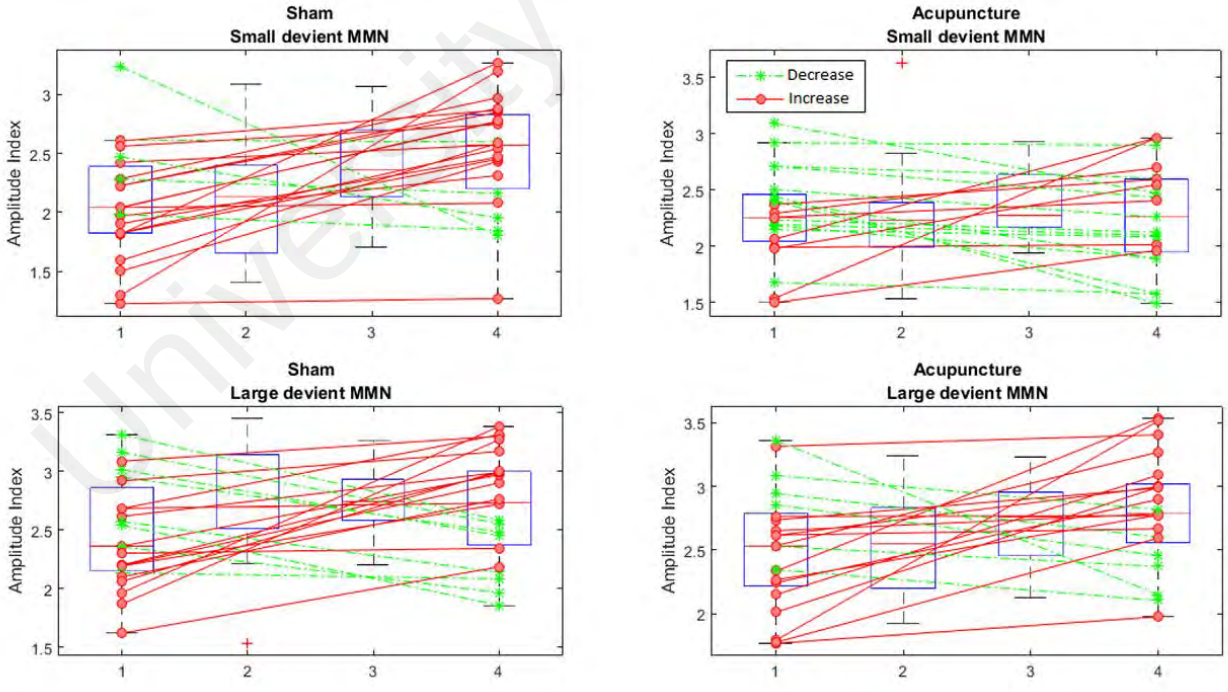


Figure 4.31: Changes in MMN amplitude index among subjects for both treatment groups for all tasks.

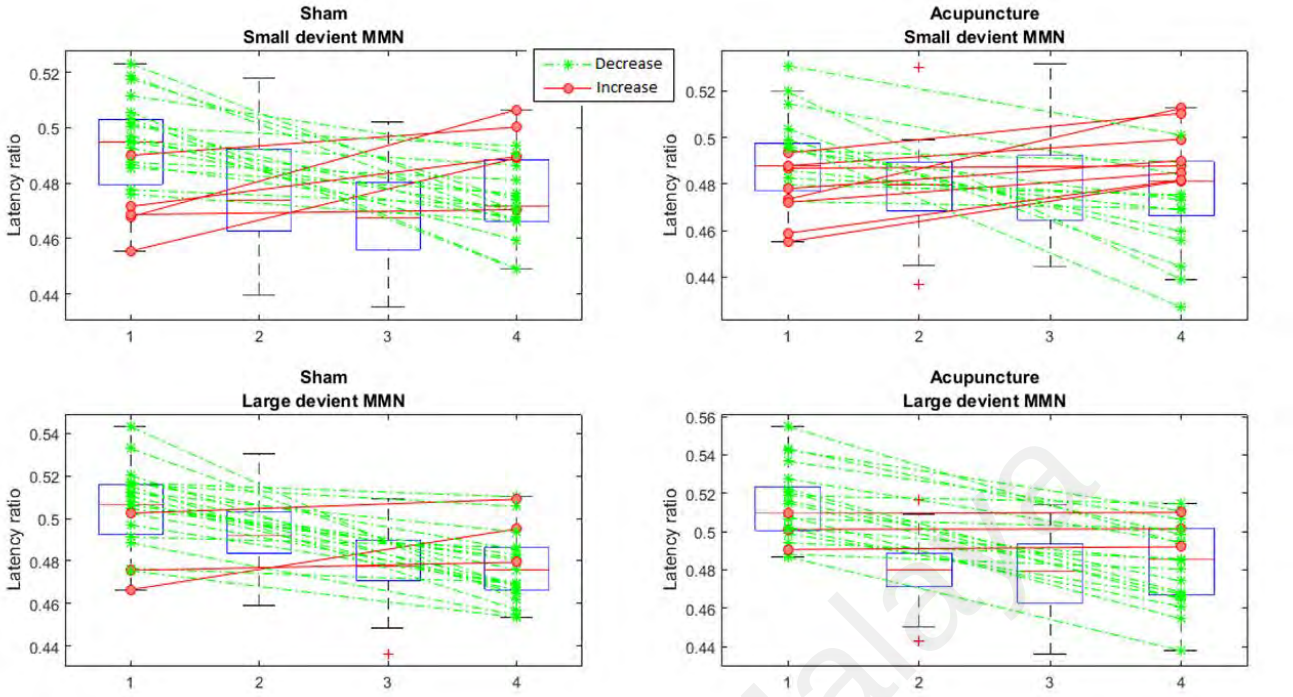


Figure 4.32: Changes in MMN latency ratio among subjects for treatment groups for all tasks. Changes are more significant for large deviant stimuli.

4.9.4 P600

Results of repeated measure ANOVA analysis of P600 features (Table 4.22) shows the significant effect of the intercept×time factor for amplitude index ($F_{(3,126)}$=11.9, p-value<0.001) with no significant effect of group. The post hoc analysis shows the enhancement of amplitude index for the groups. The paired observations of P600 features were depicted in Figure 4.33 and Figure 4.34.

Table 4.21: Table shows the repeated measure ANOVA results for P600 features (amplitude and latency). The post hoc analysis for P600 features of both groups at different tasks. The effect shows the main study factor and "A" and "B" are the first and second set of variables to be compared.

Amplitude							Latency				
P300		**F_statistics**	**P_value**	**P_GG**	**P_HF**	**P_LB**	**F_statistics**	**P_value**	**P_GG**	**P_HF**	**P_LB**
Intercept/Time		$F_{(3,126)}$=11.9	0.000	0.000	0.000	0.001	$F_{(3,126)}$=2.44	0.06	0.07	0.07	0.12
Group/Time		$F_{(3,126)}$=1.46	0.229	0.232	0.230	0.234	$F_{(3,126)}$=0.19	0.90	0.88	0.89	0.66
Location	**Interval A**	**Interval B**	**Diff**	**StdErr**	**P_value**	**Location**	**Interval A**	**Interval B**	**Diff**	**StdErr**	**P_value**
Acupuncture	Baseline	Second	-0.73	0.26	0.033						
Sham	Baseline	First	-0.68	0.22	0.017						
Sham	Baseline	Second	-1.20	0.25	0.000						
Sham	First	Third	0.54	0.20	0.049						
Sham	Second	Third	1.06	0.28	0.002						

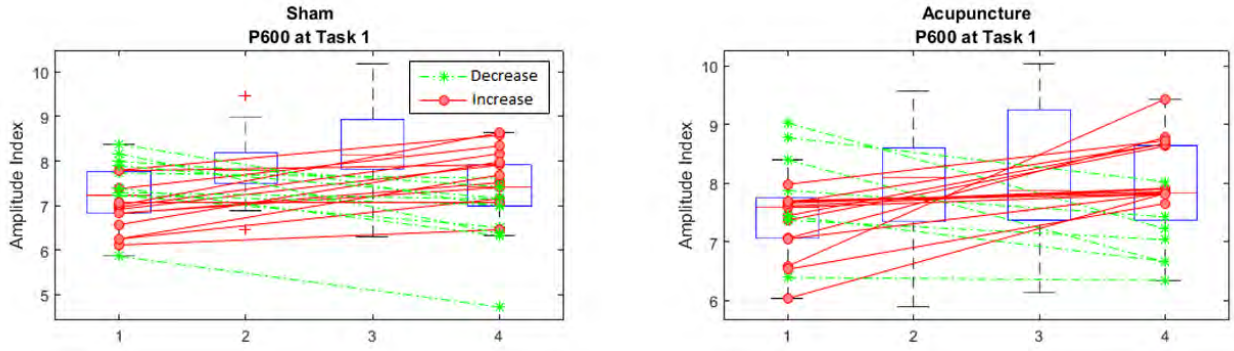


Figure 4.33: Changes in P600 amplitude index among subjects for both treatment groups.

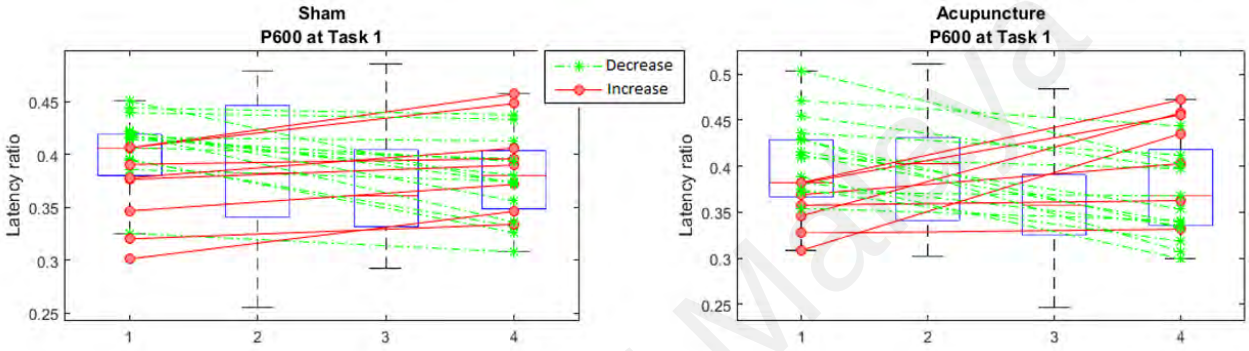


Figure 4.34: Changes in P600 amplitude index among subjects for both treatment groups.

4.10 Acute Effects

After the second month of therapy, 28 subjects of the sham group, as well as 19 subjects of EA group who did not differ in their withdrawal scales and their methadone dosage, were selected to measure their brain electrophysiological properties before and after the treatment session. Following sections describe the results of repeated measure ANOVA model for neurophysiological data. Since the initial group differences might affect the results, therefore in post hoc analysis the group's differences at pre and post dosage measurements are reflected in ANOVA tables as well. Detailed results of statistical analysis and the paired observation can be found in Appendix F.

4.10.1 PSD of Resting-state EEG

4.10.1.1 Delta

Analysis of pre-dosage and post-dosage measurements of delta PSD values showed no significant effect of group×time while intercept×time×location interaction was significant for both mean and relative values. Further analysis shows significant differences only at frontal location with an increase for mean and decreases for relative PSD values (Table 4.23). Although statistical analysis does not confirm any significant effect of group factor, Paired observations shows that while most of the subjects of the sham group have an increase in their mean delta at the frontal location, most of the subjects of EA group has a decrease of the mean delta.

Table 4.22: Table shows the repeated measure ANOVA results for mean and relative PSD values of Delta band. The post hoc analysis for mean and relative PSD values of Delta band for both groups at different locations. Factor shows the main study factor and "A" and "B" are the first and second set of variables to be compared.

Mean PSD							Relative PSD				
Delta		**F_statistics**	**P_value**	**P_GG**	**P_HF**	**P_LB**	**F_statistics**	**P_value**	**P_GG**	**P_HF**	**P_LB**
Intercept/Time		$F_{(1,45)}$=1.96	0.169				$F_{(1,45)}$=1.96	0.169			
Group/Time		$F_{(1,45)}$=0.98	0.327				$F_{(1,45)}$=0.98	0.327			
Intercept/Location/Time		$F_{(3,135)}$=2.92	0.036	0.066	0.064	0.094	$F_{(3,135)}$=2.92	0.036	0.066	0.064	0.094
Group/Location/Time		$F_{(3,135)}$=0.43	0.729	0.625	0.632	0.514	$F_{(3,135)}$=0.43	0.729	0.625	0.632	0.514
Factor	**Interval A**	**Interval B**	**Diff**	**StdErr**	**P_value**		**Diff**	**StdErr**	**P_value**		
Pre	EA	Sham	0.43	0.67	0.52		-0.04	0.03	0.18		
Post	EA	Sham	0.99	0.58	0.10		-0.02	0.02	0.38		
Central	Pre	Post	-0.22	0.33	0.50		0.01	0.02	0.71		
Frontal	Pre	Post	-0.87	0.37	0.03		0.03	0.01	0.02		
Occi-parietal	Pre	Post	-0.29	0.30	0.34		0.02	0.02	0.39		
Temporal	Pre	Post	-0.21	0.29	0.48		0.01	0.02	0.42		

4.10.1.2 Theta

Repeated measure ANOVA results for theta band shows a significant effect of time for subjects regardless of their treatment group for both mean ($F_{(1,45)}$=11.38, p<0.005) and relative ($F_{(1,45)}$=6.99, p<0.05) PSD values. The follow-up analysis showed an increase of theta band activity at all regions (Table 4.24).

Table 4.23: Table shows the repeated measure ANOVA results for mean and relative PSD values of theta band. In the post hoc analysis, factor shows the main study factor and "A" and "B" are the first and second set of variables to be compared.

Mean PSD							Relative PSD				
Theta		**F_statistics**	**P_value**	**P_GG**	**P_HF**	**P_LB**	**F_statistics**	**P_value**	**P_GG**	**P_HF**	**P_LB**
Intercept/Time		$F_{(1,45)}$=11.38	0.002				$F_{(1,45)}$=6.99	0.011			
Group/Time		$F_{(1,45)}$=0.17	0.680				$F_{(1,45)}$=0.20	0.656			
Intercept/Location/Time		$F_{(3,135)}$=3.44	0.019	0.043	0.041	0.070	$F_{(3,135)}$=0.65	0.582	0.557	0.566	0.423
Group/Location/Time		$F_{(3,135)}$=1.22	0.306	0.298	0.299	0.276	$F_{(3,135)}$=1.10	0.353	0.347	0.350	0.301
Factor	**Interval A**	**Interval B**	**Diff**	**StdErr**	**P_value**		**Diff**	**StdErr**	**P_value**		
Pre	EA	Sham	0.35	0.44	0.43		-0.01	0.02	0.69		
Post	EA	Sham	0.51	0.57	0.38		-0.02	0.03	0.52		
Central	Pre	Post	-0.94	0.27	0.00		-0.03	0.01	0.01		
Frontal	Pre	Post	-0.61	0.25	0.02		-0.02	0.01	0.06		
Occi-parietal	Pre	Post	-0.56	0.17	0.00		-0.02	0.01	0.02		
Temporal	Pre	Post	-0.42	0.14	0.00		-0.03	0.01	0.01		

4.10.1.3 Alpha

Repeated measure ANOVA results for alpha band shows no significant effect of group comparison while the post hoc analysis shows the groups differences for both pre-dosage and post-dosage measurements (Table 4.25). Therefore, PSD values of the alpha band were analyzed using an ANCOVA model (pre-measurements as covariates). The ANCOVA with the condition as the independent variable and the pre-measure as the covariate and post measure as the dependent variable answers the question of whether the post-test means, adjusted for pre-test scores, differ between the two groups. The adjustment for the pre-test score in ANCOVA has two benefits. One is to make sure that

any post-test differences truly result from the treatment, and aren't some left-over effect of (usually random) pre-test differences between the groups. The other is to account for variation around the post-test means that comes from the variation in where the patients started at pretest. ANCOVA first conducts a regression of the independent variable (i.e., the covariate) on the dependent variable. The residuals (the unexplained variance in the regression model) are then subject to an ANOVA. Thus the ANCOVA tests whether the independent variable still influences the dependent variable after the influence of the covariate(s) has been removed.

The *AOC* tool of *MATLAB* was used to perform the ANCOVA analysis, and the results are shown in Table 4.26 where the slopes of the regressions are tested. The analysis shows a significant increase of both mean and relative PSD values of the alpha band in all locations. The effect of group was only significant for the mean value of PSD at central and temporal locations. For this two locations, the regression lines are shown in Figure 4.35 and Figure 4.36. These figures in line with the ANCOVA results and paired observations shows that subjects in EA group had more increase in the mean of alpha PSD at central location while this increase is more for sham group subjects at temporal locations.

