INTERTWINED STUDY ON BRAIN BIO-ELECTRICAL SIGNAL AND SALIVARY PROTEIN FOR MENTAL STRESS LEVEL INDICATOR

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A thesis submitted in fulfilment of the

requirements for the award of the degree of

Master of Engineering (Bio-medical)

Faculty of Biosciences and Medical Engineering

Universiti Teknologi Malaysia

DECEMBER 2016

Dedicated, in thankful appreciation for support, encouragement and understandings to my beloved mother, father, brothers and sisters

ACKNOWLEDGEMENT

First and foremost, I would like to express my heartily gratitude and sincere appreciation to my supervisor, Dr. Nugraha Priya Utama for the advices, aid, enthusiasm and guidance when needed throughout the whole academic year in order to realize the development and implementation of this project.

My appreciation also goes to my family who has been so tolerant and supports me all these years. Thanks for their encouragement, love and emotional supports that they had given to me.

I would also like to thank my friends Siti Norhayati, Chiko Wong, Nabila and others who had given moral support and helping me during difficulties in order to finish this project.

Nevertheless, my great appreciation dedicated to those whom involve directly and indirectly with this project. There is such no meaningful word than thank you so much

ABSTRACT

Lifetime exposure to stress can have significant impact on health that increase the risk of having diseases that may lead to death. In this project, inter-relationship between brain activity and salivary alpha-amylase (sAA) as stress marker is investigated in response to induced mental-stress activities. By using psychophysics experiment to alter normal mental condition, several mental tasks with different levels of difficulty (baseline level: 100% correct answer, easy level: 65-75% correct answer; medium level: 20-40% correct answer, and hard level: 0% correct answer) are presented to 20 healthy participants (10 male; 10 female). In this study, participants' normal mental condition is defined using results from perceived stress scale (PSS-10) questionnaire given to them before experimental procedure with total score of 40. Participant with PSS-10 total score lower than 30 is considered in their normal mental condition. Brain activity from participants were recorded using electroencephalogram (EEG) and their saliva samples were also collected during experimental procedure. The characteristic of sAA is a biomarker for autonomic nervous system reacting sensitively to stress-related changes. A correlation analysis was conducted to see the significant correlation between specific activated brain area and sAA concentration level during alpha and beta frequency band specifically focusing on superior frontal gyrus, middle frontal gyrus, inferior frontal gyrus, orbital gyrus, anterior cingulate, and insula areas. Positive correlation that was statistically significant between brain activities in beta frequency band and sAA concentration level was found in insula area ($r= 0.998$, $n= 3$, $p=0.042<0.05$) for β_2 band and anterior cingulate ($r= 0.997$, $n= 3$, $p=0.047<0.05$) and insula area ($r= 0.999$, $n= 3$, $p= 0.031<0.05$) for β_3 band. However, alpha-band activities and sAA level did not show any statistically significant correlation in all six desired brain area. These areas were significantly more active under stimuli with highest level of difficulties compared with that of the lowest level. Saliva analysis using ANOVA also showed statistically significant difference in concentration level of sAA for 4 different groups: ($F (3, 8) =55.76509$, $p=0.00001<0.05$) where only medium ($M=110.79$, $SD=3.97$); $t (4) = 2.78$, $p= 0.002<0.0083$ (two-tailed) and hard level ($M=123.56$, $SD=2.50$); $t (4) = 2.78$, $p=0.0001<0.0083$ (two-tailed) has significantly differ from baseline level (control group). In conclusion, brain activity in anterior cingulate and insula area within beta frequency band and sAA level showed statistically positive correlation, as it altered normal mental condition in regards to different levels of stress.

