# A QUANTITATIVE STUDY ON THE PHYSIOLOGICAL CHANGES AND EFFECTS OF DIFFERENT DEEP BREATHING DURATIONS ON THE COGNITIVE CONTROL

CHENG KOK SUEN

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## A QUANTITATIVE STUDY ON THE PHYSIOLOGICAL CHANGES AND EFFECTS OF DIFFERENT DEEP BREATHING DURATIONS ON THE COGNITIVE CONTROL

By

CHENG KOK SUEN

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#### ABSTRACT

## A QUANTITATIVE STUDY ON THE PHYSIOLOGICAL CHANGES AND EFFECTS OF DIFFERENT DEEP BREATHING DURATIONS ON THE COGNITIVE CONTROL

## **Cheng Kok Suen**

Deep breathing brings positive effects on the physiological state of the body, however, the current literature does not have a consensus on how long it is necessary. Furthermore, there is no study linking deep breathing to the cognitive control. In this study, questionnaires, cerebral oxygen delivery (CDO<sub>2</sub>), heart rate variability (HRV), electroencephalogram (EEG) and event related potential (ERP) in a Go/NoGo paradigm to quantify the cognitive control were investigated for different deep breathing durations. 50 participants were recruited and randomised into one of the four groups of control (Con, n = 12), Deep breathing for 5 minutes (DB5, n = 12), 7 minutes (DB7, n = 13) and 9 minutes (DB9, n = 13). The period of interest included the baseline (R1), first Go/NoGo task (T1), during deep breathing (INT), post deep breathing (R2), second Go/NoGo task (T2), follow-up baseline (R3) and third Go/NoGo task (T3) during the follow-up session. During R3, a positive trend between the CDO<sub>2</sub> and the deep breathing duration was evident. For the HRV indices during INT, all three DB groups had a significantly larger SDNN (all three p < 0.05) and nLF (all three p < 0.001) and a significantly smaller nHF (all three p < 0.001) compared to Con. This indicated that the DB groups had a greater activation of the parasympathetic nervous system. For the EEG, DB5 and DB9 had a

significantly larger frontal relative theta power as compared to that of Con (both p < 0.05) whereas DB7 and DB9 groups achieved a centrally dominant topography. The overall beta power was lower in all three DB groups (all three p < 0.05). These showed that the DB groups' participants achieved a 'focused yet not anxious' state of mind. For the ERP, results showed that during T3, the NoGo N2 amplitude of the DB5 group was significantly larger than that of Con (p < 0.05) and an inverse relationship between the NoGo N2 amplitude and the deep breathing duration was observed. This indicated that the DB5 group had an enhanced conflict monitoring ability. Regarding the optimum deep breathing duration, the current study revealed that the optimum duration is either 5 or 9 minutes.

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## **APPROVAL SHEET**

## This dissertation/thesis entitled "<u>A QUANTITATIVE STUDY ON THE</u> <u>PHYSIOLOGICAL CHANGES AND EFFECTS OF DIFFERENT DEEP</u> <u>BREATHING DURATIONS ON THE COGNITIVE CONTROL</u>" was

prepared by CHENG KOK SUEN and submitted as partial fulfillment of the requirements for the degree of Master of Science at Universiti Tunku Abdul Rahman.

Approved by:

(DR LEE POH FOONG) Date:..... Supervisor Department of Mechatronics & Biomedical Engineering Lee Kong Chian Faculty of Engineering & Science Universiti Tunku Abdul Rahman

(DR CHANG YUN FAH) Date:..... Co-supervisor Department of Mathematical & Actuarial Sciences Lee Kong Chian Faculty of Engineering & Science Universiti Tunku Abdul Rahman

### LEE KONG CHIAN FACULTY OF ENGINEERING & SCIENCE

### UNIVERSITI TUNKU ABDUL RAHMAN

Date: \_\_\_\_\_

#### SUBMISSION OF DISSERTATION

It is hereby certified that <u>Cheng Kok Suen</u> (ID No: <u>17UEM00248</u>) has completed this dissertation entitled "A QUANTITATIVE STUDY ON THE PHYSIOLOGICAL CHANGES AND EFFECTS OF DIFFERENT DEEP BREATHING DURATIONS ON THE COGNITIVE CONTROL" under the supervision of Dr Lee Poh Foong (Supervisor) from the Department of Mechatronics & Biomedical Engineering, Lee Kong Chian Faculty of Engineering & Science, and Dr Chang Yun Fah (Co-Supervisor) from the Department of Mathematical & Actuarial Sciences, Lee Kong Chian Faculty of Engineering & Science.

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I hereby declare that the dissertation is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UTAR or other institutions.

Name Cheng Kok Suen

Date \_\_\_\_\_

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## LIST OF ABBREVIATIONS

ACC	Anterior Cingulate Cortex
ACS	Attentional Control Scale
ANS	Autonomic Nervous System
ANT	Attentional Network Task
bpm	Breaths per Minute
CAMS - R	Cognitive and Affective Mindfulness Scale - Revised
CBF	Cerebral Blood Flow
CBFV	Cerebral Blood Flow Velocity
CDO <sub>2</sub>	Cerebral Oxygen Delivery
CE	Commission Error
Con	Control Group
DB5	Deep Breathing for 5 Minutes
DB7	Deep Breathing for 7 Minutes
DB9	Deep Breathing for 9 Minutes
ECG	Electrocardiogram
EEG	Electroencephalogram
ERP	Event Related Potential
Go RT	Go Reaction Time
Hb	Deoxygenated Heamoglobin
HBO <sub>2</sub>	Oxygenated Heamoglobin
HF	High Frequency
HRV	Heart Rate Variability
IMBT	Integrative Body-mind Training

LF	Low Frequency
MAAS	Mindfulness Attention Awareness Scale
MBRP	Mindfulness-based Relapse Prevention
MBSR	Mindfulness-based Stress Reduction
MCAv	Middle Cerebral Artery Velocity
nHF	Normalised High Frequency
nLF	Normalised Low Frequency
OA	Overall Accuracy
OE	Omission Error
PaCO <sub>2</sub>	Arterial Partial Pressure of Carbon Dioxide
PetCO <sub>2</sub>	End-tidal Partial Pressure of Carbon Dioxide
PPG	Photoplethysmographm
PSD	Power Spectrum Density
RCT	Randomised Controlled Trial
RF	Respiration Frequency
RMSSD	Square Root of the Mean Squared Differences of
	Successive NN Intervals
RSA	Respiration Sinus Arrhythmia
RTV	Reaction Time Variability
SDNN	Standard Deviation of the NN Interval
$S_pO_2$	Oxygen Saturation Level
ULF	Ultra Low Frequency
VLF	Very Low Frequency

#### CHAPTER 1.0

## **INTRODUCTION**

## 1.1 Background

Breathing, which is the process of exchanging air in the body, is one of the most important biological processes for survival. However, in today's fastpaced lifestyle, there is a tendency for the breathing pattern to be moving towards the chest breathing in which the breathing is fast and shallow. This breathing pattern draws a minimum amount of air into the lungs and can cause hyperventilation (too little carbon dioxide in the blood), anxiety (Conrad *et al.*, 2007) and it is associated with panic attacks (Nardi, Freire and Zin, 2009). At the opposite end of chest breathing is the deep breathing, whereby the engagement of the diaphragm muscle is greater and thus, letting more air to enter the lungs. In contrast to chest breathing, deep breathing has been found to alleviate stress (Paul, Elam and Verhulst, 2007), anxiety and fatigue (Hayama and Inoue, 2012).

Changing the respiration rate voluntarily during deep breathing is not a natural process and it involves a higher cognitive process. In such, deep breathing will inevitably bring changes to the physiological state of the body. One of the physiological changes deep breathing brings is the alteration of the oxygen saturation level ( $S_pO_2$ ) and cerebral oxygen delivery (CDO<sub>2</sub>). The

oxygen saturation level is related to the amount of oxygen present in the blood whereas the cerebral oxygen delivery is describing how much oxygen is being delivered to the brain. By allowing more air to enter the lungs to mix with the residual air and to reduce the alveolar dead space during deep breathing (Bindu, Dharwadkar and Dharwadkar, 2013), the level of oxygen and carbon dioxide in the blood can be altered, which in turn will affect the  $S_pO_2$  and CDO<sub>2</sub>. An adequate oxygen supply to the brain is important especially under high cognitive demanding tasks where there is a higher demand for oxygen in the brain (Herff *et al.*, 2013).

Besides altering the cerebrovascular parameters, deep breathing also brings changes to the cardiovascular parameters which can be indicated by the heart rate variability (HRV). Heart rate variability refers to the inconsistency in the interbeat interval for each heart beat and this is caused the by the phenomenon known as the respiration sinus arrhythmia (RSA). The heart beat is faster during the inhalation process whereas the heart beat is slower during the exhalation process. The HRV is useful in quantifying the autonomic nervous system and higher HRV is associated with a better quality of life (Gonçalves *et al.*, 2015) and lower cardiovascular risks (Falcone *et al.*, 2014). Studies have shown that patients with cardiovascular diseases that cause sympathovagal imbalance are known to have a lower HRV measurement as compared to their healthy counterpart, as seen in patients with myocardial infarction (Katz *et al.*, 1999; Carney *et al.*, 2001; Buccelletti *et al.*, 2009), congested heart failure (Ponikowski *et al.*, 1997; Işler and Kuntalp, 2007; Patel *et al.*, 2016) and diabetic neuropathy (Chessa *et al.*, 2002; Sridhar *et al.*, 2010). Recently, breathing is proposed as a fundamental oscillating process and changing the respiration rate can affect the neurophysiology of the human brain (Heck *et al.*, 2017). In order to probe the neurophysiology, the electroencephalogram (EEG) is often used due to its excellent temporal resolution and it's non-invasiveness (Teplan, 2002). The raw EEG signal can be transformed into the frequency domain to obtain the power in each frequency band (delta, theta, alpha, beta and gamma) and by examining these power, information regarding the state of mind can be extracted.

Lastly, given the fact that deep breathing can change the neurophysiology of the brain, it makes sense that deep breathing can also bring changes to the cognitive domain. Recent research has concreted the role of deep breathing in retaining a newly learned motor skill (Yadav and Mutha, 2016), decreasing pain perception (Busch *et al.*, 2012) and reducing cognitive decline in healthy aging (Ferreira *et al.*, 2015). Thus far, there is a lack of report investigating the effects of deep breathing on the cognitive control, which is the ability to change and adapt according to the current situation. The rationale in linking deep breathing and the cognitive control is due to the fact that practitioners of mindfulness such as yoga, tai chi and Mindfulness-based Stress Reduction (MBSR) are known to have a better cognitive control abilities (Zeidan *et al.*, 2010; Quaglia, Goodman and Brown, 2016) and deep breathing is a common exercise in many mindfulness practices (Brown and Gerbarg, 2009). It seems plausible that just by performing the deep breathing an improvement can be done on the cognitive control since deep breathing is one

of the fundamental practices. In order to quantify the cognitive control ability, one method is to employ the event related potential (ERP). It is very much similar to the EEG, but the ERP is the brainwave specifically for a particular event or stimulus. The components' amplitude and latency can be used to represent different cognitive processes depending on what type of stimuli were used.

## **1.2 Problem Statement**

Although deep breathing is known to be beneficial for human physiologically, however, there is no consensus on how long the deep breathing should be. The current literature has reported a deep breathing duration ranging from 3 minutes (for example Gaurav *et al.*, (2016)) to 30 minutes (for example Tharion *et al.*, (2012)) and the optimum duration for achieving the best effects is not known. Another problem yet to be answered is whether can deep breathing alone bring improvement to the cognitive control ability, instead of having to perform a combination of exercises as seen in many mindfulness practices.

## **1.3 Research Objective**

There are two main objectives in this research and are as follows:

- 1. To quantify and compare the physiological changes brought by three different deep breathing durations during the deep breathing, immediately after the deep breathing and after 7 days of practicing consecutively in a follow-up session.
- 2. To investigate the effects of deep breathing in increasing the cognitive control, specifically the conflict monitoring, response inhibition and sustained attention level immediately after a deep breathing section as well as after 7 days of practicing consecutively in a follow-up session.

## 1.4 Significance of Study

From the physiological point of view, this study will further concrete the usefulness of deep breathing intervention by investigating the physiological effects as a function of the deep breathing duration. The optimum duration for the best effect can then be known. Due to the various physiological parameters being studied here, the obtained result would be beneficial to multiple fields, namely the field of cerebral hemodynamics (cerebral oxygen delivery, CDO<sub>2</sub>), autonomic nervous system (heart rate variability, HRV) and the neurophysiology (EEG).

From the cognitive point of view, this is the first study to investigate how deep breathing is related to the cognitive control, particularly the conflict monitoring, response inhibition and sustained attention level via event related potential (ERP) in a Go/NoGo paradigm. Similar as above, the optimum duration for the greatest enhancement of the cognitive control would be known as well. This will extends the current literature on the cognitive effects of deep breathing.

## **1.5 Dissertation Structure**

The structure of the remaining dissertation is as follows:

Chapter 2.0 entails an extensive literature review on the presence of deep breathing in many mindfulness practices, explanations and acquisition methods of each physiological parameters (CDO<sub>2</sub>, HRV, EEG and ERP) along with how deep breathing affects each one of them.

Chapter 3.0 provides the methodology employed in this research, specifying the participants, deep breathing guiding video, questionnaires used, experimental procedure, data acquisition and processing of each parameter and statistical methods used to analyse each parameter.

Chapter 4.0 states the results obtained for each parameter.

Chapter 5.0 discusses the significance of the obtained results from the physiological and cognitive point of view. The limitations of this study and some further recommendations are presented here as well.

Lastly, Chapter 6.0 concludes this dissertation.

### **CHAPTER 2.0**

#### LITERATURE REVIEW

## 2.1 Deep Breathing Exercise in Mindfulness Practices

One of the oldest mindfulness practice is without a doubt the yoga which can be traced back as far as 8000 years ago (Georg, 2001). Breath control or also known as *pranayama* is an integrated part of the whole Hindu yoga philosophy as it is one of the eight limbs of yoga written by the Hindu sage Patanjali in the second century B.C (Kimberlee Bethany, 2011). Pranayama which can be translated as the "control of energy or life force" involves voluntary controlling one's breathing pattern and respiration rate while recognising the connection between the breath and also the mind. There are various practices in *pranayama* which includes both fast *pranayama* and slow pranayama. As the fast pranayama is related to breathing rapidly and forcefully that can go up to 30 breaths per minute (Brown and Gerbarg, 2005a), thus, only the slow *pranayama* that involves a reduction in the respiration rate is reviewed. One of the forms of the slow pranayama is the dirgha pranayama or also known as the yogic breathing (Sengupta, 2012). This form of deep breathing exercise involves taking a full breath in three separate inhalations, with a short pause between each inhalation. By doing so, the lungs will be filled with more air as compared to the normal spontaneous breathing. For the exhalation part, the same procedure is taken whereby the exhalation is done in three separate steps

with a pause in between each exhalation. The second form of the slow pranayama is the ujjayi pranayama (victorious breath) and this type of breathing control is widely practised in many schools of yoga. The philosophy behind this *ujjayi pranayama* is to make the breath to be both long and smooth, typically reaching a 2 to 4 breaths per minute respiration rate (Brown and Gerbarg, 2005a). Lastly is the sama vritti (balanced breathing) practice. This deep breathing exercise comprises of inhaling and exhaling slowly in equal ratio, hence the name of balanced breathing. The normal breathing rate for this practice ranges from 5 to 8 breaths per minute. The time frame at which one practises these breathing control exercise varies from one yoga school to another, but literature has seen the period from as short as 5 minutes (Telles and Desiraju, 1992) up to more than 30 minutes (Peck et al., 2005; Sharma et al., 2014). The physiological changes from practising prayanama include increasing the percentage of oxygen intake, adjusting the autonomic nervous system and even reducing emotional disorders (Telles and Desiraju, 1991; Janakiramaiah, Gangadhar and Naga Venkatesha Murthy, 1998; Janakiramaiah et al., 2000).

Moving towards the Chinese civilisation, the two most popular mindfulness practice will be the Qi-gong and the Tai Chi Chuan, although both of them can be considered as the same thing (Jahnke *et al.*, 2010). Tai Chi Chuan is a form of ancient Chinese martial art that put emphasis on the movement of Qi or energy, focused deep breathing, graceful patterns and weight shifts. Since the Qing dynasty, the practise of Tai Chi Chuan is evident and there exists various forms of Tai Chi Chuan with differences in terms of length, posture, meditation and others. It is not until the year of 1956 that a unified form of the Tai Chi Chuan - the simplified 24 form Tai Chi Chuan was created, which become a standard practise of almost of China today (Mark, 1979). This simplified version of the practise is easier to learn and also take much less time than the traditional ones, whereby one session only takes about 6 minutes to complete (Shou-Yu and Wen-Ching, 2014). In general, the simplified 24 form Tai Chi Chuan begins with the relaxation of the body by bringing the thoughts inward, followed by the sequence of movements in a slow fashion. At the same time of performing the 24 moves, deep, diaphragmatic breathing is done in synchronisation with the movements. The main point of the deep breathing exercise is to let the breath to be deep and smooth at the same time without withholding any breaths. One slight difference between Tai Chi Chuan and yogic breathing is that the attention placed on the breathing is less in Tai Chi Chuan as compared to yoga. This is why at the beginning of practising Tai Chi Chuan the breathing is not emphasised. It is not until the movement is done smooth enough should the breathing be focused on (Shou-Yu and Wen-Ching, 2014). Some of the proven benefits of performing Tai Chi Chuan regularly include reducing pain due to osteoarthritis (Song et al., 2003), reducing stress and improving the quality of life (Sandlund and Norlander, 2000; Li, Hong and Chan, 2001).