Table 4.24: Table shows the repeated measure ANOVA results for mean and relative PSD values of the alpha band. In the post hoc analysis, factor shows the main study factor and "A" and "B" are the first and second set of variables to be compared.

		Mean PSD					Relative PSD				
Alpha		**F_statistics**	**P_value**	**P_GG**	**P_HF**	**P_LB**	**F_statistics**	**P_value**	**P_GG**	**P_HF**	**P_LB**
Intercept/Time		$F_{(1,45)}$=2.43	0.126				$F_{(1,45)}$=3.00	0.090			
Group/Time		$F_{(1,45)}$=0.00	0.946				$F_{(1,45)}$=0.48	0.494			
Intercept/Location/Time		$F_{(3,135)}$=3.41	0.019	0.039	0.037	0.071	$F_{(3,135)}$=1.77	0.156	0.166	0.163	0.190
Group/Location/Time		$F_{(3,135)}$=1.96	0.124	0.150	0.148	0.169	$F_{(3,135)}$=1.20	0.313	0.311	0.311	0.279
Factor	**Interval A**	**Interval B**	**Diff**	**StdErr**	**P_value**		**Diff**	**StdErr**	**P_value**		
Pre	EA	Sham	1.93	0.56	0.00		0.09	0.03	0.01		
Post	EA	Sham	1.89	0.57	0.00		0.07	0.03	0.02		
Central	Pre	Post	-0.85	0.31	0.01		-0.04	0.02	0.02		
Frontal	Pre	Post	-0.11	0.33	0.75		-0.02	0.01	0.19		
Occi-parietal	Pre	Post	-0.39	0.26	0.15		-0.02	0.02	0.38		
Temporal	Pre	Post	-0.09	0.21	0.66		-0.03	0.02	0.07		

Table 4.25: Table shows the ANOVA results for regression values of ANCOVA test while pre-dosage measurements were used as covariates.

Alpha	**Mean PSD**				**Relative PSD**			
Term	**Sum Sq**	**Mean Sq**	**$F_{(1,43)}$**	**P_value**	**Sum Sq**	**Mean Sq**	**$F_{(1,43)}$**	**P_value**
Frontal	13.62	13.62	4.69	0.04	0.09	0.09	20.17	0.0005
Group/Frontal	0.56	0.56	0.19	0.66	0.002	0.002	0.521	0.47
Central	162.59	162.59	41.96	0.0001	0.21	0.21	26.49	0.0006
Group/Central	20.57	20.57	5.31	0.03	0.02	0.02	3.02	0.09
Temporal	70.99	70.99	47.48	0.0001	0.09	0.09	10.86	0.002
Group/Temporal	7.44	7.44	4.98	0.03	0.001	0.001	0.14	0.71
Occi-parietal	121.67	121.67	43.42	0.0001	0.15	0.15	11.60	0.001
Group/ Occi-parietal	1.753	1.7533	0.63	0.43	0.004	0.004	0.33	0.567

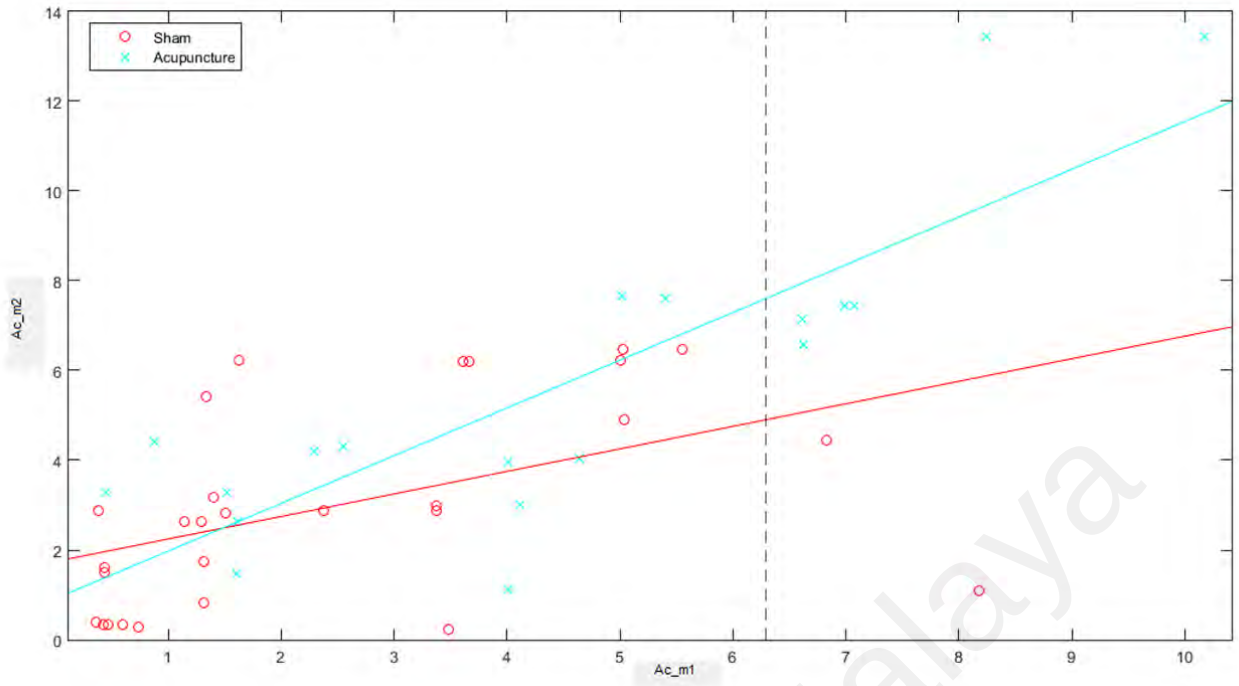


Figure 4.35: Figure depicts the regression lines of alpha mean PSD at central location for both groups.

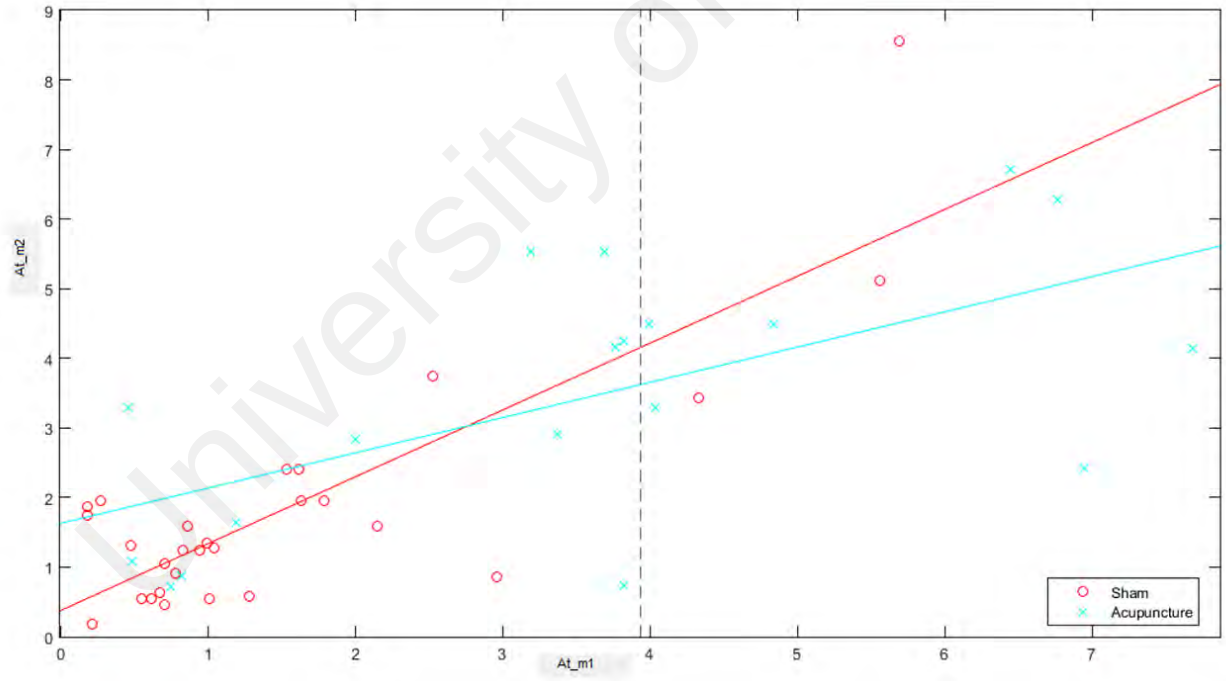


Figure 4.36: Figure depicts the regression lines of alpha mean PSD at the temporal location for both groups.

4.10.1.4 Beta

Repeated measure ANOVA results for beta band shows no significant effect of time for mean PSD values (Table 4.27). However, the intercept×time interaction had a significant effect ($F_{(1,45)}$=4.30, p<0.04) for relative PSD values regardless of the treatment group. The post hoc analysis shows decreases in relative PSD of the beta band for all locations which are statistically significant at central and temporal locations.

Table 4.26: Table shows the repeated measure ANOVA results for mean and relative PSD values of the beta band. In the post hoc analysis, factor shows the main study factor and "A" and "B" are the first and second set of variables to be compared.

Mean PSD							Relative PSD				
Beta		**F_statistics**	**P_value**	**P_GG**	**P_HF**	**P_LB**	**F_statistics**	**P_value**	**P_GG**	**P_HF**	**P_LB**
Intercept/Time		$F_{(1,45)}$=1.07	0.306				$F_{(1,45)}$=4.30	0.044			
Group/Time		$F_{(1,45)}$=0.01	0.915				$F_{(1,45)}$=0.00	0.982			
Intercept/Location/Time		$F_{(3,135)}$=0.32	0.809	0.683	0.690	0.573	$F_{(3,135)}$=6.36	0.000	0.002	0.001	0.015
Group/Location/Time		$F_{(3,135)}$=0.38	0.764	0.641	0.648	0.538	$F_{(3,135)}$=0.91	0.439	0.419	0.423	0.346
Factor	**Interval A**	**Interval B**	Diff	StdErr	P_value		Diff	StdErr	P_value		
Pre	EA	Sham	-0.01	0.09	0.88		-0.03	0.02	0.14		
Post	EA	Sham	-0.02	0.10	0.81		-0.03	0.02	0.13		
Central	Pre	Post	-0.03	0.06	0.61		0.05	0.01	0.00		
Frontal	Pre	Post	-0.06	0.06	0.29		0.00	0.01	0.90		
Occi-parietal	Pre	Post	-0.06	0.04	0.18		0.01	0.01	0.32		
Temporal	Pre	Post	-0.04	0.04	0.32		0.04	0.01	0.01		

4.10.2 P300

Statistical analysis revealed that interaction of factors with time was only significant for intercept×time of amplitude index ($F_{(1,45)}$=25.88, $p_{\text{-value}}$<0.001). While the post hoc analysis shows no significant difference of pre-measurement differences, the P3 amplitude index of subjects was seen to be significantly increased after the dosage for both groups at all task (Table 4.28).

Table 4.27: Table shows the repeated measure ANOVA results for amplitude index and latency ratio of P3 component. In the post hoc analysis, factor shows the main study factor and "A" and "B" are the first and second set of variables to be compared.

Amplitude							Latency				
P300		**F_statistics**	**P_value**	**P_GG**	**P_HF**	**P_LB**	**F_statistics**	**P_value**	**P_GG**	**P_HF**	**P_LB**
Intercept/Time		$F_{(1,45)}$=25.881	0.000				$F_{(1,45)}$=2.786	0.102			
Group/Time		$F_{(1,45)}$=3.690	0.061				$F_{(1,45)}$=0.089	0.766			
Intercept/Task/Time		$F_{(3,135)}$=0.536	0.658	0.637	0.648	0.468	$F_{(3,135)}$=2.19	0.09	0.097	0.09	0.146
Group/ Task /Time		$F_{(3,135)}$=0.229	0.876	0.854	0.866	0.634	$F_{(3,135)}$=0.08	0.97	0.963	0.97	0.776
Task	**Interval A**	**Interval B**	**Diff**	**StdErr**	**P_value**		**Diff**	**StdErr**	**P_value**		
Pre	EA	'Sham'	-0.23	0.17	0.201		0.00	0.01	0.929		
Post	EA	'Sham'	0.32	0.21	0.139		0.00	0.00	0.676		
Att	Pre	Post	-0.59	0.20	0.006		0.03	0.01	0.004		
Aud	Pre	Post	-0.82	0.30	0.010		0.00	0.01	0.771		
Rec	Pre	Post	-0.93	0.27	0.002		0.00	0.01	0.989		
Vis	Pre	Post	-0.55	0.24	0.026		0.01	0.01	0.552		

4.10.3 Auditory MMN

The results regarding the alteration of MMN properties are shown in Table 4.29 which confirmed the significant effect of intercept×time for both amplitude index ($F_{(1,45)}$=38.39, $p_{\text{-value}}$<0.001) and latency ratio ($F_{(1,45)}$=21.99, $p_{\text{-value}}$<0.001) with no significant effect of group on the results. However, the post hoc analysis shows the increase of amplitude for both deviant stimuli regardless of treatment group as well as a decrease in the latency ratio of large deviant stimuli among the subjects. The strip-charts of paired observations (Figure 4.37) shows the alteration of latency ratio and amplitude index for all subjects of both groups.

Table 4.28: Table shows the repeated measure ANOVA results for amplitude index and latency ratio of MMN component.

MMN	Amplitude							
	F_statistics	**P_value**	**Task**	**Interval A**	**Interval B**	**Diff**	**StdErr**	**P_value**
Intercept/Time	$F_{(1,45)}$=38.39	0.000	Pre	EA	Sham	0.02	0.08	0.78
Group/Time	$F_{(1,45)}$=0.011	0.916	Post	EA	Sham	0.01	0.07	0.87
Intercept/ Task /Time	$F_{(1,45)}$=4.776	0.034	Large	Pre	Post	-0.41	0.06	0.00
Group/ Task /Time	$F_{(1,45)}$=3.086	0.086	Small	Pre	Post	-0.21	0.07	0.01
	Latency							
Intercept/Time	$F_{(1,45)}$=21.99	0.000	Pre	EA	Sham	-0.01	0.00	0.14
Group/Time	$F_{(1,45)}$=0.28	0.598	Post	EA	Sham	-0.01	0.00	0.02
Intercept/ Task /Time	$F_{(1,45)}$=5.99	0.018	Large	Pre	Post	0.02	0.00	0.00
Group/ Task /Time	$F_{(1,45)}$=3.24	0.078	Small	Pre	Post	0.01	0.00	0.09

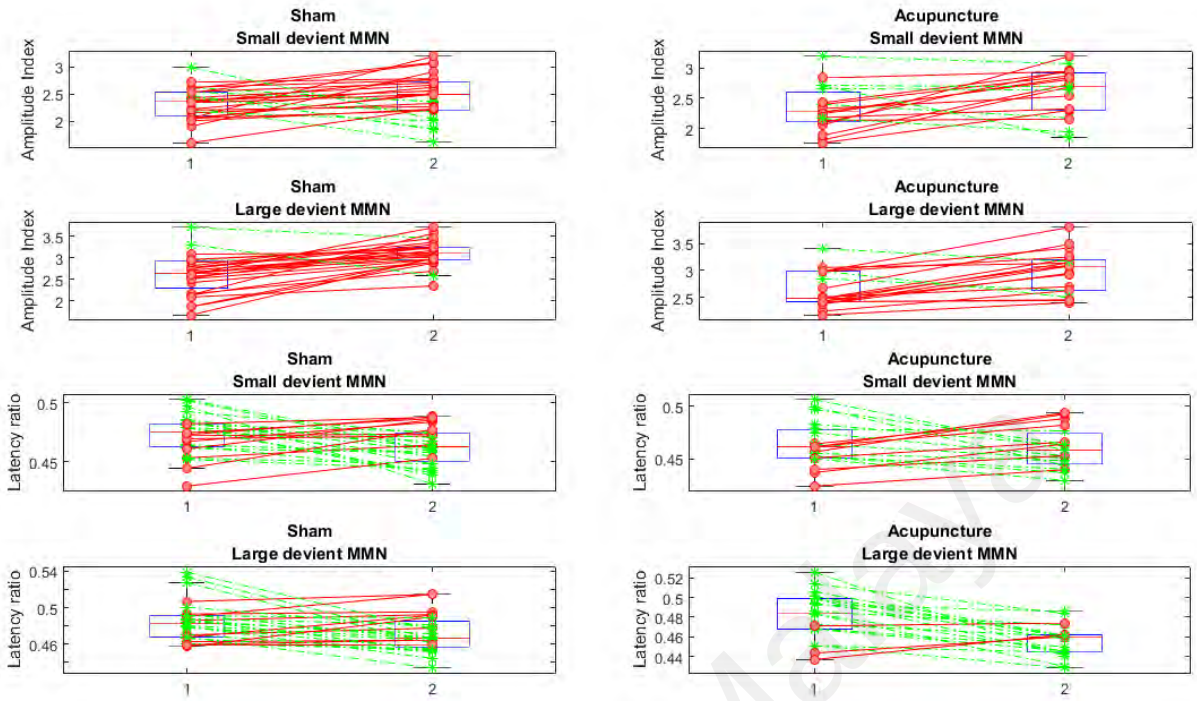


Figure 4.37: Paired observation of MMN features changes of subjects before and after the daily dosage compared for both treatment groups.