ABSTRAK

Pendedahan jangka panjang kepada tekanan boleh membawa pelbagai kesan untuk kesihatan yang boleh meningkatkan risiko untuk mempunyai penyakit yang teruk sehingga membawa maut. Dalam projek ini, satu kajian yang saling berkaitan antara aktiviti otak dan "salivary alpha-amylase" (sAA) dijadikan sebagai penanda untuk tekanan disiasat sebagai tindak balas disebabkan oleh aktiviti mental berkaitan dengan tekanan. Dengan menggunakan ujikaji "psychophysics" untuk mengubah keadaan normal mental, beberapa tugas mental dengan tahap kesukaran yang berbeza (baseline level: 100% correct answer, easy level: 65-75% correct answer; medium level: 20-40% correct answer, and hard level: 0% correct answer) telah diberikan kepada 20 orang peserta yang sihat untuk diselesaikan (10 lelaki; 10 perempuan). Keputusan daripada PSS-10 akan digunakan sebagai penanda aras keadaan normal mental setiap peserta di mana keseluruhan markah PSS-10 di bawah 30/40 dianggap dalam keadaan normal. Aktiviti otak daripada peserta telah dirakamkan menggunakan Electroencephalogram (EEG) dan sampel air liur peserta telah dikumpulkan kerana sifat sAA sebagai penanda bio untuk sistem saraf autonomi dan ia juga sensitif terhadap perubahan yang berkaitan dengan tekanan. Analisis korelasi dijalankan untuk melihat hubungan yang signifikan di antara kawasan tertentu otak yang diaktifkan dan tahap kepekatan sAA semasa jalur frekuensi, alpha dan beta khususnya tertumpu kepada "superior frontal gyrus", "middle frontal gyrus", "inferior frontal gyrus", "orbital gyrus", "anterior cingulate", dan "insula". Korelasi positif yang ketara secara statistik antara aktiviti otak dalam jalur frekuensi beta dan tahap kepekatan sAA ditemui di kawasan "insula" ($r = 0.998$, $n = 3$, $p = 0.042<0.05$) untuk β_2 band dan "anterior cingulate" ($r = 0{,}997$, $n = 3$, $p = 0{,}047<0.05$) dan kawasan "insula" ($r = 0.999$, $n = 3$, $p = 0.031<0.05$) untuk β_3 band. Walau bagaimanapun, aktiviti jalur frekuensi alpha dan tahap SAA tidak menunjukkan korelasi statistik yang signifikan dalam kesemua enam kawasan otak yang dikehendaki. Kawasan-kawasan tertentu otak adalah jauh lebih aktif di bawah rangsangan tugas yang mempunyai kesukaran tahap tertinggi berbanding dengan tahap yang paling rendah. Analisis air liur menggunakan ANOVA juga menunjukkan perbezaan statistik yang signifikan dalam tahap kepekatan sAA untuk 4 kumpulan yang berbeza: ($F(3, 8) = 55{,}76509$, $p = 0.00001<0.05$) di mana hanya tahap "Medium" ($M = 110{,}79$, $SD = 3.97$); $t(4) = 2.78$, $p = 0.002<0.0083$ (two-tailed) dan tahap "Hard" ($M = 123.56$, $SP = 2.50$); $t(4) = 2.78$, $p = 0.0001<0.0083$ (two-tailed) banyak berbeza secara signifikan daripada tahap "Baseline" (kumpulan kawalan). Kesimpulannya, aktiviti otak pada "cingulate anterior" dan kawasan "insula" dalam jalur frekuensi beta dan tahap sAA menunjukkan korelasi statistik yang positif, kerana ia mengubah keadaan normal mental berdasarkan kepada tahap tekanan yang berbeza.

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LIST OF ABBREVIATIONS

sAA	-	Salivary Alpha-Amylase
EEG	-	Electroencephalogram
ANOVA	-	Analysis Of Variance
PTSD	-	Post-Traumatic Stress Disorder
DASS-21	-	Depression Anxiety Stress Scale-21
ECG	-	Electrocardiogram
TSST	-	Trier Social Stress Test
BP	-	Blood Pressure
HRV	-	Heart Rate Variability
EDA	-	Electro Derma Response
ANS	-	Autonomic Nervous System
CNS	-	Central Nervous System
PNS	-	Parasympathetic Nervous System
SNS	-	Sympathetic Nervous System
PSS	-	Perceived Stress Scale
SRI	-	Stress Response Inventory
LECI	-	Life Event and Coping Inventory
GSR	-	Galvanic Skin Response

MEG	-	Magneto encephalography
ICA	-	Independent Component Analysis
GUI	-	Graphic User Interface
TFA	-	Time Frequency Analysis
fMRI	-	Functional Magnetic Resonance Imaging
PET	-	Positron Emission Tomography
LORETA	-	Low Resolution Brain Electromagnetic Tomography
sLORETA	-	standardized Low Resolution Brain Electromagnetic Tomography
FBME	-	Faculty of Biosciences and Medical Engineering
LPF	-	Low Pass Filter
HPF	-	High Pass Filter
TW	-	Time Window
BA	-	Broadmann Area