The mindfulness practise officially entered the Western world due to the work developed by Jon Kabat-Zinn at the University of Massachusetts Medical School. He had taken the Buddhist mindfulness practise and transformed it into a secular meditation method. This secular meditation that he had created, called the mindfulness-based stress reduction (MBSR) is probably the most popular mindfulness practise in the West. MBSR consists of an 8-week-program which comprises of weekly 2.5 to 3.5 hours classes under the guidance of an instructor, practicing daily at home following an audio guide (about 45 minutes) and a full day retreat session lasting 7.5 hours. Throughout these 8 weeks, there are a few meditation methods being practised, namely the body scan meditation, Hatha yoga, sitting meditation and walking meditation (Kabat-zinn, 1996). Besides these 'formal' meditation, MBSR is also practised during daily life by placing the awareness onto the breathing, pleasant and unpleasant events. Most of the mentioned meditation methods involve a slow and deep breathing while placing the awareness onto the present moment, experiencing them without any judgment or predisposition (Kabat-Zinn, 1982). There exist different variation of the MBSR, for example the mindfulness based cognitive therapy to treat depression and mindfulness-based relapse prevention (MBRP) to treat drug addiction (Bowen *et al.*, 2014).

# 2.2 Effects of Deep Breathing on Respiration and Cerebrovascular Parameters

Oxygen saturation is a measurement of the ratio of the oxygen-saturated haemoglobin to the total haemoglobin (both saturated and unsaturated) and it is normally reported in term of percentage. The normal range of the oxygen saturation is from 95 % to 100 % and anything lower than 90 % is considered as hypoxemia (Urschitz, 2005). Another parameter relating to the oxygen saturation is the oxygen delivery. Oxygen delivery is the measure of how much

oxygen is being delivered to a particular place (i.e. the whole body or only the brain) per minute. One of the factors in determining the oxygen delivery is the oxygen saturation such that a greater oxygen saturation will lead to an increase in the oxygen delivery, given that the other parameters are kept constant. An adequate oxygen delivery is important as the cells use oxygen as a fuel for the metabolic processes and a deficient of oxygen reaching to the cells can cause a decrement in the metabolic rate or even cellular injury and death. Oxygen delivery to the brain is known as the cerebral oxygen delivery (CDO<sub>2</sub>) and a sufficient CDO<sub>2</sub> is important in order to support the underlying cognitive tasks of the brain.

A pulse oximeter is a device to measure noninvasively an individual's oxygen saturation through the measurement of peripheral oxygen saturation (SpO<sub>2</sub>). Using a pulse oximeter is a cheap, convenient and noninvasive way to measure the oxygen saturation in a clinical setting. The working principle of the pulse oximeter is based on the difference in the absorption spectra of oxygenated haemoglobin (HbO<sub>2</sub>) and deoxygenated haemoglobin (Hb) for red and infrared wavelengths. The probe consists of two LEDs and a photodiode across the LEDs (one emitting red light and the other emitting infrared light). The probe is then applied to a location where the skin is thin, like the earlobe or the fingertip. As the light shines through the body part, different amount of each wavelength is absorbed by HbO<sub>2</sub> and Hb. For HbO<sub>2</sub>, more infrared light is absorbed while for Hb, more red light is absorbed (Barker and Tremper, 1987). The transmitted light for each wavelength is measured and normalised signals are produced. Since a large volume of blood will surge in during each beginning

of the pulse, the signal produced will be of a sinusoidal. The minimum transmitted light will then be subtracted from the peak transmitted light so that the signal is purely due to the arterial blood (Tramper and Barker, 1989). Then, the ratio of the red light measurement to the infrared measurement is calculated and converted into SpO<sub>2</sub> reading using the Beer-Lambert law (Severinghaus and Astrup, 1986).

The mechanism in which deep breathing can alter the level of CDO<sub>2</sub> lies in the autoregulation of the cerebral blood flow (CBF), which is directly related to the CDO<sub>2</sub>, responding to the arterial partial pressure of carbon dioxide (P<sub>a</sub>CO<sub>2</sub>) in the blood. It is known that the P<sub>a</sub>CO<sub>2</sub> can alter the CBF by a large margin, such that just a small rise in the PaCO<sub>2</sub> level can lead to a large increase in the CBF in order to bring back the PaCO2 level to normal (Gersten, 2011). The level of P<sub>a</sub>CO<sub>2</sub> can be altered consciously by changing the respiration frequency, for example by deep breathing, the P<sub>a</sub>CO<sub>2</sub> level can fall. Following this, the effects of simple deep breathing at 6 breaths per minute on the cerebrovascular system have been investigated by Eames, Potter and Panerai (2004) and Lucas, Lewis, Sikken, Thomas and Ainslie (2013). They had found that the end-tidal partial pressure of carbon dioxide (PetCO<sub>2</sub>; a surrogate for PaCO<sub>2</sub>) level was not different as compared to that during spontaneous respiration but the cerebral blood flow velocity (CBFV) and middle cerebral artery velocity (MCAv) were significantly smaller. Both CBFV and MCAv were used as a surrogate index for the CBF in the respective studies. From the above-cited studies, the changes in the CBF during slow deep breathing are well documented but the longitudinal effect of deep breathing still remains unclear, especially the CBF and CDO<sub>2</sub> changes during rest after long-term practicing of deep breathing. Furthermore, the effect of the deep breathing duration has not been studied as well.

## 2.3 Effects of Deep Breathing on Heart Rate Variability

HRV is the phenomenon of variation in the time interval between heartbeats (Figure 2.1) and it is modulated by the autonomic nervous system (ANS) which consists of two branches, the sympathetic nervous system and the parasympathetic nervous system (Kazmi *et al.*, 2016). The activation of the sympathetic nervous system will stimulate the body into the 'flight-or-fight' response and there will be a heightened organ's functioning, for example, the heart rate and blood pressure will increase. Whereas for the parasympathetic nervous system, or also known as the vagal nervous system, this branch functions in a totally opposite way with the sympathetic branch. The activation of the vagal branch will calm the body down into the 'rest-and-digest' state which will cause a decrease in the organ's functioning. The HRV reflects the balance and interplay of the two branches of the ANS and information regarding the autonomic function can be extracted from the HRV.

There are two main ways of analysing HRV data, the time domain analysis and the frequency domain analysis. In the time domain analysis, it is further split into two categories: simple and statistical time domain variables (Task Force of The European Society of Cardiology and The North American Society of Pacing and Electrophysiology, 1996). For the simple time domain variables, normally the mean of the whole variable is calculated and this includes the mean heart rate, mean interbeat interval and others. For the statistical time domain variables, statistical analysis is done on either the interbeat intervals or the difference between the interbeat intervals (NN intervals). Two of the most common statistical time domain variable is the standard deviation of the NN interval (SDNN) and the square root of the mean squared differences of successive NN intervals (RMSSD). These two variables represent the long term and short term variability, respectively.

The second analysis method involves transforming the raw interbeat interval data into the frequency domain to obtain the power spectrum density (PSD) along with its amplitude. Hence, the name of frequency domain analysis. The main frequency bands of HRV are as follows: ultra low frequency (ULF; 0 - 0.003 Hz), very low frequency (VLF; 0.003 - 0.04 Hz), low frequency (LF; 0.04 - 0.15 Hz) and high frequency (HF; 0.15 - 0.4 Hz) (Task Force of The European Society of Cardiology and The North American Society of Pacing and Electrophysiology, 1996). The powers are normally reported in absolute values or in a normalised form, where the absolute power is divided by the total power minus the VLF power. The HF power is associated with parasympathetic activations.

The interbeat interval can be measured using an electrocardiogram (ECG) machine which measures the cardiac wave repeatedly. The interbeat interval is then determined by the difference in time between the two successive

R phase of the cardiac wave. Another method of obtaining the interbeat interval is to measure the photoplethysmography (PPG) signal, which is the volumetric changes in the blood. Using a PPG sensor offers a procedural advantage as the ECG electrodes need to be in direct contact with the skin near the heart and this may cause problems when applying to female subjects. On the other hand, a PPG sensor is normally clipped to the fingertip or the earlobe of the subject. Besides that, the HRV measured from PPG signal is found to be as reliable and accurate as the one obtained from ECG signals (Bolanos, Nazeran and Haltiwanger, 2006; Selvaraj *et al.*, 2008).

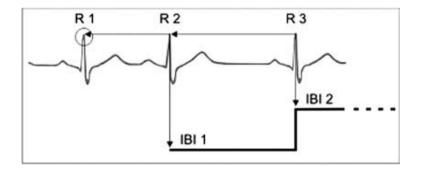


Figure 2.1: Visualisation of the heart rate variability whereby the time intervals between each heart beat are different. In this case a heart beat is defined as the interval between two consecutive R wave peaks (Shaffer and Venner, 2013).

Changes in the HRV can be done voluntarily through alteration in the respiration. Respiration and the heart rate is related through the respiration sinus arrhythmia (RSA) such that the heart rate increases during inhalation whereas

the heart rate decreases during exhalation (Hirsch and Bishop, 1981). Song & Lehrer (2003) had investigated the effects of breathing for 5 minutes at specific respiratory rates of 3, 4, 6, 8, 10, 12, and 14 breaths per minute on the HRV indices. They had found that there were no changes in terms of the mean heart rate between each respiratory rate but there was a significant difference in the LF and HF power. The lower the respiratory rate, the LF power was greater and the inverse relationship was seen in the HF power. A similar study by Guzik *et al.* (2007) who had also utilized a 5 minute breathing time had revealed that at the respiratory rate of 6 breaths per minutes, the SDNN, LF, and HF power was significantly different from 9, 12, and 15 breaths per minute. Additionally, the RMSSD indicated no difference between each respiratory rate.

On the other hand, the carry-over effects of deep breathing on the HRV measurement have been studied as well. Prinsloo *et al.* (2013), by using an HRV biofeedback-induced deep breathing intervention, had evaluated the changes in the HRV indices during baseline, intervention, and a post-intervention period and reported that during the intervention (lasting for 10 minutes), both the SDNN, total power and LF power was significantly larger than the control group whereas during the post-intervention period, the intergroup differences disappeared. Meanwhile, Tharion *et al.* (2012) had investigated the changes in the HRV measurement after one month of practicing deep breathing at 6 breaths per minute, once every day for 30 minutes. As compared to the control group with no deep breathing, there was a significant increase in the HF and total power while a decrease was observed for the mean arterial pressure and the respiration rate in the intervention group after one month of practicing. From

the above-cited studies, it can be seen that there is no consensus on the breathing duration, which can range from 5 minutes to 30 minutes. Furthermore, there is a lack of study reporting on the effect deep breathing duration on the HRV indices.

## 2.4 Effects of Deep Breathing on Electroencephalogram

Electroencephalogram (EEG) is the physiological measurement of the brain's electrical activity. The activation of the neurons in the brain cause local current to be produced (Teplan, 2002). The activation of a single neuron is extremely small and it is impossible to measure it. However, when there is synchronisation in the firing of the neurons, the sum of the current will produce a readable voltage across the scalp and this is the voltage being recorded by the EEG. Even though the voltage is readable, it is to be noted that the reading is still considerably small, only in the microvoltage range. The electrical signal is passed through an amplifier and then to a recording device to record the EEG signal. The most common EEG electrodes used is the flat type electrode that is placed on the scalp of the subject and the advantage of the flat type electrode is that it is noninvasive as compared to the needle type electrode. Besides that, EEG is also a cheap imaging modality and has a high temporal resolution.

The human brain wave oscillation consists of a vast range of frequencies, and through the use of Fourier transform or wavelet transform, the PSD of the raw EEG signal can be extracted. There are five frequency bands associated with EEG, namely the delta band (0.5 - 4 Hz), theta band (4 - 8 Hz), alpha band (8 - 13 Hz), beta band (13 - 30 Hz) and gamma band (> 30 Hz) (Teplan, 2002), as shown in Figure 2.2. Each of these bands is associated with a certain characteristic state of mind. For example, the beta state is the most common state during normal awake moment and is associated with alertness, attentive and concentration. For the alpha state, it is associated with a relaxed state of mind and is most prevalent when the eyes are closed and also during meditation process (Ahani *et al.*, 2014). Going down to the theta band, the theta state of mind is related to sleepiness or drowsiness. A heightened activity in the theta band, which is the lowest band of all five, is observed in deep sleep and the deeper the sleep, the greater the activity in the delta band (Neil R, 2012). Lastly is the gamma band. The gamma band activity plays an important role in information processing, movement control, memory and even attention (Bressler, 1990).

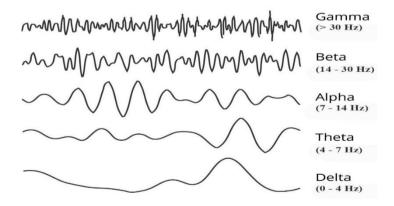


Figure 2.2: EEG waveforms in the delta, alpha, beta and gamma wavebands (Pineda, Juavinett and Datko, 2014).

One of the early studies on deep breathing and EEG was done by Stancak et al. (1993), who investigated how different breathing frequencies affects the theta, alpha and beta mean power and their variabilities. They found that at the lower end of the breathing frequencies (0.14 Hz, 0.10 Hz and 0.06 Hz) that there was no difference in terms of the mean power for all three bands; however, at 0.10 Hz there was a decrease in the variability of the alpha power in the parietal and occipital locations. The presence of a more regular alpha power was interpreted as a lower activity of the brain and hence, this supports the notion that deep breathing can be used to reducing stress. A similar study by (Bušek and Kemlink, 2005) further established the relationship between the breathing frequency and the mean power in the theta, alpha and beta bands, whereby a decrease in the breathing frequency led to an increase in the mean power and vice versa. Fumoto et al. (2004) and Yu et al. (2011) utilized a modified study by fixing the breathing frequency at approximately 3 to 4 breaths per minute to mimic the breathing technique employed in the Zen meditation and investigated the effects on the log-transform relative power for the same three bands. Further, they investigated the effects of the breathing duration, including comparing the log-transform relative power at different intervals (e.g. 5, 10, 15 and 20 minutes) to an initial resting period. From their results, the breathing duration plays an important role in the theta and alpha bands such that the initial theta power was significantly larger than at the 15th and 20th-minute mark and the alpha power showed a marked increment that was evident starting at the 5th-minute mark and remaining the same until the 20th-minute mark.

Thus far, the majority of the studies on deep breathing and EEG either did not utilized a randomised controlled trial (RCT), for example the studies by Fumoto et al. (2004) and Yu et al. (2011) or had utilized an RCT but with a cross-over design (e.g. Stancak et al., 1993; Bušek & Kemlink, 2005; Gaurav et al., 2016). RCT serves as the golden standard for evaluating the effectiveness of an intervention (Mills et al., 2007) and within an RCT, there are several experimental designs being employed. Two of the most popular designs are the parallel group design and the cross-over design. It is relatively simple to implement the parallel group design but its statistical power is not as high as the other designs; whereas the required sample size for the cross-over design may be smaller because each participant serves as his/her own control. However, the carry-over of the previous experimental condition's effect onto the next condition is a significant problem (Stedman et al., 2011). Since each participant in the cross-over design goes through the control and intervention condition in a random manner, the possible carry-over effects of the intervention can confound the results obtained in the later control condition. In studies that investigated the immediate effects of a deep breathing session, the measurements at the post-intervention period of 5 minutes are significantly different from that of the baseline and this is an indication of a carry-over effect lasting even after 5 minutes (Sherlin, Muench and Wyckoff, 2010; Gabriell E Prinsloo et al., 2013). Thus, the use of a cross-over design with a resting period (typically 3 to 5 minutes) between each breathing conditions seems to be inappropriate.