4.10.4 P600

Results of repeated measure ANOVA analysis of P600 features (Table 4.30) shows the significant increase of amplitude index as well as decrease in latency ratio in post-dosage measurements compared to the pre-measurements regardless of treatment group. The paired observations of P600 features were depicted in Figure 4.38.

Table 4.29: Table shows the repeated measure ANOVA results for amplitude index and latency ratio of P600 component.

P600	F_statistics	P_value	Task	Interval A	Interval B	Diff	StdErr	P_value
Intercept/Time	6.82	0.012	Pre	EA	Sham	0.01	0.11	0.961
Group/Time	1.56	0.219	Post	EA	Sham	-0.21	0.15	0.156
Intercept/Type/Time	8.43	0.006	Latency	Pre	Post	0.03	0.01	0.015
Group/ Type /Time	1.64	0.21	Amplitude	Pre	Post	-0.49	0.18	0.008

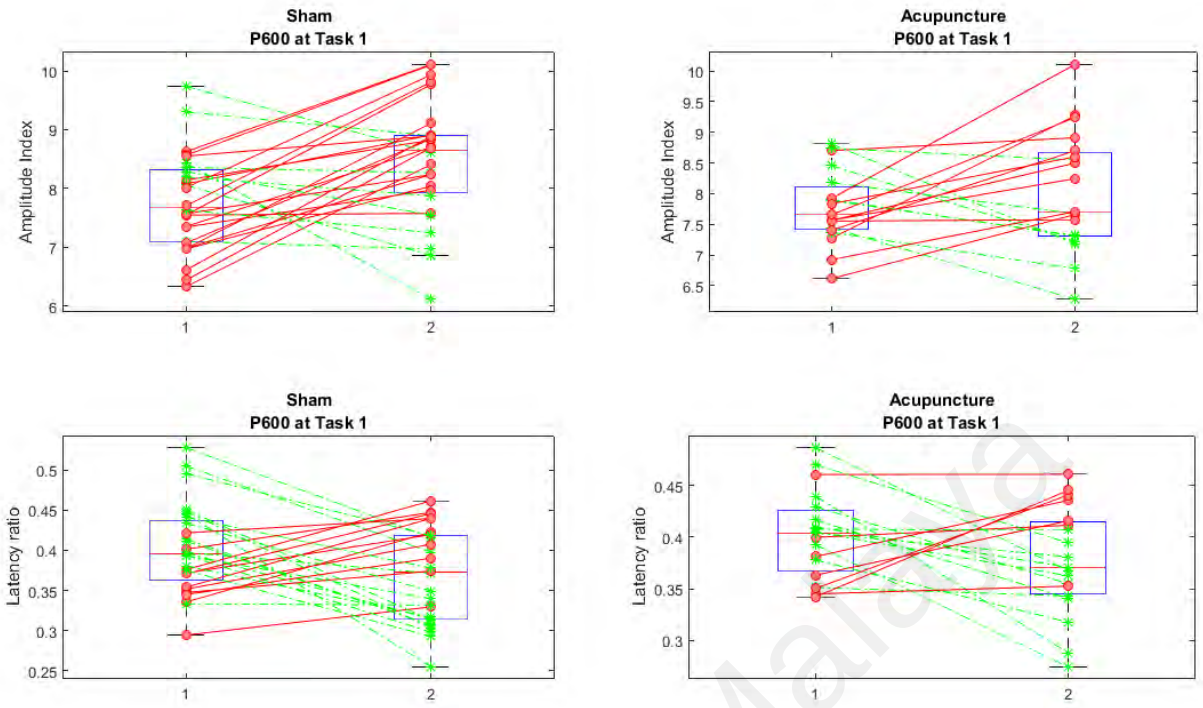


Figure 4.38: Paired observation of P600 features changes of subjects before and after the daily dosage compared for both treatment groups.

4.11 Discussion

In this longitudinal study, the power spectrum of EEG activity and event related potentials of chronic heroin addicts were monitored through 3 months of MMT in the form of two groups of with and without EA as adjunctive therapy. Furthermore, the single-trial ERP detection was compared to the conventional averaging method for the first time in this field, and the related resulted were discussed in section 4.5. The neurophysiological findings of this study based on single-trial detection method were shown earlier in the previous section, and the related discussion is summed up in this section.

The analysis of PSD showed the higher activity of beta and lower activity of delta, theta, and alpha among heroin addicts when compared with healthy subjects. The mean PSD differences were shown to be significant across all cortex locations for all bands.

While relative PSD differences were significant at frontal and temporal locations for delta and theta bands, as well as all locations of alpha and beta bands. The increase of beta band activity as an indicator of the central nervous system functionality was also reported in previous studies as the most dominant abnormal activity among opioid and nicotine users (Costa & Bauer, 1997; I. H. Franken *et al.*, 2004; Kish *et al.*, 2001; Roemer *et al.*, 1995) as well as alcohol addicts (Ceballos *et al.*, 2009; Rangaswamy *et al.*, 2002). Lower activity of alpha, delta and theta bands were also reported in previous studies in addiction field (Saletu *et al.*, 2002).

Notably, cognitive dysfunctions measured by alteration of EEG spectral power are believed to be associated with chronic heroin-related thoughts, heroin craving, and heroin abuse history (Davydov & Polunina, 2004; I. H. A. Franken *et al.*, 2004; Polunina & Davydov, 2004). Polunina and Davydov also investigated the associations between Wechsler adult intelligence scale (WAIS) performance and resting EEG power spectral properties among heroin addicts with different length of heroin addiction compared with healthy volunteers. Slower mean frequencies at right lateral frontal/temporal locations were shown to be associated with WAIS scores. Performance on subtests of long-term memory was related to delta, working/short-term memory subtests were related to temporal theta mean frequency, problem-solving abilities associated with alpha, and psychomotor speed is associated with beta band properties (Polunina & Davydov, 2006).

It is thought that low-frequency activities (delta and theta) are inhibitory, while alpha activity as an expression of normal brain functioning and beta activities is excitatory (Sokhadze *et al.*, 2008). Alpha activity thought to reflect semantic processes associated with cognitive tasks performance (Klimesch *et al.*, 1998), and problem-solving (Polunina & Davydov, 2006), whereas its frontal region activity is predictive of antisocial personality disorder diagnosis among heroin addict (Deckel *et al.*, 1996). Furthermore,

differences of alpha mean at frontal and central regions was suggested to be associated with duration of heroin addiction (Davydov & Polunina, 2004; Polunina & Davydov, 2004). While functional connections in posterior sites had shown an inverse correlation with duration of addiction.

The increase of beta power has been attributed to psychomotor speed (Polunina & Davydov, 2006), and found to be superior in gauging illness severity (Bauer, 1994; Costa & Bauer, 1997). However, some researchers believed that beta band differences could be associated with premorbid differences (Costa & Bauer, 1997; Van Baal *et al.*, 1996) and cognitive or emotional activation (Ray & Cole, 1985) rather than brain function alterations caused by drug use. It is worth mentioning that results of this study and alteration of beta band activity during the treatment course give ground to the association of high beta band activity with addiction traits.

Results of this study show that after one month of treatment the relative PSD of the delta, theta, and alpha increased while the relative value of beta band activity decreased at all locations. Comparing this results with the baseline findings indicates the enhancement of brain activity proportion among addicts. Furthermore, results indicate increase of mean PSD of delta (at frontal location), theta (at all locations), alpha (at central, frontal and occi-parietal regions), while beta decreased at all locations. The results of treatment group differences show that EA significantly benefits the enhancement of alpha PSD mean compared to the sham group while the decline of beta activity among sham groups are considerably more compared to the EA group. These results suggest that EA has increased both alpha and beta activity among the subjects.

It is noteworthy that brain electrophysiological alteration within the first month of treatment was suggested to be affected by many factors. The results of this study show that besides the association of age factor with delta, alpha, and beta spectral values,

withdrawal syndromes are significantly correlated with delta, theta and alpha bands dysregulations as well as P3 and P600 amplitude. The findings of previous studies suggesting alteration of EEG oscillations during early stages of withdrawal (Davydov & Polunina, 2004; Fingelkurts *et al.*, 2008; Polunina & Davydov, 2004), and association of the severity of opioid withdrawal symptoms with brain functional connectivity and percentage of theta-beta and beta (A. A. Fingelkurts *et al.*, 2007). It is suggested that withdrawal-induced neural dysregulation influences cognitive deficits during early opioid abstinence in the prefrontal cortex (Rapeli *et al.*, 2006).

By integrating the results after three months of treatment which shows the progress of increasing the delta (at frontal and temporal), theta and alpha (at all location), as well as the decrement of beta activity, it indicates the reorganization of brain activities to be comparable with healthy controls. The 3-month treatment results show no significant effect between EA and sham group which suggests the ability of methadone to normalize the composition of EEG activities and to restore the temporal structure of brain oscillations especially beta band activity.

In addition to qEEG, the ERP results confirmed diminished amplitude and prolonged latency of MMN, P3, and P600 components among heroin addicts compared to healthy subjects. These results confirmed decrement of attentional processing and cognitive dysfunctions induced by chronic heroin abuse. MMN as an attention-independent component is related to neural aspects of pre-attentive auditory processing and assesses discrimination abilities of subjects. The amplitude of MMN correlates with the degree of difference between standard and deviant stimuli, while its latency inversely reflects the speed of deviance detection (Stefanics *et al.*, 2014). Therefore, the diminished amplitude of MMN among chronic heroin users might be related to their pre-attentive processing deficits. In addition, MMN latency inversely reflects the speed of deviance detection

(Stefanics *et al.*, 2014), and prolonged occurrence of MMN among opioid addicts was in line with previous studies, and it was suggested to be related to deficit of attentional bias (Kivisaari *et al.*, 2007; Morie *et al.*, 2014; Yang *et al.*, 2009).

MMN elicits by shifting attention to deviant stimuli among standard ones, which is followed by P3 component when the stimulus is novel. The P3 component is believed to comprise the activation of inhibitory processes over widespread cortical areas (Tomberg & Desmedt, 1998), and may reflect the decisional process of memory updating (Polich & Herbst, 2000) and cognitive closure (Verleger, 1988). Bauer suggested evaluation of P3 amplitude among opioid addicts as an index of CNS recovery from opiates (Bauer, 2001). P3 also has been advocated to be a neurophysiological marker in correlation with addiction properties (Lubman *et al.*, 2007; Lubman *et al.*, 2009). P3 is also related to selective attention, stimulus significance (Sommer & Matt, 1990), activation of inhibitory processes (Tomberg & Desmedt, 1998), and attentional operations (Donchin, 1981; Donchin & Coles, 1988; McEvoy *et al.*, 2001; Polich, 1998). P3 and N2 investigation among heroin addicts (Bauer, 2001; Kivisaari *et al.*, 2007; Marques-Teixeira & Barbosa, 2005; Morie *et al.*, 2014; Singh *et al.*, 2009; Yang *et al.*, 2009) and smokers (Anokhin *et al.*, 2000; Evans & Drobes, 2009; Guney *et al.*, 2009; Mobascher *et al.*, 2010; Neuhaus *et al.*, 2006) confirmed significant decrease of their amplitude. Although, the diminished amplitude of these ERPs may be a consequence of low delta band power activity, MMN and P3 prolonged latency is a signature of deficiencies in extracting relevant information from sensory stimuli, as well as in directing attentional resources to environmental stimuli which reflect failure in assigning attention to stimulus sources (Polich, 1998) and neurodegenerative processes (O'Donnell *et al.*, 1995).

The P3 and MMN prolonged latency and diminished amplitudes suggest cognitive dysfunctions and the diminished ability of information processing that are argued to be

related to the failure of addicts to pay attention to stimulus sources. These cognitive deficiencies can be concluded from the abnormalities found in alpha rhythm as an indicator of the general level of excitability and health within the central nervous system which reflects nonspecific selective attention and processing of new information (Doppelmayr *et al.*, 1998; Klimesch *et al.*, 1998). Results suggest deficiencies of addicts in extracting relevant information from sensory stimuli, as well as in directing attentional resources to environmental stimuli, and inhibitory control dysfunction in heroin addicts thus reflecting gray matter abnormalities (Bauer, 2001; Marques-Teixeira & Barbosa, 2005; Singh *et al.*, 2009).

The results of the digit-span task, and with regard to the differences of P3 and P6 related to working memory (Frisch *et al.*, 2003), diminished amplitude and prolonged latency of P3 and P600 among heroin addicts reflect their difficulty in second-pass parsing processes of information processing in association with working memory systems. These abnormalities have been suggested to be related to the impairment of working memory and attention involving the right prefrontal areas of the brain (Papageorgiou *et al.*, 2003; Papageorgiou *et al.*, 2004). It is worth mentioning that the amplitude of P3 diminishes with increasing working memory load (Gaspar *et al.*, 2011); therefore, lower amplitude of P3 in the working memory task can be attributed to both attentional deficits and task difficulty. It is in agreement with the correlation of P3 amplitude index at both attention and recall phases of working memory test. The P600 component, which is modulated by basal ganglia and the cingulate area has been considered an index of the completion of any synchronized operation after target detection, rather similar to working memory operation. Results showed that although P600 amplitude recovered for MMT subjects, abnormal P600 latencies may manifest the irregular second-pass parsing processes (Papageorgiou *et al.*, 2001). These results are in agreement with the research done by Papageorgiou *et al*, which evaluated the P600 and

P3 components after six months of heroin abstinence during a working memory test, which suggested these abnormalities to be related to the impairment of working memory and attention involving the right prefrontal areas of brain (Papageorgiou *et al.*, 2003; Papageorgiou *et al.*, 2004).

MMN and P3 amplitudes were also found to be enhanced during the process of treatment. By continuing the treatment the amplitude, as well as the latency of these ERP components, enhanced during treatment. The P3 amplitude can be suggested as a CNS recovery measurement based on the positive correlation of its amplitude and the duration of therapy. Moreover, the amplitude of P3 component has been thought to increase when more attention is oriented by the participant or required by the task. As such, a higher amplitude of P3 among addicts encountering heroin-related stimuli shows the incline of their attention toward this type of stimuli compared to standard novel stimuli. However, prolonged P3 latency in cue-reactivity condition results in a deficiency of attentional processing speed among addicts. Although increment of visual cue reactivity P3 amplitude gives ground to the postulation of using this cognitive marker as an index for opioid carving, integrative neuropsychiatric studies are needed to test this hypothesis. Although, with regards to the findings of previous studies that shows lower performance of attention, memory, and executive function was explored in patients going through MMT (Prosser *et al.*, 2006; Specka *et al.*, 2000), MMT was shown to normalize the ERP amplitudes and enhance their latency to some extent which represents the increase of cognitive functions performance compared with heroin users.

Besides, the results of cue-reactivity P3 and the impact of drug cues on central attentional processing can be manifest the motivational significance of drug cues among MMT subjects which was also suggested to be related to the craving index (Lubman *et al.*, 2007, 2008; Lubman *et al.*, 2009). Based on findings, hedonic responses of cue-

reactivity and targeting anhedonic symptoms within clinical treatment settings can be so informative since heroin-related stimuli acquire motivational salience in addicts and automatically capture attentional resources (Lubman *et al.*, 2007, 2008). Hence, the enhancement of P3 in cue-reactivity conditions may be utilized as a neurophysiological marker in correlation with craving and heroin abuse (Lubman *et al.*, 2007; Lubman *et al.*, 2009).

