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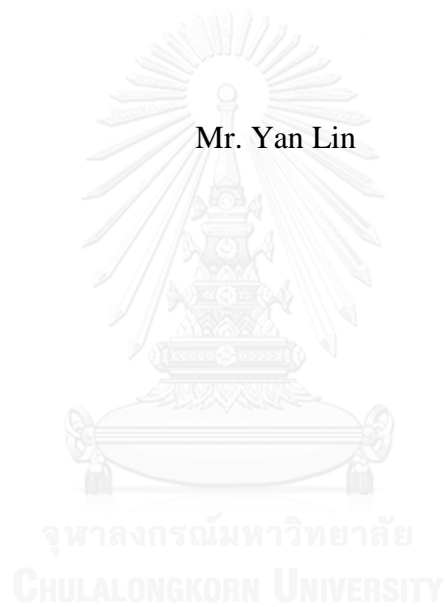
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Analysis of Physiological and Psychological Responses to Odor Stimulation

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A Dissertation Submitted in Partial Fulfillment of the Requirements
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Department of Electrical Engineering
Faculty of Engineering
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งานวิจัยนี้ใช้ความแปรปรวนของอัตราการเต้นหัวใจ(HRV)และแบบสอบถามการตอบสนองต่อความเครียด(SRS-18) เพื่อประเมินผลของการดมกลิ่นมะลิต่อความเครียดของอาสาสมัครเพศชายและเพศหญิง โดยมีอาสาสมัครสุขภาพดีเพศชาย 15 ราย(อายุ 26.3 ± 3.7 ปี) และเพศหญิง 15 ราย(อายุ 30.1 ± 3.4 ปี) เข้าร่วมในการทดลอง ข้อมูล ECG แบบ Standard Lead-II ได้ถูกเก็บในสภาวะขณะพัก สภาวะควบคุม และสภาวะที่กระตุ้นด้วยกลิ่น เพื่อหาสเปกตรัมกำลังของ HRV ได้ใช้เทคนิคการถอดแตรกจุดข้อมูล แบบ cubic spline กับทาโคกราฟของ HRV ร่วมกับการใช้เทคนิคการแปลงฟูรีเยร์อย่างรวดเร็ว(Fast Fourier Transform, FFT)

พารามิเตอร์สำหรับการวิเคราะห์ในโดเมนความถี่ของ HRV คือ ผลรวมช่วงความถี่ต่ำ (LF: 0.04 - 0.15 เฮิร์ตซ์) ผลรวมช่วงความถี่สูง (HF: 0.15 - 0.4 เฮิร์ตซ์) และอัตราส่วนของค่าที่ทำให้เป็นบรรทัดฐานของช่วงความถี่ต่ำและช่วงความถี่สูง(nLF/nHF) และพารามิเตอร์สำหรับการวิเคราะห์ในโดเมนเวลาของ HRV ได้แก่ อัตราการเต้นของหัวใจ (ครั้งต่อนาที, bpm) ค่าเบี่ยงเบนมาตรฐานของช่วง RR ปกติ(SDNN) และรากกำลังสองเฉลี่ยของความแตกต่างระหว่างช่วง RR ที่ติดกัน (RMSSD)

ผลการทดลองแสดงให้เห็นว่าภายหลังการดมกลิ่น ความเครียดของอาสาสมัครทั้งเพศชายและเพศหญิงลดลง ($p < 0.05$) และสำหรับชุดข้อมูลการดมกลิ่น (odor sets) พบว่า อัตราการเต้นของหัวใจที่ลดลงในเพศชายจะลดลงเร็วกว่าที่พบในเพศหญิง ส่วนค่า SDNN และ RMSSD จะเพิ่มขึ้นเล็กน้อยทั้งในชุดข้อมูลการดมกลิ่นและชุดข้อมูลควบคุม โดยไม่แตกต่างกันมากนักระหว่างเพศชายและเพศหญิง พบว่าค่า nLF/nHF ของอาสาสมัครเพศชายจะสูงกว่าของเพศหญิงอย่างมีนัยสำคัญ($p < 0.05$) ในทุกช่วงการวัด ซึ่งคาดว่าเป็นลักษณะเฉพาะของเพศชายและเพศหญิง และในเพศชายพบว่าค่า nLF/nHF จะลดลงเล็กน้อยหลังจากดมกลิ่น ในขณะที่ค่านี้จะเพิ่มขึ้นเล็กน้อยตลอดช่วงของกลุ่มควบคุม ค่า nLF/nHF ที่ลดลงอาจเกี่ยวข้องกับความเครียดที่ลดลง ส่วนในอาสาสมัครเพศหญิงพบว่าทั้งในช่วงการดมกลิ่นและช่วงควบคุม ค่า nLF/nHF จะไม่เปลี่ยนแปลงมากนัก โดยมีแนวโน้มเพิ่มขึ้นเล็กน้อย ผลการศึกษาแสดงให้เห็นว่าสามารถนำเอาการตรวจวัดค่า ECG ไปใช้ประเมินสภาวะเครียดในเชิงปริมาณได้ การตรวจวัดนี้จึงเป็นเทคนิคทางเลือกสำหรับการประเมินความเครียด นอกเหนือจากการตรวจวัดเชิงคุณภาพที่ใช้แบบสอบถามการตอบสนองต่อความเครียด (SRS)

ภาควิชา วิศวกรรมไฟฟ้า

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5671457821 : MAJOR ELECTRICAL ENGINEERING

KEYWORDS: HRV ANALYSIS / LEAD II ECG / SRS-18 / JASMINE ODOR / CUBIC SPLINE INTERPOLATION

YAN LIN: Analysis of Physiological and Psychological Responses to Odor Stimulation. ADVISOR: ASSOC. PROF.MANA SRIYUDTHSAK, Ph.D., CO-ADVISOR: PROF.TAKAMICHI NAKAMOTO,DR.ENG., 99 pp.

Heart rate variability (HRV) analyses as well as Stress Response Scale-18 (SRS-18) questionnaire were used to evaluate the effect of Jasmine-odor inhalation in males and females. Healthy 15 males (26.3 ± 3.7 years) and 15 females (30.1 ± 3.4 years) participated in the experiment. Standard Lead-II ECG data were collected for resting, control and odor stimulation conditions. The instantaneous heart rate variability tachogram was interpolated with the use of cubic spline interpolation, and then, FFT was performed to find the HRV power spectrum.

HRV frequency domain analysis parameters were low frequency range (LF: 0.04 - 0.15Hz), high frequency range (HF: 0.15 - 0.4 Hz) and the ratio of normalized low frequency to high frequency (nLF/nHF). HRV time domain analysis parameters in this research were heart rate (bpm), the standard deviation of all normal to normal RR intervals (SDNN) and root mean square of successive RR intervals difference (RMSSD).

Experimental results suggest that stress of both males and females decreased after exposing to odor ($p < 0.05$). It was also found that heart rate decreasing rate of males was faster than that of females for odor sets. SDNN and RMSSD parameter were slightly increased in both odor and control experiment but not so much difference in both males and females. The nLF/nHF ratio of males are significantly higher than females in all measurement intervals ($p < 0.05$). This might be intrinsic character of males and females. After odor exposure, the nLF/nHF ratio was slightly decreased in male subjects while it was slightly increased for all control intervals. Decreasing the nLF/nHF ratio might link to decreasing the stress. The nLF/nHF ratio in female subjects was not so much changed in both odor and control intervals with slightly increased trend. The results indicate that ECG measurement could be used to evaluate the stress state quantitatively. It will be alternative technique for stress evaluation other than using the qualitative measurement with SRS scale.

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Field of Study: Electrical Engineering

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CHAPTER 1

INTRODUCTION

Nowadays, people have many problems in their daily life, and then some traumatic stress due to the competitive society is unavoidable to bring some illnesses on the people. J. F. Thayer et al. [1] and J. Taelman et al. [2] indicated that the stress was also related to heart problems. Many researchers have widely investigated the reduction of some stress. As a consequence, some natural odors have been used to relieve both psychological and physiological stresses. X. Duan et al. [3] and T. Koike et al. [4] reported that some pleasant odors, such as lavender, could show the apparent effect on the emotional state as well as autonomic nervous system activities. Generally, odor molecules have the direct influence on brain activities through the olfactory system. D. Öngür and J. L. Price [5] confirmed that the olfactory system in human had strong links to the limbic system in which the emotion and nervous system were under the autonomic control. A good odor can, therefore, make people calm down and relieve some stress. Under a certain circumstance, it is clear that an aromatherapy can exhibit some potential to solve psychological problems such as reducing some stress and irritation [6]. Nonetheless, although researchers have reported applications of some pleasant odors for the treatment of stress, the real impact of odors on the human body is still ambiguous. In essence, there is a lack of evidence for a relation between odors and physiological effects on human body.

The autonomic nervous system function is physiologically linked to the human body and reflected via some physical body responses such as heart rate variability (HRV) [7]. Measuring heart rate variability is a non-invasive method to assess an autonomic nervous system function and also a stress condition [8]. Generally, a specific electrical signal, called electrocardiogram (ECG), is generated to control the heart in contracting and releasing heart muscles rhythmically and repeatedly [9]. Any irregularity in the shape of ECG reflects some abnormality of the heart rhythm or some damage to the heart muscle [10]. Any impact on the autonomic nervous system directly affects ECG signals, and eventually, leads to the change of HRV.

Recently, the investigations on the relation between odors and the olfactory system have been increasing gradually [11]. After receiving some molecules of odors,

the neurotransmission signal is generated and transmitted directly to the cerebral limbic system that controls the emotion, nervous system and memory [12]. To relieve the stress and to investigate the autonomic responses, S. Dayawansa et al. [13] examined the effect of a Cedrol odor inhalation and found that the odor effect was related to the autonomic parameter and heart rate variability. Still, there is a lack of the extensive research into the mechanism of the reaction of the human body to the odor stimulation.

1.1. Motivation

Based on the data from the survey, S. Béjean and H. Sultan-Taïeb [14] stated that some cardiovascular diseases were highly related to the stress. In addition, according to the survey on the stress of people in industrialized countries, the statistical results have revealed that stress-related cardiovascular diseases (CVD) were highly increased. Stress could also lead to the other health problems [14] such as musculoskeletal disease (MSD), back pain, depression, and burnout. Increasing the trend of work-related stress also leads to some dangerous behaviors, such as drinking alcohol and using drugs. Unhealthy and high-stress people may leave their job, and sometimes, even commit suicide. Also, there were other losses in the society and people performance because of stress.

In many European countries, people tried to assess the stress and stress-related health problems among workers [15]. J. Choi and R. Gutierrez-Osuna [8] found that mental stress can also affect the heart and especially the heart rate variability. During the past decade, an HRV measurement method has been used as a non-invasive method to assess the autonomic nervous system function and stress condition [7, 8].

A spectral analysis of (heart rate variability) HRV analysis was used to assess the effect of stress on HRV by J. Taelman et al. [2] The two main interesting frequency bands from the HRV spectral analysis are a low-frequency (LF) band and a high-frequency (HF) band. An LF/HF ratio has been considered as sympathovagal balance [2]. This LF/HF ratio index can reflect the stress condition because when people have stress, a parasympathetic nervous system, as a part of an autonomic nervous system (ANS), is deactivated, but a sympathetic nervous system is activated, as reported in the work of J. Taelman et al. [2] Although stress did not have a significant effect on the LF/HF ratio, it have been widely accepted that the stress was certainly related to an

autonomic nervous system and heart rate variability. S. Akselrod et al. [16] reported the power spectral analysis of heart rate variability and demonstrated the contribution of sympathetic and parasympathetic nervous system activities in the HRV power spectrum.

In order to tackle the stress problems, R. N. Jackson and K. Roberts [17] investigated some odor applications such as aromatherapy. They found some effects of the odor on depression, insomnia, anxiety, and some emotional diseases such as irritability and loneliness. Also, the odor could decrease the stress and make people calm and relaxed [17]. J. Chebat and R. Michon [18] reported that the floral, spice, wood, citrus, ginger, lavender, and mint odors influence on the nervous system, mood, and cognition. As mentioned above, odors influence on an ANS condition and hence leads to the indirect effect on the stress. In essence, the odor has an effect on the heart rate variability since the states of stress and autonomic nervous system in human are a key factor in controlling the heartbeat rhythmically.

Jasmine is a native flower in Asia, especially in Southeast Asia. Its odor has widely used in many ways. In some fashion, people have traditionally used its aroma to relax the body and mind since the ancient time [19]. However, it is still unclear to explain how the jasmine odor affects our body, especially on an electrocardiogram response, in quantitative point of view. Therefore, investigating the effect of jasmine odor on electrocardiogram response is an interesting topic. This work has thus focused on the investigation of the effect of jasmine odor inhalation on the change of a stress condition based on the self-report rating, and the influence on the electrocardiogram signal relied on the HRV analysis.

1.2. Experimental Setup

The experimental setup for the odor supplying system and data acquisition system is shown in Figure 1.1. An air compressor tank was used to supply the air flow through a small flexible plastic pipe (3 mm in diameters). The airflow was maintained at the constant flow rate of 3.0 SCFH using a flow meter. The air entered a bottle which was filled with a jasmine odor solution (10ml), and then come out to a plastic cone. The volume of the bottle was about 50.89 cm^3 . A plastic cone with an aperture diameter of 9.5 cm was used to expose the odor molecules to a subject. A plastic cone was placed

in a range of 5-10 cm from the subject's nose. A breathing rate data acquisition system was also integrated with the ECG data acquisition system.

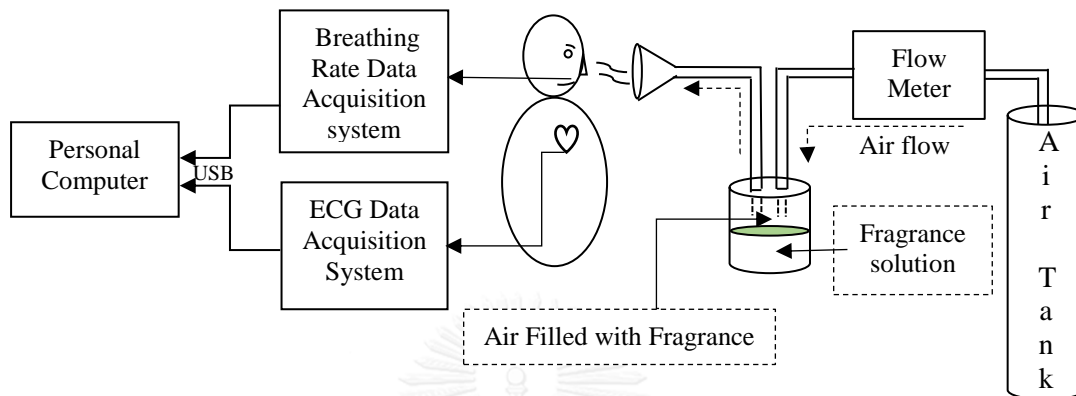


Figure 1.1: Experimental setup

Heart rate variability was widely accepted that it has circadian rhythm [20]. The variation comes from the changes of an autonomic nervous system balance depending on a different time such as morning, afternoon, evening or night. All experiments were undertaken between 9:30 a.m. and 12:30 p.m. to avoid any deviation in ECG signals from the circadian rhythm in HRV. The experiment was conducted in a silent room (length=11ft, width=8ft, height=8ft), and the light was adjusted for comforts of the subject. The room temperature was also maintained at about 24°C. To prevent an accumulation of the odorant molecules from the prior experiment, the experiment room was ventilated using a fan. Subjects wore comfortable clothing during the experiment to avoid any variation in heart rate variability from the pressure of clothing as reported by Y. Ling and Z. Wen-bin [21].