## 2.5 Effects of Deep Breathing on Cognitive Control and Event Related Potential

Event related potential (ERP) is the brain wave associated with a certain time-locked event or stimulus. ERP is important in the research of behavioural and cognitive science as ERP has the ability to review how the brain functions towards a particular event or stimulus due to its high temporal resolution of up to the millisecond range. But, the amplitude of such potential is usually much smaller than the normal recorded brain wave such that it is almost 10 times smaller than the normal recorded voltage. The normal range for ERP is about 10  $\mu$ V while the normal brain wave can go up to 100  $\mu$ V (Teplan, 2002). In order to extract the ERP, one method is to repeatedly present the stimulus and record at which time point did the stimulus occurs in the brain wave. After that, the raw EEG signal is epoched into many segments around the stimulus and all of the segments are then averaged out to obtain the ERP waveform. This averaging process works because ideally, only the desired waveform associated with the stimulus will survive the averaging process and all other signals due to noise are averaged to zero. Mathematically speaking, if the raw EEG signal, x(t,k) is given as

$$x(t,k) = s(t) + n(t,k),$$
 (2.1)

where t is the time, k is the number of stimulus trial, s is the desired ERP signal and n is the noise of the raw EEG signal, then the average of the EEG signal,  $\bar{x}(t,k)$  will be given as

$$\bar{x}(t,k) = s(t) + \sum_{k=1}^{N} n(t,k),$$
 (2.2)

where N is the total number of stimulus trial. In an ideal case, the second term in Eq. (2.2) will equal to zero and the obtained signal are equal to the ERP waveform. This is conceptually shown in Figure 2.3 along with an example ERP waveform.

The components in the ERP waveform are labelled as positive (P) or negative (N) followed by either the number of peaks or the latency of the component. For example, the first positive peak of the ERP waveform can be labelled as P1 or P100, with the 100 meaning the 100 ms latency after the presentation of the stimulus. So for the second trough of the waveform, it is labelled as N2 or N200. Different components of the ERP review different processes of the brain, with normally the earlier components like P1, N1 representing the recognition of the stimulus whereas the later component (N2, P3) reviews the cognitive control functions and reaction towards the stimulus (Woodman, 2010). With every component, there are two variables that are measured regularly, which are the amplitude and the latency. The amplitude of a particular component represents the degree of activation of a particular cognitive process while the latency represents the cognitive processing speed (Polich and Criado, 2006).

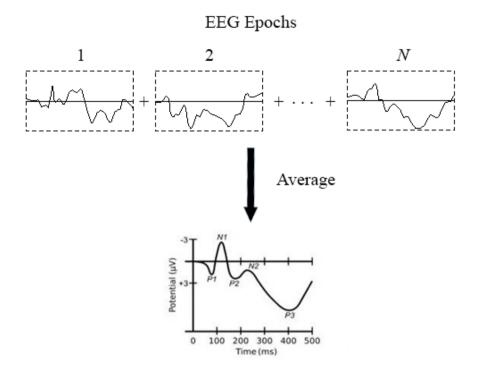


Figure 2.3: Conceptualisation of the averaging process to obtain an ERP waveform. *N* number of epochs representing *N* number of stimuli are averaged together in order to extract the relatively small ERP waveform from the background EEG waveform. The first five components are also labelled on the ERP waveform (Luck, Woodman and Vogel, 2000).

Humans possess the ability to flexibly change their behaviours according to the current situation, reflecting the need to maintain the goals for that particular situation. This ability, known as cognitive control, is vital in everyday life given that our behaviours need to be altered accordingly when facing unknown situations (Davidson *et al.*, 2006). Among the different cognitive processes that build up the cognitive control, *conflict monitoring* and *response inhibition* are of most interest due to their relevance to mental disorders. Conflict in the cognitive sense occurs when two or more incompatible processes compete for the same attentional resources simultaneously (Botvinick *et al.*, 2001). One example of conflict would be response overriding, whereby a prepotent response towards a normal stimulus needs to be inhibited. After the conflicting information has been monitored then the part of response inhibition comes into play so that the prepotent response can be successfully inhibited. A third component that comes into play is the sustained attention, defined as the long-term placement of one's attention on a particular task or process (Sarter, Givens and Bruno, 2001). Sustained attention is characterized by the need to be vigilance enough to pick up and react to any unexpected stimuli or conflicts and thus, modulates the process of conflict monitoring and response inhibition (Barkley, 1997).

Conflict monitoring and response inhibition are needed in a Go/NoGo task which normally consists of two stimuli: a majority Go stimulus that requires a certain action, whether covert or overt, and a minority NoGo stimulus that requires no actions (See *et al.*, 1995). The commission error would reflect in these processes as a successful inhibition would lead to lower commission error (Roche *et al.*, 2005). Furthermore, given the huge skewing of probability towards the Go stimulus, the norm would be in responding to the majority of Go stimulus and an autopiloting action might come in (Robertson *et al.*, 1997). The mind will start to wander around instead of being fully focused on the task (Robertson *et al.*, 1997; Manly *et al.*, 1999) and this act of mind-wandering will reduce the performance in the task (Thomson, Besner and Smilek, 2015).

requires sustained attention and is indexed by the omission error and reaction time variability (O'Halloran *et al.*, 2011). To further elucidate the cognitive process during this task, event-related potentials (ERP) are employed due to its high temporal resolution. In the context of the Go/NoGo task, two components are elicited, namely the N2 and P3 components for each Go and NoGo trials. The NoGo N2 and P3 are used to reflect the conflict monitoring and cancellation of preplanned response towards the Go stimulus, respectively (Donkers and Van Boxtel, 2004; Smith, Johnstone and Barry, 2007), whereas the Go P3 can serve as a biomarker for the sustained attention level (Datta *et al.*, 2007; Hart *et al.*, 2012).

Practitioners of mindfulness are shown to have greater sustained attention, conflict monitoring and response inhibition compared to control groups with no experience in mindfulness (Zeidan *et al.*, 2010; Teper and Inzlicht, 2013; Quaglia, Goodman and Brown, 2016). By definition, mindfulness is the placement of one's attention to the present moment and accepting any thoughts that occur without any judgment. An operational definition of mindfulness given by Bishop *et al.* (2004) suggests that mindfulness can be separated into two facets: self-regulation of attention and orientation of one's experience in the present moment. Sustained attention is required for the first facet such that the mind is kept in the present and would not wander around whereas for the second facet, it involves an awareness and acceptance of any conflicts or judgmental thoughts that are distracting. The first facet is directly relating to the cognitive control while the second facet of awareness and acceptance is also shown to be related to the cognitive control

(Teper, Segal and Inzlicht, 2013). By practicing mindfulness, the cognitive control would be enhanced through improvements in the above two facets. Even though the benefits of mindfulness practices are established, there are some potential barriers that make the community reluctant to embrace such practices. One such barrier is the existence of different exercises being employed in each mindfulness practice. For example, mindfulness-based stress reduction (MBSR) has the element of mindful walking whereas for the Zen meditation there is an element of Tandem Breathing, both of which are not present in the other practice. Besides, the high demand in time and the need for guidance from a coach can discourage the community from taking up these practices as well (Carmody *et al.*, 2008). For a full MBSR course it can take up to eight weeks, with weekly meetings that last for about three hours and daily practice of about an hour (Kabat-zinn, 1996). This can be difficult to adhere to especially in the current fast-paced lifestyle of young people.

Deep breathing is a common element in most mindfulness practices, for instance yoga, tai chi and meditation (Brown and Gerbarg, 2009). Recent interest has surged in the investigation of the effects of deep breathing on human cognition. The literature has reported the effects of deep breathing on the retention of newly learned motor skills (Yadav and Mutha, 2016) and the agingrelated cognitive decline (Ferreira *et al.*, 2015). Yadav and Mutha (2016) had provided the first evidence to link deep breathing to motor memory by showing that a 30-minute deep breathing intervention led to a greater performance in a motor skill. This better retention of the motor skill was evident both immediately after the breathing session, as well as, after a one-day break. The study by Ferreira et al. (2015) on the other hand, investigated the cognitive decline due to aging in three interventions, namely an aerobic exercise group, a respiratory training group, and a control group. The respiratory training group had undergone seven forms of respiratory training among 20 of them, in which one of them was the deep breathing. At the end of the study, the attention level (indexed by the Wechesler Adult Intelligence Symbol Search subscale) of the respiratory training group was stable showing no significant difference between the pre- and post-measurement, whereas for the control and aerobic exercise groups, there was a significant decline in the attention level. Combining the above results and the fact that deep breathing is a common element in many mindfulness practices, a possible linkage between deep breathing, conflict monitoring and sustained attention is expected. A plausible mechanism underlying how deep breathing can lead to a better cognitive control is that deep breathing will alter the activation of the anterior cingulate cortex (ACC) which facilitates cognitive control (Sridharan, Levitin and Menon, 2008). A greater activation of the ACC would be required to resolve any conflicts such as distracting thoughts in order to maintain the attention placed on the deep breathing process (Holzel et al., 2011). This greater activation of the ACC, in turn, can be inferred from the ERP measurements obtained from a Go/NoGo task (Bokura, Yamaguchi and Kobayashi, 2001).

#### 2.6 Summary

From the above discussion, it can be seen that the current literature has extensively studied the effect of deep breathing on the cerebrovascular parameters, HRV and neurophysiology. However, the majority of the studies had arbitrarily chosen the deep breathing duration, which ranged from 5 minutes to 30 minutes and hence, the effect of the deep breathing duration on the physiological changes is still not clear. Further, the majority of the studies only investigated the physiological changes during the deep breathing intervention without considering the carry-over effect of deep breathing after the intervention or after training for a certain period.

In terms of the cognitive domain, research is still ongoing to reveal the possible effects of deep breathing on the cognitive domain. It is now known that deep breathing aids in the retention of newly learned motor skill (Yadav and Mutha, 2016) and prevent attention decline (Ferreira *et al.*, 2015), but the effect of deep breathing on the cognitive control is still not known. This study will be first study to investigate the possible linkage between deep breathing and cognitive control through a Go/NoGo paradigm and ERP measurements.

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#### **CHAPTER 3.0**

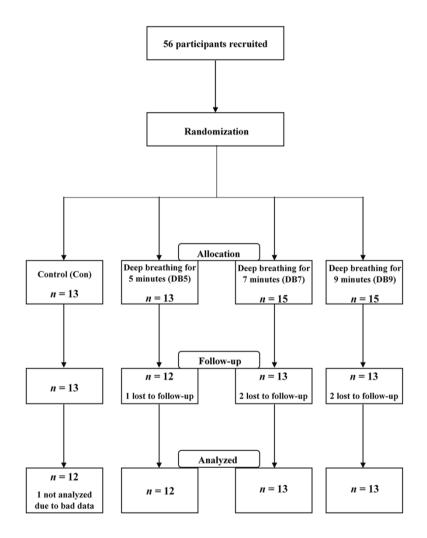
#### METHODOLOGY

#### 3.1 Participants

56 undergraduates as participants from the university were recruited via the distribution of flyers for the study. Three exclusion criteria were used to select the participants: 1) those who have been ill for the past two weeks prior to the experiment, 2) those on long-term medication or are on drug prescriptions and 3) those who are unable to perform deep breathing for 5 minutes or more. In accordance to self-reported questionnaires, all participants had a normal or corrected-to-normal vision and no respiratory diseases or psychiatric disorder. Further, all of the participants do not smoke as confirmed through verbal confirmation, however, their drinking habit was not known. Five participants did not come for the follow-up session and hence, the total number of participants completed the protocol was 51. Furthermore, one participant's data was not analysed due to having too many artefacts. Thus, the total final participants were 50 (age:  $22.04 \pm 1.65$ , 22% females), with 92\% Malaysian Chinese, 4% Malaysian Indian, 2% Aryan and 2% Sino-Kadazan. The demographic information and flowchart of the participants are shown in Table 3.1 and Figure 3.1, respectively.

	Con (n = 12)	DB5 (n = 12)	DB7 (n = 13)	DB9 (n = 13)
Age (years)	22.0 (2.0)	22.4 (1.4)	22.2 (1.8)	21.9 (1.5)
Age range (years)	20 - 27	20 - 24	20 - 24	20 - 24
Height	168.8 (8.3)	167.6 (8.7)	171.3 (9.2)	169.3 (7.5)
Weight	61.3 (15.0)	60.3 (13.8)	65.9 (12.8)	58.8 (12.0)

 Table 3.1: Demographic information of the participants.



**Figure 3.1: Flow chart of the participants.** 

#### **3.2** Description of the Deep Breathing Guiding Video

The participants were guided to perform deep breathing through a video as shown in Figure 3.2. The breathing frequency was set to 0.1 Hz to achieve a resonance between respiration and heartbeat, producing the largest amplitude in the respiration sinus arrhythmia (RSA) (Vaschillo, Vaschillo and Lehrer, 2006). The top-right corner of the video showed the number of completed breathing cycles while the bottom-left showed how much time had passed. At the centre of the video, there was a yellow smiley face with five appearing and disappearing petals, with each petal lasting for one second. When the petals appear, the participants were required to inhale and exhale when the petals disappear. Both inhalation and exhalation were done continuously without breaks. The participants were also instructed to focus on the video and to feel the air going in and out of their body. The rationale behind using a visual guidance instead of an auditory guidance is that it is easier to follow a video guide than an auditory guide whenever the surrounding noise level is high. Hence, this video enables one to perform the deep breathing exercise easily at anywhere at any time.

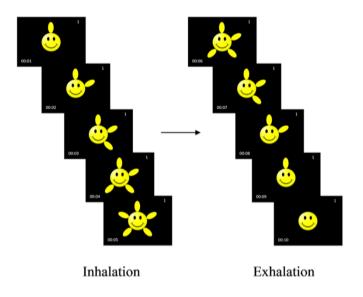


Figure 3.2: Screenshots from the deep breathing video. The video has two sections, one with appearing petals for the inhalation and one for the exhalation with disappearing petals. The breathing rate was set to 6 breaths per minute.

### 3.3 Questionnaire

### 3.3.1 Attentional Control Scale (ACS)

The Attentional Control Scale (ACS) consists of 20 items measuring individual differences in attentional control. Attentional control refers to the ability to focus on a specific thing while ignoring others. The items are in a Likert scale form, with each item ranges from 1 (almost never) to 4 (always) and hence, a total score which ranges from 20 to 80. A higher score represents a greater attentional control of an individual.

#### 3.3.2 Cognitive and Affective Mindfulness Scale - Revised (CAMS-R)

The Cognitive and Affective Mindfulness Scale – Revised (CAMS-R) contains 12 items that assess four factors that constitute to mindfulness: attention, present-focus, awareness and acceptance/non-judgment of thoughts and feelings. This scale is designed to be used with general language and it does not require any type of previous meditation training. The scoring ranges from 1 (rarely/not at all) to 4 (almost always) with the exception of item 6 where it is reversed scored. The final score will be the summation of the individual score. A higher score reflects a greater mindful quality.

#### 3.3.3 Mindfulness Attention Awareness Scale (MAAS)

The Mindful Attention Awareness Scale (MAAS) is a self-reported questionnaire that only measures the attention facet of mindfulness, as compared to CAMS-R which measures a broader sense of mindfulness. This MAAS has 15 items in a Likert scale format ranging from 1 (almost always) to 6 (almost never). A mean score is calculated and a higher score represents greater mindfulness characteristic.

#### **3.4** Experimental Procedure

The research procedures have been approved by the university's Scientific and Ethical Review committee (Ref. No: U/SERC/04/2017). The participants understood the whole procedure and an informed consent was obtained prior to the experiment. The participants were randomised based on their chosen time slot for the experiment into one of the four groups: Control group (Con, n = 12), deep breathing for 5 minutes (DB5, n = 12), deep breathing for 7 minutes (DB7, n = 13), and deep breathing for 9 minutes (DB9, n = 13).

The experiments took place in a laboratory room with ample ambient lighting. On arrival at the laboratory, the participants were rested for 15 minutes to ensure their physiological state was stable. A baseline reading of 5 minutes (R1) was taken, followed by a Go/NoGo task (T1). After performing the task, the participants in the DB groups underwent the deep breathing session (INT) following the video for either 5, 7 or 9 minutes. For the Con group, they were instructed to rest for 9 minutes without showing them any video. After the deep breathing session, all the participants were requested to rest again for another 5 minutes (R2). For the last session, the Go/NoGo task was performed again (T2) to be the post-deep breathing effect outcome. Throughout the experiment (R1, T1, INT, R2, and T2), the participants were required to open their eyes while recording of the variables were done. This concluded the first session.

During the one-week gap between the first session and the follow-up session, the DB participants were instructed to practice the deep breathing

following the video guide every day once at any time convenient for them. Messages were sent to remind participants on a daily basis to practice the deep breathing and a reply message was requested to confirm the practice. At day 7, participants returned to the laboratory for the follow-up session EEG recording. During the follow-up session, participants were first rested for 15 minutes while the recording equipment was being applied. After that, a baseline reading of 5 minutes (R3) was recorded followed by a third Go/NoGo task (T3) at openedeyes conditions. The whole procedure was summarised in Figure 3.3.