Craving is a conditioned dysphoric state, the main feature of drug dependence, and contributes to relapse (McKay, 1999). Anhedonia is associated with craving intensity (Hatzigiakoumis *et al.*, 2011; Whelan *et al.*, 2012) and is a fundamental element of addiction (Goldstein & Volkow, 2002; Jentsch & Taylor, 1999), particularly during periods of acute and long-term withdrawal (Heinz *et al.*, 1994), for both abstinent and active opioid abusers (Aguilar de Arcos *et al.*, 2005; Dunning *et al.*, 2011; Fox *et al.*, 2007; Fox *et al.*, 2011).

The comparison of electrophysiological measurements before and after the daily methadone intake indicate the acute effects of methadone induction on EEG PSD and cognitive responses. The mean PSD results show that methadone can significantly increase delta (at frontal location), theta and alpha (at all locations) bands activity for both groups. However, EA could enhance the alpha mean PSD at central regions more compared to sham group. Therefore, acupuncture was shown to have positive immediate effects as a potential therapy for the treatment of opiate addiction. While the beta mean PSD has not changed significantly, the relative PSD values of beta (central and temporal locations) and delta (all locations) declined, and conversely theta and alpha band activity proportions increased. These changes give ground to the postulation that after methadone intake and by reducing the craving of opiate among MMT subjects their brain activity dysregulations can be moderated.

Moreover, the results showed an enhancement in ERP amplitudes after methadone intake regardless of their group. Methadone intake also declines the latency of P600 and enhance the MMN latency for large deviant stimuli compared to the small one. However, it has been confirmed that auditory evoked potentials are associated with alpha and theta band oscillations (Başar & Schürmann, 1994). These findings suggest that methadone like fentanyl can significantly increase the amplitude of theta activity over the central midline region (Greenwald & Roehrs, 2005). Therefore, theta band may significantly influence the enhancement of ERP amplitudes after methadone intake. It is important to note that the acute effects of methadone on neurophysiological traits found to be similar to the results of acute nicotine administration which shows increase of alpha power (Fisher *et al.*, 2012a, 2012b) and enhancement of the P3 and MMN amplitudes (Houlihan *et al.*, 2001; Logemann *et al.*, 2014a, 2014b; Shah *et al.*, 2011), thus indicating that methadone rapidly enhances preattentive processing (enhancement of MMN amplitude). Hence, these electrophysiological alterations after methadone intake support the postulation that methadone treatment per se affects the cognitive performance, which might be related to the general state of arousal (Porjesz *et al.*, 2002) and activation of cortical neuronal networks (Lopes da Silva *et al.*, 1980; Pfurtscheller & Klimesch, 1990) suggesting an increase in alertness (Bonnet & Arand, 2001; Knyazeva & Vil'davskii, 1986).

Aside from the discussion provided here, it should be considered that EEG properties can be influenced by ongoing cognitive processes such as memory processes, chronic craving, and drug use, and initial emotional state of subjects could affect the cognitive process of the brain. On the other hand, the prevalence of smoking is higher in methadone-maintained patients rather than in the general population. According to L.D. Chait et al., 1984, methadone therapy is shown to increase the tobacco smoking among these group of patients depends on their dose. According to A.K. Elkader et al., 2009, smoking

attenuated opioid withdrawal syndrome among methadone patients. Therefore, nicotine as a highly prevalent cognitive stimulant (Clarke *et al.*, 2001; Clemmey *et al.*, 1997) affects ERP components. Nicotine has positive acute effects on learning and memory functions (Levin *et al.*, 2006) and P3 properties (Domino, 2003; Houlihan *et al.*, 1996; Knott *et al.*, 1999; Muller *et al.*, 2007) which should be considered in future studies.

CHAPTER 5: CONCLUSION

5.1 Conclusion

In this longitudinal study, EEG power spectrum and event related potentials of chronic heroin addicts were monitored through 3 months of MMT in the form of two groups of with and without EA as adjunctive therapy. This study confirmed that investigation of cognitive traits associated with striking features of addiction in a single study could benefit the understanding of neurological features of addiction. In addition, the comprehensive investigation of cognitive traits speculated that ERP properties could be used as an index for severity of addiction and a reliable objective assessment for recovery evaluation. Investigating EEG spectral activity, MMN, P3, and P600 properties can lead to a better understanding of brain neurobiological features alteration among heroin addicts and offer more insight for a reliable analysis of brain-behavior relations related to pre-attentive processing, attentional deficit and response inhibition related to the development and maintenance of addictive behaviors.

Brain electrophysiological approaches in heroin addiction studies provide an excellent medium to understand neurobiological dysregulation and identify the location of cognitive responses in the brain. EEG and ERP provide accurate information regarding brain functionality and attentional biases, which offer a more direct measure to evaluate information processing and cognition.

The precision of ERP detection provides cognitive resolution attributes of the brain to identify affection or disruption of information processing properties. In this study, a method of measurement also was evaluated, and the results have further confirmed that method of measurement has a significant effect on the ERP latency ratio and amplitude index. This should be pointed out that single-trial method provides features with lower interquartile value for each group, which results in higher variability of ERP

characteristics between groups over channel location. Therefore, single-trial analysis can be a beneficial tool to investigate this variability of ERP features relative to their spatial source domains which provide more accurate and reliable values compared to the averaging method.

Results of this study confirm that chronic heroin abuse alters brain functioning and cause information processing ability abnormalities. The findings lead to the conclusion that various brain activity abnormalities and dysregulation related to information processing are presented among chronic heroin addicts, which are attributed to a variety of cognitive dysfunctions. Comparison of various neurophysiological attributes of both groups confirmed dysregulation of brain activity and cognitive abnormalities, response inhibition, the decrement of pre-attentive processing, and central executive working memory among addicts when compared to healthy subjects. Moreover, attentional bias associated with P3 properties was found among addicts confronting heroin-related stimuli. Dysfunction of these brain structures may contribute to the identification of basic cognitive processes that could account for the cognitive deficits in addiction.

This is the first study to explore the acute effects of methadone induction on both the resting EEG spatial-spectral and ERP properties. Acute effects of methadone significantly alter the spectral pattern over the central and frontal midline region. Monitoring the immediate and short-term effects of methadone induction confirms the electrophysiological effects of methadone on subjects during MMT which may be influential in reorganizing their brain functions. It was also demonstrated that evoked potentials enhancement after methadone administration was associated with alpha and theta activity, which shows that methadone rapidly enhances preattentive and attentional processing. During the MMT period, cognitive dysfunctions moderates and P3 and P6 amplitudes are comparable to healthy subjects, but their latency has not fully recovered.

On the other hand, temporal-spatial spectral changes proved that although there are significant enhancements in normalizing the EEG oscillation compositions during MMT.

Furthermore, the results confirm the lack of MMT in complete reorganization of EEG activities which acute effects of EA suggest the efficiency of alternative addiction treatment overcome the methadone deficiencies. However, EA has not shown any long-term effect on brain electrophysiological properties, and it can be used only during the withdrawal period of treatment. In addition, the results indicated that EA effects on EEG spectral power alterations are more significant at frontal and central regions of the brain.

5.2 Contribution

In this study, for the first time in the addiction field, a comprehensive paradigm was used, and an advanced signal processing technique was utilized for single-trial analysis of ERP components in an addiction-based experimental study. The paradigm which was designed in this study together with advanced signal processing techniques could provide more robust electrophysiological features during withdrawal and abstinence periods associated with addiction traits such as craving and anhedonia. The spatiotemporal source variability as a major characteristic of ERPs can be more efficiently assessed by utilizing the proposed single-trial ERP analysis technique.

This study has further confirmed that single-trial assessment of ERPs can be more informative compared to traditional averaging techniques for detailed investigation of brain cognitive responses. The precise investigation of ERP properties of heroin addicts can give further details about neuro-electrophysiological abnormalities and cognitive dysregulations caused by chronic heroin abuse and promote a better understanding of drug effects on brain compared to healthy subjects as well as defining new neuro-electrophysiological characteristics of addiction properties.

This is the first study to explore the acute effects of methadone induction on both the resting EEG spatial-spectral and ERP properties and evaluate EA as an adjunctive therapy to MMT. The current study proposes the possibility of using neurophysiological properties as biomarkers of addiction traits instead of conventional subjective methods. Simultaneous evaluation of P3 and N2 components, as a sensitive measure of impaired cognitive control, can improve neurophysiological elucidation of addiction. It is speculated that these ERP properties can be used as an index for severity of substance abuse and a reliable objective assessment for recovery evaluation. MMN does not require participant's attention to be acquired, which makes it appropriate to serve as an index of cognitive deterioration among addicts. P3 as an index of information processing is suitable as a sensitive index for the ability to allocate attentional resources. Furthermore, based on the findings of this study, integration of resting EEG spectral activity at frontal and central regions as well as MMN and P3 latency can be used as an index of addiction severity.

5.3 Limitations

Besides the common problems in psychiatric clinical trials, there have been some methodological limitation in this study. EEG properties can be influenced by ongoing cognitive processes such as memory processes, while the initial emotional state of subjects affects the cognitive process of the brain; for instance, the anxiety of the subjects can cause disinhibition of alpha activity, which leads to increase its activity within the limbic system or nucleus cerelus. Aside from the discussion provided here, it should be considered that EEG properties can be influenced by ongoing cognitive processes such as memory processes, chronic craving, and drug use, and initial emotional state of subjects could affect the cognitive process of the brain. On the other hand, the prevalence of smoking is higher in methadone-maintained patients rather than in the general population and methadone therapy is shown to increase the tobacco smoking among these

group of patients depends on their dose. Smoking suggests attenuating opioid withdrawal syndrome among methadone patients. Therefore, nicotine as a highly prevalent cognitive stimulant affects ERP components and influence the results of MMT subjects' cognitive traits.

In addition, there were some controversies between the statistical analysis and individual observations of subjects regarding the PSD changes, which due to confounding factors and limitations of this study, a matched-pair experimental study among subjects with no symptoms of withdrawal is required to arrive at a robust conclusion on this matter. Furthermore, recovery process among abstinent subjects who did not use substitute drugs should be compared with methadone users for a better elaboration of methadone effects on the brain.

5.4 Recommendations for Future work

As it explains in the limitation of the study regarding the influential effects of anxiety, the author suggests that a study of the anxiety level and other psychological withdrawal effects simultaneously with brain electrophysiological analysis during methadone treatment is required to prove this hypothesis.

In addition, the association of brain oscillations properties and ERP characteristics with withdrawal symptoms and addiction treatment features suggests the need for future investigation of the opioid withdrawal symptoms and other addiction treatment features associated with EEG and ERP properties.

It is important to note that the spatiotemporal dynamics of ERPs and their relationship with their latency remain relatively little studied and single-trial analysis of cognitive responses. Furthermore, ERPs are produced by the synchronous field activity in small cortical domains, and this approach of single-trial analysis can contribute to the

integration of this reliable technique, and a larger number of recording channels spread over scalp with fMRI results can provide a reliable measure for modulations of specific ERP components.

REFERENCES

Aguilar de Arcos, F., Verdejo-García, A., Peralta-Ramírez, M. I., Sánchez-Barrera, M., & Pérez-García, M. (2005). Experience of emotions in substance abusers exposed to images containing neutral, positive, and negative affective stimuli. *Drug and alcohol dependence, 78*(2), 159-167.

Alho, K. (1995). Cerebral generators of mismatch negativity (MMN) and its magnetic counterpart (MMNm) elicited by sound changes. *Ear and hearing, 16*(1), 38-51.

Altman, D. G., & Bland, J. M. (1983). Measurement in Medicine: The Analysis of Method Comparison Studies. *Journal of the Royal Statistical Society. Series D (The Statistician), 32*(3), 307-317. doi:10.2307/2987937

Andreassi, J. L. (2000). *Psychophysiology: Human behavior & physiological response*: Psychology Press.

Anokhin, A. P., Vedeniapin, A. B., Sirevaag, E. J., Bauer, L. O., O'Connor, S. J., Kuperman, S., . . . Rohrbaugh, J. W. (2000). The P300 brain potential is reduced in smokers. *Psychopharmacology, 149*(4), 409-413. doi:10.1007/s002130000387

Ashton, H., Nodiyal, A., Green, D., Moore, B., & Heather, N. (2009). Acupuncture or counselling: outcomes and predictors of treatment choice in a non-statutory addiction service. *J Subst Use, 14*(3-4), 151-160. doi:10.1080/14659890802659002

Avants, S. K., Margolin, A., Holford, T. R., & Kosten, T. R. (2000). A randomized controlled trial of auricular acupuncture for cocaine dependence. *Arch Intern Med, 160*(15), 2305-2312. doi:10.1001/archinte.160.15.2305

Ball, J. C., & Ross, A. (1991). *The effectiveness of methadone maintenance treatment: Patients, programs, services, and outcome*: Springer-Verlag Publishing.

Başar, E., & Schürmann, M. (1994). Functional aspects of evoked alpha and theta responses in humans and cats. *Biological cybernetics, 72*(2), 175-183.

Bauer, L. O. (1994). Electroencephalographic and Autonomic Predictors of Relapse in Alcohol‐Dependent Patients. *Alcoholism: Clinical and Experimental Research, 18*(3), 755-760.

Bauer, L. O. (2001). CNS recovery from cocaine, cocaine and alcohol, or opioid dependence: a P300 study. *Clinical Neurophysiology, 112*(8), 1508-1515. doi:10.1016/s1388-2457(01)00583-1

Baxter, M. G., Parker, A., Lindner, C. C., Izquierdo, A. D., & Murray, E. A. (2000). Control of response selection by reinforcer value requires interaction of amygdala and orbital prefrontal cortex. *The journal of Neuroscience, 20*(11), 4311-4319.

Bearn, J., Swami, A., Stewart, D., Atnas, C., Giotto, L., & Gossop, M. (2009). Auricular acupuncture as an adjunct to opiate detoxification treatment: Effects on

withdrawal symptoms. *J Subst Abuse Treat, 36*(3), 345-349. doi:10.1016/j.jsat.2008.08.002

Bergdahl, L., Berman, A. H., & Haglund, K. (2014). Patients' experience of auricular acupuncture during protracted withdrawal. *J Psychiatr Ment Health Nurs, 21*(2), 163-169. doi:10.1111/jpm.12028

Berman, A. H., Lundberg, U., Krook, A. L., & Gyllenhammar, C. (2004). Treating drug using prison inmates with auricular acupuncture: A randomized controlled trial. *J Subst Abuse Treat, 26*(2), 95-102. doi:10.1016/s0740-5472(03)00162-4

Bernat, E., Shevrin, H., & Snodgrass, M. (2001). Subliminal visual oddball stimuli evoke a P300 component. *Clinical Neurophysiology, 112*(1), 159-171.

Bernstein, K. S. (2000). The experience of acupuncture for treatment of substance dependence. *J Nurs Scholarsh.* Retrieved from http://search.ebscohost.com/login.aspx?direct=true&db=mdc&AN=12462821&site=ehost-live

Black, S., Carey, E., Webber, A., Neish, N., & Gilbert, R. (2011a). Determining the efficacy of auricular acupuncture for reducing anxiety in patients withdrawing from psychoactive drugs. *Journal of Substance Abuse Treatment, 41*(3), 279-287. doi:10.1016/j.jsat.2011.04.001

Black, S., Carey, E., Webber, A., Neish, N., & Gilbert, R. (2011b). Determining the efficacy of auricular acupuncture for reducing anxiety in patients withdrawing from psychoactive drugs. *J Subst Abuse Treat, 41*(3), 279-287. doi:10.1016/j.jsat.2011.04.001

Bonnet, M. H., & Arand, D. L. (2001). Impact of activity and arousal upon spectral EEG parameters. *Physiology & behavior, 74*(3), 291-298.

Bonnette, M. L. (2008). Auricular transcutaneous electrical neuro-stimulation, addiction education, behavioral training, coaching support and the nicotine addiction treatment process. *J Addict Nurs, 19*(3), 130-140. doi:10.1080/10884600802305976

Bostanov, V., & Kotchoubey, B. (2006). The t-CWT: A new ERP detection and quantification method based on the continuous wavelet transform and Student's t-statistics. *Clinical Neurophysiology, 117*(12), 2627-2644. doi:http://dx.doi.org/10.1016/j.clinph.2006.08.012

Boutros, N., Nasrallah, H., Leighty, R., Torello, M., Tueting, P., & Olson, S. (1997). Auditory evoked potentials, clinical vs. research applications. *Psychiatry research, 69*(2), 183-195.

Brickman, A. M., Paul, R. H., Cohen, R. A., Williams, L. M., MacGregor, K. L., Jefferson, A. L., . . . Gordon, E. (2005). Category and letter verbal fluency across the adult lifespan: relationship to EEG theta power. *Archives of Clinical Neuropsychology, 20*(5), 561-573.