Silver/silver chloride (Ag/AgCl) electrodes were attached to a right arm (RA), a left leg (LL), and a right leg (RL) for recording the Lead-II ECG data. Analog ECG data were measured and amplified using an instrumentation amplifier. The noise was rejected from the signal using an analog band-pass filter (0.05 Hz – 50 Hz). The gain of this filter was controlled and adjusted. The overall gain of the ECG data acquisition system was constant at 900. An offset level shifter was used to shift the output signal

from the band-pass filter before sending the signal to a microcontroller. The microcontroller digitized the analog ECG data with the 180 Hz sampling frequency and 10-bit resolution. A breathing rate was also recorded using a small thermistor, placed close to the nose. Respiratory data were then digitized by the microcontroller at the same sampling rate and resolution as applied to the ECG data. Both ECG and respiratory data were stored in PC simultaneously via the USB interfacing with the baud rate of 38400 bps.

1.2.1. Data Analysis Flow Chart

The procedure for analyzing the acquired ECG to investigate the odor responses is shown in Figure 1.2. There are two main HRV analyses, i.e. time-domain analysis and frequency-domain analysis. Selected time-domain parameters are

- (i) an average heart rate (HR),
- (ii) a standard deviation of all normal to normal intervals (SDNN) and
- (iii) a root mean square of successive RR intervals difference (RMSSD).

Selected frequency-domain parameters are

- (i) a normalized low-frequency (nLF),
- (ii) a normalized high-frequency (nHF) and
- (iii) a normalized low-frequency to normalized high-frequency ratio (nLF/nHF).

All parameters were carefully chosen according to the Task Force of the European Society Guidelines [22] so that they can be used to reflect the state of an autonomic nervous system and a stress condition.

After digitizing the raw ECG data, a digital notch filter was used to remove the 50 Hz line frequency noise.

A differentiator was then used to obtain a slope information of a QRS complex and to remove a baseline drift in some certain circumstances. If the output signal from the differentiator was higher than a threshold level, an R-peak was identified.

Once the R-peaks were extracted, an RR time interval for each consecutive an R-peak was calculated to obtain a tachogram of RR time intervals. Time-domain analysis parameters (mean heart rate, SDNN, and RMSSD) were calculated from this

tachogram. Also, an instantaneous heart rate variability was calculated from each RR time interval.

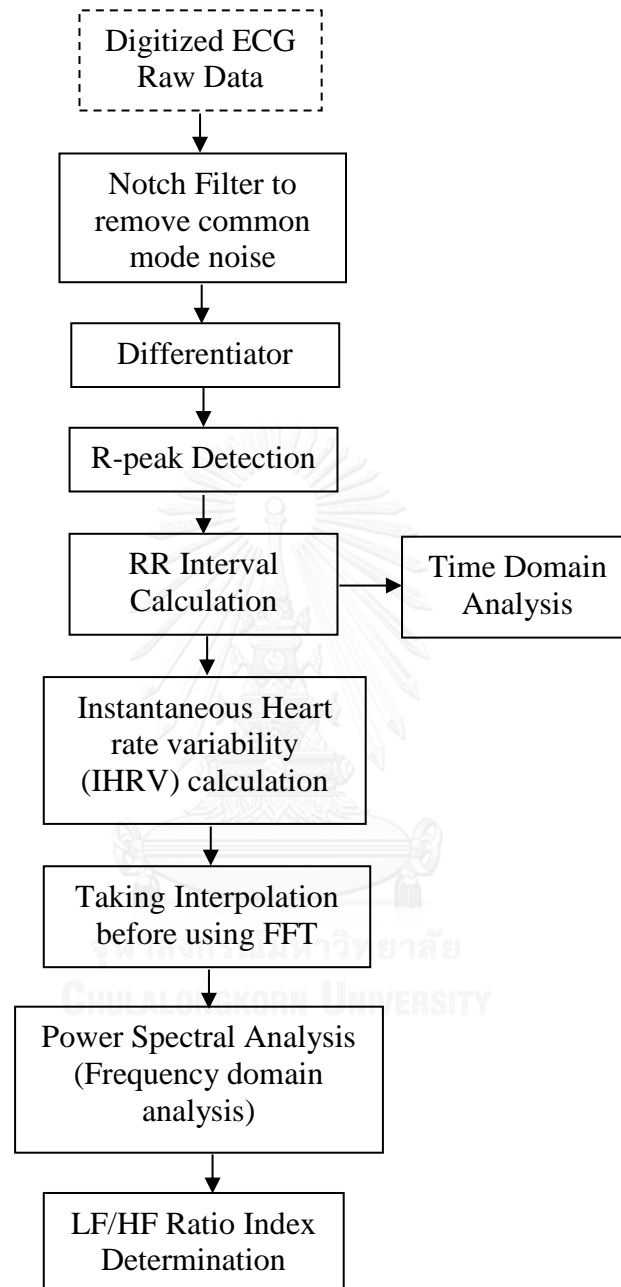


Figure 1.2: Data analysis procedure

The instantaneous HRV tachogram was not adequately sampled, and hence, it was impractical to apply the Fast Fourier Transform to analyze the tachogram in the frequency domain. With an interpolation technique, some intermediate data points within the discrete set of the known data can be constructed to increase the sample

group of the tachogram. Instead of a linear piecewise interpolation, a cubic spline interpolation technique was applied in this work to smooth curves of the first and second derivatives. The instantaneous HRV tachogram in this work was thus resampled at the frequency of 4 Hz before the transformation into the frequency domain. The discrete Fourier transformation was applied to calculate using the Fast Fourier Transform (FFT) with the data size of 1024 points. According to the suggestions in the Guidelines [22], the power spectral density was divided into two main frequency bands, i.e. the low-frequency band (0.04 Hz to 0.15 Hz) and the high-frequency band (0.15 Hz to 0.5 Hz). In essence, this work employed the LF/HF ratio index to investigate the odor effect on HRV.

1.3. Outlines of Thesis

The thesis is composed of five chapters as follows.

Chapter 1 includes the introduction and focuses on the motivation of the work. In addition, the overview of the work is summarized, including the concept of data analyses in this work and the contribution of the work.

In chapter 2, some background knowledge of electrocardiogram, heart rate variability and autonomic nervous system functions are mentioned. Also, some key related studies are summarized to show the general view of the research on the odor effect on human.

Chapter 3 mainly provides the details of the experimental setup, materials, and methods. Additionally, the inclusion criteria and data analysis procedures are explained.

Chapter 4 shows the key results and discussions based on the experiments and data analyses. This chapter principally concentrates on the influences of the jasmine scent on both psychological and physiological responses in human.

The key effects of the odor on human are summarized in chapter 5. To clarify some ambiguous effects, some further work is required and suggested at the end of the chapter.

CHAPTER 2

BACKGROUND AND LITERATURE REVIEW

Background knowledge on the morphology and function of the heart, the features of electrocardiogram (ECG) and the orientation of 12-Lead ECG were introduced in this chapter. Moreover, some recent publications were reviewed to show the essential concept of electrocardiogram, stress, and effect of odors on a human. Later on, the concepts of heart rate variability, an autonomic nervous system function, and the olfactory system were provided in this chapter.

2.1. Morphology and Function of the Heart

The heart is physiologically constituted with muscles. An electrical signal is generated to make heart muscles contract and release. The graphical representation of the electrical signal that is generated from the heart is called Electrocardiogram [9]. A heart has four chambers composed of the two upper chambers and the two lower chambers. The upper chambers are a left atrium and a right atrium. The lower chambers are a left ventricle and a right ventricle.

Oxygen-poor blood from the whole body enters a right atrium through the vena cava (the two large veins). As the right atrium contracts, the oxygen-poor blood is then pumped into a lower right ventricle through a tricuspid valve. The right ventricle pumps this oxygen-poor blood through a pulmonary valve into a pulmonary artery, and then, the blood flows to the lungs where the exchange of oxygen and carbon dioxide takes place between air and blood. After receiving the oxygen, the blood becomes oxygen-rich and return to a left atrium via a pulmonary vein. The left atrium then pumps this oxygen-rich blood into a left ventricle through a mitral valve. Subsequently, the blood is pumped via an aortic valve into the aorta, and carry the oxygen to the rest of the body [9]. The valves in a heart regulate the one-way flow of blood.

Figure 2.1 shows the morphology and the blood flow in the heart. In a healthy heart, heartbeats are initiated by a small electrical signal that occurs from a small area in the heart called the Sino-Atrial (SA) node. The high pressure is needed to pump the blood to the whole body, while a certain lower pressure is needed to convey the blood

between a heart and lungs. Typically, a muscular wall of a left ventricle is three times thicker than that of a right ventricle. [9].

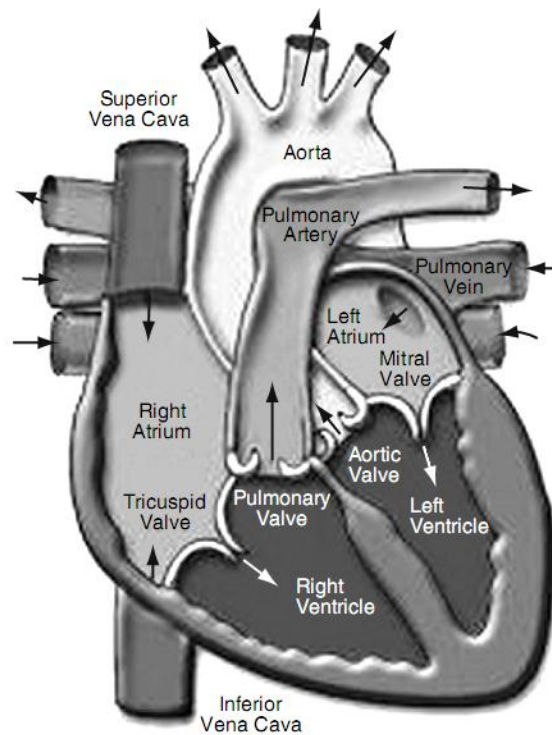


Figure 2.1: Morphology and blood flow in the heart [9]

2.1.1. Sino-Atrial (SA) node and Atrial-Ventricular (AV) node

A Sino-Atrial node, located at the top of a right atrium, is commonly known as a natural pacemaker of the heart. An electrical excitation for the heart activity is initiated from the SA node [23]. This signal rapidly spreads throughout the atria to make all the heart muscle in these areas contract simultaneously to pump the blood into the ventricles. On the other hand, a signal from an atrial-ventricular (AV) node is generated at the slower rate than the SA signal. As the consequence, the AV signal causes the contraction of the ventricles, shortly after pumping the blood from the atria into the ventricles. This is the sinus rhythm, which is named from its origin at the SA node.

A SA node is stimulated by both parasympathetic nervous system and sympathetic nervous system. Those nervous systems are the branches of the autonomic nervous system [24]. By regulating the action potential from the SA node, the

parasympathetic nervous system has a major role in decreasing the heart rate, while the sympathetic nervous system plays a direct role in increasing the heart rate [24].

2.2. Typical Amplitude and Period of ECG

Figure 2.2 shows the typical ECG signal corresponding to the standard time interval and amplitude. The PQ interval (also known as the PR interval) is a time interval from the beginning of the P complex to the QRS complex. This represents the period between the beginning of the atrial contraction and the beginning of the ventricular contraction. The normal duration of this PQ interval is approximately between 0.12 and 0.2 sec. Similarly, the QT interval is the time between ventricular contraction (ventricular depolarization) and ventricular releasing (ventricular repolarization). This is defined from the beginning of the Q wave to the end of the T wave and the typical value of this QT interval is between 0.3 and 0.42 sec. The QRS duration is approximately 0.09 sec and the R-R interval is approximately 0.6 to 1 sec [25].

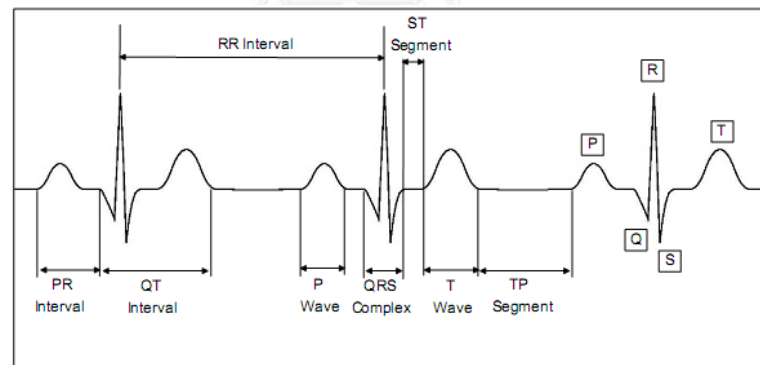


Figure 2.2: A typical ECG wave [26]

The voltage of the ECG signal can also be changed depending on the location of the electrodes which are placed on the body. If the electrodes are placed close to the heart, the recorded potentials can be as high as 5 mV. However, if the electrodes are placed further from the heart, such as at the wrists, a typical value is 1 mV from the peak of the R wave to the bottom of the S wave. A P-wave is approximately 0.1 to 0.25 mV and 0.09 sec period. A T-wave is approximately in a range of 0.1 to 0.5 mV and its period is approximately 0.12 to 0.18 sec [26].

2.3. Depolarization and Repolarization of Cardiac Cells

Generally, a cell membrane of the cardiac muscle cells separates the cells from the surrounding solution. The solutions, both inside and outside the membrane, are composed of various ions such as sodium, potassium, and calcium, but possess different concentrations. In the resting state of the cardiac muscle cells, the cardiac muscle cells are polarized and the ECG signal is not generated. The action potential is generated from the cardiac cells when the ion exchange across the cell membrane takes place. This is called depolarization. Depolarization of the heart is a part of the cardiac activation process. When the atria are stimulated and atrial depolarization occurs, cardiac muscle cells contract and the P-wave appears in the ECG signal. The QRS complex can be detected when the ventricles are stimulated and ventricle depolarization occurred. Depolarization spreads as a wave through the heart corresponding to the contraction of heart muscle cells [27].