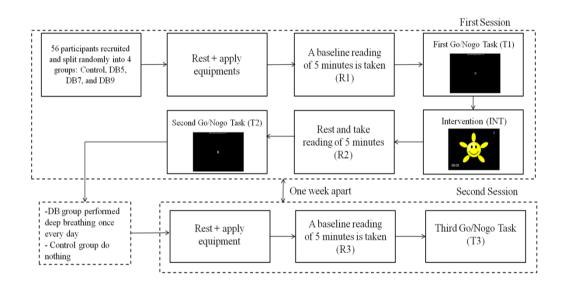


Figure 3.3: Summary of the whole experimental procedure which consisted of 7 main parts: baseline (R1), first Go/NoGo task (T1), intervention (INT), post-intervention (R2), second Go/NoGo task (T2), follow-up baseline (R3) and the third Go/NoGo task (T3).

#### 3.5 Go/NoGo Task Paradigm

In this study, the Go/NoGo task was implemented using The Psychology Experiment Building Language (PEBL; Mueller & Piper 2014), which is an open source software. The two stimuli in this test were the letters 'P' and 'R', with a stimulus time of 500 ms, followed by a blue star for 500 ms and thus, an interstimulus time of 1000 ms (Figure 3.4). In total, there were 480 Go 'P's (80%) and 120 NoGo 'R's (20%). The stimuli were given in two separate blocks with 300 stimuli in each block with a short break between the blocks. The participants were required to click on the mouse pad whenever a 'P' appeared and to refrain from any action whenever an 'R' appeared. Prior to the test, there was a practice session with 20 stimuli (16 'P's and 4 'R's) for the participants to get familiarise with the task. At the end of the task which lasts about 10 minutes, the overall accuracy (OA), Go reaction time (Go RT), omission error (OE), commission error (CE) and reaction time variability (RTV) were recorded.

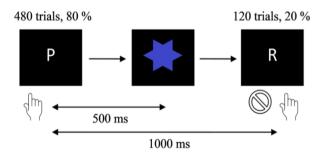


Figure 3.4: Stimuli in the Go/NoGo task whereby 'P's need to be responded whereas 'R's do not need to be responded.

## 3.6 Respiration Frequency, Oxygen Saturation and Cerebral Oxygen Delivery Data Acquisition and Processing

The oxygen saturation ( $S_pO_2$ ) and respiration frequency (RF) were measured by a pulse oximeter (Model: Contec CMS501) and a smartphone app named 'RRate' (Karlen *et al.*, 2014), respectively.

The value of the blood oxygen saturation level,  $S_pO_2$  obtained from the pulse oximeter was first converted into the arterial partial pressure of oxygen,  $P_aO_2$  using the formula by Severinghaus (1979)

$$P_a O_2 = (B+A)^{\frac{1}{3}} \cdot (B-A)^{\frac{1}{3}}, \qquad (2.1)$$

where A and B are

$$A = 11700(S_p O_2^{-1} - 1)^{-1}, \qquad (2.2)$$

$$B = (50^3 + A^2)^{\frac{1}{2}},$$
 (2.3)

respectively. Next, the alveolar-arterial gradient,  $AaPO_2$  was calculated based on the regression formula by Crapo *et al.* (1999) given as

$$AaPO_2 = 33.3652 + 0.1996y - 0.2005H + 0.1906W - 0.0190P_B, \quad (2.4)$$

whereby y is the age in years, H is the height in cm, W is the weight in kg, and  $P_B$  is the barometric pressure in mm Hg, set at sea level of 760 mm Hg. Using the  $AaPO_2$  and  $P_aO_2$ , the arterial partial pressure of carbon dioxide,  $P_aCO_2$  was then calculated using the alveolar gas equation

$$P_a O_2 = F_I O_2 \left( P_{atm} - P_{H_2 O} \right) - \frac{P_a C O_2}{RER},$$
(2.5)

where  $F_1O_2$  is the fraction of inspired oxygen gas set to 0.21,  $P_{atm}$  is the atmospheric pressure set to 760 mm Hg,  $P_{H_2O}$  is the saturated vapor pressure of water set to 47 mm Hg, and *RER* is the respiratory exchange ratio set to 0.8.

The cerebral blood flow, *CBF* was calculated next using the hierarchical cerebrovascular model containing a total of 19 levels of arteries and veins proposed by Piechnik, Chiarelli and Jezzard (2008). First, the radius of each artery or vein in the *i*th level,  $r_i$  was given by

$$r_i = r_i^o (1 + c_i \Delta P_a C O_2),$$
 (2.6)

where  $r_i^o$  is the radius of the artery/vein in the *i*th level when  $P_aCO_2 = 40$  mm Hg,  $c_i$  is the  $CO_2$  reactivity of the *i*th level, and  $\Delta P_aCO_2$  is the change in the arterial partial pressure of carbon dioxide relative to a level of 40 mm Hg. The calculated radius was then fed into the following equation to calculate the *CBF* 

$$CBF = (P_a - P_v) \left( \sum_{i=1}^{M} \frac{8\mu l_i}{\pi m_i r_i^4} \right)^{-1}, \qquad (2.7)$$

where  $P_a$  and  $P_v$  are the arterial and venous pressure, respectively,  $\mu$  is the dynamic viscosity of blood,  $l_i$  is the length of the artery/vein at the *i*th level,  $m_i$  is the number of vessels at the *i*th level and *M* is the total number of levels, which in this case is equal to 19. The values of  $r_i^o$ ,  $c_i$ ,  $l_i$ , and  $m_i$  were obtained from Piechnik, Chiarelli and Jezzard (2008) whereas  $P_a$ ,  $P_v$ , and  $\mu$  was set to 100 mm Hg, 10 mm Hg and  $2.63 \times 10^{-5}$  mm Hg s, respectively, following Lampe *et al.* (2014).

The arterial oxygen content,  $C_a O_2$  was calculated using the following equation

$$C_a O_2 = 1.34 \times Hb \times S_p O_2 + 0.0031 \times P_a O_2, \tag{2.8}$$

where *Hb* is the hemoglobin level, which was set to 15.7 g/dL for males and 13.8 g/dL for females (MecPherson and Pincus, 2011). Lastly, the cerebral oxygen delivery,  $CDO_2$  was calculated as

$$CDO_2 = C_a O_2 \times CBF. \tag{2.9}$$

#### 3.7 Heart Rate Variability Data Acquisition and Processing

A photoplethysmograph (PPG) was obtained using a KYTO Mobile Heart Rate Monitor (Model no: HRM-2935; http://kytofitness.com) which transmitted the raw interbeat interval data to a smartphone app named Elite HRV (https://www.elitehrv.com). During INT, the data recording lasted for 5 minutes for each deep breathing duration. For the DB5 group, the reading was taken from the 0<sup>th</sup> minute to the 5<sup>th</sup> minute; DB7 group was from 2<sup>nd</sup> minute to the 7<sup>th</sup> minute; DB9 group was from 4<sup>th</sup> minute to the 9<sup>th</sup> minute. These recording periods provided the recommended 5 minutes reading for HRV proposed by the Task Force of The European Society of Cardiology and The North American Society of Pacing and Electrophysiology (1996). In the Elite HRV app, the raw R-R interval data was exported and analyzed using HRVAS (Ramshur, 2010), which is an open source software designed to perform HRV analysis using MATLAB.

The raw R-R interval data was first to be checked for ectopic beats. Two filters were used to detect the ectopic beats: when an interval was 20% more than the previous interval and also intervals that lay beyond 3 standard deviations from the mean interbeat interval. Following that, a wavelet detrending method was used to detrend the data. In the time domain analysis, the mean heart rate (HR), SDNN and RMSSD was obtained while in the frequency domain, the normalised power in the LF (nLF; 0.04 - 0.15 Hz) and HF (nHF; 0.15 - 0.4 Hz) bands was obtained. A Lomb-Scargle Periodogram

that uses a least squares fit of sinusoids to the data was used as this method do not need the waveform to be stationary (Van Dongen *et al.*, 1999).

#### 3.8 Electroencephalogram Data Acquisition and Processing

The NCC Medical 32 Channels Type A Routine EEG System (Model no.: Nation 7128W-A32) was used to acquire EEG signals. The 32 Ag/AgCl electrodes in the electrode cap was placed in accordance with the International 10-20 system (site: Fp1, Fp2, AF3, AF4, F7, F3, Fz, F4, F8, FT7, FC3, FC4, FT8, T3, C3, Cz, C4, T4, CP7, CP3, CP4, CP8, P3, Pz, P4, PO3, PO4, T5, O1, Oz, O2, T6) with the reference electrode at Cz and the ground electrode at Fpz. The electrode cap was then connected to Type-A EEG amplifier with a sampling rate of 256 Hz and the signals stored in a computer. The raw EEG signals are processed for bad channels and artefacts using FASTER (Nolan, Whelan & Reilly, 2010) which acts as a plugin in EEGLAB (Lopez-Calderon & Luck, 2014). The high-pass, low-pass and notch filter frequencies were 1 Hz, 30 Hz and 50 Hz, respectively. Bad channels were detected and interpolated whereas artefacts in the channels, epochs, decomposed independent components and single-channel single-epochs were removed using a statistical thresholding of z $= \pm 3$ . Subsequently, any epochs that contained signals with an amplitude greater than 75  $\mu$ V were removed as these signals are likely due to the movement artefacts. The processed EEG signals were then segmented into epochs of 1 s and 200 epochs were randomly selected using a random number generator for analysis. The mean power of each electrode was extracted using the Welch periodogram method with 50 % overlap and a resolution of 1 Hz, followed by the computation of the relative mean power (power in a particular band/total power in all three bands). In order to reduce the data and to study the EEG topography, the processed signals were grouped into 6 different locations: frontal (Fp1, Fp2, AF3, AF4, Fz), central (C3, C4, FC3, FC4, Cz), parietal (P3, P4, CP3, CP4, Pz), occipital (O1, O2, PO3, PO4, Oz), left temporal (F3, F7, T3, T5, FT7, CP7) and right temporal (F4, F8, T4, T6, FT8, CP8). The placement and groupings of the 32 electrodes are shown in Figure 3.5.

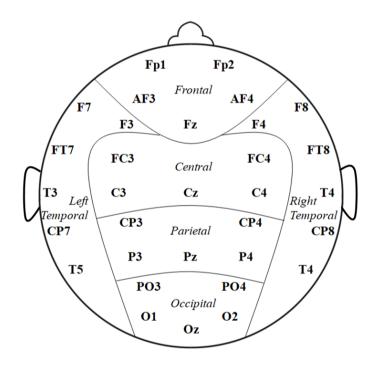


Figure 3.5: The placement and groupings of the 32 electrodes. The electrodes are group into the frontal, central, parietal, occipital, left temporal or right temporal location.

#### 3.9 Event Related Potential Data Acquisition and Processing

The EEG raw data was again processed by using FASTER (Nolan, Whelan & Reilly, 2010). For ERP analysis, only the signals from the midline electrodes (Fz, Cz and Pz) were analysed. The EEG raw data were segmented into epochs ranging from 200 ms before the stimulus and 800 ms post-stimulus, with baseline correction from -200 ms to 0 ms (0 ms representing the stimulus onset). Two major components of the ERP were focused on, namely the N2 (peaks at approximately 100 ms - 300 ms after stimulus) and P3 (peaks at approximately 300 ms - 600 ms). The time windows for the peak detection were determined from the grand average waveform of the ERP of the Go and NoGo trials. Only correct trials were used for the ERP waveform and the Go and NoGo trials waveforms were obtained separately. An area based measurement for the amplitude was selected to analyze the negative or positive area under the ERP waveform in the two time windows for N2 and P3 as to reduce the effect of latency jittery (Luck, 2014). The peak latency was used to quantify the latency.

#### 3.10 Statistical Analysis

The statistical analysis for each parameter was done separately using SPSS (ver. 20). A two-sided p < 0.05 was considered as statistically significant and a p < 0.10 was reported as a trend. Values were reported as means and

standard errors in parenthesis when normally distributed or as medians and interquartile ranges in parenthesis when not normally distributed.

#### 3.10.1 Questionnaire

The scores from the ACS, MAAS and CAMS-R at R3 were analysed using a one-way ANCOVA with the Group (Con, DB5, DB7 and DB9) as the between-subject variable and the scores at R1 as each respective covariates. A planned contrast comparing each of the DB groups to the Con group with Bonferroni correction was performed as the post-hoc test.

## 3.10.2 Respiration Frequency, Oxygen Saturation and Cerebral Oxygen Delivery

The RF was not normally distributed as tested using the Shapiro-Wilk test for normality, thus a nonparametric method was used. For between groups difference, a Kruskal-Wallis test was used and if significance was found, multiple Mann-Whitney U tests with Bonferroni correction were performed to locate the specific changes. For within time period difference in each group, a Friedman test was used to find the overall difference. For post-hoc analysis, multiple Wilcoxon signed-rank test with Bonferroni correction (corrected p = 0.008) was performed.

The S<sub>p</sub>O<sub>2</sub> and CDO<sub>2</sub>, being normally distributed as tested using the Shapiro-Wilk test for normality, were analyzed using a  $4 \times 3$  repeated measure ANCOVA with the Group (Con, DB5, DB7, and DB9) as the between-subject factor, Time (INT, R2, and R3) as the within-subject factor and the baseline readings during R1 as covariates. For sphericity correction, the Huynh-Feldt method was applied. A planned contrast was carried out when any significance was found for the Group variable. The value of the Con group was compared to each of the DB groups with Bonferroni correction and no comparison was done among the DB groups. For the Time variable, a pairwise comparison was done with Bonferroni correction to find the specific changes.

#### 3.10.3 Heart Rate Variability

For the HRV measurements (mean HR, SDNN, nLF and nHF), they were analyzed using a 4 × 3 repeated measure ANCOVA with the Group (Con, DB5, DB7, and DB9) as the between-subject factor, Time (INT, R2, and R3) as the within-subject factor and the baseline reading at R1 as the covariate. Whenever the sphericity condition was violated, the Greenhouse-Geisser (when  $\varepsilon < 0.75$ ) or Huynh-Feldt (when  $\varepsilon > 0.75$ ) correction was applied depending on the epsilon. A planned contrast was carried for any significant Group difference such that the Con group was compared to each of the DB groups with Bonferroni correction but no comparisons were done among the DB groups For the Time variable, a pairwise comparison with Bonferroni correction was used to find the specific change.

#### 3.10.4 Electroencephalogram

The mean powers were analyzed using a  $4 \times 3 \times 6$  repeated ANCOVA with the Group (Con, DB5, DB7, and DB9) as the between-subject factor and the Time (INT, R2 and R3) and Location (frontal, central, parietal, occipital, left temporal and right temporal) as the within-subject factor. The baseline readings at R1 were used as covariates. The sphericity correction using the Greenhouse-Geisser (when  $\varepsilon < 0.75$ ) or Huynh-Feldt (when  $\varepsilon > 0.75$ ) method was applied whenever necessary. For post-hoc tests, a planned contrast with Bonferroni correction was used to find specific significance for any Group main effects or interactions such that the Con was compared to each DB groups and no comparison was made between the DB groups. For the other factors, a pairwise comparison with Bonferroni correction was used to find the specific significant changes.

#### 3.10.5 Go/NoGo Behavioural Result and Event Related Potential

The behavioural results from the Go/NoGo task were analysed using a  $4 \times 2$  repeated ANCOVA with the Group (Con, DB5, DB7, and DB9) as the between-subject factor and the Time (T2 and T3) as the within-subject factor. For the ERP result (N2 and P3 amplitudes and latencies), they were analysed using a  $4 \times 2 \times 3 \times 2$  repeated ANCOVA with the Group (Con, DB5, DB7, and DB9) as the between-subject factor and Time (T2 and T3), Site (Fz, Cz and Pz)

and Condition (Go and NoGo) as the within-subject factors. The respective baseline readings at T1 were used as the covariates for each ANCOVAs. The Greenhouse-Geisser (when  $\varepsilon < 0.75$ ) or Huynh-Feldt (when  $\varepsilon > 0.75$ ) correction was applied when necessary. For any significant finding involving the Group, a planned contrast of comparing the Con to each of the DB groups was done with Bonferroni correction. For the rest of the variables, a pairwise comparisons with Bonferroni correction were used to find the specific changes.

### **CHAPTER 4.0**

## RESULT

## 4.1 Questionnaire

The scores from the ACS, MAAS and CAMS-R are shown in Figure 4.1. There was no Group main effect for the ACS (F(3,45) = 1.930, p = 0.138,  $\eta_p^2 = 0.114$ ), MAAS (F(3,45) = 1.210, p = 0.317,  $\eta_p^2 = 0.075$ ) and CAMS-R (F(3,45) = 0.147, p = 0.931,  $\eta_p^2 = 0.010$ ).

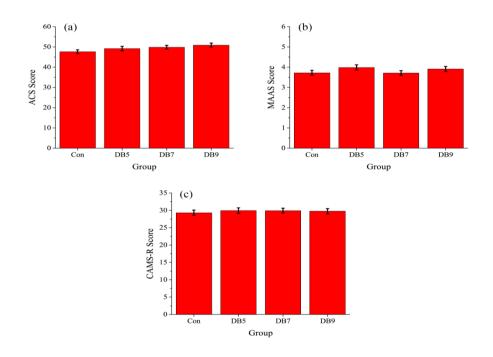


Figure 4.1: The scores of (a) ACS, (b) MAAS and (c) CAMS-R questionnaires. The error bars represent the standard errors.