Bullock, M. L., Kiresuk, T. J., Sherman, R. E., Lenz, S. K., Culliton, P. D., Boucher, T. A., & Nolan, C. J. (2002). A large randomized placebo controlled study of auricular acupuncture for alcohol dependence. *J Subst Abuse Treat, 22*(2), 71-77. doi:10.1016/s0740-5472(01)00217-3

Buzzell, G. A., Fedota, J. R., Roberts, D. M., & McDonald, C. G. (2014). The N2 ERP component as an index of impaired cognitive control in smokers. *Neurosci Lett, 563*, 61-65. doi:10.1016/j.neulet.2014.01.030

Cabioglu, M. T., Ergene, N., & Tan, U. (2007). Smoking cessation after acupuncture treatment. *International Journal of Neuroscience, 117*(5), 571-578. doi:10.1080/00207450500535289

Cai, X., Song, X., Li, C., Xu, C., Li, X., & Lu, Q. (2012). Acupuncture inhibits cue-induced heroin craving and brain activation. *Neural Regen Res, 7*(33), 2607-2616. doi:10.3969/j.issn.1673-5374.2012.33.006

Ceballos, N. A., Bauer, L. O., & Houston, R. J. (2009). Recent EEG and ERP Findings in Substance Abusers. *Clinical Eeg and Neuroscience, 40*(2), 122-128.

Chae, Y., Kang, O. S., Lee, H. J., Kim, S. Y., Lee, H., Park, H. K., . . . Park, H. J. (2010). Effect of acupuncture on selective attention for smoking-related visual cues in smokers. *Neurol Res, 32*, S27-S30. doi:10.1179/016164109x12537002793805

Chae, Y., Park, H.-J., Kang, O. S., Lee, H.-J., Kim, S.-Y., Yin, C.-S., & Lee, H. (2011). Acupuncture attenuates autonomic responses to smoking-related visual cues. *Complement Ther Med, 19*, S1-S7. doi:10.1016/j.ctim.2010.09.003

Chae, Y., Yeom, M., Han, J.-H., Park, H.-J., Hahm, D.-H., Ac, I. S., . . . Lee, H. (2008). Effect of acupuncture on anxiety-like behavior during nicotine withdrawal and relevant mechanisms. *Neurosci Lett, 430*(2), 98-102. doi:10.1016/j.neulet.2007.10.026

Chae, Y. Y., Yang, C. H., Kwon, Y. K., Kim, M. R., Pyun, K. H., Hahm, D. H., . . . Shim, I. (2004). Acupuncture attenuates repeated nicotine-induced behavioral sensitization and c-Fos expression in the nucleus accumbens and striatum of the rat. *Neurosci Lett, 358*(2), 87-90. doi:10.1016/j.neulet.2003.12.121

Chan, Y. Y., Lo, W. Y., Li, T. C., Shen, L. J., Yang, S. N., Chen, Y. H., & Lin, J. G. (2014). Clinical Efficacy of Acupuncture as an Adjunct to Methadone Treatment Services for Heroin Addicts: A Randomized Controlled Trial. *Am J Chin Med, 42*(3), 569-586. doi:10.1142/s0192415x14500372

Chang, B.-H., & Sommers, E. (2014). Acupuncture and relaxation response for craving and anxiety reduction among military veterans in recovery from substance use disorder. *Am J Addict, 23*(2), 129-136. doi:10.1111/j.1521-0391.2013.12079.x

Chang, B. H., Sommers, E., & Herz, L. (2010). Acupuncture and relaxation response for substance use disorder recovery. *J Subst Use, 15*(6), 390-401. doi:10.3109/14659890903580466

Chu, N.-n., Xia, W., Yu, P., Hu, L., Zhang, R., & Cui, C.-L. (2008a). Chronic morphine-induced neuronal morphological changes in the ventral tegmental area in rats are reversed by electroacupuncture treatment. *Addict Biol, 13*(1), 47-51. doi:10.1111/j.1369-1600.2007.00092.x

Chu, N.-n., Xia, W., Yu, P., Hu, L., Zhang, R., & Cui, C.-L. (2008b). Chronic morphine-induced neuronal morphological changes in the ventral tegmental area in rats are reversed by electroacupuncture treatment. *Addiction Biology, 13*(1), 47-51. doi:10.1111/j.1369-1600.2007.00092.x

Clarke, J. G., Stein, M. D., McGarry, K. A., & Gogineni, A. (2001). Interest in smoking cessation among injection drug users. *The American Journal on Addictions, 10*(2), 159-166.

Clemmey, P., Brooner, R., Chutuape, M. A., Kidorf, M., & Stitzer, M. (1997). Smoking habits and attitudes in a methadone maintenance treatment population. *Drug and alcohol dependence, 44*(2), 123-132.

Costa, L., & Bauer, L. (1997). Quantitative electroencephalographic differences associated with alcohol, cocaine, heroin and dual-substance dependence. *Drug and alcohol dependence, 46*(1-2), 87-93.

Courbasson, C. M. A., de Sorkin, A. A., Dullerud, B., & Van Wyk, L. (2007). Acupuncture treatment for women with concurrent substance use and anxiety/depression - An effective alternative therapy? *Fam Community Health, 30*(2), 112-120.

Cowan, D. (2011). Methodological issues in evaluating auricular acupuncture therapy for problems arising from the use of drugs and alcohol. *Acupuncture in Medicine, 29*(3), 227-229. doi:10.1136/aim.2010.003772

Cui, C. L., Wu, L. Z., & Luo, F. (2008). Acupuncture for the treatment of drug addiction. *Neurochemical Research, 33*(10), 2013-2022. doi:10.1007/s11064-008-9784-8

Cui, G. H., Ren, X. W., Wu, L. Z., Han, J. S., & Cui, C. L. (2004a). Electroacupuncture facilitates recovery of male sexual behavior in morphine withdrawal rats. *Neurochemical Research, 29*(2), 397-401. doi:10.1023/B:NERE.0000013743.53827.ad

Cui, G. H., Ren, X. W., Wu, L. Z., Han, J. S., & Cui, C. L. (2004b). Electroacupuncture facilitates recovery of male sexual behavior in morphine withdrawal rats. *Neurochem Res, 29*(2), 397-401. doi:10.1023/B:NERE.0000013743.53827.ad

Darke, S., Sims, J., McDonald, S., & Wickes, W. (2000). Cognitive impairment among methadone maintenance patients. *Addiction, 95*(5), 687-695.

Daubechies, I. (1990). The wavelet transform, time-frequency localization and signal analysis. *IEEE transactions on information theory, 36*(5), 961-1005.

Davydov, D. M., & Polunina, A. G. (2004). Heroin abusers' performance on the Tower of London Test relates to the baseline EEG alpha2 mean frequency shifts.

Progress in Neuro-Psychopharmacology & Biological Psychiatry, 28(7), 1143-1152. doi:10.1016/j.pnpbp.2004.06.006

Deckel, A. W., Hesselbrock, V., & Bauer, L. (1996). Antisocial personality disorder, childhood delinquency, and frontal brain functioning: EEG and neuropsychological findings. *Journal of Clinical Psychology, 52*(6), 639-650.

Delorme, A., & Makeig, S. (2004). EEGLAB: an open source toolbox for analysis of single-trial EEG dynamics including independent component analysis. *Journal of neuroscience methods, 134*(1), 9-21.

Delorme, A., Sejnowski, T., & Makeig, S. (2007). Enhanced detection of artifacts in EEG data using higher-order statistics and independent component analysis. *Neuroimage, 34*(4), 1443-1449.

Domino, E. F. (2003). Effects of tobacco smoking on electroencephalographic, auditory evoked and event related potentials. *Brain Cogn, 53*(1), 66-74. doi:10.1016/s0278-2626(03)00204-5

Donchin, E. (1981). Surprise!… surprise? *Psychophysiology, 18*(5), 493-513.

Donchin, E., & Coles, M. G. (1988). Is the P300 component a manifestation of context updating? *Behavioral and brain sciences, 11*(03), 357-374.

Doppelmayr, M., Klimesch, W., Schwaiger, J., Auinger, P., & Winkler, T. (1998). Theta synchronization in the human EEG and episodic retrieval. *Neuroscience Letters, 257*(1), 41-44.

dos Santos, J. G., Filev, R., Coelho, C. T., Yamamura, Y., Mello, L. E., & Tabosa, A. (2009). Electroacupuncture Inhibits Ethanol-Induced Locomotor Sensitization and Alters homer1A mRNA Expression in Mice. *Alcohol Clin Exp Res, 33*(8), 1469-1475. doi:10.1111/j.1530-0277.2009.00977.x

Duncan, C. C., Barry, R. J., Connolly, J. F., Fischer, C., Michie, P. T., Näätänen, R., . . . Van Petten, C. (2009). Event-related potentials in clinical research: guidelines for eliciting, recording, and quantifying mismatch negativity, P300, and N400. *Clinical Neurophysiology, 120*(11), 1883-1908.

Dunning, J. P., Parvaz, M. A., Hajcak, G., Maloney, T., Alia-Klein, N., Woicik, P. A., . . . Goldstein, R. Z. (2011). Motivated attention to cocaine and emotional cues in abstinent and current cocaine users - an ERP study. *Eur J Neurosci, 33*(9), 1716-1723. doi:10.1111/j.1460-9568.2011.07663.x

Escosteguy-Neto, J. C., Fallopa, P., Varela, P., Filev, R., Tabosa, A., & Santos, J. G. (2012). Electroacupuncture inhibits CB1 upregulation induced by ethanol withdrawal in mice. *Neurochem Int, 61*(2), 277-285. doi:10.1016/j.neuint.2012.05.014

Evans, D. E., & Drobes, D. J. (2009). Nicotine self-medication of cognitive-attentional processing. *Addict Biol, 14*(1), 32-42. doi:10.1111/j.1369-1600.2008.00130.x

Fallopa, P., Escosteguy-Neto, J. C., Varela, P., Carvalho, T. N., Tabosa, A. M. F., & Santos, J. G. (2012a). Electroacupuncture reverses ethanol-induced locomotor sensitization and subsequent pERK expression in mice. *International Journal of Neuropsychopharmacology, 15*(8), 1121-1133. doi:10.1017/s1461145711001325

Fallopa, P., Escosteguy-Neto, J. C., Varela, P., Carvalho, T. N., Tabosa, A. M. F., & Santos, J. G. (2012b). Electroacupuncture reverses ethanol-induced locomotor sensitization and subsequent pERK expression in mice. *Int J Neuropsychopharmacol, 15*(8), 1121-1133. doi:10.1017/s1461145711001325

Farrell, M., Ward, J., Mattick, R., Hall, W., Stimson, G. V., Des Jarlais, D., . . . Strang, J. (1994). Methadone maintenance treatment in opiate dependence: a review. *BMJ: British Medical Journal, 309*(6960), 997.

Fingelkurts, A., Kahkonen, S., Fingelkurts, A., Kivisaari, R., Borisov, S., Puuskari, V., . . . Autti, T. (2008). Reorganization of the composition of brain oscillations and their temporal characteristics during opioid withdrawal. *Journal of Psychopharmacology, 22*(3), 270-284. doi:10.1177/0269881108089810

Fingelkurts, A. A., Fingelkurts, A. A., Kivisaari, R., Autti, T., Borisov, S., Puuskari, V., . . . Kahkonen, S. (2006). Increased local and decreased remote functional connectivity at EEG alpha and beta frequency bands in opioid-dependent patients. *Psychopharmacology, 188*(1), 42-52. doi:10.1007/s00213-006-0474-4

Fingelkurts, A. A., Fingelkurts, A. A., Kivisaari, R., Autti, T., Borisov, S., Puuskari, V., . . . Kahkonen, S. (2007). Opioid withdrawal results in an increased local and remote functional connectivity at EEG alpha and beta frequency bands. *Neuroscience Research, 58*(1), 40-49. doi:10.1016/j.neures.2007.01.011

Fingelkurts, A. A., Fingelkurts, A. A., Kivisaari, R., Autti, T., Borisov, S., Puuskari, V., . . . Kahkonen, S. (2009). METHADONE RESTORES LOCAL AND REMOTE EEG FUNCTIONAL CONNECTIVITY IN OPIOID-DEPENDENT PATIENTS. *International Journal of Neuroscience, 119*(9), 1469-1493. doi:10.1080/00207450903007985

Fingelkurts, A. A., Kähkönen, S., Fingelkurts, A. A., Kivisaari, R., Borisov, S., Puuskari, V., . . . Autti, T. (2007). Composition of EEG oscillations and their temporal characteristics: Methadone treatment. *International Journal of Psychophysiology, 64*(2), 130-140. doi:http://dx.doi.org/10.1016/j.ijpsycho.2007.01.001

Fisher, D. J., Daniels, R., Jaworska, N., Knobelsdorf, A., & Knott, V. J. (2012a). Effects of acute nicotine administration on behavioral and neural (EEG) correlates of working memory in non-smokers. *Brain Res, 1429*, 72-81. doi:10.1016/j.brainres.2011.10.029

Fisher, D. J., Daniels, R., Jaworska, N., Knobelsdorf, A., & Knott, V. J. (2012b). Effects of Acute Nicotine Administration on Resting EEG in Nonsmokers. *Exp Clin Psychopharmacol, 20*(1), 71-75. doi:10.1037/a0025221

Fletcher, T. (2009). Support vector machines explained. *Tutorial paper*.

Folstein, J. R., & Van Petten, C. (2008). Influence of cognitive control and mismatch on the N2 component of the ERP: a review. *Psychophysiology, 45*(1), 152-170. doi:10.1111/j.1469-8986.2007.00602.x

Foster, K. R., Koprowski, R., & Skufca, J. D. (2014). Machine learning, medical diagnosis, and biomedical engineering research-commentary. *Biomedical engineering online, 13*(1), 94.

Fox, H., Axelrod, S., Paliwal, P., Sleeper, J., & Sinha, R. (2007). Difficulties in emotion regulation and impulse control during cocaine abstinence. *Drug Alcohol Depend, 89*(2), 298-301. doi:10.1016/j.drugalcdep.2006.12.026

Fox, H. C., Bergquist, K. L., Casey, J., Hong, K. A., & Sinha, R. (2011). Selective Cocaine‐Related Difficulties in Emotional Intelligence: Relationship to Stress and Impulse Control. *Am J Addict, 20*(2), 151-160. doi:10.1111/j.1521-0391.2010.00108.x

Franken, I. H., Stam, C. J., Hendriks, V. M., & van den Brink, W. (2004). Electroencephalographic power and coherence analyses suggest altered brain function in abstinent male heroin-dependent patients. *Neuropsychobiology, 49*(2), 105-110.

Franken, I. H. A., Stam, C. J., Hendriks, V. M., & van den Brink, W. (2003). Neurophysiological evidence for abnormal cognitive processing of drug cues in heroin dependence. *Psychopharmacology, 170*(2), 205-212. doi:10.1007/s00213-003-1542-7

Franken, I. H. A., Stam, C. J., Hendriks, V. M., & van den Brink, W. (2004). Electroencephalographic power and coherence analyses suggest altered brain function in abstinent male heroin-dependent patients. *Neuropsychobiology, 49*(2), 105-110. doi:10.1159/000076419

Freunberger, R., Klimesch, W., Doppelmayr, M., & Höller, Y. (2007). Visual P2 component is related to theta phase-locking. *Neuroscience Letters, 426*(3), 181-186.

Frisch, S., Kotz, S. A., von Cramon, D. Y., & Friederici, A. D. (2003). Why the P600 is not just a P300: the role of the basal ganglia. *Clinical Neurophysiology, 114*(2), 336-340. doi:http://dx.doi.org/10.1016/S1388-2457(02)00366-8

Fu, L.-p., Bi, G.-h., Zou, Z.-t., Wang, Y., Ye, E.-m., Ma, L., & Yang, Z. (2008). Impaired response inhibition function in abstinent heroin dependents: an fMRI study. *Neuroscience Letters, 438*(3), 322-326.

Gaspar, P. A., Ruiz, S., Zamorano, F., Altayó, M., Pérez, C., Bosman, C. A., & Aboitiz, F. (2011). P300 amplitude is insensitive to working memory load in schizophrenia. *Bmc Psychiatry, 11*(1), 1-6. doi:10.1186/1471-244x-11-29

Gates, S., Smith, L. A., & Foxcroft, D. R. (2006). Auricular acupuncture for cocaine dependence. *Cochrane Database of Systematic Reviews*(1). doi:10.1002/14651858.CD005192.pub2

Gatt, J. M., Kuan, S. A., Dobson-Stone, C., Paul, R. H., Joffe, R. T., Kemp, A. H., . . . Williams, L. M. (2008). Association between BDNF Val66Met polymorphism and trait depression is mediated via resting EEG alpha band activity. *Biological psychology, 79*(2), 275-284.