After the depolarization period, cardiac muscle cells will return to the resting state. Repolarization is the process of the heart muscle cells to return to their resting condition, and also relaxed state. Ventricular repolarization appears as components in the ECG signal, i.e. T-wave, and U-wave [27]. Figure 2.3 shows the action potential during the depolarization and repolarization.

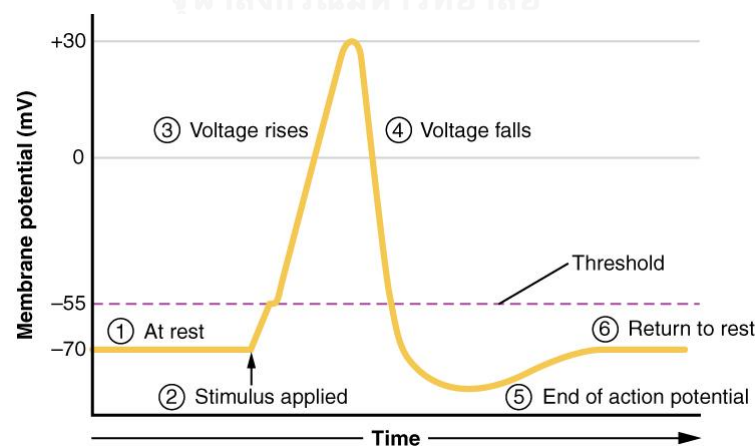


Figure 2.3: The action potential [27]

2.4. Standard 12-Lead ECG Measurement

ECG measurement is generally performed in the standard 12-leads protocol which uses 10 input electrodes. Input electrodes are placed on the skin according to the specific lead configuration. With a certain configuration, a specific signal waveform of electrocardiogram will be measured.

Einthoven was the main person for the remarkable of electrocardiograph invention [28]. He introduced the string galvanometer to detect the electrical signal generated from the heart. The electronic device used to measure the electrical signal of the heart is called an electrocardiograph. The rate and the regular changes of the heartbeat can be monitored from an electrocardiogram. Because of the temporal, morphological and statistical features [29], applications of ECG have been widely investigated and used for some medical diagnoses. The temporal features of ECG wave are an R-R interval, an average R-R, a QRS duration, a T-wave duration and a P-wave flag. The morphological features are beat area, beat power, beat minimum, beat maximum and beat max-min ratio. The statistical features are a QRS variance, a QRS histogram variance, a beat mean and a beat variance [29].

A single lead ECG measurement system typically uses three electrodes: the positive electrode, the negative electrode, and the reference electrode or ground. An ionic current because of the depolarization and repolarization of the cardiac cells flows through the resistive tissue. Additionally, a small potential difference appears between two different spots on the skin surface [9]. This small potential difference is detected by the electrodes and then amplified to a certain level for the clear identification. A representation of 12-Leads ECG in a particular orientation is indicated as follow. Mainly, there are three types of leads. They are:

- (1) Bipolar limb leads
- (2) Augmented unipolar limb leads
- (3) Unipolar chest leads or precordial leads

2.4.1. Bipolar limb leads

According to the bipolar limb leads, the electrodes are attached to the limbs of the body, and thus, these leads are called bipolar limb leads. There are three bipolar limb leads. They are:

- (i) Lead I: RA (-) and LA (+)
- (ii) Lead II: RA (-) and LL (+)
- (iii) Lead III: LA (-) and LL (+)

Each of, Lead I, Lead II and Lead III needs two electrodes to retrieve the signal. An electrode is set as a positive electrode and another electrode is a negative electrode. Therefore, it is commonly named as a bipolar lead.

For the Lead I, a right arm (RA) electrode is used as the negative electrode and a left arm (LA) electrode is used as the positive electrode.

For the Lead II, a right arm electrode is referred to as the negative electrode and a left leg (LL) electrode is referred to as the positive electrode.

For the Lead III, the negative electrode is attached to a left arm and the positive electrode is attached to a left leg. The positive electrode refers to the positive input terminal of the amplifier and the negative electrode refers to the negative input terminal of the amplifier, respectively. A right leg (RL) is always connected to ground as a reference electrode for all leads.

In the electrocardiograph, three electrodes placed on a right arm, a left arm, and a left leg are employed to detect signals from the heart activities. Their location is analogously at a corner of a triangle and forms an imaginary triangle as shown in Figure 2.4. The center of the triangle is analogous to the location of the heart in a human body. This is called the Einthoven's triangle. Table 2.1 shows the bipolar limb leads and measurement calculation for each lead.

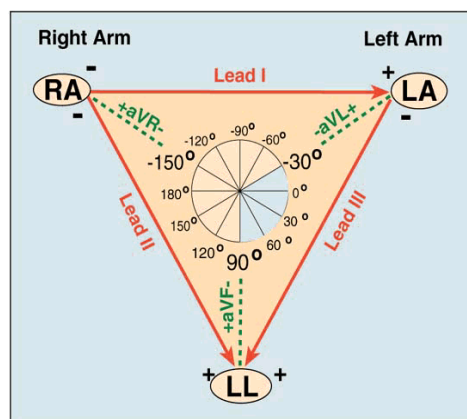


Figure 2.4: Einthoven's triangle [30]

Table 2.1: Bipolar limb leads and measurements

Lead Name	Calculation	Remark
Lead_I	LA-RA	The voltage between a left arm and a right arm. Actual lead
Lead_II	LL-RA	The voltage between a left leg and a right arm. Actual lead
Lead_III	LL-LA (lead-II minus lead-I)	The voltage between a left leg and a left arm. Actual lead

2.4.2. Augmented unipolar limb leads

In augmented unipolar limb leads, two limb leads are combined and used as a negative electrode. An another electrode is defined as a positive electrode. Based on the augmented unipolar limb lead, there are three leads configurations.

They are namely: (i) aVR, (ii) aVL and (iii) aVF.

(i) aVR: RA (+) to [LA & LL] (-)

In aVR Lead, the negative electrode receives an average signal from a left arm with respect to a left leg. The positive electrode is placed on a right arm.

(ii) aVL: LA (+) to [RA & LL] (-)

In aVL Lead, the negative electrode is placed on a right arm with respect to a left leg. This configuration is employed to measure the average signal from both limbs. Meanwhile, the positive electrode is placed on a left arm.

(iii) aVF: LL (+) to [RA & LA] (-)

The aVF Lead is used to measure the average signal between a right arm and a left arm. The electrodes on the arm set up a negative electrode, while the electrode placed on a left leg is used as a positive electrode.

Table 2.2: Augmented unipolar limb leads and measurements

Lead Name	Calculation	Remark
aVR	$-(\text{Lead_I} + \text{Lead_II})/2$	Derived lead
aVL	$\text{Lead_I} - (\text{Lead_II})/2$	Derived lead
aVF	$\text{Lead_II} - (\text{Lead_I})/2$	Derived lead

2.4.3. Unipolar chest leads or precordial leads

There are six chest electrodes V1, V2, V3, V4, V5 and V6 for the unipolar chest leads or precordial leads. Positive electrodes for the precordial leads are on the chest point, i.e. V1, V2, V3, V4, V5 and V6 respectively, as shown in Figure 2.5. The Wilson's central terminal is used as a negative electrode. The terminal is a virtual combination and its potential is an average of the signals from three electrodes on specific parts of the body, i.e. a left arm, a right arm and a left leg. Table 2.3 shows the precordial lead names and measurement calculation for each lead.

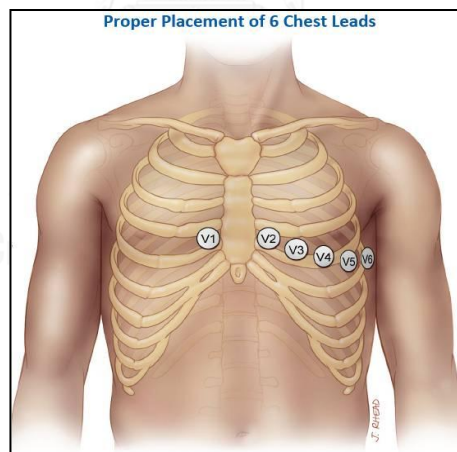


Figure 2.5: Placement of six chest leads [30]

Table 2.3: Precordial leads and measurements

Lead Name	Calculation	Remark
V_w (Wilson's central terminal)	$1/3(LA+RA+LL)$	This is not used for display of ECG trace.
V1	$(Vc1-V_w)$	Actual lead is shown in ECG trace
V2	$(Vc2-V_w)$	Actual lead is shown in ECG trace
V3	$(Vc3-V_w)$	Actual lead is shown in ECG trace
V4	$(Vc4-V_w)$	Actual lead is shown in ECG trace
V5	$(Vc5-V_w)$	Actual lead is shown in ECG trace
V6	$(Vc6-V_w)$	Actual lead is shown in ECG trace

2.5. Characteristics of ECG and various noises in a system

An ordinary ECG signal has a low amplitude voltage with three basic waves pattern, namely P-wave, QRS complex, and T-wave. Additionally, an ECG signal also consists of various noises. The main sources of noises in ECG are a power line interference, an electrode contact noise, a motion artifact, an electromyography (EMG), a baseline wander noise and the other noise such as a high-frequency RF noise. A power-line interference is common in an ECG signal and coupled to a system via an inductive path. In Thailand, a power line interference is 50 Hz but in some parts of the world is 60 Hz noises. Figure 2.6 shows the ECG signal with a power line interference compared to that with 50 Hz noise rejection.

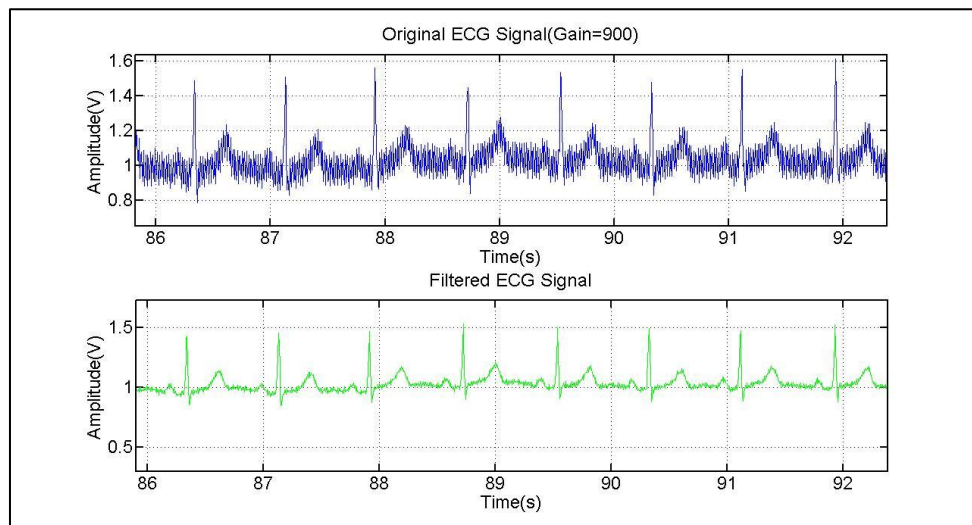


Figure 2.6: A power line interference and a filtered ECG signal

A loss at the electrode contact between the electrode and the skin surface can also cause the electrode contact noise and it is similar to a transient interference. The motion of the human body leads to change of the impedance at an electrode/skin contact, and hence the motion artifact can also occur. The time duration for this kind of an artifact occurring can be varied from 100 ms to 500 ms while the peak amplitude is typically five times higher than that of the ECG signal [9]. Contraction of muscles can cause the electromyography (EMG) noise. Estimated time duration for this kind of noise is approximately 50 ms, and its amplitude is around 10% of peak to peak ECG signal [9]. The baseline wander or a low-frequency noise in ECG signal is also one of the most problematic noises in ECG signal measurement to detect the R peak. Respiration, as well as subject movement, can cause this baseline wander. In average, the frequency of this is around 0.15 to 0.3 Hz and baseline variation is around 15 % of ECG amplitude [9].

The most common electrode in ECG measurement system is silver-silver chloride (Ag/AgCl) electrode [31]. Because of the half-cell potential at the electrode, the higher offset level voltage can also appear. In practice, a typical ECG signal has the amplitude of around $\pm 1\text{mV}$. However, the electrode may have higher voltage offset level than the actual ECG signal. Normally, the offset voltage level from the electrode is around $\pm 300\text{ mV}$. Figure 2.7 shows the comparison between the signals, the electrode offset level, and the common mode signal.

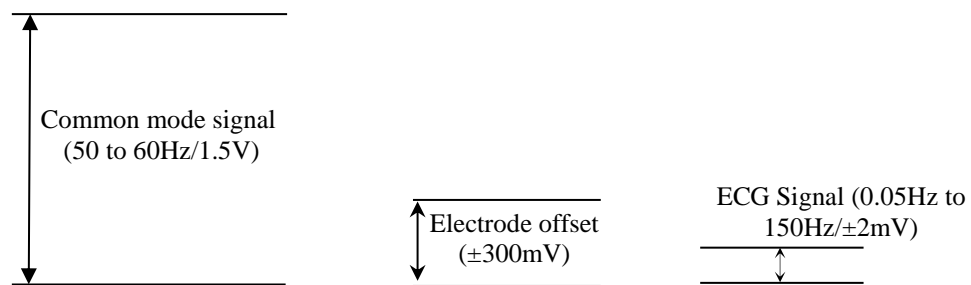


Figure 2.7: ECG, the electrode offset, and the common mode signal voltage

2.5.1. Filtering Noise in ECG Signal

Using an instrumentation amplifier with high common mode rejection ratio and the use of notch filter at 50Hz/60Hz can remove the common mode noise and the power line interference. A notch filter is simply called band-stop filter or band-rejection filter. A software-based digital filter is also a powerful tool for removing noise in the ECG data acquisition system [32]. Figure 2.8 shows the basic twin-T active notch filter design and Figure 2.9 shows frequency response curve of a notch filter. Most of the frequency can be passed without attenuating with the use of notch filter but it can attenuate the specific frequency value.