# 4.2 Respiration Frequency, Oxygen Saturation and Cerebral Oxygen Delivery

For the RF (Table 4.1 and Figure 4.2), there was a significant group difference during INT ( $\chi^2(3) = 47.603$ , p < 0.001) and R2 ( $\chi^2(3) = 9.511$ , p = 0.023). Post-hoc analysis revealed that during INT, the RF of Con was larger than all three DB groups (all three p < 0.001) whereas during R2, the RF of Con was only statistically larger than DB7. Within the time period of each group, a significant result was obtained for all three DB groups (DB5:  $\chi^2(3) = 21.194$ , p < 0.001; DB7:  $\chi^2(3) = 30.157$ , p < 0.001; DB9:  $\chi^2(3) = 20.784$ , p < 0.001). Post-hoc analysis revealed that for DB5, the RF during INT was smaller than R1, R2 and R3 with all three p = 0.003. A similar result was obtained for DB7 and DB9 as well (DB7: all three p = 0.001; DB9: p = 0.002, p = 0.003, and p = 0.003, respectively).

Table 4.1: The respiration rate (RF), oxygen saturation  $(S_pO_2)$  and cerebral oxygen delivery (CDO<sub>2</sub>) for each group at each time period. The values are represented as mean (median) and standard error (interquartile range) in parenthesis.

Variable	Group -	Time				
		R1	INT	R2	R3	
Respiration rate (bpm)	Con	17.0	18.0	19.0	18.0	
	Con	(14.0 - 19.0)	(14.0 - 19.0)	(16.0 - 21.0)	(14.0 - 20.0)	
	DB5	16.0	6.0	12.0	17.0	
		(14.0 - 18.0)**	(6.0 - 6.0)###	(9.5 - 16.5)**	(14.0 - 17.0)**	
	DB7	14.5	6.0	13.0	14.5	
		(14.0 - 17.0)**	(6.0 - 6.0)###	(11.0 - 13.8)##, **	(13.0 - 16.0)**	
	DB9	15.5	6.0	14.5	14.5	
		(13.3 - 16.8)**	(6.0 - 6.0)###	(10.3 - 16.5)**	(12.3 - 16.0)**	
S <sub>p</sub> O <sub>2</sub> (%)	Con	97.1 (0.3)	96.9 (0.2)	96.7 (0.3)	96.8 (0.3)	
	DB5	97.3 (0.3)	97.6 (0.2)	96.3 (0.3)**	96.3 (0.3)**	
	DB7	96.7 (0.3)	98.1 (0.2)##	96.6 (0.3)***	96.1 (0.3)***	
	DB9	96.5 (0.3)	98.3 (0.2)##	96.7 (0.3)***	96.8 (0.3)**	
CDO2 (ml/100g/min)	Con	7.0 (0.8)	7.7 (0.6)	7.9 (0.6)	7.9 (0.5)	
	DB5	6.9 (0.7)	5.7 (0.6)	8.5 (0.7)***	8.5 (0.6)*	
	DB7	8.1 (0.6)	4.5 (0.6)##	7.9 (0.6)***	9.3 (0.5)***	
	DB9	8.7 (0.8)	4.0 (0.6)##	7.7 (0.6)***	8.1 (0.6)***	

bpm - breaths per minute; \* - p < 0.05 as compared to INT; \*\* - p < 0.01 as compared to INT; \*\*\* - p < 0.001 as compared to INT ; ## - p < 0.01 as compared to Con; ### - p < 0.001 as compared to Con

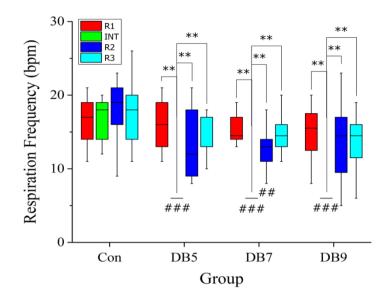


Figure 4.2: The median and interquartile range of the respiration rate for each group during each time period. ## - p < 0.01 as compared to the Con group; ### - p < 0.001 as compared to the Con group; \*\* - p < 0.01; \*\*\* - p < 0.001.

For the S<sub>p</sub>O<sub>2</sub> (Table 4.1 and Figure 4.3(a)), there was a significant Time main effect (F(2,92) = 24.755, p < 0.001,  $\eta_p^2 = 0.350$ ) and a significant Time × Group interaction (F(6,90) = 2.657, p = 0.020,  $\eta_p^2 = 0.150$ ). Post-hoc analysis revealed that during INT, the S<sub>p</sub>O<sub>2</sub> of Con was smaller than that of DB7 and DB9 with p = 0.001 and p < 0.001, respectively. For the DB5 group, the S<sub>p</sub>O<sub>2</sub> value during INT was larger than that during R2 and R3 with p = 0.001 and p <0.006, respectively. The same result was observed in DB7 and DB9 as well (DB7: both p < 0.001; DB9: p < 0.001 and p = 0.001, respectively). For CDO<sub>2</sub> (Table 4.1 and Figure 4.3(b)), there was a significant Time main effect (F(2,92)= 31.640, p < 0.001,  $\eta_p^2 = 0.408$ ) and a significant Time × Group interaction  $(F(6,90) = 3.709, p = 0.002, \eta_p^2 = 0.198)$ . Post-hoc analysis showed a significantly larger CDO<sub>2</sub> for the Con group than DB7 (p < 0.001) and DB9 (p < 0.001). Furthermore, the CDO<sub>2</sub> for all three DB groups during INT was significantly smaller than that during R2 and R3, with p value of < 0.001 and 0.010 for DB5; both p < 0.001 for DB7 and both p < 0.001 for DB9.

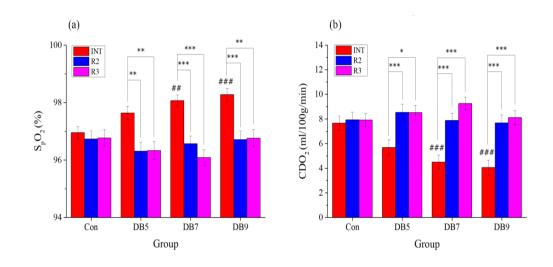


Figure 4.3: The adjusted mean of the (a) oxygen saturation level,  $S_pO_2$  and (b) cerebral oxygen delivery, CDO<sub>2</sub> for each group during each time period. The error bars represent the standard errors. ## - p < 0.01 as compared to the Con group; ### - p < 0.001 as compared to the Con group; \* p < 0.05; \*\* - p < 0.01; \*\*\* - p < 0.001.

#### 4.3 Heart Rate Variability

The adjusted mean and the standard error for each parameter is shown in Table 4.2. For the mean heart rate, there was a significant Time main effect  $(F(1.336,61.465) = 5.779, p = 0.012, \eta_p^2 = 0.112)$ , indicating the mean heart rate during INT was larger than R2 (p < 0.001). For the SDNN, there was a significant Time main effect  $(F(1.773,81.570) = 23.802, p < 0.001, \eta_p^2 = 0.341)$ and a significant Time × Group interaction (F(5.532,82.982) = 2.990, p = 0.013, $\eta_p^2 = 0.166)$ . Post-hoc analysis with Bonferroni correction revealed that during INT, the SDNN of Con group was smaller than DB5, DB7 and DB9 (p = 0.012,p = 0.033, and p = 0.033, respectively). Furthermore, the SDNN value during INT was larger than R2 and R3 for the DB5 group (p < 0.001 and p = 0.001, respectively). The same result was observed in both DB7 and DB9 groups as well (DB5: p < 0.001 and p = 0.004; DB9: p = 0.002 and p = 0.016). For the RMSSD, there was only a significant Time main effect (F(1.746,73.191) = $4.829, p = 0.014, \eta_p^2 = 0.095)$ , indicating the RMSSD was larger during INT than R2 (p = 0.003). These results are summarized in Figure 4.4(a) and 4.4(b).

	0			
	Group	INT	R2	R3
	Con	74.6 (1.4)	72.1 (1.2)	76.2 (2.3)
Mean Heart Rate (bpm)	DB5	76.4 (1.5)	71.9 (1.3)	74.9 (2.6)
Mean Heart Nate (opin)	DB7	76.4 (1.3)	73.3 (1.2)	73.7 (2.2)
	DB9	76.8 (1.5)	72.7 (1.3)	71.4 (2.5)
	Con	56.3 (7.5)	60.5 (5.0)	53.1 (3.9)
SDNN (ms)	DB5	90.6 (8.3)	60.4 (5.5)	52.7 (4.3)
SDIVIN (IIIS)	DB7	83.9 (7.3)	57.7 (4.8)	55.9 (3.8)
	DB9	85.1 (7.8)	62.6 (5.2)	59.3 (4.1)
	Con	41.9 (5.9)	43.9 (3.6)	41.7 (4.2)
RMSSD (ms)	DB5	59.4 (6.5)	47.4 (4.0)	42.3 (4.6)
RWISSD (IIIS)	DB7	53.3 (5.7)	38.0 (3.5)	45.6 (4.1)
	DB9	56.3 (6.1)	45.7 (3.7)	46.9 (4.4)
	Con	0.60 (0.03)	0.59 (0.04)	0.49 (0.04)
nLF (n.u)	DB5	0.88 (0.04)	0.63 (0.04)	0.46 (0.05)
	DB7	0.89 (0.03)	0.62 (0.04)	0.51 (0.04)
	DB9	0.83 (0.04)	0.56 (0.04)	0.46 (0.05)
	Con	0.40 (0.03)	0.41 (0.04)	0.51 (0.04)
nHF (n.u)	DB5	0.12 (0.04)	0.37 (0.04)	0.54 (0.05)
11111' (11 <b>.</b> u)	DB7	0.11 (0.03)	0.39 (0.04)	0.49 (0.04)
	DB9	0.17 (0.04)	0.44 (0.04)	0.54 (0.05)

Table 4.2: HRV indices for each group during each time period. The values are reported as the adjusted mean and the standard error in parenthesis.

For the nLF, there was a significant Time main effect (F(2.92) = 69.849, p < 0.001,  $\eta_p^2 = 0.603$ ), a significant Group main effect (F(3,45) = 3.391, p = 0.014,  $\eta_p^2 = 0.208$ ) and a significant Time × Group interaction (F(6,90) = 4.483, p = 0.001,  $\eta_p^2 = 0.230$ ). Post-hoc analysis with Bonferroni correct showed that during INT, the nLF of the Con group was smaller than that of DB5, DB7 and DB9 (all three p < 0.001). Within the DB5 group, the magnitude of the nLF during INT was larger than both R2 and R3 (both p < 0.001) and the magnitude during R2 was larger than R3 as well (p = 0.007). For DB7 and DB9, the magnitude of nLF during INT was larger than both R2 and R3 (DB7: both p < 0.007).

0.001; DB9: both p < 0.001). For the nHF, there was a significant Time main effect (F(2,92) = 69.849, p < 0.001,  $\eta_p^2 = 0.603$ ), a significant Group main effect (F(3,45) = 3.948, p = 0.014,  $\eta_p^2 = 0.208$ ) and a significant Time × Group interaction (F(6,90) = 4.466, p = 0.001,  $\eta_p^2 = 0.229$ ). Post-hoc analysis with Bonferroni correct showed that during INT, the nHF of the Con group was larger than that of DB5, DB7 and DB9 (all three p < 0.001). Within the DB5 group, the magnitude of the nHF during INT was smaller than both R2 and R3 (both p < 0.001) and the magnitude during R2 was smaller than R3 as well (p = 0.007). For DB7 and DB9, the magnitude of nHF during INT was smaller than smaller than both R2 and R3 (DB7: both p < 0.001; DB9: both p < 0.001). These results are summarized in Figure 4.4(c) and 4.4(d).

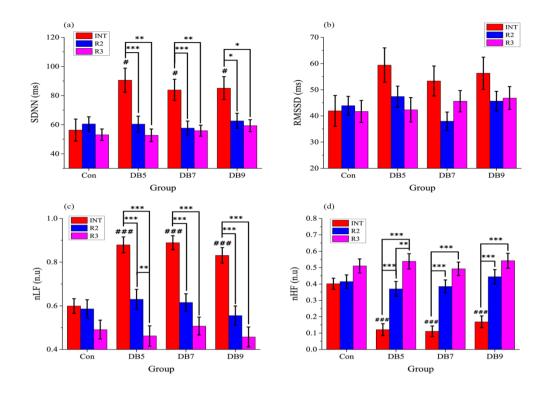


Figure 4.4: The adjusted mean of (a) SDNN, (b) RMSSD, (c) nLF and (d) nHF for each group during each time period. The error bars represent the standard errors. # - p < 0.05 as compared to the Con group; ### - p < 0.001 as compared to the Con group; \* - p < 0.05; \*\* - p < 0.01; \*\*\* - p < 0.001

# 4.4 Electroencephalogram

The topography of the mean relative power of the theta, alpha, and beta bands for each group during each time section is shown in Table 4.3 and Figure 4.5

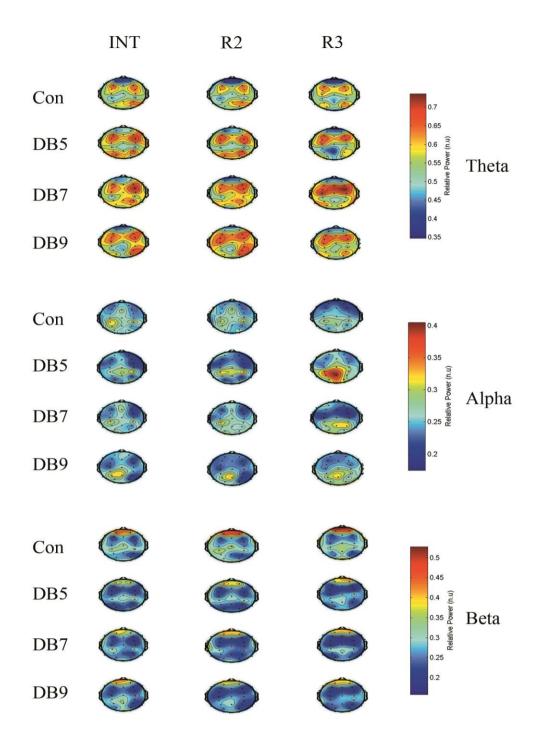


Figure 4.5: The topography of the relative theta, alpha and beta for each group during each time section.

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Table 4.3: The adjusted relative mean theta, alpha and beta power for each groups at each time sections. The values are reported as the adjusted means and standard deviations in parenthesis.