Goldstein, R. Z., & Volkow, N. D. (2002). Drug addiction and its underlying neurobiological basis: neuroimaging evidence for the involvement of the frontal cortex. *Am J Psychiatry, 159*(10), 1642-1652. doi:10.1176/appi.ajp.159.10.1642

Goldstein, R. Z., & Volkow, N. D. (2011). Dysfunction of the prefrontal cortex in addiction: neuroimaging findings and clinical implications. *Nat Rev Neurosci, 12*(11), 652-669. doi:http://www.nature.com/nrn/journal/v12/n11/suppinfo/nrn3119_S1.html

Gorricho, M. D., & Uson, J. R. V. (2008). QEEG mapping and methadone. *European Journal of Psychiatry, 22*(1), 29-37.

Greenwald, M. K. (2006). Early impact of methadone induction for heroin dependence: differential effects of two dose sequences in a randomized controlled study. *Exp Clin Psychopharmacol, 14*(1), 52-67. doi:10.1037/1064-1297.14.1.52

Greenwald, M. K., & Roehrs, T. A. (2005). Mu-opioid self-administration vs passive administration in heroin abusers produces differential EEG activation. *Neuropsychopharmacology, 30*(1), 212-221. doi:10.1038/sj.npp.1300596

Gritz, E. R., Shiffman, S. M., Jarvik, M. E., Haber, J., Dymond, A. M., Coger, R., . . . Schlesinger, J. (1975). Physiological and psychological effects of methadone in man. *Archives of General Psychiatry, 32*(2), 237-242.

Guillem, F., Rougier, A., & Claverie, B. (1999). Short-and long-delay intracranial ERP repetition effects dissociate memory systems in the human brain. *Journal of Cognitive Neuroscience, 11*(4), 437-458.

Guney, F., Genc, B. O., Kutlu, R., & Ilhan, B. C. (2009). Auditory P300 event-related potential in tobacco smokers. *J Clin Neurosci, 16*(10), 1311-1315. doi:10.1016/j.jocn.2008.11.025

Handy, T. C. (2005). *Event-related potentials: A methods handbook*: MIT press.

Haselhorst, R., Dürsteler–MacFarland, K., Scheffler, K., Ladewig, D., Müller–Spahn, F., Stohler, R., . . . Seifritz, E. (2002). Frontocortical N-acetylaspartate reduction associated with long-term iv heroin use. *Neurology, 58*(2), 305-307.

Hatzigiakoumis, D. S., Martinotti, G., Di Giannantonio, M., & Janiri, L. (2011). Anhedonia and substance dependence: clinical correlates and treatment options. *Front Psychiatry, 2*. doi:10.3389/fpsyt.2011.00010

Hazewinkel, M. (2001). Chebyshev inequality in probability theory. *Encyclopedia of Mathematics. Springer*.

Heinz, A., Schmidt, L., & Reischies, F. (1994). Anhedonia in schizophrenic, depressed, or alcohol-dependent patients--neurobiological correlates. *Pharmacopsychiatry, 27*, 7-10. doi:10.1055/s-2007-1014317

Hou, X. R., Zhang, R. J., Lv, H., Cai, X. H., Xie, G. C., & Song, X. G. (2014). Acupuncture at Baihui and Dazhui reduces brain cell apoptosis in heroin readdicts. *Neural Regen Res, 9*(2), 164-170. doi:10.4103/1673-5374.125345

Houlihan, M. E., Pritchard, W. S., & Robinson, J. H. (1996). Faster P300 latency after smoking in visual but not auditory oddball tasks. *Psychopharmacology, 123*(3), 231-238.

Houlihan, M. E., Pritchard, W. S., & Robinson, J. H. (2001). Effects of smoking/nicotine on performance and event-related potentials during a short-term memory scanning task. *Psychopharmacology (Berl), 156*(4), 388-396. doi:10.1007/s002130100751

Hu, A. R., Lai, M. J., Wei, J. Z., Wang, L. N., Mao, H. J., Zhou, W. H., & Liu, S. (2013). The effect of electroacupuncture on extinction responding of heroin-seeking behavior and FosB expression in the nucleus accumbens core. *Neurosci Lett, 534*, 252-257. doi:10.1016/j.neulet.2012.12.021

Hu, L., Chu, N. N., Sun, L. L., Zhang, R., Han, J. S., & Cui, C. L. (2009a). Electroacupuncture treatment reverses morphine-induced physiological changes in dopaminergic neurons within the ventral tegmental area. *Addiction Biology, 14*(4), 431-437. doi:10.1111/j.1369-1600.2009.00163.x

Hu, L., Chu, N. N., Sun, L. L., Zhang, R., Han, J. S., & Cui, C. L. (2009b). Electroacupuncture treatment reverses morphine-induced physiological changes in dopaminergic neurons within the ventral tegmental area. *Addict Biol, 14*(4), 431-437. doi:10.1111/j.1369-1600.2009.00163.x

Hyvärinen, A., Karhunen, J., & Oja, E. (2004). *Independent component analysis* (Vol. 46): John Wiley & Sons.

Janssen, P. A., Demorest, L. C., Kelly, A., Thiessen, P., & Abrahams, R. (2012). Auricular acupuncture for chemically dependent pregnant women: a randomized controlled trial of the NADA protocol. *Subst Abuse Treat Prev Policy, 7*. doi:10.1186/1747-597x-7-48

Janssen, P. A., Demorest, L. C., & Whynot, E. M. (2005). Acupuncture for substance abuse treatment in the downtown Eastside of Vancouver. *J Urban Health, 82*(2), 285-295. doi:10.1093/jurban/jti054

Jentsch, J. D., & Taylor, J. R. (1999). Impulsivity resulting from frontostriatal dysfunction in drug abuse: implications for the control of behavior by reward-related stimuli. *Psychopharmacology (Berl), 146*(4), 373-390. doi:10.1007/PL00005483

Jiang, Y.-p., Liu, H., Xu, P., Wang, Y., & Lu, G.-h. (2011). Effect of Electro-acupuncture Intervention on Cognition Attention Bias in Heroin Addiction Abstinence-A Dot-probe-based Event-related Potential Study. *Chinese Journal of Integrative Medicine, 17*(4), 267-271. doi:10.1007/s11655-011-0698-y

Jordan, J. B. (2006a). Acupuncture treatment for opiate addiction: A systematic review. *Journal of Substance Abuse Treatment, 30*(4), 309-314. doi:10.1016/j.jsat.2006.02.005

Jordan, J. B. (2006b). Acupuncture treatment for opiate addiction: A systematic review. *J Subst Abuse Treat, 30*(4), 309-314. doi:10.1016/j.jsat.2006.02.005

Kalatzis, I., Piliouras, K., Ventouras, E., Papageorgiou, C. C., Liappas, I. A., Nikolaou, C. C., . . . Cavouras, D. D. (2005). Design and implementation of a multi-PNN structure for discriminating one-month abstinent heroin addicts from healthy controls using the P600 component of ERP signals. *Pattern Recognition Letters, 26*(11), 1691-1700. doi:10.1016/j.patrec.2005.01.012

Kang, O. S., Kim, S. Y., Jahng, G. H., Kim, H., Kim, J. W., Chung, S. Y., . . . Chae, Y. (2013). Neural substrates of acupuncture in the modulation of cravings induced by smoking-related visual cues: an FMRI study. *Psychopharmacology (Berl), 228*(1), 119-127. doi:10.1007/s00213-013-3015-y

Kaptchuk, T. J. (2002). Acupuncture: Theory, efficacy, and practice. *Annals of internal medicine, 136*(5), 374-383.

Karst, M., Passie, T., Friedrich, S., Wiese, B., & Schneider, U. (2002). Acupuncture in the treatment of alcohol withdrawal symptoms: a randomized, placebo-controlled inpatient study. *Addict Biol, 7*(4), 415-419. doi:10.1080/1355621021000006017

Kim, J. H., Chung, J. Y., Kwon, Y. K., Kim, K. J., Yang, C. H., Hahm, D. H., . . . Shim, I. (2005). Acupuncture reduces alcohol withdrawal syndrome and c-Fos expression in rat brain. *Am J Chin Med, 33*(6), 887-896. doi:10.1142/s0192415x0500348x

Kim, M. R., Kim, S. J., Lyu, Y. S., Kim, S. H., Lee, Y. K., Kim, T. H., . . . Yang, C. H. (2005). Effect of acupuncture on behavioral hyperactivity and dopamine release in the nucleus accumbens in rats sensitized to morphine. *Neurosci Lett, 387*(1), 17-21. doi:10.1016/j.neulet.2005.07.007

Kish, S. J., Kalasinsky, K. S., Derkach, P., Schmunk, G. A., Guttman, M., Ang, L., . . . Haycock, J. W. (2001). Striatal dopaminergic and serotonergic markers in human heroin users. *Neuropsychopharmacology, 24*(5), 561-567.

Kivisaari, R., Lehtinen, R., Autti, T., Puuskari, V., Jokela, O., Ahveninen, J., . . . Kahkonen, S. (2007). Impaired pre-attentive auditory processing in opioid dependence with and without benzodiazepine co-dependence revealed by combined magnetoencephalography and electroencephalography. *Progress in Neuro-Psychopharmacology & Biological Psychiatry, 31*(7), 1378-1386. doi:10.1016/j.pnpbp.2007.06.001

Klimesch, W., Doppelmayr, M., Russegger, H., Pachinger, T., & Schwaiger, J. (1998). Induced alpha band power changes in the human EEG and attention. *Neuroscience Letters, 244*(2), 73-76.

Knott, V., Bosman, M., Mahoney, C., Ilivitsky, V., & Quirt, K. (1999). Transdermal nicotine: single dose effects on mood, EEG, performance, and event-related potentials. *Pharmacology Biochemistry and Behavior, 63*(2), 253-261.

Knyazeva, M., & Vil'davskii, V. (1986). Correspondence of spectral characteristics of EEG and regional blood circulation in 9-14 years old children. *Physiologija Cheloveka (Human Physiology), 12*(3), 387-394.

Knyazeva, M. G., & Innocenti, G. M. (2001). EEG coherence studies in the normal brain and after early-onset cortical pathologies. *Brain research reviews, 36*(2), 119-128.

Kraus, N., McGee, T., Carrell, T. D., & Sharma, A. (1995a). NEUROPHYSIOLOGIC BASES OF SPEECH-DISCRIMINATION. *Ear and hearing, 16*(1), 19-37. doi:10.1097/00003446-199502000-00003

Kraus, N., McGee, T., Carrell, T. D., & Sharma, A. (1995b). Neurophysiologic bases of speech discrimination. *Ear Hear, 16*(1), 19-37. doi:10.1097/00003446-199502000-00003

Kujala, T., Tervaniemi, M., & Schröger, E. (2007). The mismatch negativity in cognitive and clinical neuroscience: theoretical and methodological considerations. *Biological psychology, 74*(1), 1-19.

Kunz, S., Schulz, M., Lewitzky, M., Driessen, M., & Rau, H. (2007). Ear acupuncture for alcohol withdrawal in comparison with aromatherapy: A randomized-controlled trial. *Alcohol Clin Exp Res, 31*(3), 436-442. doi:10.1111/j.1530-0277.2006.00333.x

Lee, B., Han, S.-M., & Shim, I. (2009). Acupuncture attenuates cocaine-induced expression of behavioral sensitization in rats: Possible involvement of the dopaminergic system in the ventral tegmental area. *Neurosci Lett, 449*(2), 128-132. doi:10.1016/j.neulet.2008.10.089

Lee, B., Shim, I., Lee, H., Yin, C. S., Park, H. K., Yang, J. S., & Hahm, D. H. (2010). Morphine-induced locomotor response and Fos expression in rats are inhibited by acupuncture. *Neurol Res, 32*, S107-S110. doi:10.1179/016164109x12537002794444

Lee, B. H., Lim, S. C., Jeon, H. J., Kim, J. S., Lee, Y. K., Lee, H. J., . . . Yang, C. H. (2013). Acupuncture suppresses reinstatement of morphine-seeking behavior induced by a complex cue in rats. *Neurosci Lett, 548*, 126-131. doi:10.1016/j.neulet.2013.05.026

Lee, B. H., Ma, J. H., In, S., Kim, H. Y., Yoon, S. S., Jang, E. Y., & Yang, C. H. (2012). Acupuncture at SI5 attenuates morphine seeking behavior after extinction. *Neurosci Lett, 529*(1), 23-27. doi:10.1016/j.neulet.2012.09.020

Lee, T.-W. (1998). Independent component analysis. In *Independent Component Analysis* (pp. 27-66): Springer.

Lee, T., Zhou, W.-h., Luo, X.-j., Yuen, K. S., Ruan, X.-z., & Weng, X.-c. (2005). Neural activity associated with cognitive regulation in heroin users: a fMRI study. *Neuroscience Letters, 382*(3), 211-216.

Levin, E. D., McClernon, F. J., & Rezvani, A. H. (2006). Nicotinic effects on cognitive function: behavioral characterization, pharmacological specification, and anatomic localization. *Psychopharmacology, 184*(3-4), 523-539.

Li, H. Y., Zhang, R., Cui, C. L., Han, J. S., & Wu, L. Z. (2011). Damage of Splenic T Lymphocyte Proliferation and Differentiation and Its Normalization by Electroacupuncture in Morphine-Dependent Mice Mode. *Evid Based Complement Alternat Med*, 1-5. doi:10.1155/2011/424092

Li, J., Sun, Y. A., & Ye, J. H. (2012). Electroacupuncture Decreases Excessive Alcohol Consumption Involving Reduction of FosB/Delta FosB Levels in Reward-Related Brain Regions. *PLoS One, 7*(7), 12. doi:10.1371/journal.pone.0040347

Li, J., Zou, Y. H., & Ye, J. H. (2011). Low frequency electroacupuncture selectively decreases voluntarily ethanol intake in rats. *Brain Res Bull, 86*(5-6), 428-434. doi:10.1016/j.brainresbull.2011.08.013

Li, Y., Ma, Z., Lu, W., & Li, Y. (2006). Automatic removal of the eye blink artifact from EEG using an ICA-based template matching approach. *Physiological measurement, 27*(4), 425.

Li, Y. J., Zhong, F., Yu, P., Han, J. S., Cui, C. L., & Wu, L. Z. (2011a). Electroacupuncture Treatment Normalized Sleep Disturbance in Morphine Withdrawal Rats. *Evid Based Complement Alternat Med*, 1-8. doi:10.1093/ecam/nep133

Li, Y. J., Zhong, F., Yu, P., Han, J. S., Cui, C. L., & Wu, L. Z. (2011b). Electroacupuncture Treatment Normalized Sleep Disturbance in Morphine Withdrawal Rats. *Evidence-Based Complementary and Alternative Medicine*, 1-8. doi:10.1093/ecam/nep133

Liang, J., Li, Y. J., Ping, X. J., Yu, P., Zuo, Y. F., Wu, L. Z., . . . Cui, C. L. (2006). The possible involvement of endogenous ligands for mu-, delta- and kappa-opioid receptors in modulating morphine-induced CPP expression in rats. *Peptides, 27*(12), 3307-3314. doi:10.1016/j.peptides.2006.08.011

Liang, J., Ping, X. J., Li, Y. J., Ma, Y. Y., Wu, L. Z., Han, J. S., & Cui, C. L. (2010). Morphine-induced conditioned place preference in rats is inhibited by electroacupuncture at 2 Hz: Role of enkephalin in the nucleus accumbens. *Neuropharmacology, 58*(1), 233-240. doi:10.1016/j.neuropharm.2009.07.007

Liddell, B. J., Paul, R. H., Arns, M., Gordon, N., Kukla, M., Rowe, D., . . . Williams, L. M. (2007). Rates of decline distinguish Alzheimer's disease and mild cognitive impairment relative to normal aging: integrating cognition and brain function. *Journal of integrative neuroscience, 6*(01), 141-174.

Light, G. A., Williams, L. E., Minow, F., Sprock, J., Rissling, A., Sharp, R., . . . Braff, D. L. (2010). Electroencephalography (EEG) and event‐related potentials (ERPs) with human participants. *Current protocols in neuroscience*, 6.25. 21-26.25. 24.

Lilly, J. M., & Olhede, S. C. (2012). Generalized Morse wavelets as a superfamily of analytic wavelets. *IEEE Transactions on Signal Processing, 60*(11), 6036-6041.