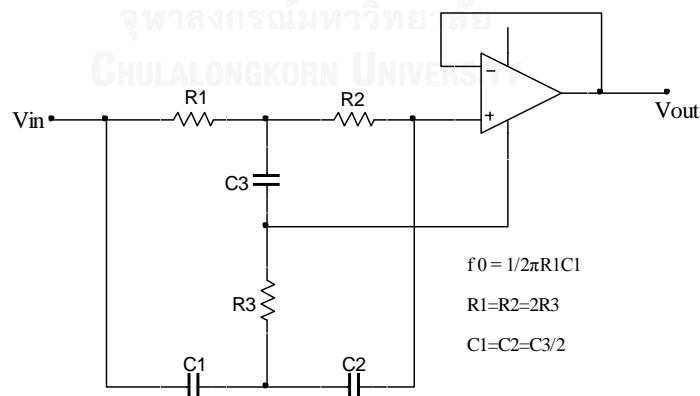


Figure 2.8: Twin-T active notch filter

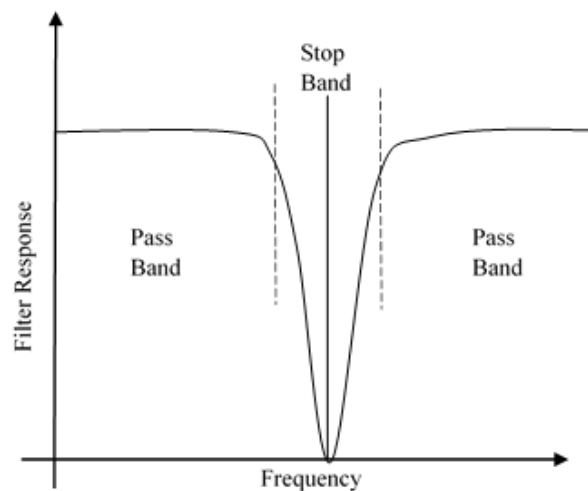


Figure 2.9: Notch filter frequency response curve

To solve the electrode contact noise and the motion artifact problem, good sticky electrode and suitable low pass filter are also used to filter the high-frequency noise. Generally, there are dead skin cells on the surface of the skin. Those dead skin cells form as the resistive elements and because of those resistive elements, there may be difficult to detect the electrical activity of ECG on the surface of the skin. To solve this problem, an electrode conductive gel is usually used under the electrode to detect the electrical activity of the ECG on the skin surface. Baseline wander is a low-frequency component, and thus, implementing a high pass filter can prevent the baseline wander. Band pass filter is generally used for the combination of a low pass and a high pass filter. Figure 2.10 shows a basic active band pass filter circuit design. DC offset from the half-cell potential between the electrode and the body surface can also saturate the signal at higher gain. An offset level shifter and high-pass capacitor can be employed to prevent the offset level saturation. High-frequency RF noise can also be prevented by using shielded cable.

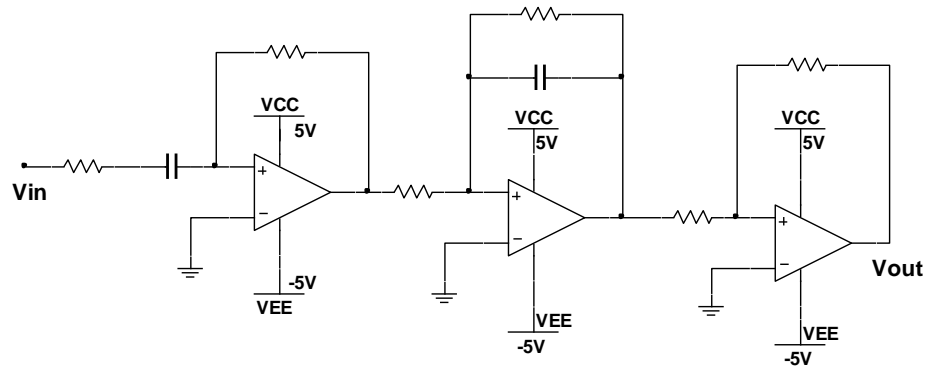


Figure 2.10: Active band pass filter [33]

2.6. Heart Rate Variability

Variation at each consecutive RR time interval is the heart rate variability. Heart rate variability measurement is calculated from an RR time interval series by defining the distance between consecutive R peaks. Heart rate variability is largely influenced by an autonomic nervous system on SA node with the changes in a sympathetic nervous system and a parasympathetic nervous system [34]. A sympathetic nervous system activity usually causes the heart rate to increase and a parasympathetic nervous system activity causes the heart rate to decrease [35]. A sympathovagal balance is a balance between the activity of sympathetic nervous system and parasympathetic nervous system. A sympathovagal balance can be represented using the ratio of absolute low-frequency power to absolute high-frequency power of heart rate variability [36]. Since the low-frequency component and high-frequency component of heart rate variability are related to an automatic nervous system condition, the LF/HF ratio index is usually used to estimate the psychological state or mental state of human [34]. In general, the frequency component of heart rate variability (HRV) can be divided to very low frequency (0-0.04Hz), low frequency (0.04-0.15Hz) and high frequency (0.15-0.4Hz) [22] [37].

An instantaneous heart rate can be determined from the inverse of RR time interval series. An instantaneous heart rate is an essential bio-signal because it possesses some features for analyses of heart rate variation and heart problem [38]. An instantaneous heart rate value tells how long one beat takes. Figure 2.11 shows instantaneous heart rate reconstruction from consecutive an RR time interval.

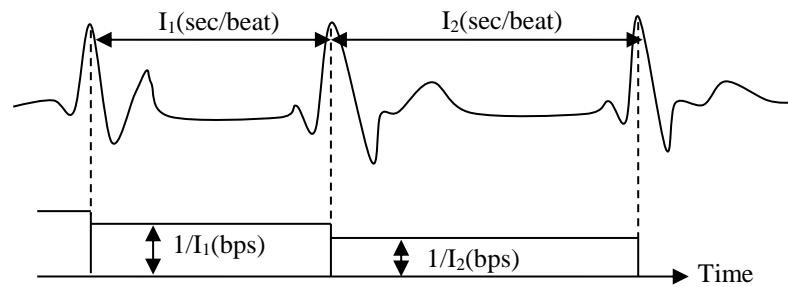


Figure 2.11: Instantaneous heart rate reconstruction

2.7. Heart Rate Variability (HRV) Analysis

During the last decades, heart rate variability (HRV) analysis has been used as a non-invasive method to assess the overall health of the cardiac and the cardiac autonomic nervous system activity [22]. Generally, an instantaneous heart rate or beat-to-beat RR interval change is mostly depending on the several different inputs to the SA node. Most of the inputs to the SA node which change the variation of RR interval are sympathetic and parasympathetic nervous system of an autonomic nervous system [39] and the other inputs are respiration, baroreflex, thermoregulation, hormones, sleep-wake cycle, meals, physical activity, and the stress [40]. Heart rate variability analysis is widely used in general medicine and provided useful information in the activation of sympathetic and parasympathetic nervous input to cardiac activity [41]. In 1981, S. Akselrod et al. [16] introduced power spectrum analysis of heart rate variation. The most widely used heart rate variability analysis methods are time domain analysis and frequency domain analysis method. An another method such as Poincaré plot [42] for a non-linear method was also proposed.

2.7.1. Time Domain Analysis for HRV

Time domain analysis method is the simplest method for the heart rate variability analysis. This method determines an instantaneous heart rate and the intervals between successive normal to normal (NN) intervals. Normal to normal (NN) intervals are all the intervals between adjacent QRS complexes. Some of the simplest time-domain measurement parameters are the mean NN interval, the mean heart rate, the difference between the longest and shortest NN interval and the difference between

day and night heart rate, and so on. The time-domain method can also be divided into a statistical method and geometrical method [22]. Commonly used time domain analysis parameters for HRV analysis are summarized in Table 2.4.

Table 2.4: Commonly used time-domain parameters for HRV analysis

Variable	Units	Description (Statistical measured parameters)
AVNN	ms	Average of all NN intervals
SDNN	ms	Standard deviation of all NN intervals
SDANN	ms	Standard deviation of the averages of NN intervals in all 5-minute segments of a 24-hour recording
SDNN index	ms	Mean of the standard deviations of NN intervals in all 5-minute segments of a 24-hour recording
SDSD	ms	Standard deviation of differences between adjacent NN intervals
RMSSD	ms	Root mean square of differences between adjacent NN intervals
pNN50	%	Percentage of differences between adjacent NN intervals that are greater than 50 ms
NN50 count		Number of pairs of adjacent NN intervals differing by more than 50 ms in the entire recording

2.7.2. Frequency Domain Analysis for HRV

Power spectral density (PSD) analysis is usually used for the frequency domain analysis of HRV analysis. There are two methods; parametric and non-parametric method to calculate PSD [22]. Frequency domain measurements are generally calculated from RR interval series. Original RR interval series are not equally sampled. Therefore, RR interval time series are generally interpolated to resample and then applying the Fast Fourier Transform in most of the cases. Three main spectral components can be classified for the short-term recordings from 2 to 5-minute. Three main spectral frequency bands are a very low-frequency band (≤ 0.04 Hz), a low-frequency band (0.04-0.15 Hz) and a high-frequency band (0.15-0.4 Hz) [22]. An Ultra-low frequency component is also provided as well as VLF, LF and HF component for long-term (24 hrs.) recordings [22]. To evaluate the HF components of HRV, the measurement period of approximately 1-minute and to assess the LF component, the measurement period of approximately 2-minute is recommended, respectively by the Task Force of the European Society of Cardiology and The North American [22]. Some

experimental designs and environmental variables are also recommended to be controlled because there are also some effects of the environment such as the nature of physical activity and the nature of emotional circumstances. The environmental condition for individual subjects and investigations design should also be in the same condition. Manual editing and checking for the RR data are also recommended to perform for getting standard correct identification and QRS complex detection. High-frequency component is mostly reflected by the vagal activity and low-frequency component is mostly considered by the effect of sympathetic and vagal activity [22]. Commonly used frequency domain analysis parameters for HRV analysis are summarized in Table 2.5 [22].

Table 2.5: Commonly used frequency-domain parameters for HRV analysis

Variable	Units	Description	Frequency Range
VLF	ms ²	Power in very low-frequency range	≤ 0.04 Hz
LF	ms ²	Power in low-frequency range	0.04 – 0.15 Hz
LF norm	n.u	LF power in normalized units	
HF	ms ²	Power in high-frequency range	0.15 – 0.4 Hz
HF norm	n.u	HF power in normalized units	
LF/HF		The Ratio of low to high-frequency power	

2.8. Literature Review about the Effect of Odor

M. Bensafi et al., (2002) [43] tried to do the research on the effect of some odors to the skin conductance and heart rate. In this research, Isovaleric acid, Thiophenol, Pyridine, Menthol, Isoamyl acetate, and Cineole were used to find the correlation between each odor dimension (intensity, pleasantness, arousal, and familiarity) and the skin conductance as well as heart rate. A positive significant correlation between the skin conductance and the odor arousal were found with the use of those odors. A negative significant correlation between heart rate and odor pleasantness was also found in that research. Moreover, a positive significant correlation between skin conductance and heart rate as well as a positive significant correlation between odor intensity and odor arousal self-report rating were found in that research article.

S. Haze et al., (2002) [44] also did the research about the effect of some odors on systolic blood pressure (SBP) and plasma catecholamine levels. Increasing in low-

frequency amplitude of SBP was found and that showed in increased sympathetic activity due to pepper oil, estragon oil, fennel oil and grapefruit oil stimulation. However, rose oil or patchouli oil decreased in both sympathetic activity and adrenaline level while pepper oil and grapefruit oil increased adrenaline level.

S. Dayawansa et al., (2003) [13] found the effect of Cedrol inhalation on systolic blood pressure, diastolic blood pressure (DBP), heart rate (HR), high-frequency component of heart rate variability and low to high-frequency ratio of HRV. Decreasing in systolic blood pressure and diastolic blood pressure were found during Cedrol inhalation. Decreasing in LF/HF ratio of SBP and HRV were also found during Cedrol inhalation. Moreover, the significant decrease in heart rate was also found during Cedrol inhalation. Respiration rate also significantly decreased during Cedrol inhalation.

X. Duan et al., (2007) [3] investigated the effect of Lavender inhalation in systolic blood pressure, diastolic blood pressure, mean blood pressure, heart rate, normalized low-frequency component and normalized high-frequency of HRV and low frequency to high frequency ratio of HRV parameters. Respiration rate and the Stress Response Scale (SRS-18) self-report rating were also investigated. No significant difference between control and lavender administration was found in systolic blood pressure, diastolic blood pressure, mean blood pressure and heart rate. Decreasing in normalized low frequency and increasing in normalized high frequency as well as decreasing in the LF/HF ratio were found after 10 min Lavender administration compared to control. Respiration was not changed and the SRS-18 scores, the self-report rating was significantly lower in lavender inhalation compared to control.

T. Matsumoto et al., (2013) [45] also investigated the effect of lavender stimulation on heart rate, the high-frequency component of HRV and the low-frequency to high-frequency ratio. In their investigations, decrease in heart rate after aroma stimulation was found and no statistically significant difference was detected in the LF/HF ratio between the two trials (lavender and control). In profile of mode state (POMS) test, scores of depression-dejections ($p=0.045$) and confusion ($p=0.049$) significantly decreased after the inhalation of lavender as compared to those of the control trial with water.

2.9. Autonomic Nervous System

Continuous electrical signal, ECG wave is starting from the Sino-atrial nodes, commonly called SA nodes. The rate of electrical impulses from SA nodes is controlled by the nerves especially an autonomic nervous system [39]. Generally, the brain stimulates the heart via the ANS. ANS can be divided into the sympathetic and parasympathetic branches. Sympathetic and parasympathetic nerves are started out from the central nervous system and connected to different organs inside a human body. Heart rate can be increased because of the sympathetic nervous system (SNS) activation. Parasympathetic nervous system (PSNS) activation can decrease the heart rate [2]. Stress is also one of the factors that affect the autonomic nervous system and heart rate variability. Therefore, it can also change the heart rate and heart rate variability. Generally, a muscle sympathetic nerve activity (MSNA) is lower in female than in male for the same age and that a muscle sympathetic nerve activity is increased with older age. If the age of subject is younger than 50, a muscle sympathetic nerve activity of male is higher than female at the same age [46]. However, there was no difference between male and female for older ages [46]. Two branches of an autonomic nervous system (sympathetic and parasympathetic) and internal human organs relations are shown in the following Figure 2.12.

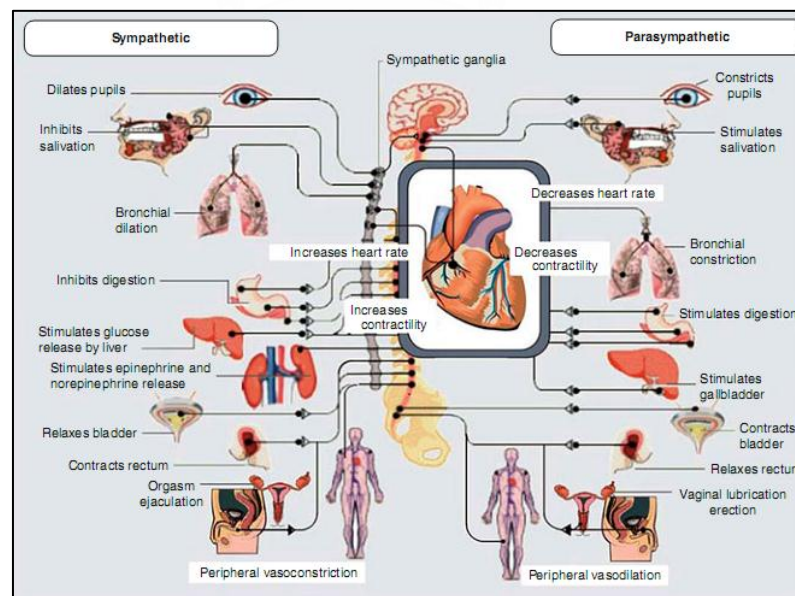


Figure 2.12: Autonomic nervous system linking with internal organs [47]

2.9.1. Sympathetic Nervous System

Sympathetic nervous system is one branch of the autonomic nervous system. Sympathetic activity can be increased by some external disturbance or mental changes. Generally, the brain stimulates the heart through the autonomic nervous system. During physical exercise, sympathetic branches of the autonomic nervous system can activate the heart to increase heart rate [2]. Activation of sympathetic division can initiate “fight” reaction.