				Γ	INT					R	R2					R3			
		Fron	Cent	Pari	Occi	LTem	RTem	Fron	Cent	Pari	Occi	LTem	RTem	Fron	Cent	Pari	Occi	LTem	RTem
	CON	0.452 (0.027)	0.587	0.538 (0.024)	0.545 (0.023)	0.550 (0.021)	0.561 (0.020)	0.414 (0.022)	0.576 (0.022)	0.538 (0.024)	0.527 (0.024)	0.513 (0.021)	0.557 (0.018)	0.410 (0.028)	0.585 (0.021)	0.530	0.508	0.531 (0.027)	0.544 (0.027)
Ē	DB5	0.525 (0.027)	0.631	0.561 (0.024)	0.584 (0.023)	0.566 (0.021)	0.594 (0.020)	0.501	0.634 (0.022)	0.556 (0.024)	0.598 (0.024)	0.541	0.572	0.472 (0.028)	0.606 (0.021)	0.501	0.522 (0.031)	0.553 (0.027)	0.586 (0.027)
Iheta	DB7	0.502 (0.026)	0.648	0.571 (0.023)	0.57 (0.022)	0.557 (0.020)	0.571 (0.020)	0.477 (0.021)	0.628	0.582	0.557 (0.023)	0.542 (0.020)	0.566 (0.018)	0.494	0.682 (0.020)	0.539 (0.027)	0.556 (0.030)	0.593	0.617 (0.025)
	DB9	0.481 (0.026)	0.626 (0.021)	0.559 (0.023)	0.553 (0.022)	0.570 (0.020)	0.597 (0.020)	0.518 (0.021)	0.640 (0.021)	0.586 (0.023)	0.572 (0.023)	0.596 (0.020)	0.619 (0.018)	0.494 (0.027)	0.622 (0.020)	0.567 (0.027)	0.540 (0.030)	0.542 (0.026)	0.565 (0.025)
		Fron	Cent	Pari	Occi	LTem	RTem	Fron	Cent	Pari	Occi	LTem	RTem	Fron	Cent	Pari	Occi	LTem	RTem
	CON	0.246	0.252	0.286	0.263	0.238	0.242	0.243	0.254	0.272	0.258	0.249	0.243	0.214	0.245	0.274	0.272	0.238	0.233
		0.234	0.230	0.269	0.260	(0.009) 0.241	(0.009) 0.217	(0.01) 0.239	(210.0) 0.228	(c10.0) 0.282	(c.10.0) 0.249	0.241	0.229	0.010)	0.261	0.333	0.308	(cluu) 0.267	0.252
Almha	CBU	(0.011)	(0.011)	(0.012)	(0.011)	(0.00)	(0.00)	(0.015)	(0.013)	(0.013)	(0.013)	(0.011)	(0.012)	(0.016)	(0.012)	(0.020)	(0.019)	(0.013)	(0.015)
pridres	DB7	0.257	0.234	0.270	0.266	0.249	0.238	0.253	0.243	0.273	0.276	0.241	0.250	0.240	0.210	0.297	0.286	0.238	0.236
		(01010)	(110.0)	(210.0)	(0.011)	(600.0)	(600.0)	(0.015)	(0.012)	(0.012)	(0.013)	(0.011)	0.011)	(0.016)	(0.012)	(610.0)	(0.018)	0.013)	(0.01)
	DB9	0.010)	0.233 (0.011)	0.273	0.011) (1100)	162.0 (600.0)	(0.009)	0.015) (0.015)	0.229	962.0 (0.012)	0.234 (0.013)	0.237 (0.011)	0.240 (0.011)	0.230 (0.016)	0.24/	0.28/ (0.019)	(0.018)	0.013) (0.013)	0.266 (0.015)
		Fron	Cent	Pari	Occi	LTem	RTem	Fron	Cent	Pari	Occi	LTem	RTem	Fron	Cent	Pari	Occi	LTem	RTem
	CON	0.397	0.259	0.284	0.291	0.306	0.292	0.436	0.27	0.294	0.313	0.335	0.296	0.458	0.265	0.299	0.317	0.325	0.315
		0.326	0.225	0.262	0.246	0.279	0.271	0.346	0.230	0.259	0.245	0.308	0.287	0.358	0.224	0.264	0.267	0.271	0.252
Doto	cau	(0.028)	(0.016)	(0.018)	(0.017)	(0.019)	(0.018)	(0.024)	(0.016)	(0.017)	(0.016)	(0.017)	(0.015)	(0.028)	(0.015)	(0.018)	(0.021)	(0.023)	(0.020)
DCIG	DB7	0.346	0.215	0.267	0.273	0.294	0.289	0.364	0.223	0.249	0.270	0.309	0.282	0.358	0.194	0.275	0.263	0.264	0.240
		0368	(010.0)	(110.0)	010.0)	(610.0)	0.256	(020.0)	(010.0)	010.0)	(010.0)	0.010)	0.738	0.350	(010.0)	(110.0)	0.268	(770.0) 0 204	0.266
	DB9	(0.027)	0.0160	(0.017)	0.016	(010)	0.017)	0.073)	0.016)	0160	0.015)	0.201	0.010.0	(2000)	0.0151	(110.0)	000007	10000	(0.010)

#### 4.4.1 Theta Band

There was a significant Location main effect (F(2.743, 126.163) =27.367, p < 0.001,  $\eta_p^2 = 0.373$ ), a significant Time  $\times$  Location interaction  $(F(5.803,266.917) = 2.316, p = 0.036, \eta_p^2 = 0.048)$  and a significant Time  $\times$ Location × Group interaction (F(17.671,235.613) = 1.782, p = 0.029,  $\eta_p^2 =$ 0.118). In terms of group differences, post-hoc analysis with Bonferroni correction revealed that during R2, the frontal theta power for DB5 was larger than that of Con (p = 0.027), whereas for DB9 there was a larger power at both frontal and left temporal as compared to Con (p = 0.006 and p = 0.021,respectively). During R3, a significant difference was evident at the central location with DB7 showing a larger theta power than Con (p = 0.006). Besides that, the central theta power for DB7 was larger in R3 than R2 (p = 0.006). The statistical testing for the Location for each Group and Time is shown in Table 4.4 along with the relative difference and the *p* values. For Con, the distribution of the relative theta power was generally stable at each time points, with the frontal theta power being smaller than all the other locations. Among the DB groups, there were differences at the frontal and central locations. As the breathing duration increases from 5 minutes to either 7 or 9 minutes, there was a greater significant difference between frontal and central with the rest of the locations. The difference between 7 and 9 minutes was not obvious. However, this trend was shown during INT and R2 only. For R3, the differences at the frontal location across the three groups disappeared but the differences at the central location remained. For DB7 and DB9, the central relative theta power was larger than the majority of the other locations, showing a central dominance locus.

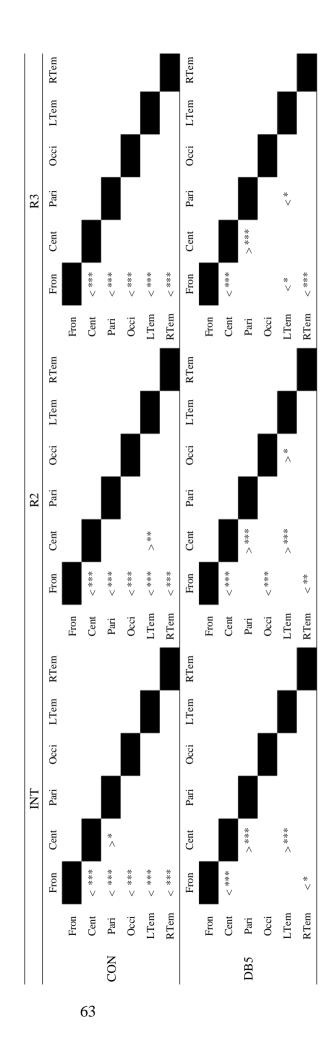
# 4.4.2 Alpha Band

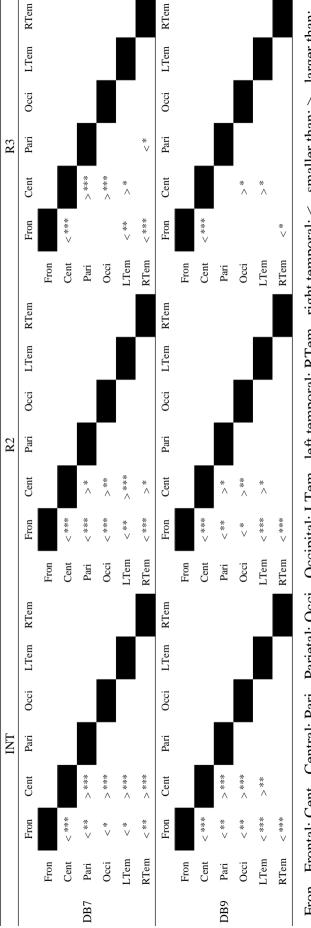
There was a significant Location main effect (F(4.856, 223.395) =23.381, p < 0.001,  $\eta_p^2 = 0.337$ ) and a significant Time × Location interaction  $(F(4.791,220.376) = 5.011, p < 0.001, \eta_p^2 = 0.098)$ . Post-hoc analysis with Bonferroni correction showed that when collapsed across the four groups, the relative alpha power at the central location was larger during R3 as compared to R2 and INT (p = 0.004 and p = 0.025, respectively). A similar result was obtained at the occipital location as well (p = 0.001 and p = 0.009, relative to R2 and INT, respectively). Besides that, there was a significant Location  $\times$ Group interaction (F(15,200) = 1.919, p = 0.023,  $\eta_p^2 = 0.126$ ). It was reported that there was no intergroup difference for the 6 brain regions but the distribution of the relative alpha power for the DB groups in general was different (Figure 4.5). For the Con group, the distribution of the alpha power was larger at the parietal location compared to the frontal (p < 0.001), central (p > 0.001)= 0.014) and left and right temporal (both p = 0.001). In comparison to the Con group, there was a shift from the central location towards the occipital region in the DB groups. For all three DB groups, the occipital relative alpha power was larger than that of the central location ( $p \le 0.001$ ). In addition, for DB5, the occipital and left temporal power were larger than the right temporal location (p = 0.040 and p = 0.030, respectively) while for DB7, the occipital power was larger than both left and right temporal locations (both p = 0.001). These topographical comparisons are summarized in Table 4.5. Lastly, there was also a Time × Group interaction trend (*F*(6,80) = 2.099, p = 0.062,  $\eta_p^2 = 0.136$ ).

#### 4.4.3 Beta Band

There was a significant Location main effect (F(2.281,104.936) = 30.891, p < 0.001,  $\eta_p^2 = 0.402$ ). Post-hoc analysis with Bonferroni correction revealed that the relative beta power at the frontal location was significantly larger than the other five locations (all p < 0.001) while the power at central location was significantly smaller than the rest (all p < 0.001). Another significant difference occurred between the left temporal and parietal, occipital and right temporal locations, with the beta power at left temporal being larger than the other three locations (p = 0.008, p = 0.025 and p = 0.022, respectively). Lastly, there is a significant Group main effect (F(3,40) = 3.412, p = 0.026,  $\eta_p^2 = 0.204$ ), showing that the relative beta power of Con was larger than DB5 (p = 0.033), DB7 (p = 0.045) and DB9 (p = 0.033).

Table 4.4: Post-hoc test of the different brain regions for different time sections (INT, R2 and R3) from all 4 groups (CON, DB5, DB7 and DB9) for the relative mean theta power. The table is interpreted starting from the column locations and then the row location. For example, the first '< \*\*\*' for the CON group during INT can be interpreted as 'the mean relative theta power of the frontal location is smaller than that of the central location with p < 0.001.



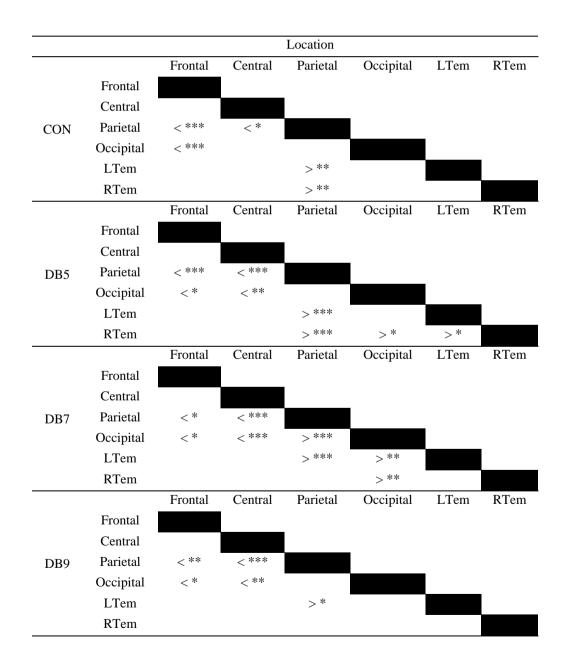


Fron - Frontal; Cent - Central; Pari - Parietal; Occi - Occipital; LTem - left temporal; RTem - right temporal; < - smaller than; > - larger than;

\* - p < 0.05; \*\* - p < 0.01; \*\*\* p < 0.001

# **Table 4.4 Continued**

Table 4.5: Post-hoc test of the different brain regions collapsed across the time sections (INT, R2 and R3) from all 4 groups (CON, DB5, DB7 and DB9) for the relative mean alpha power. The table is interpreted starting from the column location and then the row location. For example, the first '< \*\*\*' for the CON group can be interpreted as 'the mean relative alpha power of the frontal location is smaller than that of the parietal location with p < 0.001'.



#### 4.5 Go/NoGo Behavioural Result and Event Related Potential

#### 4.5.1 Behavioural Result

The means and standard error of the behavioural data are shown in Table 4.6. For the Go reaction time (RT), there was a significant Time main effect (F(1,48) = 10.313, p = 0.002,  $\eta_p^2 = 0.177$ ) and a Group × Time interaction (F(3, 47) = 3.040, p = 0.038,  $\eta_p^2 = 0.162$ ). Post-hoc analysis showed that the reaction time for T3 was shorter than that of T2 for DB5 and DB9 (DB5: 414.8 (6.9) vs 430.0 (7.6), p = 0.039; DB9: 412.5 (6.3) vs 436.8 (7.0), p = 0.001), presented in Figure 4.6. There was no significance for the Group main effect (F(3,47) = 0.425, p = 0.736,  $\eta_p^2 = 0.026$ ). For OE and RTV, both had a significant Time main effect (F(1,48) = 6.575, p = 0.014,  $\eta_p^2 = 0.120$  and F(1,48) = 5.801, p = 0.020,  $\eta_p^2 = 0.108$ , respectively) with the value at T3 being smaller than that of T2. However, no significance was reached for the Group main effect (OE: F(3,47) = 0.024, p = 0.995,  $\eta_p^2 = 0.002$ ; RTV: (F(3,47) = 0.724, p = 0.543,  $\eta_p^2 = 0.044$ ) and Group × Time interaction (OE: F(3,47) = 0.659, p = 0.581,  $\eta_p^2 = 0.040$ ; RTV: (F(3,47) = 1.668, p = 0.187,  $\eta_p^2 = 0.096$ ). For OA and CE, there were no significant main effects nor interaction.

Table 4.6: The adjusted Go/NoGo overall accuracy, omission error, commission error, Go reaction time and reaction time variability. The values are represented as the adjusted means and standard errors in parenthesis.

	Group	T2	Т3
	Con	92.3 (2.8)	92.6 (3.4)
Mean Overall Accuracy	DB5	93.1 (2.8)	92.3 (3.5)
(%)	DB7	92.4 (2.8)	92.8 (3.4)
	DB9	92.9 (2.8)	92.2 (3.4)
	Con	1.2 (1.5)	0.2 (0.6)
Omiggion Ernor (9/)	DB5	0.5 (1.5)	0.4 (0.6)
Omission Error (%)	DB7	0.9 (1.5)	0.5 (0.6)
	DB9	0.9 (1.5)	0.4 (0.6)
	Con	34.3 (10.5)	34.5 (15.0)
Comission Ennon (9/)	DB5	31.3 (10.7)	35.4 (15.2)
<b>Comission Error</b> (%)	DB7	35.8 (10.6)	35.2 (15.0)
	DB9	33.3 (10.6)	39.0 (15.1)
	Con	429.0 (25.1)	427.4 (22.8)
Co Depotion Time (mg)	DB5	430.0 (26.3)	414.8 (23.9) *
Go Reaction Time (ms)	DB7	431.6 (25.1)	430.7 (22.8)
	DB9	436.8 (25.3)	412.5 (22.9) **
	Con	75.5 (14.9)	70.4 (14.5)
<b>Reaction Time</b>	DB5	69.5 (14.9)	67.9 (14.6)
Variability (ms)	DB7	70.7 (14.9)	71.0 (14.6)
	DB9	70.4 (14.9)	60.7 (14.6)

\* - p < 0.05; \*\* - p < 0.001 as compared to T2

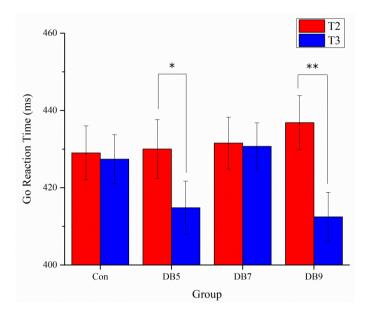


Figure 4.6: The adjusted mean Go reaction time for T2 and T3. The error bars represent the standard errors. \* - p < 0.05; \*\* - p < 0.005.

# 4.5.2 Event Related Potential

The *p* values for each of the main effects and the interactions of the N2 and P3 latency and amplitude are summarized in Table 4.7. The grand average waveforms for each condition (Go/NoGo) averaged across three midline electrodes (Fz, Cz and Pz) at T1, T2 and T3 are plotted in Figure 4.7 whereas the grand average waveforms for each electrode site individually are plotted in Figure 4.8.

Table 4.7: The *p* value of the main effects and interactions for the N2 and P3 amplitude and latency. The significant effects/interactions (p < 0.05) are bolded while trends (p < 0.10) are reported as well.

	<i>p</i> value					
Effects/Interactions	N2	2	P3			
	Amplitude	Latency	Amplitude	Latency		
Т	0.064	ns	ns	ns		
S	0.040	ns	0.062	ns		
С	< 0.001	ns	< 0.001	ns		
G	ns	0.074	ns	ns		
$\mathbf{T}  imes \mathbf{S}$	ns	ns	0.084	ns		
$T\times C$	ns	ns	ns	0.051		
$T\times G$	ns	ns	ns	0.045		
$\mathbf{S} \times \mathbf{C}$	ns	ns	0.059	0.022		
$\mathbf{S}  imes \mathbf{G}$	ns	ns	ns	ns		
$\mathbf{C}  imes \mathbf{G}$	0.029	0.090	ns	ns		
$T\times S\times C$	ns	ns	0.043	ns		
$T\times S\times G$	0.072	ns	ns	ns		
$T\times C\times G$	0.040	0.093	ns	ns		
$S \times C \times G$	ns	ns	ns	ns		
$T\times S\times C\times G$	ns	ns	ns	ns		

T - Time; S - Site; C - Condition; G - Group; ns - not significant.