LIST OF SYMBOLS

δ	-	Delta (1.5-6) Hz
θ	-	Theta (6.5-8) Hz
α_1	-	Alpha band 1 (8.5-10) Hz
α_2	-	Alpha band 2 (10.5-12) Hz
β_1	-	Beta band 1 (12.5-18) Hz
β_2	-	Beta band 2 (18.5-21) Hz
β_3	-	Beta band 3 (21.5-30) Hz
r	-	Pearson Correlation Coefficient
p	-	p-value
F	-	F-test value
M	-	Mean
SD	-	Standard Deviation
t	-	t-test value
A	-	Absorbance
ε	-	Absorptivity
c	-	Constant
l	-	Length
T	-	Transmission

I	-	Intensity
λ	-	Wavelength (lambda)
℃	-	Celsius
w / v	-	weight over volume
C	-	Concentration
V	-	Volume
m	-	Mass

LIST OF APPENDICES

CHAPTER 1

INTRODUCTION

This chapter provides brief introduction on background of study, followed by problem statements, objectives, and scopes of project.

1.1 BACKGROUND INFORMATION

Stress is known to be persistent and insidious because it stem from psychological rather than physical threats. Even though, there is no accurate definition for stress, it can be briefly explained as the feeling of being under too much of mental or emotional pressure that can make stress arises when an individual perceive that, they feel unable to cope with the demands being made on them or threats to their well-being (Lazarus 1966). In addition, stress also explains as an external stimulus that threaten normal balance of body function known as homeostasis condition. Stress can be good as it helps body to control stress responses properly and provide extra strength and energy needed when confronted with crucial physical challenge.

In addition, acute physiological responses to stress can defend the body and brain in establishing and maintaining the homeostasis. However, if stress responses continue for prolonged periods, it can continuously promote physiological stress responses and soon to be hard to shut them off when they are not needed. When this happen, it will upset the body's biochemical balance which can accelerate the disease development. As an example, releasing of hormones can help to improve memory, boost immune function and enhance muscular activity. But if these hormone continue to be released for a long period, it can be harmful as it will impairing the memory, suppressing immune function and leading to weakened muscles. Epinephrine is a hormone secreted by the medulla of the adrenal glands which play an important role in fight or flight responses. It stimulate to release epinephrine hormone after adrenal gland receives input from nervous system. When stress occurred, this hormone will give an effect of alertness or energetic state as it prepares body for the action. However, too much released of epinephrine can cause inflammation and lead to arthritis and accelerate aging of the brain which promotes neurological diseases. This is why stress are known as silent killer as it happen silently without any noticeable sign.

Generally, stress can be divided into two: acute stress (short-term) or chronic stress (long-term) which acute stress known to be not a risk or threat to health while chronic stress portrayed an important issue in today's social life because it promotes a wide range of health problems. Based on the world wide statistical analysis, about 60% to 80% of outpatient visits may be related to stress and it may be associated with development of most major health problem such as heart disease, cancer, stroke, post-traumatic stress disorder (PTSD) and mental disorder (Cohen, Janicki-Deverts et al. 2007, Marin, Lord et al. 2011).

Most of the previous research study on stress was done based on the healthy participant as which it measure acute stress using different type of stressor. Based on the study done by Takai et al., the study using the video of corneal transplant surgery on healthy participant to measure their acute stress level while Rohleder et al. using the Trier Social Stress Test (TSST) as the results revealed that sAA activity is increased by the effect of acute stress (Rohleder, Nater et al. 2004, Takai, Yamaguchi et al. 2004). In addition, Noto and friends using mental arithmetic stress task in

inducing the acute stress level which the results from State Trait Anxiety Inventory (STAI) was significantly correlated to sAA but not to salivary chromogranin-A or cortisol (Noto, Sato et al. 2005). Furthermore, Stroop Colour Word Stressor was applied in research study done by Pehlivanoglu et al. and Svetlak et al. but on different parameter. Pehlivanoglu measured using the Electrocardiogram (ECG) and blood pressure and the results showed strong relation between ECG and blood pressure while Svetlak measuring the acute stress using the electro derma activity (EDA) as the responses was increased during stress event on majority of the subjects.