Sympathetic nervous system is related to cardiovascular function including heart rate, heart rate variability, hypertension, myocardial infarction and chronic heart failure. When the sympathetic nervous system is activated, heart rate as well as blood pressure and some hormone excretion increase. Sympathetic nerves are started out from the area close to the middle of the spinal cord and it connects to some internal organs [47].

2.9.2. Parasympathetic Nervous System

Parasympathetic nervous system is a branch of the autonomic nervous system. Its action is reverse to the sympathetic nervous system. Generally, parasympathetic activation has opposite effects to the target organs. This is a way that our body balances the sympathetic activation. During sleeping, parasympathetic activity can decrease the heart rate [2] and it can also affect the heart rate variability. Parasympathetic regulation can change the psychophysical mode of the human body. A parasympathetic nerve is related to a vagus nerve. A vagus nerve is one of the 12 cranial nerves in the human body. An Origin of vagus nerve is found in medulla oblongata [47]. This nerve is responsible for the parasympathetic nerve activation. Vagus nerve stimulation is generally used for the treatment of depression [48].

2.10. Olfactory Bulb and Sense of Odor

Olfaction is one of the five senses (vision, hearing, olfaction, tasting, and touching). The human can recognize about 10,000 odors while dogs can recognize more than human [49]. Olfaction occurs when odorant molecules come in touch to the olfactory receptors in the olfactory bulb.

An olfactory bulb (OB) is the main organ in the processing for the sense of odor or olfaction. An olfactory bulb receives the odor information directly from the axons of the receptor cells and sends the output information to the olfactory cortex in the brain [50]. Odor information is generally odor molecule. Figure 2.13 shows some parts of the olfactory system.

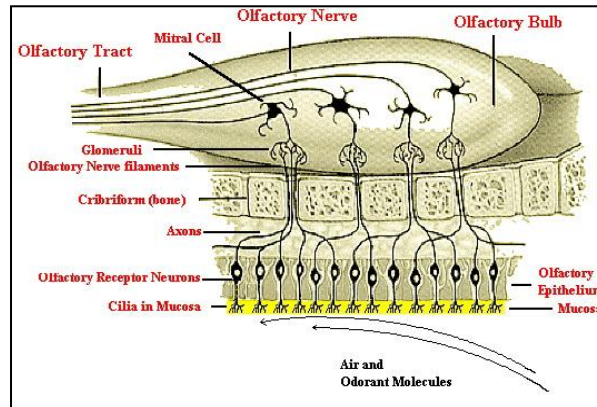


Figure 2.13: Parts of the olfactory system [51]

Olfactory receptor neurons are located in the upper part of the two nasal cavities and covered by a mucous layer [51]. Each olfactory cell has some specific types of the odorant receptor protein. Each receptor can detect the limited number of odorants. The receptor neurons transmit odor information directly to glomeruli in the olfactory bulb [49]. Figure 2.14 shows the olfactory system related to the parts of the brain.

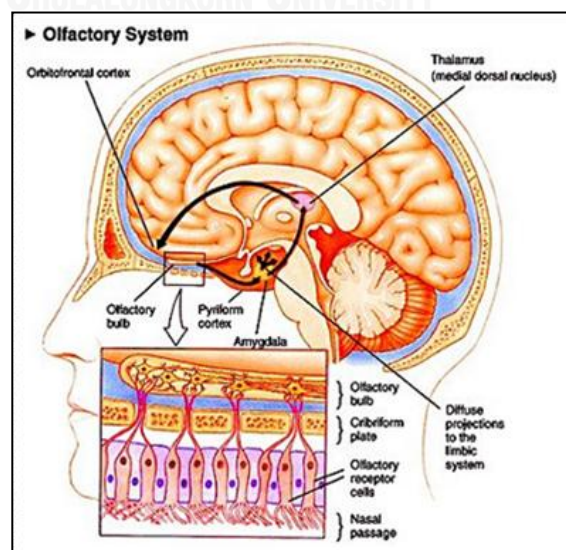


Figure 2.14: Olfactory system and part of the brain [52]

In mammals including human, olfaction can stimulate the primary olfactory cortex. The piriform cortex of the brain can receive the input signal directly from the olfactory bulb through the olfactory tract [52]. In the study of R. J. Zatorre et al. [53] with positron emission tomography (PET), odorant stimulation can activate the cerebral blood flow (CBF) corresponding to the piriform cortex, the insula cortex, and the right orbital frontal cortex. N. Sobel et al. [54] reported that odor molecules can also activate various parts of the limbic system, the ventral temporal region and some part of the posterior and orbitofrontal cortex.

2.10.1. Olfactory Nerve

Olfactory nerve, also simply called CN I or the first cranial nerve is one of the twelve cranial nerves located in the human forehead. Figure 2.15 shows the CN I olfactory nerve which is situated above the nasal cavity. Olfactory nerve senses the smell and transfers the smell data to the brain through the olfactory tract. Olfactory nerve has olfactory receptor neurons that transmit the smell information to the central nervous system. The original terminal of the olfactory nerves or olfactory receptor neuron is located on the olfactory mucous which is in the upper part of the nasal cavity. Very small smell molecules stimulate the olfactory receptor neurons. Stimulated information is transformed into an electrical impulse signal in the olfactory nerve and transferred the impulses to the olfactory bulb. Olfactory tract carried these impulse signals from the olfactory bulb to the central nervous. Cranial nervous are set out directly from the brain [55].

All the 12-cranial nervous are namely:

- (i) the olfactory nerve, CN I
- (ii) the optic nerve, CN II
- (iii) the oculomotor nerve, CN III
- (iv) the trochlear nerve, CN IV
- (v) the trigeminal nerve, CN V
- (vi) the abducens nerve, CN VI
- (vii) the facial nerve, CN VII
- (viii) the vestibulocochlear nerve, CN VIII
- (ix) the glossopharyngeal nerve, CN IX

- (x) the vagus nerve, CN X
- (xi) the accessory nerve, VN XI and
- (xii) the hypoglossal nerve, CN XII

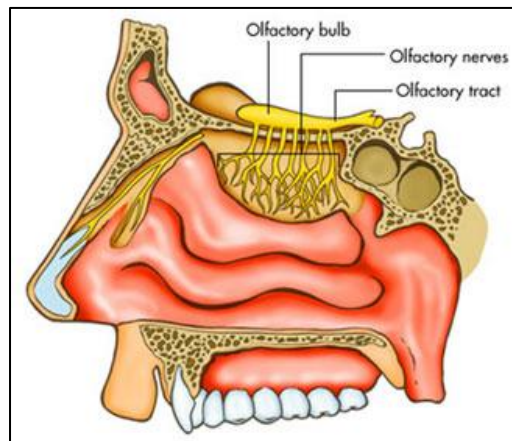


Figure 2.15: CN I olfactory nerve [55]

CHAPTER 3

EXPERIMENTAL SETUP AND METHODOLOGY

In this chapter, experimental setup and hardware test system were explained in detail. Participated subjects, the use of material and method were also presented. Detail explanation for inclusion criteria, exclusion criteria, and experimental procedure were also introduced in this chapter. A compact ECG data acquisition was constructed to collect the lead-II ECG data. Actual measurement of lead-II ECG data was used to evaluate the effect of jasmine odor stimulation because it is the standard lead for measuring heartbeat and arrhythmia classification [56]. Measurement parameters which were extracted from lead-II ECG were heart rate, a standard deviation of all normal to normal intervals, a root mean square of successive RR intervals difference, a normalized low-frequency, a normalized high-frequency and a ratio of a normalized low-frequency to a normalized high-frequency component from heart rate variability power spectral analysis.

3.1. ECG Data Acquisition System

A low-cost portable ECG data acquisition system was used to take the lead-II ECG signal in this research. An ECG data acquisition system was generally composed of two parts, an analog data acquisition unit and a digital data processing unit. The data acquisition unit was divided into three parts: (i) an input circuit, (ii) an amplifier and (iii) a band-pass filter. Disposable silver/silver chloride (Ag/AgCl) electrodes were used in the measurement. Typical ECG electrodes used in the system are shown in Figure 3.1.



Figure 3.1: ECG electrodes

Electrodes were attached to a right arm, a right leg, and a left leg for recording the lead-II ECG data. The lead-II is one of the most common and popular configurations for the QRS-complex detection[56]. A block diagram of the ECG data acquisition system is shown in Figure 3.2.

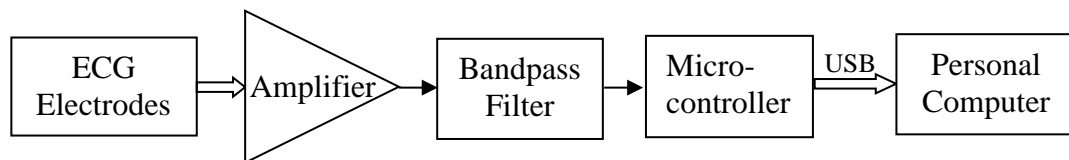


Figure 3.2: System block diagram

In this work, an INA128 was used to magnify a signal retrieving from the Ag/AgCl electrodes. This device is a compact user-friendly amplifier which is designed to be easily adaptable to various applications. As demonstrated in Figure 3.3, the internal circuit configuration makes the INA128 possess a high input impedance. As a consequence, the fluctuation in the input bias current is kept low, when the input voltage varies during measurement. Three operational amplifiers are connected to the well-matched resistors. In this manner, its common-mode rejection ratio (CMRR) is boosted up to 120 dB when the gain is above 100. Also, this device has high accuracy with a wide range of the power supply, from ± 2.25 to ± 18 V. In essence, the INA128 is perfectly matched to an ECG measurement in this study.

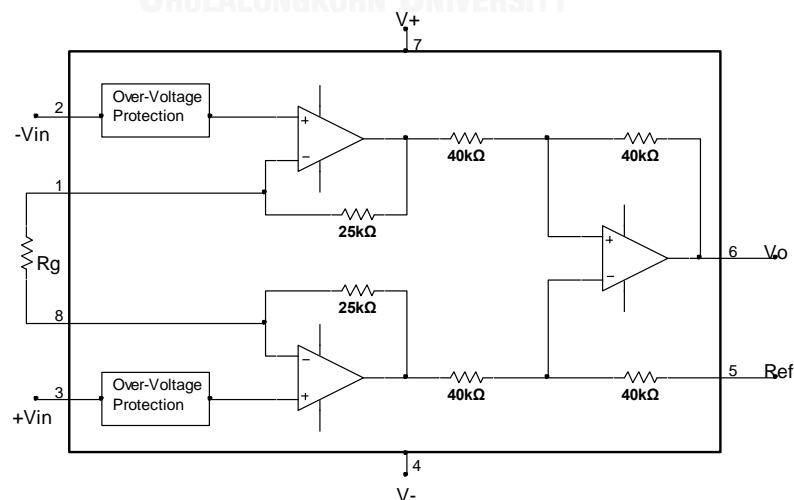


Figure 3.3: A typical instrumentation amplifier internal design [57]

The internal circuit of the INA128 can be divided into two parts, input and output circuits. The input circuit is comprised of non-inverting amplifiers that have a buffer characteristic. This can prevent a loading effect when measuring ECG signals. Meanwhile, the output circuit is a differential amplifier. As schematically shown in the figure, the resistance of the resistors in the differential amplifier is designed to make a unity gain. However, the gain for the input circuit is not dependent on only the internal resistors but also the external resistor R_G . The overall gain of the INA128 is calculated as shown in equation (3.1).

$$G = 1 + \frac{50k\Omega}{R_G} \quad (3.1)$$

where, G is an overall gain of the INA128.

Therefore, it is obvious that we can manage the gain of the circuit conveniently by selecting an appropriate resistor, R_G . The datasheet [57] suggests that the gain can be varied from 1 to 10,000. However, it is essential in practice that R_G should be below 100 k Ω for noise suppression on one hand, but above 100 Ω to avoid the operation at a high current. In this work, the INA128 was designed to have the overall gain less than 500.

3.1.1. Band-pass Filter

In this work, a band-pass filter was required to reject both low-frequency and high-frequency noises. A band-pass filter was implemented using a simple RC circuit as shown in Figure 3.4. Its cut-off frequencies and components are listed in Table 3.1.

Table 3.1: Resistances and capacitances in a band-pass filter

Filter Type	R	C	3dB cut-off frequency, $F_c = \frac{1}{2\pi RC}$
High-pass filter	300 k Ω	10 μ F	≈ 0.05 Hz
Low-pass filter	75 k Ω	33 nF	≈ 64 Hz

In practice, most of the noises in an ECG measuring system are in the high-frequency regime. The band-pass filter was designed asymmetrically in relation to the noises, and hence, composed of two main circuits, i.e. a first order high-pass filter and

a second order low-pass filter. For simplicity, two cascading the first order low-pass was employed to approximate the second order low-pass filter.