For the N2 amplitude, there was a significant Condition main effect  $(F(1,46) = 47.423, p < 0.001, \eta_p^2 = 0.508)$ , showing the expected larger amplitude in the NoGo condition. Besides that, a significant Site main effect  $(F(2,92) = 3.344, p = 0.040, \eta_p^2 = 0.068)$  was observed but the post-hoc analysis failed to find any significance after Bonferroni correction. Significant Condition × Group interaction  $(F(3,40) = 3.342, p = 0.029, \eta_p^2 = 0.200)$  and Time × Condition × Group interaction  $(F(3,40) = 3.048, p = 0.040, \eta_p^2 = 0.186)$  were also evident. Post-hoc analysis showed that the NoGo N2 amplitude was larger

at T3 as compared to T2 for the DB5 group (0.166 (0.027) vs 0.096 (0.022), p = 0.027) and was larger than the control group at T3 (0.166 (0.037) vs 0.069 (0.024), p = 0.040), as shown in Figure 4.9. Several trends were observed as well which included a Time main effect (F(1,46) = 3.596, p = 0.064,  $\eta_p^2 = 0.073$ ) and a Time × Site × Group interaction (F(6,80) = 2.023, p = 0.072,  $\eta_p^2 = 0.132$ ). With regards to the latency, there were only three trends involving the Group, namely the Group main effect (F(3,40) = 2.487, p = 0.074,  $\eta_p^2 = 0.157$ ), Condition × Group interaction (F(3,40) = 2.322, p = 0.090,  $\eta_p^2 = 0.148$ ) and Time × Condition × Group interaction (F(3,40) = 2.288, p = 0.093,  $\eta_p^2 = 0.146$ ).

For the P3 amplitude, significant Condition main effect ( $F(1,46) = 41.868, p < 0.001, \eta_p^2 = 0.476$ ) and Time × Site × Condition interaction ( $F(2,92) = 3.261, p = 0.043, \eta_p^2 = 0.066$ ) were found, showing that the NoGo P3 having a fronto-central distribution was larger than the Go condition which had a fronto-parietal distribution. Three trends were observed: Site main effect ( $F(2,92) = 2.873, p = 0.062, \eta_p^2 = 0.059$ ), Time × Site interaction ( $F(2,92) = 2.546, p = 0.084, \eta_p^2 = 0.052$ ) and Site × Condition interaction ( $F(2,92) = 2.918, p = 0.059, \eta_p^2 = 0.060$ ). No main effect or interaction involving the Group was observed. As for the latency, there was a significant Time × Group interaction ( $F(3,40) = 2.930, p = 0.045, \eta_p^2 = 0.180$ ) whereby the latency at T3 was shorter than that of T2 for the DB7 group. Besides that, a significant Site × Group interaction ( $F(6,80) = 3.256, p = 0.006, \eta_p^2 = 0.196$ ) was found as well. Posthoc analysis revealed that at Fz, the latency for the Con group was shorter than DB7 (430.9 (14.0) vs 490.2 (14.2), p = 0.018) whereas at Pz, the latency of DB5

was shorter than Con (421.1 (12.8) vs 480.5 (12.0), p = 0.006). Lastly, there was also a Time × Condition interaction trend (F(1,46) = 4.001, p = 0.051,  $\eta_p^2 = 0.080$ ).

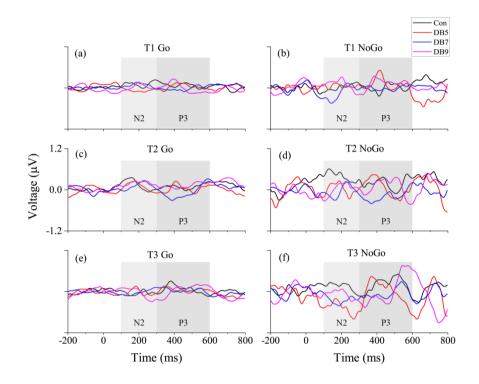


Figure 4.7: The grand average waveform averaged across the three midline electrodes for (a) T1 Go, (b) T1 NoGo, (c) T2 Go, (d) T2 NoGo, (e) T3 Go and (f) T3 NoGo.

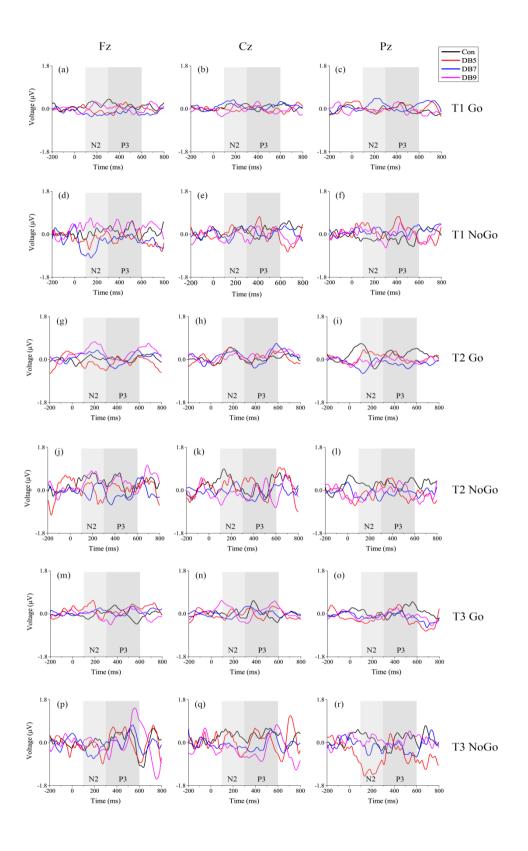


Figure 4.8: The grand average waveform at each electrode site (Fz, Cz and Pz). Both Go and NoGo condition ERP waveforms are shown at each Time (T1, T2 and T3) for the four groups.

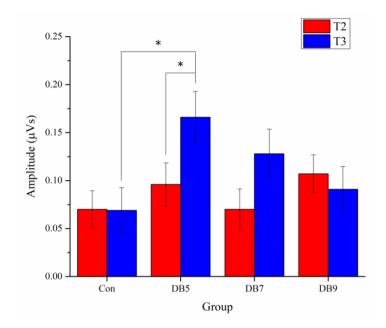


Figure 4.9: The adjusted amplitude of the NoGo N2 component. The error bars represent the standard errors. \* - p < 0.05.

#### **CHAPTER 5.0**

#### DISCUSSION

# 5.1 Questionnaire

This study revealed that there was no group effect on the scores from the Attentional Control Scale (ACS), Mindful Attention Awareness Scale (MAAS) and Cognitive Affective Mindfulness Scale - Revised (CAMS-R) after controlling for the baseline scores. This suggests that by deep breathing at 6 breaths per minute for 7 days consecutively there was no improvement regarding the attention level nor the mindfulness level. The lack of improvement in the self-perceived attention level is consistent with the lack of an enhanced P3 Go amplitude for the Go/NoGo task in the three DB groups, which will be discussed further in section 5.5.

In terms of the mindfulness level, just by practicing the fundamental of deep breathing, the result showed that it did not increase the mindfulness level. This suggests that it is the combination of the different exercises in each mindfulness practices such as yogic body posture (Sengupta, 2012), mindful walking (Kabat-zinn, 1996), martial arts (Shou-Yu and Wen-Ching, 2014) and others with the deep breathing that leads to an increase in the mindfulness level. Another possibility is that the practice duration of just 7 days is not long enough to induce a noticeable effect on the mindfulness state of mind, and hence, the use of questionnaires was not sensitive enough to detect it.

# 5.2 Respiration Frequency, Oxygen Saturation and Cerebral Oxygen Delivery

The result profoundly shows that during INT, the RF of 6 breaths per minutes for the DB groups was significantly smaller than that of Con and the level of  $S_pO_2$  was significantly larger than the control group. Due to the greater depth of exhalation and lower RF during deep breathing, more carbon dioxide was removed and hence, the P<sub>a</sub>CO<sub>2</sub> level of the DB groups was lower. This reduction in P<sub>a</sub>CO<sub>2</sub> level led to the vasoconstriction of the cerebral vessels which in turns limits the CBF and CDO<sub>2</sub> to the brain in order to restore to a normal P<sub>a</sub>CO<sub>2</sub> level, consistent with previous reports (Eames, Potter and Panerai, 2004; Lucas *et al.*, 2013b).

For the period after the deep breathing and during the follow-up session after 7 days, there was no statistically significant difference in  $S_pO_2$  and  $CDO_2$ between the DB groups and the control group. However, from a qualitative point of view, the second hypothesis was supported, at least during R3. During the follow-up session, the median resting RF was lower in all three DB groups (Figure 4.2), and this trend was reflected in the CDO<sub>2</sub> as well. All three CDO<sub>2</sub> values were larger than that of Con (Figure 4.3(b)) and this suggests that constant practice of deep breathing can lower the RF, which leads to an increase in the CDO<sub>2</sub>. This is similar to the study by Tharion *et al.* (2012) who found that after practicing deep breathing at 6 breaths per minute for 30 minutes per day for a month, the RF was significantly lower statistically as compared to a baseline reading.

Regarding the deep breathing duration, a negative trend between the  $S_pO_2$  and the breathing duration during INT was observed such that by increasing the deep breathing duration, the  $S_pO_2$  level will be higher as seen in Figure 4.3(a). Another trend was observed between the CDO<sub>2</sub> and the deep breathing duration during INT such that the CDO<sub>2</sub> decreases with the deep breathing duration, shown in Figure 4.3(b). There is only one other study by Bilo *et al.* (2012) that investigated the effect of deep breathing at 6 breaths per minute on the oxygen saturation at two high altitudes (4559 m and 5400 m). The  $S_pO_2$  level was found to increase steadily throughout the deep breathing period, which is consistent with our result at near sea level of approximately 100 m. Furthermore, this is the first study to establish a relationship between the deep breathing duration and the CDO<sub>2</sub> through calculation.

# 5.3 Heart Rate Variability

This study investigated the effects of different deep breathing durations on the HRV indices in both the time domain (mean heart rate, SDNN, and RMSSD) and the frequency domain (nLF and nHF). Deep breathing at 6 breaths per minute for either 5, 7 or 9 minutes had significantly increased the SDNN and nLF when compared to the control group during the intervention. The magnitude of the nHF for the DB groups was significantly smaller than that of the control group during the intervention as well. There was no difference between the control and DB groups during the post-intervention period as well as the follow-up session after 7 days.

Breathing at 6 breaths per minutes for either 5, 7 or 9 minutes had resulted in a higher HRV measurement compared to the control group which was performing spontaneous breathing. In terms of the time domain variables, the increase in the SDNN but not the mean HR and RMSSD is consistent with previously reported literature (Wang *et al.*, 2010; Gabriell E. Prinsloo *et al.*, 2013; Lin, Tai and Fan, 2014; Kim, Bae and Park, 2016). From Figure 4.4(b), it can be seen that the RMSSD value for the DB groups was larger than that of Con although they were not statistically different. This result provides evidence that deep breathing at a rate of 6 breaths per minutes caused an incline of the ANS towards the parasympathetic side, consistent with the relaxation feeling obtained during deep breathing (Kjellgren *et al.*, 2007; Prinsloo *et al.*, 2011; Lin, Tai and Fan, 2014).

Regarding the frequency domain, the huge difference observed in the nLF and the nHF between the DB groups and the Con group was due to the breathing frequency of 0.1 Hz (6 breaths per minute). The power spectrum density of the HRV is modulated by the breathing frequency such that the peaks will shift towards and be centred at around the breathing frequency (Sanderson *et al.*, 1996). In this study, the breathing frequency of 0.1 Hz led to a huge peak at around 0.1 Hz, which lies in the LF range (0.04 Hz to 0.15 Hz) and caused a

mixture of the LF and HF power. Even though the nLF and nHF are associated with the sympathetic and parasympathetic activation, respectively, this explanation could not be applied here as the LF and HF components are indissociable (Malliani, 2005). However, this problem was solved by Aysin and Aysin (2006) who had utilized an enhanced HRV analysis that takes into account the breathing frequency for the frequency domain analysis. Using this enhanced method, they had successfully isolated the LF and HF regions when the participants were breathing at 6 breaths per minutes and had found that the HF power was larger than the LF power. This result provided further evidence that during deep breathing there was a greater activation of the parasympathetic nervous system.

Regarding the deep breathing duration, from the results here it suggested that deep breathing for 5 minutes was the optimum duration as it produced the largest change in HRV as compared to the control group, contrary to the hypothesis. During the post-intervention period and also the follow-up session, there was no significant difference between the DB groups and the Con group, which was in agreement with several studies that reported a similar result (Tharion *et al.*, 2012; Gabriell E. Prinsloo *et al.*, 2013).

#### 5.4 Electroencephalogram

One of the major findings in this study is the significantly larger frontal theta power in the DB5 and DB9 groups, but not DB7 during the period after the deep breathing (R2). The larger frontal theta power is not present after 7 days of consecutive practice. Since deep breathing forms an elemental part in the majority of mindfulness meditations (Brown and Gerbarg, 2005b) the increase in frontal theta could be interpreted as a greater focused attention (Nakashima and Sato, 1993; Aftanas and Golocheikine, 2001; Park et al., 2002). The result of increased theta power in the various literature on the EEG study of deep breathing and meditation practices is recreated here (Bušek and Kemlink, 2005; Lagopoulos et al., 2009; Chan et al., 2011; Park and Park, 2012; Henz and Schollhorn, 2017). Further, the frontal theta power is inversely correlated to anxiety such that a greater frontal theta power indicates a lower anxiety (Inanaga, 1998). Collectively, the video-guided deep breathing is able to act as a meditation technique for achieving the state of 'focused yet not anxious' that is common in most mindfulness meditations (Tomasino, Chiesa and Fabbro, 2014). However, one interesting point to note here is that only DB5 and DB9 achieved a statistically significant difference from the control group, whereas for DB7, even though there is an increase of the relative theta power (Figure 4.5), the increment is not statistically significant. The reason for this discontinuity in trend is unknown and this entails a further investigation into deep breathing for 7 minutes.

Another novel finding in this study is the different topographical distribution of the relative theta power of the DB groups compared to the control group. Focusing on DB7 and DB9, the distribution of the relative theta power is a central dominance for all three-time points compared to DB5 and Con. In a study by Tang et al. (2009), the theta power from the Fz, FCz and Cz locations had a positive correlation with the high frequency heart rate variability power, which indexes the parasympathetic nervous system activity (Task Force of The European Society of Cardiology and The North American Society of Pacing and Electrophysiology, 1996). Following this, the central dominance of the theta power obtained in this result can be interpreted as a shift from the sympathetic to the parasympathetic nervous system. The latter gives a state of feed-andbreed, whereas the former gives a state of fight-or-flight (McCorry, 2007). Hence, the greater activation of the parasympathetic activity will, in general, be more relax and less anxious (Pavlenko, Chernyi and Goubkina, 2009) and the result observed here is consistent with the greater frontal theta and also, later in the beta band. From Table 4.3, the central-largest topographical distribution of the relative theta power is only observed in DB7 and DB9. This is suggestive of a time factor in play for the shifting of the autonomic nervous system such that a 5 minutes deep breathing duration was not sufficient in shifting towards the parasympathetic side. However, the lack of difference between DB7 and DB9 suggests that there the parasympathetic activation would not be different when the breathing duration further increases as the topographies of the theta power are relatively similar.

Analysis on the relative alpha power showed that there was no group difference between the control and the deep breathing groups during INT, R2, and R3. In terms of the topography, a shift of power spectrum from the central location towards the occipital location is evident in the DB groups but not in the Con group. The lack of difference between two groups for the relative alpha power magnitude contrasts markedly with literature reports of a heightened alpha power after deep breathing (Arambula et al., 2001; Fumoto et al., 2004; Sherlin, Muench and Wyckoff, 2010; Yu et al., 2011; Park and Park, 2012) that is normally interpreted as an induction of a relaxation state. This discrepancy can be explained by the fact that the control participants are not shown in any video during the deep breathing period, unlike the DB groups that followed the video guide. The visual stimulant perceived by the DB participants is greater than the control group and the presence of a greater visual stimulation could result in a reduction of the alpha power (Barry et al., 2009). Another possibility for the absence of the relaxed alpha wave could be due to the act of focusing on the video and also, on the breath. The alpha wave has an inverse relationship with the cortical activation of the brain (Alexander, O'Boyle and Benbow, 1996) and is modulated by the attention (Ray and Cole, 1985; Klimesch et al., 1998; Connell *et al.*, 2008) such that the suppression of the alpha oscillation is evident with the engagement of attention. The act of focusing on the video and the breath caused a heightened brain activity needed for the extra active focusing instead of passively watching the video. Thus, the guidance for deep breathing via a video and the active focusing on the video may have levelled the relative alpha power and caused the power to be the same between the control and deep breathing groups.