Lin, J. G., Chan, Y. Y., & Chen, Y. H. (2012). Acupuncture for the Treatment of Opiate Addiction. *Evidence-Based Complementary and Alternative Medicine*. doi:10.1155/2012/739045

Liu, S., Zhou, W. H., Liu, H. F., Yang, G. D., & Zhao, W. K. (2005). Electroacupuncture attenuates morphine withdrawal signs and c-Fos expression in the central nucleus of the amygdala in freely moving rats. *Brain Res, 1044*(2), 155-163. doi:10.1016/j.brainres.2005.02.075

Liu, S., Zhou, W. H., Ruan, X. Z., Li, R. H., Lee, T. T., Weng, X. C., . . . Yang, G. D. (2007). Activation of the hypothalamus characterizes the response to acupuncture stimulation in heroin addicts. *Neurosci Lett, 421*(3), 203-208. doi:10.1016/j.neulet.2007.04.078

Liu, S., Zhu, F. L., Lai, M. J., Sun, L. M., Liu, Y. J., & Zhou, W. H. (2012). Electroacupuncture Suppresses Discrete Cue-Evoked Heroin-Seeking and Fos Protein Expression in the Nucleus Accumbens Core in Rats. *Evid Based Complement Alternat Med*. doi:10.1155/2012/286404

Logemann, H. N. A., Bocker, K. B. E., Deschamps, P. K. H., Kemner, C., & Kenemans, J. L. (2014a). The effect of enhancing cholinergic neurotransmission by nicotine on EEG indices of inhibition in the human brain. *Pharmacol Biochem Behav, 122*, 89-96. doi:10.1016/j.pbb.2014.03.019

Logemann, H. N. A., Bocker, K. B. E., Deschamps, P. K. H., Kemner, C., & Kenemans, J. L. (2014b). The effect of the augmentation of cholinergic neurotransmission by nicotine on EEG indices of visuospatial attention. *Behav Brain Res, 260*, 67-73. doi:10.1016/j.bbr.2013.11.044

London, E. D., Ernst, M., Grant, S., Bonson, K., & Weinstein, A. (2000). Orbitofrontal cortex and human drug abuse: functional imaging. *Cerebral Cortex, 10*(3), 334-342.

Lopes da Silva, F. H., Vos, J., Mooibroek, J., & Van Rotterdam, A. (1980). Relative contributions of intracortical and thalamo-cortical processes in the generation of alpha rhythms, revealed by partial coherence analysis. *Electroencephalography and Clinical Neurophysiology, 50*(5), 449-456.

Louria, D. B., Hensle, T., & Rose, J. (1967). The major medical complications of heroin addiction. *Annals of internal medicine, 67*(1), 1-22.

Lua, P. L., & Talib, N. S. (2013). Auricular acupuncture for drug dependence: an open-label randomized investigation on clinical outcomes, health-related quality of life, and patient acceptability. *Altern Ther Health Med, 19*(4), 28-42.

Lua, P. L., Talib, N. S., & Ismail, Z. (2013). Methadone maintenance treatment versus methadone maintenance treatment plus auricular acupuncture: impacts on patient satisfaction and coping mechanism. *J Pharm Pract, 26*(6), 541-550. doi:10.1177/0897190013489574

Lubman, D. I., Allen, N. B., Peters, L. A., & Deakin, J. F. W. (2007). Electrophysiological evidence of the motivational salience of drug cues in opiate addiction. *Psychological Medicine, 37*(8), 1203-1209. doi:10.1017/s0033291707009932

Lubman, D. I., Allen, N. B., Peters, L. A., & Deakin, J. F. W. (2008). Electrophysiological evidence that drug cues have greater salience than other affective stimuli in opiate addiction. *Journal of Psychopharmacology, 22*(8), 836-842. doi:10.1177/0269881107083846

Lubman, D. I., Yucel, M., Kettle, J. W. L., Scaffidi, A., MacKenzie, T., Simmons, J. G., & Allen, N. B. (2009). Responsiveness to Drug Cues and Natural Rewards in Opiate Addiction Associations With Later Heroin Use. *Archives of General Psychiatry, 66*(2), 205-213.

Luck, S. J. (2005). Ten simple rules for designing ERP experiments. *Event-related potentials: A methods handbook, 262083337*.

Luck, S. J. (2014). *An introduction to the event-related potential technique*: MIT press.

Luck, S. J., & Hillyard, S. A. (1994). Electrophysiological correlates of feature analysis during visual search. *Psychophysiology, 31*(3), 291-308.

Luck, S. J., Woodman, G. F., & Vogel, E. K. (2000). Event-related potential studies of attention. *Trends in cognitive sciences, 4*(11), 432-440.

Lyoo, I. K., Pollack, M. H., Silveri, M. M., Ahn, K. H., Diaz, C. I., Hwang, J., . . . Renshaw, P. F. (2006). Prefrontal and temporal gray matter density decreases in opiate dependence. *Psychopharmacology, 184*(2), 139-144.

Makeig, S., Bell, A. J., Jung, T.-P., & Sejnowski, T. J. (1996). Independent component analysis of electroencephalographic data. *Advances in neural information processing systems*, 145-151.

Manfredonia, J. F. (2005). Prescribing methadone for pain management in end-of-life care. *JAOA: Journal of the American Osteopathic Association, 105*(3 suppl), 18S-21S.

Mangun, G. R. (1995). Neural mechanisms of visual selective attention. *Psychophysiology, 32*(1), 4-18. doi:10.1111/j.1469-8986.1995.tb03400.x

Mangun, G. R., & Hillyard, S. A. (1991). Modulations of sensory-evoked brain potentials indicate changes in perceptual processing during visual-spatial priming. *J Exp Psychol Hum Percept Perform, 17*(4), 1057. doi:10.1037/0096-1523.17.4.1057

Margolin, A., Avants, S. K., & Arnold, R. (2005). Acupuncture and spirituality-focused group therapy for the treatment of HIV-positive drug users: A preliminary study. *J Psychoactive Drugs, 37*(4), 385-390.

Margolin, A., Avants, S. K., & Holford, T. R. (2002). Interpreting conflicting findings from clinical trials of auricular acupuncture for cocaine addiction: Does treatment context influence outcome? *J Altern Complement Med, 8*(2), 111-121. doi:10.1089/107555302317371398

Margolin, A., Kleber, H. D., Avants, S. K., Konefal, J., Gawin, F., Stark, E., . . . Vaughan, R. (2002). Acupuncture for the treatment of cocaine addiction - A randomized controlled trial. *JAMA, 287*(1), 55-63. doi:10.1001/jama.287.1.55

Marques-Teixeira, J. E., & Barbosa, M. F. S. (2005). Emotional states and informational brain processing in drug addicts free of drugs: an ERPs study. *International Journal of Psychiatry in Clinical Practice, 9*(3), 213-220. doi:10.1080/13651500510029101

Marsch, L. A. (1998). The efficacy of methadone maintenance interventions in reducing illicit opiate use, HIV risk behavior and criminality: a meta‐analysis. *Addiction, 93*(4), 515-532.

McEvoy, L. K., Pellouchoud, E., Smith, M. E., & Gevins, A. (2001). Neurophysiological signals of working memory in normal aging. *Cognitive Brain Research, 11*(3), 363-376.

McKay, J. R. (1999). Studies of factors in relapse to alcohol, drug and nicotine use: a critical review of methodologies and findings. *J Stud Alcohol, 60*(4), 566.

Meade, C. S., Lukas, S. E., McDonald, L. J., Fitzmaurice, G. M., Eldridge, J. A., Merrill, N., & Weiss, R. D. (2010). A randomized trial of transcutaneous electric acupoint stimulation as adjunctive treatment for opioid detoxification. *J Subst Abuse Treat, 38*(1), 12-21. doi:10.1016/j.jsat.2009.05.010

Mintzer, M. Z., & Stitzer, M. L. (2002). Cognitive impairment in methadone maintenance patients. *Drug and alcohol dependence, 67*(1), 41-51.

Mobascher, A., Brinkmeyer, J., Warbrick, T., Wels, C., Wagner, M., Grunder, G., . . . Winterer, G. (2010). The P300 event-related potential and smoking - A population-based case-control study. *Int J Psychophysiol, 77*(2), 166-175. doi:10.1016/j.ijpsycho.2010.06.002

Montazeri, K., Farahnakian, M., & Saghaei, M. (2002). The effect of acupuncture on the acute withdrawal symptoms from rapid opiate detoxification. *Acta Anaesthesiol Sin, 40*(4), 173-177.

Morie, K. P., Garavan, H., Bell, R. P., De Sanctis, P., Krakowski, M. I., & Foxe, J. J. (2014). Intact inhibitory control processes in abstinent drug abusers (II): A high-density electrical mapping study in former cocaine and heroin addicts. *Neuropharmacology, 82*, 151-160. doi:10.1016/j.neuropharm.2013.02.023

Motlagh, F., Tang, S., & Motlagh, O. (2013). *Optimal accuracy and runtime trade-off in wavelet based single-trial P300 detection.* Paper presented at the Signal and Image Processing Applications (ICSIPA), 2013 IEEE International Conference on.

Motlagh, F. E., Ibrahim, F., Rashid, R. A., Seghatoleslam, T., & Habil, H. (2016). Acupuncture therapy for drug addiction. *Chinese medicine, 11*(1), 1.

Muller, B. W., Specka, M., Steinchen, N., Zerbin, D., Lodemann, E., Finkbeiner, T., & Scherbaum, N. (2007). Auditory target processing in methadone substituted opiate addicts: The effect of nicotine in controls. *Bmc Psychiatry, 7*. doi:10.1186/1471-244x-7-63

Naatanen, R. (1992). The role of attention in auditory information processing as revealed by event-related potentials and other brain measures of cognitive function. *BEHAV BRAIN SCI*, 102-210. doi:10.1017/S0140525X00078407

Neuhaus, A., Bajbouj, M., Kienast, T., Kalus, P., von Haebler, D., Winterer, G., & Gallinat, J. (2006). Persistent dysfunctional frontal lobe activation in former smokers. *Psychopharmacology (Berl), 186*(2), 191-200. doi:10.1007/s00213-006-0366-7

Noel, M. B., & Gratton, A. (1995). Electrochemical evidence of increased dopamine transmission in prefkontal cortex and nucleus accumbens elicited by ventral tegmental μ - opioid receptor activation in freely behaving rats. *Synapse, 21*(2), 110-122.

Nolan, H., Whelan, R., & Reilly, R. (2010). FASTER: fully automated statistical thresholding for EEG artifact rejection. *Journal of neuroscience methods, 192*(1), 152-162.

O'Donnell, B. F., Faux, S. F., McCarley, R. W., Kimble, M. O., Salisbury, D. F., Nestor, P. G., . . . Shenton, M. E. (1995). Increased rate of P300 latency prolongation with age in schizophrenia: Electrophysiological evidence for a neurodegenerative process. *Archives of General Psychiatry, 52*(7), 544-549.

Onton, J., & Makeig, S. (2006). Information-based modeling of event-related brain dynamics. *Progress in brain research, 159*, 99-120.

Oostenveld, R., & Praamstra, P. (2001). The five percent electrode system for high-resolution EEG and ERP measurements. *Clinical Neurophysiology, 112*(4), 713-719.

Organization, W. H. (2002). WHO traditional medicine strategy 2002-2005.

Organization, W. H. (2014). Drug-resistant TB: surveillance and response: supplement to global tuberculosis report 2014.

Overstreet, D. H., Cui, C. L., Ma, Y. Y., Guo, C. Y., Han, J. S., Lukas, S. E., & Lee, D. Y. W. (2008a). Electroacupuncture reduces voluntary alcohol intake in alcohol-preferring rats via an opiate-sensitive mechanism. *Neurochemical Research, 33*(10), 2166-2170. doi:10.1007/s11064-008-9791-9

Overstreet, D. H., Cui, C. L., Ma, Y. Y., Guo, C. Y., Han, J. S., Lukas, S. E., & Lee, D. Y. W. (2008b). Electroacupuncture reduces voluntary alcohol intake in alcohol-

preferring rats via an opiate-sensitive mechanism. *Neurochem Res, 33*(10), 2166-2170. doi:10.1007/s11064-008-9791-9

Papageorgiou, C., Liappas, I., Asvestas, P., Vasios, C., Matsopoulos, G. K., Nikolaou, C., . . . Rabavilas, A. (2001). Abnormal P600 in heroin addicts with prolonged abstinence elicited during a working memory test. *Neuroreport, 12*(8), 1773-1778. doi:10.1097/00001756-200106130-00051

Papageorgiou, C., Rabavilas, A., Liappasa, L., & Stefanis, C. (2003). Do obsessive-compulsive patients and abstinent heroin addicts share a common psychophysiological mechanism? *Neuropsychobiology, 47*(1), 1-11. doi:10.1159/000068868

Papageorgiou, C. C., Liappas, I. A., Ventouras, E. M., Nikolaou, C. C., Kitsonas, E. N., Uzunoglu, N. K., & Rabavilas, A. D. (2004). Long-term abstinence syndrome in heroin addicts: indices of P300 alterations associated with a short memory task. *Progress in Neuro-Psychopharmacology & Biological Psychiatry, 28*(7), 1109-1115. doi:10.1016/j.pnpbp.2004.05.049

Park, H. J., Kim, S. T., Yoon, D. H., Jin, S. H., Lee, S. J., Lee, H. J., & Lim, S. (2005). The association between the DRD2 TaqI A polymorphism and smoking cessation in response to acupuncture in Koreans. *J Altern Complement Med, 11*(3), 401-405. doi:10.1089/acm.2005.11.401

Penetar, D. M., Burgos-Robles, A., Trksak, G. H., MacLean, R. R., Dunlap, S., Lee, D. Y. W., & Lukas, S. E. (2012). Effects of transcutaneous electric acupoint stimulation on drug use and responses to cue-induced craving: a pilot study. *Chin Med, 7*. doi:10.1186/1749-8546-7-14

Percival, D. B., & Walden, A. T. (1993). *Spectral analysis for physical applications*: cambridge university press.

Pfurtscheller, G., & Klimesch, W. (1990). Topographical display and interpretation of event-related desynchronization during a visual-verbal task. *Brain Topography, 3*(1), 85-93.

Picton, T. W. (1992). The P300 wave of the human event-related potential. *Journal of clinical neurophysiology, 9*(4), 456-479.

Polich, J. (1998). P300 clinical utility and control of variability. *Journal of clinical neurophysiology, 15*(1), 14-33.

Polich, J., & Herbst, K. L. (2000). P300 as a clinical assay: rationale, evaluation, and findings. *Int J Psychophysiol, 38*(1), 3-19. doi:10.1016/S0167-8760(00)00127-6

Polunina, A. G., & Davydov, D. M. (2004). EEG spectral power and mean frequencies in early heroin abstinence. *Progress in Neuro-Psychopharmacology & Biological Psychiatry, 28*(1), 73-82. doi:10.1016/j.pnpbp.2003.09.022

Polunina, A. G., & Davydov, D. M. (2006). EEG correlates of Wechsler adult intelligence scale. *International Journal of Neuroscience, 116*(10), 1231-1248. doi:10.1080/00207450600550287

Polunina, A. G., Davydov, D. M., & Kozlov, A. A. (2007). Brain Disintegration in Heroin Addicts: The Natural Course of the Disease and the Effects of Methadone Treatment. *eroin ddiction and elated linical roblems*, 17.

Porjesz, B., Almasy, L., Edenberg, H. J., Wang, K., Chorlian, D. B., Foroud, T., . . . Rohrbaugh, J. (2002). Linkage disequilibrium between the beta frequency of the human EEG and a GABAA receptor gene locus. *Proceedings of the National Academy of Sciences, 99*(6), 3729-3733.

Prosser, J., Cohen, L. J., Steinfeld, M., Eisenberg, D., London, E. D., & Galynker, I. I. (2006). Neuropsychological functioning in opiate-dependent subjects receiving and following methadone maintenance treatment. *Drug and alcohol dependence, 84*(3), 240-247.

Quiroga, R. Q., & Garcia, H. (2003). Single-trial event-related potentials with wavelet denoising. *Clinical Neurophysiology, 114*(2), 376-390.

Rabinstein, A. A., & Shulman, L. M. (2003). Acupuncture in clinical neurology. *Neurologist, 9*(3), 137-148. doi:10.1097/00127893-200305000-00002

Rangaswamy, M., Porjesz, B., Chorlian, D. B., Wang, K., Jones, K. A., Bauer, L. O., . . . Reich, T. (2002). Beta power in the EEG of alcoholics. *Biological psychiatry, 52*(8), 831-842.

Rapeli, P., Kivisaari, R., Autti, T., Kahkonen, S., Puuskari, V., Jokela, O., & Kalska, H. (2006). Cognitive function during early abstinence from opioid dependence: a comparison to age, gender, and verbal intelligence matched controls. *Bmc Psychiatry, 6*. doi:10.1186/1471-244x-6-9

Ray, W. J., & Cole, H. W. (1985). EEG alpha activity reflects attentional demands, and beta activity reflects emotional and cognitive processes. *Science, 228*(4700), 750-752.