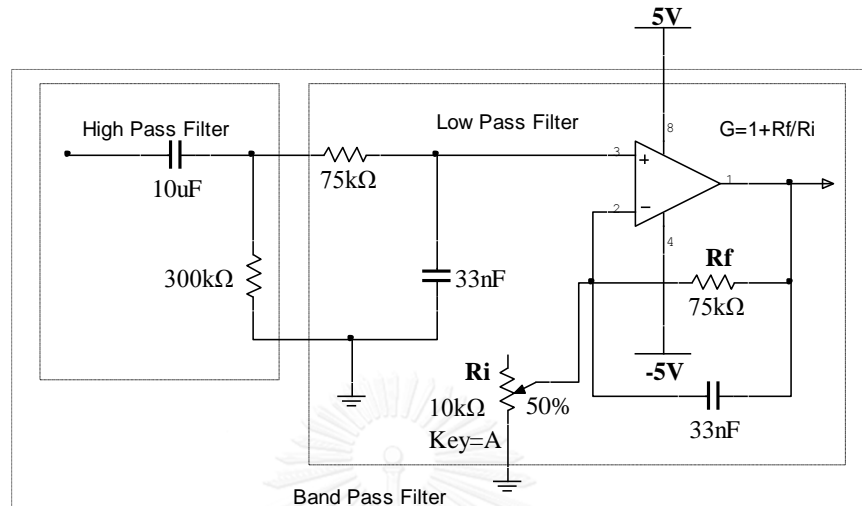


Figure 3.4: Band-pass filter with amplification

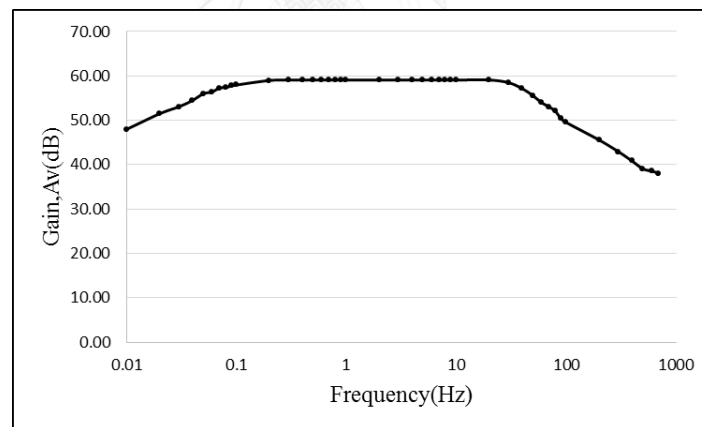


Figure 3.5: Band-pass filter frequency response

The filters were designed to have a cut-off frequency at 0.05 Hz for the low cut-off and 64 Hz for a high cut-off frequency. Nonetheless, the test results show that the actual (-3dB) cut-off frequency of the high-pass filter was 0.06 Hz, while the low-pass filter had the cut-off frequency at 50 Hz. With the limit on generating a very low-frequency sinusoidal signal in the experiment, the cut-off frequency of the high-pass filter might drift from the designed value. Meanwhile, the simplicity of the low-pass filter led to the repeated poles on the real axis. Unlike the Butterworth filter, the

frequency response of the designed low-pass filter with the repeated poles has a non-maximally flat magnitude response. This is a reason why the cut-off frequency of the low-pass filter moved down to 50 Hz, and the bandwidth of the band-pass filter was narrower than that expected from the calculation. The magnitude plot of the band-pass filter is shown in Figure 3.5. Despite the deviance of the cut-off frequency in the band-pass filter, the bandwidth of the filter was adequate for the noise rejection in the ECG measurement.

3.1.2. DC Offset Level Shifter

The output signal from the amplifier and filter was in the range of -5 to +5 V. However, analog-to-digital circuits in the microcontroller can convert only the signal with an amplitude between 0 and +5 V. Therefore, a DC offset level shifter is needed to shift an ECG signal to a certain level between 0 and +5V. Based on the configuration of an op-amp voltage adder, the shifter was realized using an op-amp and a variable or a potentiometer as shown in Figure 3.6. In this work, a TL082, a JFET operational amplifier, was used in the shifter circuit due to its high input impedance.

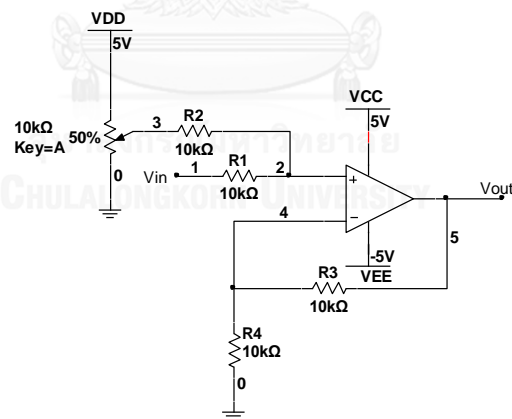


Figure 3.6: DC offset level shifter

3.1.3. Analog Data Acquisition Unit

In this work, an instrumentation amplifier, a band-pass filter, and a DC offset level shifter were integrated to implement an analog ECG data acquisition unit as shown in Figure 3.7. Since the circuits were connected to each other in a cascade configuration, the overall gain of the unit can be analyzed separately.

Based on the equation 3.1, the gain resistor R_G was 330Ω . Therefore, the gain value for this stage was approximately 150. The gain equation of the band-pass filter stage is shown in equation (3.2).

$$G = 1 + \frac{R_f}{R_i} \quad (3.2)$$

where, $R_f = 75 \text{ k}\Omega$. R_i was a variable resistor ($75 \text{ k}\Omega$). It was preset to be $15 \text{ k}\Omega$. Therefore, the gain value for the band-pass filter was 6, then the overall gain for the system was approximately 900. However, the overall gain can be controlled by adjusting the variable resistor (preset), R_i . The output from the system was connected to the analog input of the Arduino Nano microcontroller.

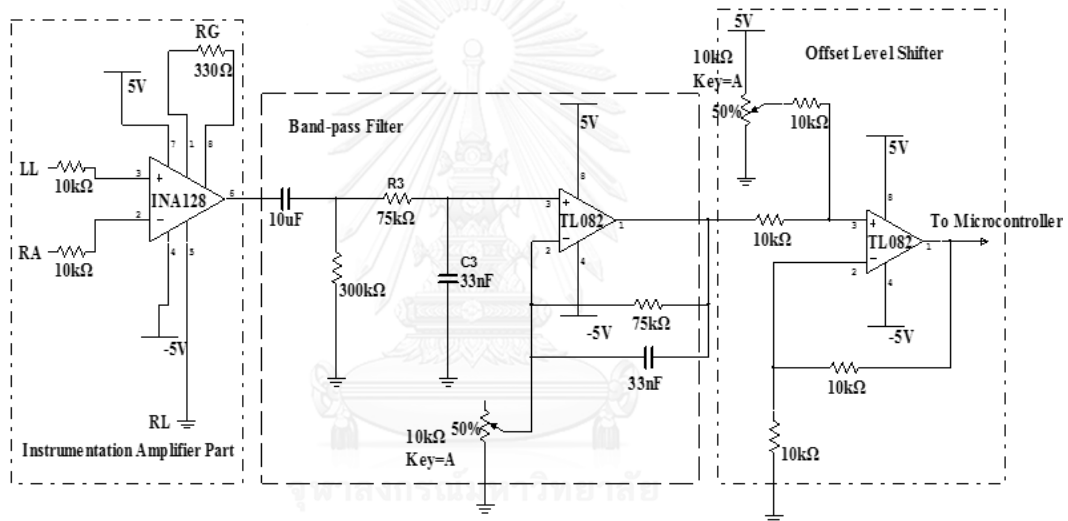


Figure 3.7: Overall circuit design

3.2. Digital Data Processing Unit

A digital data processing unit composed of a microcontroller and a PC. An Arduino Nano V3.0 board based on Atmega 328 microcontroller was used as a pre-processing unit. The Arduino Nano V3.0 pin diagram is shown in Figure 3.8 and a description for each pin number is listed in Table 3.2. The Atmega328 has 32 KB of a flash memory for storing a code. It also has 2 KB of SRAM and 1 KB of EEPROM. The Arduino Nano is the smallest design in Arduino board products and it uses surface mount component with integrated USB. The Arduino Nano has a USB power connection for +5V power supply. It has 14 digital input/output pins and 8 analog input

pins. DC current per I/O pin is 40 mA [58]. An Arduino programming language was used to program the microcontroller on the board.

Microcontroller digitized the analog ECG data with the sampling frequency of 180 Hz at 10-bit resolution. A respiration rate was also recorded by using a small thermistor which was put closely in front of a nose. A respiratory data was also digitized at the same sampling rate of 180 Hz at 10-bit resolution. ECG and respiratory data were stored in PC simultaneously via the USB interfacing with a baud rate of 38400 bps. Analog pin A0 and A1 were used to receive the ECG data and respiratory data, respectively.

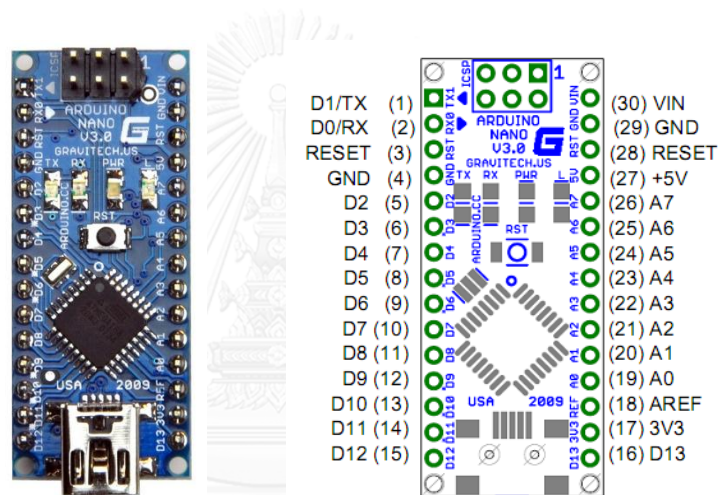


Figure 3.8: Arduino Nano V3.0 pin diagram [58]

Table 3.2: Arduino Nano V3.0 pin description

Pin No.	Name	Description
1-2, 5-16	D0-D13	Digital Input/Output port 0 to 13
3, 28	RESET	Reset (Active low)
4, 29	Gnd	Ground
17	3V3	+3.3V Output
18	AREF	ADC Reference
19-26	A0-A7	Analog Input Channel 0 to 7
27	+5V	+5V output from onboard regulator (or) +5V output from external power supply
30	VIN	Supply Voltage

3.3. Material and Subjects

Jasmine essence was chosen for the experiment because it is common and widely used as an anti-depressant, aphrodisiac and even as a medicine to sleep better in most of Southeast Asia and Asia countries. The participated subjects in our experiment were also from Southeast Asia and Asia countries. Jasmine can be found easily in many places around India, Thailand and Southeast Asia and Asia countries [19]. Jasmine oil is the natural essential oil which was extracted from jasmine flowers and it has a sweet floral aroma. The main chemical components included in jasmine are linalool and benzyl acetate [59]. In this thesis, jasmine essence from Siam perfumes (Bangkok, Thailand) was used. A preference concentration survey was done by asking the odor of each dilution in advance to find the optimal dilution. Isopropyl alcohol was used to dilute the original stock. According to the pretest, the most preferred concentration with the dilution ratio of 3:2 (6 ml of solvent and 4 ml of stock) was used for the laboratory experiment.

Total 15 males (average age = 26.3 ± 3.7 years and average weight = 67.2 ± 10.3 kg) and 15 females (average age = 30.1 ± 3.4 years and average weight = 58.8 ± 10.4 kg) joined in the experiment. Subjects were students at Chulalongkorn University, Thailand. Subjects were also asked about their general information before starting the experiment. General information was age, height, weight, and blood pressure on current condition, one-night sleeping hours before the experiment, what kind of food and drink that they had before the experiment, what medication that they took.

3.3.1. Inclusion Criteria

Inclusion criteria for this experiment were no exercise at least 2-hours before the experiment, no smoking, no drinking coffee and alcohol at least one day before the experiment, no fever, no chronic diseases, no cardiac problem. Subjects with normal blood pressure and no pregnancy female were selected. Inclusion criteria for normal blood pressure (BP) were systolic and diastolic BP in the range of 90-130 and 50-80 mmHg, respectively.

To evaluate the stress condition, 18 items of the stress response scale, SRS-18 were used. This modified stress response scale was consulted with Asst. Prof. Dr. Apitchaya Chaiwutikornwanich, Faculty of Psychology from Chulalongkorn

University. Detail explanation and items for SRS-18 are listed in Table 3.3. These items were modified from S. Suzuki et al. [60]. The subjects were asked to rate their condition by giving a mark from 1 (definitely not/no), 2 (slightly not/no), 3 (moderately), 4 (rather true/yes) to 5 (definitely true/yes). The subjects were asked to rate their stress condition just before the experiment and after odor exposure for 1-minute, 2-minute, and 3-minute at each protocol.

Table 3.3: Stress response scale -18

No.	Items	Definitely Not/No	Slightly Not/No	Moderately	Rather True/Yes	Definitely True/Yes
eg,	Tired easily	1	2	3	4	5
1	Get angry easily	1	2	3	4	5
2	Feeling sad	1	2	3	4	5
3	Worry about something	1	2	3	4	5
4	Feeling angry	1	2	3	4	5
5	Crying feeling	1	2	3	4	5
6	Cannot control feeling	1	2	3	4	5
7	Mortifying	1	2	3	4	5
8	Unhappy	1	2	3	4	5
9	Feeling down	1	2	3	4	5
10	Irritate nervous	1	2	3	4	5
11	Cannot believe in many things	1	2	3	4	5
12	Don't like everything	1	2	3	4	5
13	Thinking bad things	1	2	3	4	5
14	Cannot conclude on talking	1	2	3	4	5
15	Need someone made me comfort	1	2	3	4	5
16	No patience	1	2	3	4	5
17	Not to be alone	1	2	3	4	5
18	Cannot concentrate on anything	1	2	3	4	5

3.3.2. Experimental Condition

An experiment laboratory room was a suitable silent room (length=11ft, width=8ft, height=8ft) with not too bright light. Experiment room temperature was also kept at about 24°C. The experiment room was also ventilated by using a fan to avoid odorant molecule accumulation in advance. Subjects were also instructed to wear comfortable clothing because the clothing pressure can also affect HRV [21].

Subjects were instructed to sit comfortably on a chair and to take a rest in the experiment room for about 10-minute before the experiment. Subjects were also told not to move and not to speak during the experiment in order to reduce the motion artifacts and to get the good accuracy data. Subjects were instructed to look at the jasmine-picture in front of them during the experiment for their attention. Subjects were also instructed to take a breath normally during the measurement as well as during breathing the odor and the air.

3.4. Experimental Procedure

Three experimental data sets (resting, control and odor data set) were carried out in sequence. Two sequences of experiments were performed. Control-odor and odor-control sequence were the measurements of control followed by odor data set, and odor data set followed by control data set, respectively. After the subjects had been rested for 10-minute, the measurement was taken for 3-minute. This data set was called resting data set. Before measuring for resting data set, subjects were asked to rate their stress response using SRS-18. A 9-minute measurement of control data set was done for three trials with a 2-minute break between the trials. The 2-minute break was typically applied in the study of autonomic parameters using the inhalation of pleasant and unpleasant odors [43].

Subjects were breathing blank room air during the control sets (1-minute, 2-minute, and 3-minute). An experimental procedure was summarized as a flow chart shown in Figure 3.9.