However, Shamsuddin and colleagues done their research study on university students as there were about 27.5% of university students that had moderate depression whereas 9.7% of them had severe and extremely severe depression. While for anxiety 34% of students had moderate anxiety and 29% of them had severe or extremely severe anxiety. However, approximately 18.6% and 5.1% had moderate and severe or extremely severe stress scores respectively based on the Depression Anxiety Stress Scale-21 (DASS-21) inventory conducted on 506 university students in Klang Valley, Malaysia (Shamsuddin, Fadzil et al. 2013). This survey disclosed how bad mental stress is as it can lead to negative consequences not only for the elderly as well as the young generation.

Therefore, it is significant to have various psychological stress assessment instruments among doctors, psychologists or clinical researchers in order to examine different level and effect of stress to an individual accurately as one of preventive measure from major health problems.

1.2 PROBLEM STATEMENT

Nowadays, the term of stress has become significant in the human life over the world. Lifetime exposure to stress can bring important consequences for health and the effect of stress on health varies across the different stages of human life (Almela, Hidalgo et al. 2011). As example, people who exposed or experienced chronic stress

can have a serious impact on their physical and mental health condition. This condition will increase the risks on having severe diseases such as cardiovascular disease, immune system disorder, mental disorder and worse cases leading to sudden death.

In accordance to that, there are many recent research studies that focus on investigating mental stress based on different scale of parameter such electrocardiography (ECG), blood pressure, electro dermal response, electroencephalography (EEG), and salivary proteins (Hjortskov, Rissén et al. 2004, Takai, Yamaguchi et al. 2004, Noto, Sato et al. 2005, Seo and Lee 2010). Therefore, there are mental stress researches that focusing on the brain signal activity in investigating the effect of psychological stress on the detrimental changes of brain structure and function (Aftanas, Pavlov et al. 2003, Aftanas and Golosheykin 2005, Lewis, Weekes et al. 2007, Oei, Veer et al. 2012).

Over the past 20 years, most of the study focus on the finding of salivary cortisol as non-invasive biomarker in indicating the stress level (Baum 1993, Hellhammer, Wüst et al. 2009, Brouwer, Neerincx et al. 2011, Obayashi 2013) because of its reliability and stability but it has low sensitivity to stress response compared to salivary alpha-amylase (sAA) after same mental event (Takai, Yamaguchi et al. 2004, Noto, Sato et al. 2005, Ali and Pruessner 2012). Based on recent study, researcher has found another non-invasive biomarker by suggesting salivary alpha-amylase (sAA) as an index of autonomic activity in indicating psychological stress. This is because it is directly produced by salivary gland in the oral mucosa which shows positive correlation with acute sympathetic nervous system and has high sensitivity to stress responses (Ali & Pruessner 2012; Almela et al. 2011; Bosch et al. 2011; Chatterton et al. 1996; D. A. Granger et al. 2007; Nater & Rohleder 2009; Schumacher et al. 2013; van Stegeren et al. 2006; Vineetha et al. 2014).

Yet there is no intertwined study on brain signal activities and salivary alpha-amylase (sAA) in measuring stress responses based on work puzzle task with different level of difficulties (psychological stressor) (**see Appendix A**). Hence this study will investigates mental stress level induced by the psychological stimuli under different level of difficulties (psychological stressor) as well as which brain area is activated

under stress response from stimuli using sLORETA method based on two different parameter: EEG signal and salivary alpha-amylase (sAA) reaction.

1.3 PROJECT OBJECTIVES

The objectives of this project are as follow:

1. To analyse the activated areas of brain regions based on the brain signal recorded in accordance to the stimuli with different degree of difficulties in healthy young adults.

2. To study the effect of salivary alpha-amylase (sAA) concentration in relation to the stimuli with different degree of difficulties given to the young healthy adults.
3. To investigate the correlation between recorded brain signal and concentration of salivary alpha-amylase (sAA) in term of different levels of mental stress based on the degree of difficulties stimuli given to the young healthy adults.