Roemer, R. A., Cornwell, A., Dewart, D., Jackson, P., & Ercegovac, D. V. (1995). Quantitative electroencephalographic analyses in cocaine-preferring polysubstance abusers during abstinence. *Psychiatry research, 58*(3), 247-257.

Rousselet, G. A., Foxe, J. J., & Bolam, J. P. (2016). A few simple steps to improve the description of group results in neuroscience. *Eur J Neurosci*. doi:10.1111/ejn.13400

Russell, L. C., Sharp, B., & Gilbertson, B. (2000). Acupuncture for addicted patients with chronic histories of arrest - A pilot study of the Consortium Treatment Center. *J Subst Abuse Treat, 19*(2), 199-205. doi:10.1016/s0740-5472(00)00118-5

Saletu, B., Anderer, P., Saletu-Zyhlarz, G., Arnold, O., & Pascual-Marqui, R. (2002). Classification and evaluation of the pharmacodynamics of psychotropic drugs by single-lead pharmaco-EEG, EEG mapping and tomography (LORETA). *Methods and findings in experimental and clinical pharmacology, 24*(Suppl. C), 97-120.

Samuels, N., Gropp, C., Singer, S. R., & Oberbaum, M. (2008). Acupuncture for psychiatric illness: A literature review. *Behavioral Medicine, 34*(2), 55-62. doi:10.3200/bmed.34.2.55-64

Scherg, M. (1990). Fundamentals of dipole source potential analysis. *Auditory evoked magnetic fields and electric potentials, 6*, 40-69.

Schlögl, A., Keinrath, C., Zimmermann, D., Scherer, R., Leeb, R., & Pfurtscheller, G. (2007). A fully automated correction method of EOG artifacts in EEG recordings. *Clinical Neurophysiology, 118*(1), 98-104.

Schupp, H. T., Cuthbert, B. N., Bradley, M. M., Cacioppo, J. T., Ito, T., & Lang, P. J. (2000). Affective picture processing: the late positive potential is modulated by motivational relevance. *Psychophysiology, 37*(2), 257-261.

Shah, D., Impey, D., Chique-Alfonzo, M., Fisher, D., Lorenzo-Lopez, L., & Knott, V. (2011). Neural effects of acute nicotinic treatment on visual spatial attention in non-smokers. *Pharmacol Biochem Behav, 100*(2), 228-236. doi:10.1016/j.pbb.2011.08.018

Sheehan, D. V., Lecrubier, Y., Sheehan, K. H., Amorim, P., Janavs, J., Weiller, E., . . . Dunbar, G. C. (1998). The Mini-International Neuropsychiatric Interview (M.I.N.I.): the development and validation of a structured diagnostic psychiatric interview for DSM-IV and ICD-10. *J Clin Psychiatry, 59 Suppl 20*, 22-33;quiz 34-57.

Shi, X. D., Ren, W., Wang, G. B., Luo, F., Han, J. S., & Cui, C. L. (2003a). Brain opioid-receptors are involved in mediating peripheral electric stimulation-induced inhibition of morphine conditioned place preference in rats. *Brain Research, 981*(1-2), 23-29. doi:10.1016/s0006-8993(03)02798-7

Shi, X. D., Ren, W., Wang, G. B., Luo, F., Han, J. S., & Cui, C. L. (2003b). Brain opioid-receptors are involved in mediating peripheral electric stimulation-induced inhibition of morphine conditioned place preference in rats. *Brain Res, 981*(1-2), 23-29. doi:10.1016/s0006-8993(03)02798-7

Shi, X. D., Wang, G. B., Ma, Y. Y., Ren, W., Luo, F., Cui, C. L., & Han, J. S. (2004a). Repeated peripheral electrical stimulations suppress both morphine-induced CPP and reinstatement of extinguished CPP in rats: accelerated expression of PPE and PPD mRNA in NAc implicated. *Brain Res Mol Brain Res, 130*(1-2), 124-133. doi:10.1016/j.molbrainres.2004.07.016

Shi, X. D., Wang, G. B., Ma, Y. Y., Ren, W., Luo, F., Cui, C. L., & Han, J. S. (2004b). Repeated peripheral electrical stimulations suppress both morphine-induced CPP and reinstatement of extinguished CPP in rats: accelerated expression of PPE and PPD mRNA in NAc implicated. *Molecular Brain Research, 130*(1-2), 124-133. doi:10.1016/j.molbrainres.2004.07.016

Silveri, M. M., Pollack, M. H., Diaz, C. I., Nassar, L. E., Mendelson, J. H., Yurgelun-Todd, D. A., . . . Kaufman, M. J. (2004). Cerebral phosphorus metabolite and transverse relaxation time abnormalities in heroin-dependent subjects at onset of

methadone maintenance treatment. *Psychiatry Research: Neuroimaging, 131*(3), 217-226.

Simpson, D. D., & Sells, S. B. (1982). Effectiveness of treatment for drug abuse: An overview of the DARP research program. *Advances in Alcohol & Substance Abuse, 2*(1), 7-29.

Singh, S. M., Basu, D., Kohli, A., & Prabhakar, S. (2009). Auditory P300 Event-Related Potentials and Neurocognitive Functions in Opioid Dependent Men and Their Brothers. *American Journal on Addictions, 18*(3), 198-205. doi:10.1080/10550490902786975

Sokhadze, T. M., Cannon, R. L., & Trudeau, D. L. (2008). EEG biofeedback as a treatment for substance use disorders: Review, rating of efficacy, and recommendations for further research. *Applied Psychophysiology and Biofeedback, 33*(1), 1-28. doi:10.1007/s10484-007-9047-5

Sommer, W., & Matt, J. (1990). Awareness of P300 ‐ Related Cognitive Processes: A Signal Detection Approach. *Psychophysiology, 27*(5), 575-585. doi: 10.1111/j.1469-8986.1990.tb01980.x

Specka, M., Finkbeiner, T., Lodemann, E., Leifert, K., Kluwig, J., & Gastpar, M. (2000). Cognitive-motor performance of methadone-maintained patients. *European Addiction Research, 6*(1), 8-19.

Stefanics, G., Kremláček, J., & Czigler, I. (2014). Visual mismatch negativity: a predictive coding view. *Frontiers in human neuroscience, 8*.

Thanavaro, J. L., & Delicath, T. A. (2010). Auricular Transcutaneous Electrostimulation Therapy and Intensive Counseling for the Treatment of Smoking Cessation in a Primary Care Practice. *J Addict Nurs, 21*(4), 215-224. doi:10.3109/10884602.2010.515694

Tian, X. W., & Krishnan, S. (2006). Efficacy of auricular acupressure as an adjuvant therapy in substance abuse treatment: A pilot study. *Altern Ther Health Med, 12*(1), 66-69.

Tomberg, C., & Desmedt, J. E. (1998). Human perceptual processing: inhibition of transient prefrontal-parietal 40 Hz binding at P300 onset documented in non-averaged cognitive brain potentials. *Neurosci Lett, 255*(3), 163-166. doi:10.1016/S0304-3940(98)00740-X

Trumpler, F., Oez, S., Stahli, P., Brenner, H. D., & Juni, P. (2003). Acupuncture for alcohol withdrawal: A randomized controlled trial. *Alcohol Alcohol, 38*(4), 369-375. doi:10.1093/alcalc/agg091

Van Baal, G., De Geus, E., & Boomsma, D. (1996). Genetic architecture of EEG power spectra in early life. *Electroencephalography and clinical Neurophysiology, 98*(6), 502-514.

Verleger, R. (1988). Event-related potentials and cognition: A critique of the context updating hypothesis and an alternative interpretation of P3. *BEHAV BRAIN SCI, 11*(3), 343-356. doi:10.1017/S0140525X00058015

Wang, B., Luo, F., Xia, Y. Q., & Han, J. S. (2000). Peripheral electric stimulation inhibits morphine-induced place preference in rats. *Neuroreport, 11*(5), 1017-1020. doi:10.1097/00001756-200004070-00024

Wang, G. B., Wu, L. Z., Yu, P., Li, Y. J., Ping, X. J., & Cui, C. L. (2011). Multiple 100 Hz electroacupuncture treatments produced cumulative effect on the suppression of morphine withdrawal syndrome: Central preprodynorphin mRNA and p-CREB implicated. *Peptides, 32*(4), 713-721. doi:10.1016/j.peptides.2010.12.006

Wang, G. Y., Kydd, R., Wouldes, T. A., Jensen, M., & Russell, B. R. (2015). Changes in resting EEG following methadone treatment in opiate addicts. *Clinical Neurophysiology, 126*(5), 943-950.

Wang, G. Y., Wouldes, T. A., Kydd, R., Jensen, M., & Russell, B. R. (2014). Neuropsychological performance of methadone-maintained opiate users. *Journal of Psychopharmacology*, 0269881114538541.

Warnke, A., Remschmidt, H., & Hennighausen, K. (1994). Verbal information processing in dyslexia--data from a follow-up experiment of neuro-psychological aspects and EEG. *Acta Paedopsychiatr, 56*(3), 203-208.

Wechsler, D. (2000). Manual for the wechsler adult intelligence scale. New York-III: psychological corporation. Dutch translation. In: Lisse, the Netherlands: Swets & Zeitlinger BV.

Wen, H., & Cheung, S. (1973). Treatment of drug addiction by acupuncture and electrical stimulation. *Asian J Med, 9*, 138-141.

Whelan, R., Conrod, P. J., Poline, J.-B., Lourdusamy, A., Banaschewski, T., Barker, G. J., . . . Cummins, T. D. (2012). Adolescent impulsivity phenotypes characterized by distinct brain networks. *Nature neuroscience, 15*(6), 920-925. doi:10.1038/nn.3092

White, A. R., Rampes, H., & Campbell, J. L. (2006). Acupuncture and related interventions for smoking cessation. *Cochrane Database of Systematic Reviews*(1). doi:10.1002/14651858.CD0000009.pub2.

White, A. R., Rampes, H., Liu, J. P., Stead, L. F., & Campbell, J. (2011). Acupuncture and related interventions for smoking cessation. *Cochrane Database of Systematic Reviews*(1). doi:10.1002/14651858.CD000009.pub3

White, A. R., Rampes, H., Liu, J. P., Stead, L. F., & Campbell, J. (2014). Acupuncture and related interventions for smoking cessation. *Cochrane Database of Systematic Reviews*(1). doi:10.1002/14651858.CD000009.pub4

White, N. M. (1989). A functional hypothesis concerning the striatal matrix and patches: Mediation of S・R memory and reward. *Life sciences, 45*(21), 1943-1957.

Whitford, T. J., Rennie, C. J., Grieve, S. M., Clark, C. R., Gordon, E., & Williams, L. M. (2007). Brain maturation in adolescence: concurrent changes in neuroanatomy and neurophysiology. *Human Brain Mapping, 28*(3), 228-237.

Wilczek, H., Haluzik, M., Svobodova, J., & Parizkova, J. (2004). [Serum leptin levels in patients with heroin addiction, the effect of one year of methadone substitution]. *Vnitrni lekarstvi, 50*(4), 282-285.

Woodman, G. F. (2010). A brief introduction to the use of event-related potentials in studies of perception and attention. *Attention, Perception, & Psychophysics, 72*(8), 2031-2046.

Xia, W., Chu, N. N., Liang, J., Li, Y. J., Zhang, R., Han, J. S., & Cui, C. L. (2011). Electroacupuncture of 2 Hz Has a Rewarding Effect: Evidence from a Conditioned Place Preference Study in Rats. *Evid Based Complement Alternat Med*. doi:10.1093/ecam/nen043

Xu, N., Gao, X., Hong, B., Miao, X., Gao, S., & Yang, F. (2004). BCI competition 2003-data set IIb: enhancing P 300 wave detection using ICA-based subspace projections for BCI applications. *IEEE transactions on biomedical engineering, 51*(6), 1067-1072.

Yang, B., Yang, S. Y., Zhao, L., Yin, L. H., Liu, X., & An, S. S. (2009). Event-related potentials in a Go/Nogo task of abnormal response inhibition in heroin addicts. *Science in China Series C-Life Sciences, 52*(8), 780-788. doi:10.1007/s11427-009-0106-4

Yang, C. H., Yoon, S. S., Hansen, D. M., Wilcox, J. D., Blumell, B. R., Park, J. J., & Steffensen, S. C. (2010a). Acupuncture Inhibits GABA Neuron Activity in the Ventral Tegmental Area and Reduces Ethanol Self-Administration. *Alcoholism-Clinical and Experimental Research, 34*(12), 2137-2146. doi:10.1111/j.1530-0277.2010.01310.x

Yang, C. H., Yoon, S. S., Hansen, D. M., Wilcox, J. D., Blumell, B. R., Park, J. J., & Steffensen, S. C. (2010b). Acupuncture Inhibits GABA Neuron Activity in the Ventral Tegmental Area and Reduces Ethanol Self-Administration. *Alcohol Clin Exp Res, 34*(12), 2137-2146. doi:10.1111/j.1530-0277.2010.01310.x

Yeh, M.-L., Chang, C.-Y., Chu, N.-F., & Chen, H.-H. (2009). A Six-Week Acupoint Stimulation Intervention for Quitting Smoking. *Am J Chin Med, 37*(5), 829-836.

Yoshimoto, K., Fukuda, F., Hori, M., Kato, B., Kato, H., Hattori, H., . . . Yasuhara, M. (2006). Acupuncture stimulates the release of serotonin, but not dopamine, in the rat nucleus accumbens. *Tohoku J Exp Med, 208*(4), 321-326. doi:10.1620/tjem.208.321

Yoshimoto, K., Kato, B., Sakai, K., Shibata, M., Yano, T., & Yasuhara, M. (2001). Electroacupuncture stimulation suppresses the increase in alcohol-drinking behavior in restricted rats. *Alcohol Clin Exp Res, 25*(6), 63S-68S.

Zajocov, A., Wilczek, H., & HOL, V. (2004). Short Communication The Alterations of Immunological Reactivity in Heroin Addicts and Their Normalization in Patients Maintained on Methadone. *Folia Biologica (Praha), 50*, 24-28.

Zalewska-Kaszubska, J., & Obzejta, D. (2004). Use of low-energy laser as adjunct treatment of alcohol addiction. *Lasers Med Sci, 19*(2), 100-104. doi:10.1007/s10103-004-0307-9

Zhang, R. J., Cai, X. H., Song, X. G., Dong, C. Y., Hou, X. R., & Lv, L. (2014). Normalization of ventral tegmental area structure following acupuncture in a rat model of heroin relapse. *Neural Regen Res, 9*(3), 301-307. doi:10.4103/1673-5374.128228

Zhao, R. J., Yoon, S. S., Lee, B. H., Kwon, Y. K., Kim, K. J., Shim, I., . . . Yang, C. H. (2006a). Acupuncture normalizes the release of accumbal dopamine during the withdrawal period and after the ethanol challenge in chronic ethanol-treated rats. *Neuroscience Letters, 395*(1), 28-32. doi:10.1016/j.neulet.2005.10.043

Zhao, R. J., Yoon, S. S., Lee, B. H., Kwon, Y. K., Kim, K. J., Shim, I., . . . Yang, C. H. (2006b). Acupuncture normalizes the release of accumbal dopamine during the withdrawal period and after the ethanol challenge in chronic ethanol-treated rats. *Neurosci Lett, 395*(1), 28-32. doi:10.1016/j.neulet.2005.10.043

LIST OF PUBLICATIONS AND PAPERS PRESENTED

Conference:

Motlagh, F. E., & Ibrahim, F. (2015, December). Developing an Optimized Single-Trial P300-Based Brain Computer Interface System. In International Conference for Innovation in Biomedical Engineering and Life Sciences (pp. 6-10). Springer Singapore.

Journals:

Motlagh, F., Ibrahim, F., Menke, J. M., Rashid, R., Seghatoleslam, T., & Habil, H. (2016). Neuroelectrophysiological approaches in heroin addiction research: A review of literatures. Journal of neuroscience research, 94(4), 297-309.

Motlagh, F., Ibrahim, F., Rashid, R., Seghatoleslam, T., & Habil, H. (2016). Investigation of brain electrophysiological properties among heroin addicts: Quantitative EEG and event-related potentials. Journal of Neuroscience Research.

Motlagh, F. E., Ibrahim, F., Rashid, R. A., Seghatoleslam, T., & Habil, H. (2016). Acupuncture therapy for drug addiction. Chinese Medicine, 11(1), 1-20.