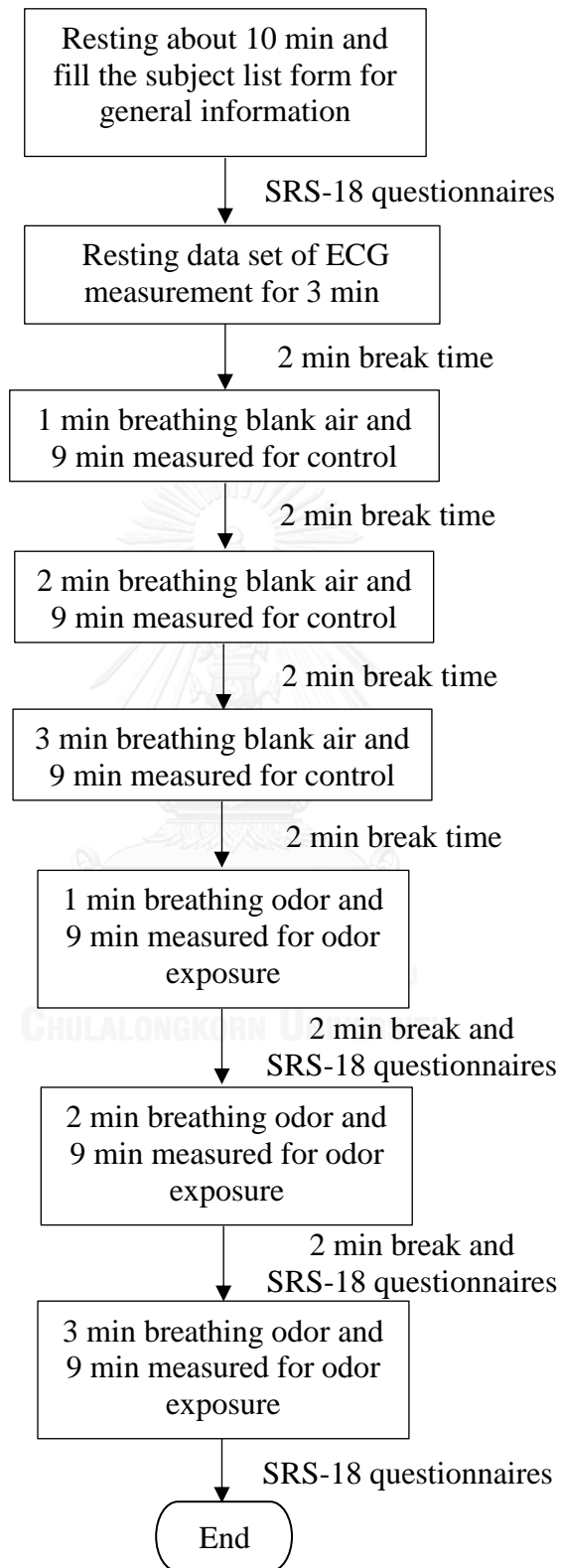


Figure 3.9: Experimental procedure flow chart

Three trials of odor data sets were recorded after exposing the jasmine odor. For the first trial, the subjects were exposed to the odor for 1-minute before data recording for 9-minute. The subjects were asked again after data recording to rate their psychological stress response using SRS-18. For the second and third trial, the similar procedure as the first trial was repeated except for the subjects were exposed to the odor for 2-minute and 3-minute, respectively. The subjects were asked to evaluate the SRS-18 after finishing each trial for 9-minute. The recommended minimum measurement time for short-term heart rate variability analysis from Task Force of the European Society of Cardiology was approximately 2-minute. Therefore, each data set in our experiment was chosen for 3-minute interval. All the 9-minute data of each trial were separated into three-time interval (I1, I2, and I3) for further processing.

3.5. Data Analysis

Time domain and frequency domain analysis were done for HRV analysis. Recorded ECG data were used for further data analysis. Firstly, the 50 Hz noise was filtered using 50 Hz notch digital filter, R-peaks were detected from the ECG data and consecutive RR time intervals were measured to plot the RR interval tachogram curve with the use of Octave 4.2.0 software programming. A typical Lead-II ECG and 50 Hz filtered ECG signal were shown in Figure 3.10 and Figure 3.11, respectively.

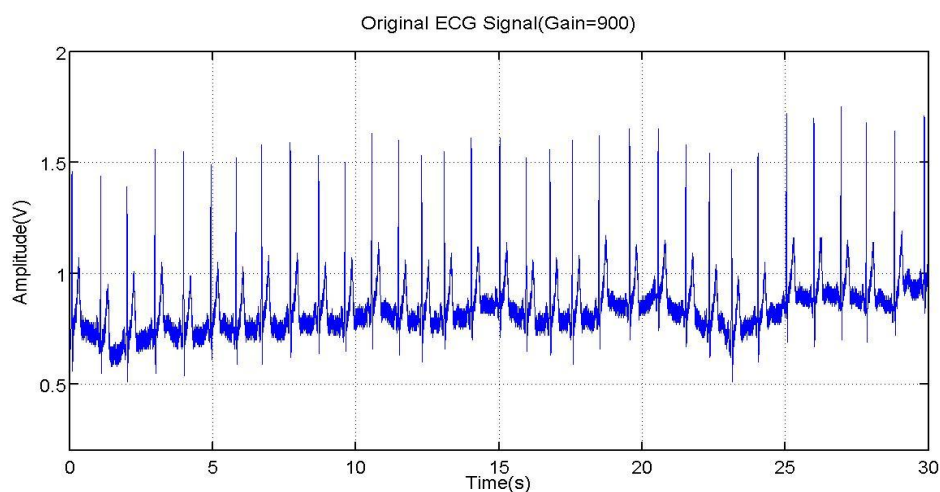


Figure 3.10: A typical lead-II ECG (Gain = 900)

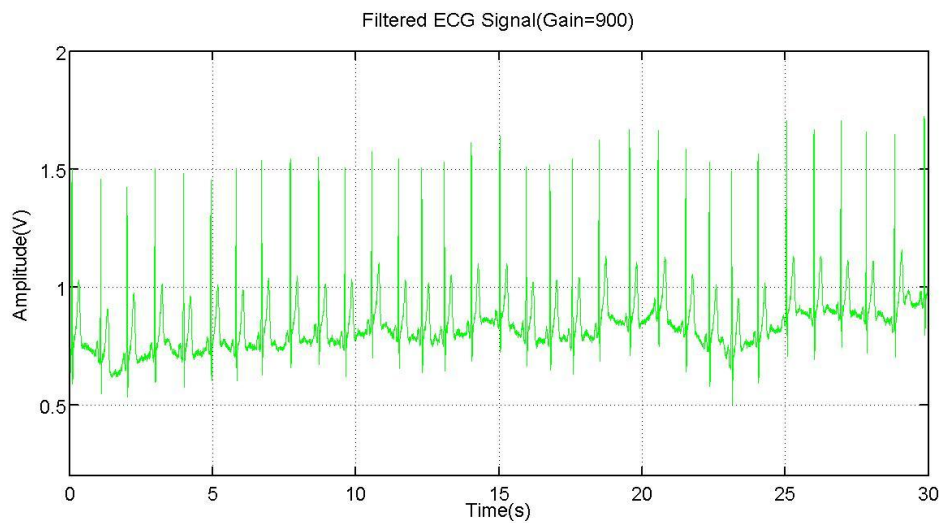


Figure 3.11: A typical 50 Hz filtered ECG signal (Gain = 900)

3.5.1. Time Domain Analysis

Time domain parameters are heart rate in beat per minute (bpm), a standard deviation of normal to normal intervals and a root mean square of successive RR interval difference.

The 50 Hz filtered ECG signal was then processed to find the R-peak position. A typical R-peak detection is shown in the following Figure 3.12.

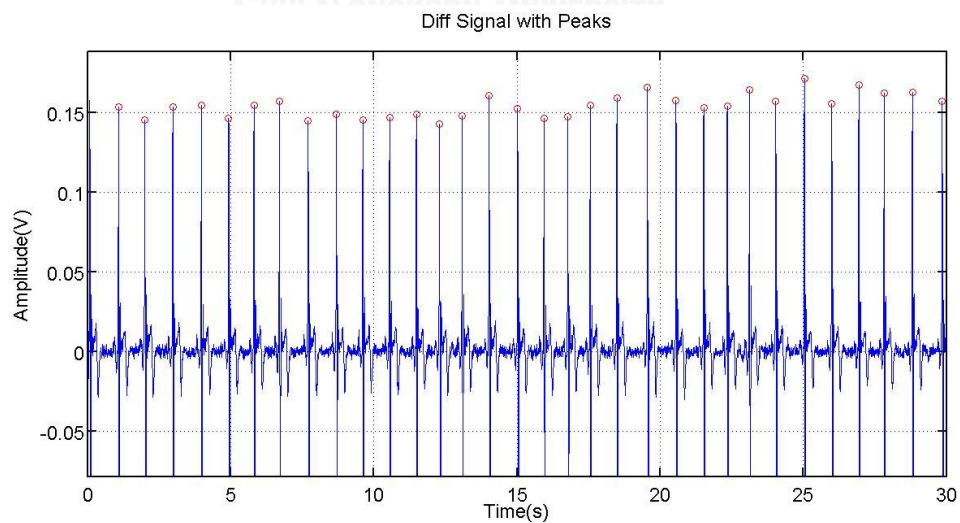


Figure 3.12: A typical R-peak detection

The number of R-peaks (N) within the measurement time duration was counted and heart rate was calculated by using equation (3.3).

$$\text{Heart Rate (bpm)} = \frac{N}{T} \quad (3.3)$$

where, N = total number of R-peak in the measurement time

T = measurement time (minute)

Consecutive RR-interval values were also calculated for every RR interval by counting the number of samples between the consecutive R-peaks. The number of samples between the consecutive R-peaks was divided by the sampling frequency, F_s (sample per second) to get the RR time interval in second. Equation (3.4) was used to compute the consecutive RR interval in second.

$$\text{RR time interval (sec)} = \frac{n}{F_s} \quad (3.4)$$

where, n = number of samples between the consecutive R-peaks

F_s = sampling frequency (sample per second)

A typical consecutive RR interval variation tachogram was plotted as shown in Figure 3.13. After getting all the RR interval values, a standard deviation of normal to normal intervals in a millisecond and a root mean square of successive normal to normal RR interval difference in a millisecond were computed by using equation (3.5) and (3.6).

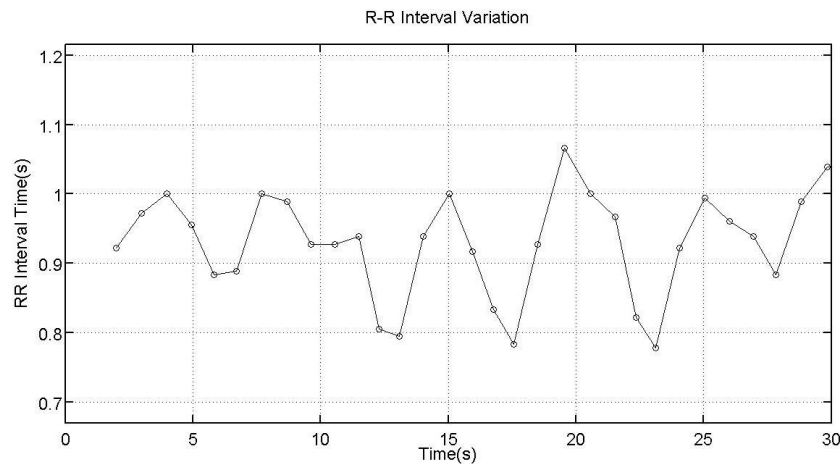


Figure 3.13: A typical RR tachogram

$$SDNN = \sqrt{\frac{1}{N-1} \sum_{i=2}^N [RR(i) - \overline{RR}]^2} \quad (3.5)$$

where, N = total number of the heartbeat within the whole measurement time

RR(i) = RR interval value at each heat beat, i

\overline{RR} = mean of all RR intervals

$$RMSSD = \sqrt{\frac{1}{N-2} \sum_{i=3}^N [RR(i) - RR(i-1)]^2} \quad (3.6)$$

where, N = total number of R-peak in the measurement time

RR(i) = RR interval value at i

RR(i-1) = previous RR interval value at i-1

An instantaneous heart rate variability was calculated from RR time interval tachogram by taking reciprocal of all RR intervals. An instantaneous heart rate variability was plotted as shown in the following Figure 3.14. A plotted IHRV tachogram was not adequately time-sampled. Therefore, the tachogram was interpolated by using a cubic spline interpolation with 4 Hz resampling rate to obtain the equal distance time series. An interpolated IHRV is shown in the Figure 3.15.

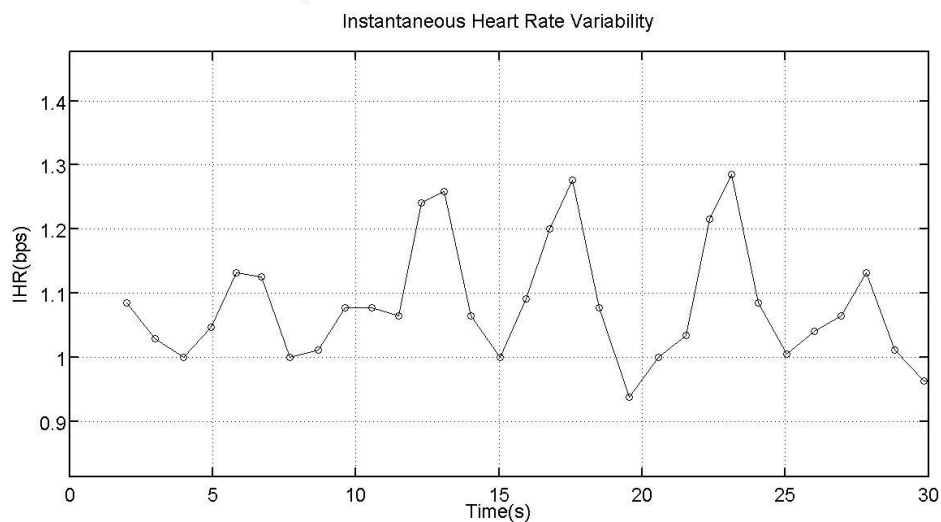


Figure 3.14: An instantaneous heart rate variability (IHRV)

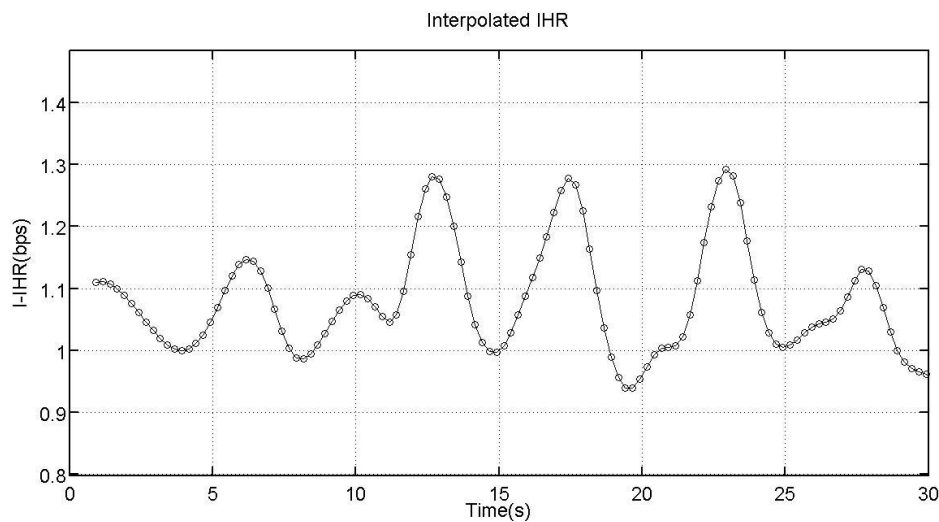


Figure 3.15: An interpolated IHRV

3.5.2. Frequency Domain Analysis

Fast Fourier Transform was used for the frequency domain analysis to find the power spectral density from the interpolated IHRV. Frequency domain analysis parameters are a low-frequency component, a high-frequency component and a ratio of low-frequency to high-frequency components. According to the standard Task Force of the European Society [22], the standard low-frequency range for heart rate variability analysis was from 0.04 to 0.15 Hz and high-frequency range was from 0.15 to 0.4 Hz. The low-frequency and high-frequency component were computed by summing the instantaneous heart rate variability power spectrum from 0.04 to 0.15 Hz and from 0.15 to 0.4 Hz, respectively. To evaluate the relative combination of LF and HF powers and to reduce the effect of total power changes, low-frequency power and high-frequency power were normalized with the following equation (3.7) and (3.8). Heart rate variability power spectrum is shown in Figure 3.16.