As for the beta power, there is a reduction in the overall relative beta power during deep breathing, immediately after and also, after a 7-day followup in the DB groups. This reduction of beta power is interpreted as a decrease in anxiety (Pavlenko, Chernyi and Goubkina, 2009) and again, this is consistent with the shifting towards to the parasympathetic nervous system during deep breathing, which reduces anxiety (Miu, Heilman and Miclea, 2009). Even though there are relatively fewer studies on the beta power, current literature has produced mixed results. In an earlier study by Stancak et al. (1993) using a within-subject experimental design, the effect of paced breathing with the eves staying closed at frequencies of 0.25, 0.20, 0.14, 0.10 and 0.06 Hz for 3 minutes on the EEG power was investigated. Their result showed that only for 0.25 and 0.20 Hz, a significant difference in the beta power was observed when compared to resting spontaneous breathing. At 0.10 Hz or 6 breaths per minute employed in this current study, there was no pronounced difference in the beta band. A similar null result on the beta power was recreated by Gaurav et al. (2016) with the breathing rate at 6 breaths per minute and a breathing duration of 3 minutes as well. However, Prinsloo et al. (2013) had shown in their study on a 10-minute single session of heart rate variability (HRV) biofeedback caused the relative beta power to decrease during the biofeedback intervention and also, during the post-intervention period. It may seem that the HRV biofeedback is methodologically different but in reality, it shares many similarities with the video-guided deep breathing used in the current study; for example, the HRV monitors the changes of heart rate variations associated with respiration across a range of frequencies. The biofeedback is done by controlling the breathing rate such that a resonant effect between the heart rate and the respiration is maximized, and this rate normally lies between 4.5 to 6.5 breaths per minute (Vaschillo, Vaschillo and Lehrer, 2006). From these discussions, there are two possibilities to explain the varying results. The first explanation is that the beta power is modulated by the opening or closing of the eyes, much like the theta power (Aftanas and Golocheikine, 2001; Henz and Schollhorn, 2017). The second possibility lies in the duration of deep breathing. It seems only when the deep breathing duration is up to 5 minutes as was done in this study that there will be an effect on the beta power, and this trend stays the same up until 10 minutes of practice.

From the above discussion, the known neurophysiological changes of deep breathing and its associated benefits have been recreated in this study. The increment of the frontal theta power, centrally-dominant theta topography and decrement of the beta power achieved by the DB groups indicates that deep breathing had shifted the autonomic nervous system to the parasympathetic side to attain a state of 'focused yet not anxious'. However, these replications were only observed for some particular deep breathing durations, and this is an indication that the mean power and topographical distribution of the different EEG frequency bands are modulated by the deep breathing duration. Combining the three bands' result, it seems that the notion of 'the long the better' is supported such that for 9 min of deep breathing all of the benefits were obtained. The results presented here may serve as a guide in determining how long it is necessary for practicing deep breathing as an intervention to bring focus, increasing the parasympathetic activity and to reduce anxiety.

#### 5.5 Go/NoGo Behavioural Result and Event Related Potential

This study investigated the effect of different lengths of deep breathing (5 minutes, 7 minutes and 9 minutes) on the behavioural and neurophysiological measurement by using a Go/NoGo paradigm. On the behavioral level in terms of the mean overall accuracy, omission and commission error, Go reaction time, and reaction time variability, there was no difference between groups at T2 or T3. However, for both DB5 and DB9, the Go RT at T3 was shorter than that of their respective T2 value (Table 4.6). The reason for the non-significant improvement of the DB7 group's Go RT is unknown. On the neurophysiological measurement of ERP, the NoGo N2 amplitude for the DB5 group was larger than that of Con at T3, indicative of an enhanced conflict monitoring towards the NoGo trials after seven days of practicing deep breathing for 5 minutes. There was no group effect on the N2 latency as well as for the P3 amplitude. Given that the omission error, reaction time variability, and P3 amplitude were not different between groups after the intervention, it suggested that deep breathing at 6 breaths per minute was not able to increase the sustained attention level. However, there was also a Time  $\times$  Group interaction for the P3 latency (Table 4.7). At Fz, the latency for Con is shorter than DB7 while the opposite was true at Pz, with the difference now between Con and DB5. Collectively, these results showed that practicing short duration of deep breathing can improve the ability to perceive conflict and possibly increase the cognitive processing speed.

Deep breathing is a common respiration exercise in many mindfulness practices including yoga (Brown and Gerbarg, 2005b), Tai Chi (Shou-Yu and Wen-Ching, 2014), MBSR (Kabat-Zinn, 1982) and many others. This is due to the fact that deep breathing gives a greater sense of body existence, which makes it easy to bring back the attention. Whenever the mind starts to wander, one would need to acknowledge and bring the attention back towards the breathing (Arch and Craske, 2006). In this study, the participants would need to place their attention on the appearing and disappearing petals in the video in order to follow the pre-set breathing frequency of 6 breaths per minute, along with the instruction of noticing their breath. This very much similar to the instructions given by teachers of mindfulness practices. Following this, literature that reports on mindfulness practices that encompass mindful breathing supports the current results of greater conflict monitoring. In a study conducted by Menezes et al. (2013), they evaluated the effects of a six-week-focused meditation training (focusing one's attention on the breath) on the regulation of emotion and attention on 74 college students and found that there were fewer omission errors in a visual discrimination task for the students in the focused meditation groups compared to the control group. A similar result was shown earlier by Tang et al. (2007) who had investigated a short-term effect of integrative body-mind training (IMBT) on the attention using 40 undergraduate students. IMBT incorporates elements from traditional Chinese medicine practices and contemporary mindfulness practices, in which one of them was the mindful breathing. By the end of the 5 days training, the participants have enhanced conflict monitoring as indexed by a better performance in the Attentional Network Task (ANT). Furthermore, studies using ERP to investigate the

cognitive process of practicing mindfulness practices have shown a similar increase in the N2 component for people with higher trait mindfulness (Quaglia, Goodman and Brown, 2016), and in adults (Moore *et al.*, 2012) and seniors (Malinowski *et al.*, 2017) as compared to control groups. The discussion above proved that in general, practitioners of mindfulness practices would have a greater conflict monitoring ability, and the current results extended the possibility of achieving the same enhancement simply by performing deep breathing at 6 breaths per minute every day without any supervision as opposed to the whole set of mindfulness practices.

In addition, studies that investigated the brain activation of mindfulness practices provided further evidence towards the current results. The neural source of the NoGo N2 in a Go/NoGo task was found to be at the anterior cingulate cortex (ACC; Bokura *et al.* 2001) and the vast research on the function of the ACC had firmed-up its role in the monitoring of conflict information (De Zubicaray *et al.*, 2000; Braver *et al.*, 2001; Durston *et al.*, 2003). The activation of the ACC will be greater under three categories of situation: response override, selection of equally possible responses and situations involving error (Botvinick, Cohen and Carter, 2004), in which all three involve conflicting information. The practitioners of mindfulness practices or meditation are found to have greater activation of the ACC along with several frontal and parietal regions of the brain through several *f*MRI studies (Brefczynski-Lewis *et al.*, 2007; Hölzel *et al.*, 2007; Short *et al.*, 2010; Dickenson *et al.*, 2013). This result implies that mindfulness practitioners are more adept in monitoring and perceiving conflicting information by engaging more attentional resources. This is in line

with the state of mindfulness, which entails the need to be aware of one's thought on the present without any judgment and to bring back one's mind whenever it is lost. This phenomenon was studied and verified by Hasenkamp *et al.* (2012) such that the activation of the ACC was the greatest during the awareness-ofmind-wandering process. Relating back to ERP, since the neural source of the N2 component is the ACC, a greater activation of the ACC will inevitably lead to a greater N2 amplitude. Thus, the greater NoGo N2 amplitude from the current result and hence, greater conflict monitoring may provide evidence that by deep breathing there would be a greater activation of the ACC, just like other mindfulness practice.

Another point that this study addressed was the effects of varying lengths of deep breathing at both the immediate and a follow-up level after practicing for 7 days. Current results showed that there was no group difference at the immediate level for both behavioural and neurophysiological results. Even though a relatively small amount of literature has reported the immediate effect following a meditation session, there is evidence showing that the immediate effect in this study would be too small. In a study by Johnson *et al.* (2013), a single session of mindfulness meditation for 25 minutes did not lead to greater performance in a battery of cognitive and memory tasks, although it did produce an improvement on the mood states; whereas Polak (2009) had assessed the attentional efficiency of 150 novice meditators assigned to 15 minutes of mindfulness training, relaxation training or neutral task immediately after the second session. His result revealed that an overall better attention was not achieved by the mindfulness group as compared to the other two groups.

Combining the above two studies and the current study, it may seem that a single session of practice is not able to improve the cognitive ability of the participants, even when the practice time is extended up to 25 minutes. Thus, the maximum deep breathing of 9 minutes in this study was not able to produce any observable difference between groups. Even though the latter study had evaluated the results at the end of the second session, two days are still considered short compared to longer mindfulness practices that can last up to weeks or even months, and hence, was included as an immediate effect.

As for a follow-up at T3, the result presented here suggests that 5 minutes of deep breathing was optimum in achieving an enhanced conflict monitoring ability. From Figure 4.9, the NoGo N2 amplitude for the DB groups was generally larger than the control group at T3 and the amplitude decreases with increase in the deep breathing duration. This trend suggests that the deep breathing duration plays a role in enhancing the conflict monitoring ability. This study is one of the few studies that investigated the effect of practice duration. In a preliminary study by Carmody & Baer (2009), they had reviewed the class contact hours of MBSR program and its corresponding effect size on psychological distress. They found that the correlation between the duration and the effect size was not significant and this suggests the fact that a shorter duration of the MBSR program can achieve the same effect. A different study conducted by Prasad et al. (2011) investigating a 5, 15 and 30 minutes of paced breathing meditation on the stress and quality of life among health care professionals concluded that the duration of practice did not affect the improvement of the stress and quality of life, despite having the 15 minutes

practice generating the greatest improvement. Although the current result is not consistent with the above study, but due to the difference in terms of methodology, study duration and assessment, the effect of the duration of practice on the end results may have been modulated.

Lastly, regarding the sustained attention and response inhibition, the current results do not support the hypothesis that deep breathing can increase the sustained attention and response inhibition level. This is evident from the fact that there was no intergroup difference in terms of the behavioural results and the P3 component between the DB groups and the control group at both T2 and T3. The absence of improvements for the behavioural result is in line with previous reports that had investigated mindfulness practices using either a neutral or emotional Go/NoGo task (Hoogland, 2011; Paul et al., 2013; Meland et al., 2015). However, the absence of improvements for the P3 component amplitude (both Go and NoGo condition) is in contrast with previous literature reporting a greater P3 amplitude for practitioners of mindfulness as compared to a control group (Moore et al., 2012; Delgado-Pastor et al., 2013; Schoenberg et al., 2014; Atchley et al., 2016). One possible explanation is that different aspect of the cognitive control enhancements (i.e. conflict monitoring, sustained attention, and respond inhibition) is associated with a particular exercise. From this study, it seems possible that only the conflict monitoring is associated with the deep breathing while the sustained attention and response inhibition are associated with other exercises.

#### 5.6 Optimum Deep Breathing Duration

Regarding the optimum deep breathing duration, the obtained results showed that the optimum duration is either 5 or 9 minutes. In terms of the HRV and ERP measurements, the 5 minutes duration is optimum as it produces the greatest change in the HRV indices, indicating a greater activation of the parasympathetic nervous system; whereas for the ERP, it produced the greatest enhancement in the conflict monitoring ability during the follow-up session. However, when looking into the CDO<sub>2</sub> and EEG parameters, the 9 minutes duration seems to be the optimum duration. For the CDO<sub>2</sub> during the follow-up session, the CDO<sub>2</sub> increases with the deep breathing duration, thus by deep breathing for 9 minutes for 7 days, the resting oxygen supply to the brain increases. As for the EEG power bands, the 9 minutes duration achieved a larger frontal relative theta power as compared to the control group and has a centrally focused topography for the relative theta power, achieving a state of focused yet not anxious state of mind. Further imaging research may further elucidate the mechanism of the deep breathing duration.

## 5.7 Limitations

One of the limitations of this study is the small sample size in each group. Further studies should have a larger sample size and extend the study to different cohorts of children, adolescents and seniors in order to have a better understanding of the physiological changes brought by deep breathing of different durations and its effect on the cognitive control.

Besides that, the maximum breathing duration in this study was limited to 9 minutes to prevent the possibility of hyperventilation. These durations are considered as short compared to the majority of the mindfulness practices. Greater durations can be investigated but precautionary steps need to be taken such as having a prior training session, using different breathing techniques such as capnometry-assisted breathing (Meuret *et al.*, 2008), pursued lips breathing (Fregonezi *et al.*, 2004) or separating the one long duration into several shorter durations. These precautions will reduce the risk of hyperventilation.

Lastly, the data can be analysed separately according to gender. In terms of the EEG and HRV, the final data may be modulated by the difference in hormonal processes (Becker *et al.*, 1982; Jensen-Urstad *et al.*, 1997; Martinović, Jovanović & Ristanović, 1998; Carrier *et al.*, 2001; Zhang, 2007). The ERP result could also be modulated by the gender given that the underlying brain structure, especially the ACC is different between a male and a female (Yücel *et al.*, 2001). In this study, the number of female participants in each group is too small to make a statistically viable comparison.

# **CHAPTER 6.0**

## CONCLUSION

# 6.1 Conclusion

This study has investigated the physiological changes in terms of the cerebral oxygen delivery (CDO<sub>2</sub>), heart rate variability (HRV) and EEG relative power as a function of three deep breathing durations of 5, 7 and 9 minutes. Besides that, the effect of short duration, video-assisted deep breathing on the cognitive control (conflict monitoring, response inhibition and sustained attention) was investigated as well using event related potential via a Go/NoGo paradigm, along with three questionnaires which include the Attentional Control Scale (ACS), Mindful Attention Awareness Scale (MAAS) and Cognitive and Affective Mindfulness Scale - Revised (CAMS-R). The period of interest included the baseline, during deep breathing, immediately after the deep breathing and a follow-up session after 7 days of practicing the deep breathing consecutively. From the physiological point of view, the resting CDO<sub>2</sub> during the follow-up session was found to be increasing with the deep breathing duration. For the HRV (SDNN, RMSSD, nLF and nHF), there was no carry-over effect into the post deep breathing and the follow-up session. However, during the deep breathing period, the DB groups had a significantly larger SDNN and nLF whereas the nHF was significantly smaller compared to the control group. This is indicative of a greater activation of the

parasympathetic nervous branch and the 5 minutes duration was optimum as it produced the greatest change in the HRV indices. For the EEG relative power, deep breathing of 5 and 9 minutes had produced a larger frontal theta power after the deep breathing whereas the theta topography showed a centrally dominant distribution. There was no difference in the alpha power but for the beta power, all three DB groups achieved a lower overall beta power compared to the Con group. Collectively, these results are interpreted as achieving a 'focused yet not anxious' state of mind. Lastly on the cognitive control side, deep breathing only affects the conflict monitoring ability but not the response inhibition and sustained attention such that only the N2 NoGo component was enhanced for the DB groups following 7 days of deep breathing. During the follow-up session, the N2 NoGo amplitude of the DB5 group was significantly larger statistically than the Con group and an inverse relationship between the N2 NoGo amplitude and the deep breathing duration was observed. The lack of improvement in the sustained attention level is consistent with the null difference between groups for the three questionnaires' scores. Overall, an optimum deep breathing duration cannot be concluded, with the result suggesting either 5 or 9 minutes depending on which physiological or cognitive parameters being investigated.

## 6.2 Future Works

A larger sample size that includes various age group needs to be recruited in order to concrete the results obtained here. Subgroup analysis can also be performed on the various age groups and gender to further reveal the different effects of deep breathing durations on the human physiology and cognitive control.

Besides that, one possible future work in this study is to perform MRI scans when the participants are performing the deep breathing exercise so that the neural activations elicited by different deep breathing durations can be investigated. Besides that, this study can be improved by considering the mental states such as depression, anxiety and stress of the participants as possible factors affecting the efficacy of deep breathing. By doing so, one can further understand the fundamental physiological and cognitive changes brought by deep breathing of different durations.

## LIST OF PUBLICATION RELATING TO THIS THESIS

- Cheng, K. S., Chang, Y. F., Han, R. P. S. and Lee, P. F. (2017) 'Enhanced conflict monitoring via a short-duration, video-assisted deep breathing in healthy young adults: an event-related potential approach through the Go/NoGo paradigm', *PeerJ*, 5, pp. e3857. doi: 10.7717/peerj.3857.
- Cheng, K. S., Han, R. P. S. and Lee, P. F. (2018) 'Neurophysiological study on the effect of various short durations of deep breathing: A randomized controlled trial', *Respiratory Physiology and Neurobiology*, 249(December 2017), pp. 23–31. doi: 10.1016/j.resp.2017.12.008.
- Cheng, K. S., Lee, P. F. (2018) 'A physiological model study on deep breathing effect on human respiration rate, oxygen saturation, and cerebral oxygen delivery', *Neurophysiology*. Accepted.
- Cheng, K. S., Lee, P. F. (2018) 'Heart rate variability with different deep breathing durations and its correlation to the mental health state in young adults', *Mindfulness*. Submitted.

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