1.4 SIGNIFICANCE OF THE PROJECT

The significance of this project are as follow:

1. The reliability and certainty indicating the different mental stress level can be increased so that it can help as precautionary measures to reduce mental-stress level.

2. The reliability and accuracy on the application of saliva as non-invasive technique in measuring the mental stress level can be increased and validated plus can help in coping with the mental-stress problem at early stage.

3. Brain area of interest related to stressful event can be found and investigated which can increasing the certainty as which part of brain is highly activated for future research references related to mental-stress.

1.5 SCOPE OF PROJECT

The scope of this project is divided into four categories as follows:

1. **Participants:** 20 young healthy students with no neurological and psychiatric illness (screening based on PSS scale questionnaire) from Faculty of Biosciences and Medical Engineering, UTM were chosen (range of ages: 19 to 30 years).

2. **Stimuli:** 12 different word tasks were categorized based on 4 different degrees of difficulties (Baseline, Easy, Medium and Hard) for 3 different sessions.

3. **Parameter:** EEG machine with 32 channels (NEURO PRAX EEG) will be used to record the brain signal data (EEG) while UV-Visible Spectrophotometer will be used to measure the salivary protein (salivary alpha-amylase).

4. **Data Analysis:** EEGLAB under Matlab software will be used to analyse the EEG data for the purpose in reducing the noises, artifacts, and cutting the epoch. sLORETA will be used to approximate the activated brain area based on those clean data. Salivary Alpha-Amylase (sAA) will be analysed based on its absorbance and concentration levels correspond to the experiment using the spectrophotometric stop-reaction.

1.6 OUTLINE OF THESIS

This report consists of five chapters. The first chapter presents the background information related to this project. It also introduces the problem statement of the project including the objectives, significances and scopes of the project. Chapter 2 elaborates the current literatures related to the study is presented which mainly on stress related responses, salivary alpha-amylase (sAA), EEG measurement system and brain signal processing. Chapter 3 provides the methodology employed in this study which includes experimental procedure during data collection, salivary amylase spectrophotometric reaction, EEG measurement, data processing and analysis. In this psychophysics experiment, several stimuli with different level of difficulties were given to the subjects for inducing different level of mental stress. The result and discussion were presented in Chapter 4. Last but not least, Chapter 5 discusses the conclusion of this project with a few remarks on possible expansion that could be done in the future.

1.7 WORK SUMMARY

At the beginning of the project, literature review is done to study on mental stress which has been discovered through previous studies. The experimental procedure was designed including the timeline of the experiment, presentation of stimuli and validation of the stimuli/experiment. Prior to data collection, detailed procedure of the experiment will be informed to the subjects.

Therefore, two different data sets will be collected during the experiment; brain signals and saliva sample on healthy university students. Electroencephalography (EEG) system will be used to record the brain signals while UV Visible Spectrophotometer will be used to measure the absorbance of salivary alpha-amylase. The data analysis on both parameters will be carried out using the EEGLAB, sLORETA source localization and Microsoft Excel. Hence, the effects of different mental stress-level will be investigated based on two different parameters; brain

signals and salivary alpha amylase. Finally, the thesis on this project is completed. Figure 1.1, 1.2, 1.3 and 1.4 provides the Gantt chart of this project for Semester 1, Semester 2, Semester 3 and Semester 4 respectively.

Month		September				October				November				December				January			
Week		1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
Problem Formulation																					
Literature Review																					
Design of the project	Methodology of the experiment																				
	Presentation of stimuli/ experiment																				
	Validation of stimuli/ experiment																				
Report Writing																					

Figure 1.1 Gantt chart for Semester 1 (2013)

Month	February				March				April				May				June			
Week	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
Literature Review																				
Validation of stimuli/ experiment																				
Preparation of experiment																				
Data collection																				
Report Writing																				

Figure 1.2 Gantt chart for Semester 2 (2014)

Month	September				October				November				December				January			
Week	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
Literature Review																				
Data Collection																				
Saliva measurement																				
Data Analysis																				
Source Localization of EEG data																				
Report writing																				

Figure 1.3 Gantt chart for Semester 3 (2014)

Month	February				March				April				May				June			
Week	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
Literature Review																				
Data analysis																				
Source Localization of EEG data																				
Finalize the Project																				
Thesis Writing																				

Figure 1.4 Gantt chart for Semester 4 (2015)

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