$$nLF = LF*100/(LF+HF) \quad (3.7)$$

$$nHF = HF*100/(LF+HF) \quad (3.8)$$

where, LF = power in the low frequency component (0.04 to 0.15 Hz) from HRV power spectrum

HF = power in the high frequency component (0.15 to 0.4 Hz) from HRV power spectrum

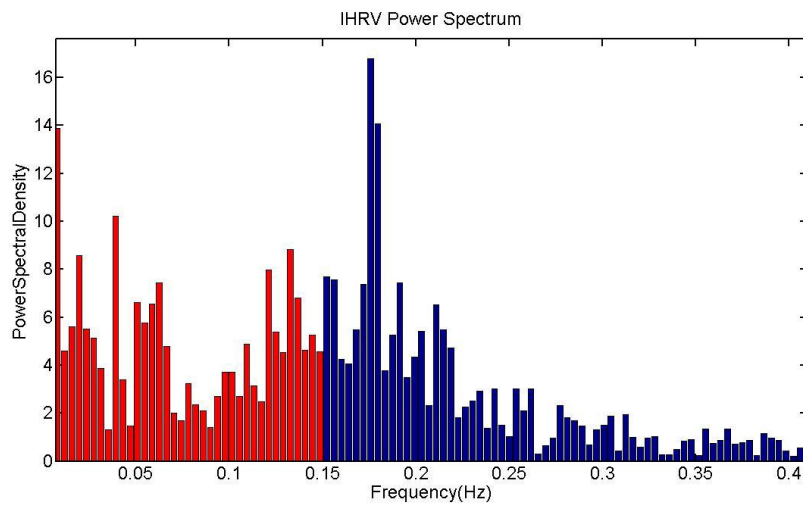


Figure 3.16: HRV power spectrum

In conclusion, the data processing was performed as shown in Figure 3.17.

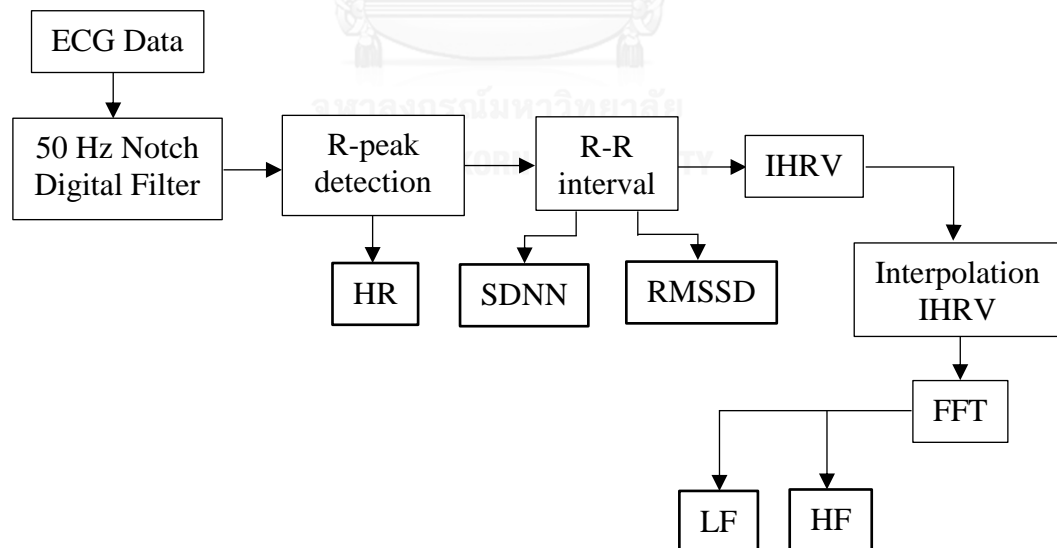


Figure 3.17: Data processing steps

CHAPTER 4

RESULTS AND DISCUSSION

In this chapter, the Lead-II ECG data were shown and discussed in detail to show the effect of the odor stimulation. A heart rate, a standard deviation of normal-to-normal intervals, and a root mean square of successive RR-interval differences were extracted from the Lead-II ECG data. The low-frequency and high-frequency components were investigated from a HRV power spectral analysis, and the ratio of the normalized low-frequency and high-frequency components was calculated as noted in chapter 3. The effect of jasmine odor inhalation on a psychological response was also discussed based on the analysis of the questionnaire with the stress response scale. The background information of the subjects was categorized into two groups, i.e. a male group and a female group, and described in detail as in Table 4.1 and 4.2, respectively.

Table 4.1: Background information of the male participants in the experiment

No.	Age (years)	Height (cm)	Weight (kg)	Blood pressure (mmHg) (Sys./Dia.)	Sleep hour (one night before exp.)
1.	20	175	72	114/71	8 hrs 30-minute
2.	26	173	60	126/70	6 hrs 30-minute
3.	24	174	62	128/67	7 hrs
4.	27	168	50	105/69	8 hrs
5.	25	175	66	124/70	8 hrs
6.	25	173	70	110/71	6 hrs
7.	31	173	70	110/71	6 hrs
8.	26	165	70	131/71	7 hrs
9.	26	172	94	120/69	6 hrs
10.	31	175	74	121/70	9 hrs
11.	24	164	58	120/73	6 hrs
12.	26	165	61	116/75	8 hrs
13.	35	162	62	112/78	8 hrs
14.	22	177	61	113/63	7 hrs
15.	26	175	78	114/67	6 hrs

Table 4.2: Background information of the female participants in the experiment

No.	Age (years)	Height (cm)	Weight (kg)	Blood pressure (mmHg) (Sys./Dia.)	Sleep hour (one night before exp.)	Menstruation finished state
1.	25	165	57	103/69	7 hrs	3 weeks ago
2.	29	159	46	107/77	6 hrs 30-minute	3 weeks ago
3.	33	160	56	95/52	8 hrs	1 week ago
4.	29	158	67	110/62	7 hrs	3 weeks ago
5.	27	155	53	108/72	7 hrs	2 weeks ago
6.	31	160	73	110/75	7 hrs	2 weeks ago
7.	32	160	57	114/72	7 hrs 30-minute	1 day ago
8.	30	168	70	104/69	6 hrs	1 week ago
9.	33	164	58	117/72	6 hrs 30-minute	2 weeks ago
10.	27	153	43	106/73	7 hrs	3 weeks ago
11.	38	150	61	115/73	5 hrs	2 days ago
12.	29	158	64	112/62	7 hrs	3 weeks ago
13.	29	158	44	106/64	6 hrs	3 weeks ago
14.	26	168	78	114/63	7 hrs	2 weeks ago
15.	34	150	53	110/68	6 hrs 30-minute	2 weeks ago

4.1. Heart-Rate Analysis

X. Duan et al. [3] found the decrease in heart rate during the lavender aroma stimulation. S. Dayawansa et al. [13] also confirmed that the heart rate decreased when the subject inhaled some natural Cedrol. However, some crucial background information about the exposed and control groups was not reported in the study. Still, the real influence of odor stimulation is ambiguous and in need of some extensive investigation.

The data from the experiment were divided into two categories, i.e. male and female groups. Meanwhile, the resting, control, and stimulation (odor) stages were followed in all experiments. During the resting period, an average heart rate was calculated from a 3-minute measurement. On the other hand, a 9-minute measurement was undertaken during the control period for three times (the first time was after 1-minute air exposure, the second time was after 2-minute air exposure and the third time was after 3-minute air exposure). In the same manner, a 9-minute measurement was also taken during the stimulation period for three times (the first time was after 1-minute odor exposure, the second time was after 2-minute odor exposure, and the third time

was after 3-minute odor exposure). The 9-minute experiment was basically divided into three 3-minute intervals, i.e. interval-1 (I1), interval-2 (I2), and interval-3 (I3). The effect of the odor was investigated using the data from each 3-minute interval. The measurement time for the experiment and separated time intervals were shown in Figure 4.1.

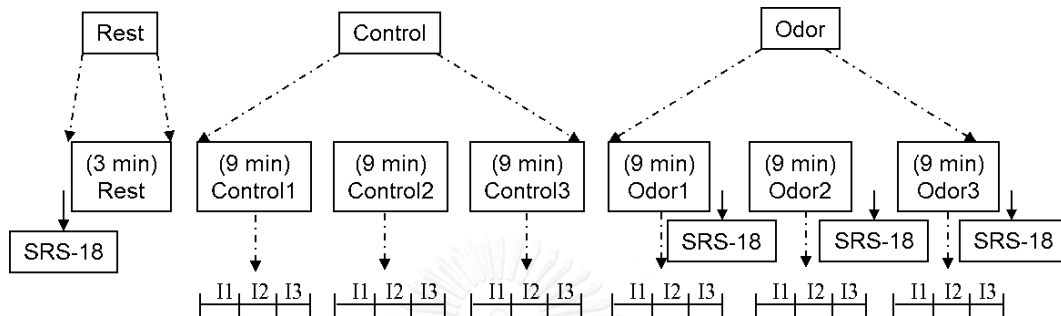


Figure 4.1: Separated measurement time intervals

In comparison between the groups, the average heart rate was plotted in the sequence of the stages in the experiment, i.e. resting, control and stimulation (odor) stages, as shown in Figure 4.2. Despite some fluctuation during the measurement, it is obvious that the decrease of the average heart rate reflected a consistent trend in both subject groups. The heart rate of the male subjects was found higher than that of the female subjects.

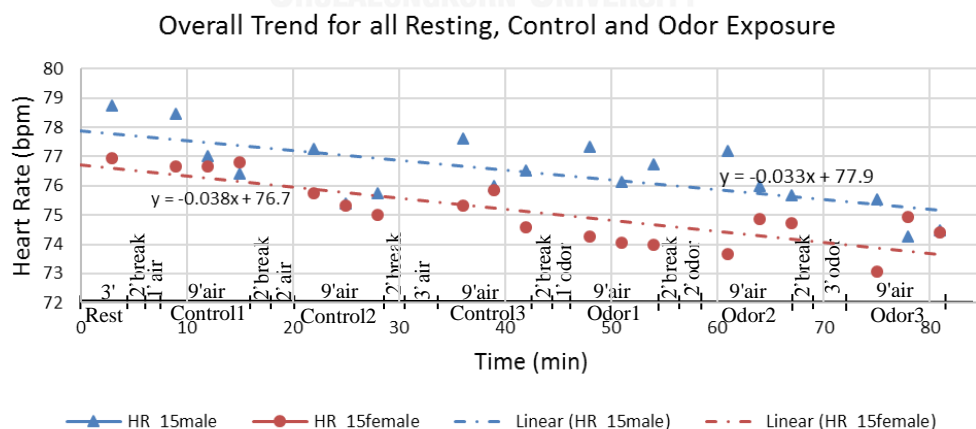


Figure 4.2: Heart rate for control-odor sequence

The average of the heart rate before the odor exposure (resting) and during the interval-1 in the control period was summarized in Table 4.3. Table 4.4 and 4.5 showed the data from the measurement during the interval-2 and interval-3 in the control period.

Table 4.3: Heart rate of interval-1 for each controlled trial and before odor exposure

Gender	Before Odor Exposure (B)	Interval-1					
		After 1-minute air Exposure (C1)		After 2-minute air Exposure (C2)		After 3-minute air Exposure (C3)	
	Mean	Mean	p-value (B-C1)	Mean	p-value (B-C2)	Mean	p-value (B-C3)
Male	78.7 ±10.3	78.5 ±11.0	p > 0.05	77.3 ±11.3	p > 0.05	77.6 ±10.0	p > 0.05
Female	76.9 ±6.4	76.7 ±5.3	p > 0.05	75.7 ±5.9	p > 0.05	75.3 ±6.1	p > 0.05

Table 4.4: Heart rate of interval-2 for each controlled trial and before odor exposure

Gender	Before Odor Exposure (B)	Interval-2					
		After 1-minute air Exposure (C1)		After 2-minute air Exposure (C2)		After 3-minute air Exposure (C3)	
	Mean	Mean	p-value (B-C1)	Mean	p-value (B-C2)	Mean	p-value (B-C3)
Male	78.7 ±10.3	78.7 ±11.7	p > 0.05	77.5 ±12.0	p < 0.05*	77.5 ±10.2	p > 0.05
Female	76.9 ±6.4	76.7 ±5.2	p > 0.05	75.3 ±5.3	p > 0.05	75.9 ±5.4	p > 0.05

*Significant difference, paired t-test: p-value < 0.05

Table 4.5: Heart rate of interval-3 for each controlled trial and before odor exposure

Gender	Before Odor Exposure (B)	Interval-3					
		After 1-minute air Exposure (C1)		After 2-minute air Exposure (C2)		After 3-minute air Exposure (C3)	
	Mean	Mean	p-value (B-C1)	Mean	p-value (B-C2)	Mean	p-value (B-C3)
Male	78.7 ±10.3	78.1 ±11.5	p > 0.05	77.5 ±12.1	p > 0.05	78.1 ±10.4	p > 0.05
Female	76.9 ±6.4	76.8 ±5.1	p > 0.05	75.0 ±5.5	p > 0.05	74.6 ±5.6	p < 0.05*

*Significant difference, paired t-test: p-value < 0.05

The comparison between the mean heart rate before the odor exposure and that during the exposure was also done. The statistical data of the interval-1, interval-2 and interval-3 are concluded in Table 4.6, 4.7, and 4.8, respectively.

Table 4.6: Heart rate of interval-1 for each odor trial and before odor exposure

Gender	Before Odor Exposure (B)	Interval-1					
		After 1-minute Odor Exposure (O1)		After 2-minute Odor Exposure (O2)		After 3-minute Odor Exposure (O3)	
		Mean	Mean	p-value (B-O1)	Mean	p-value (B-O2)	Mean
Male	78.7 ±10.3	77.3 ±10.1	p > 0.05	77.2 ±9.6	p > 0.05	75.5 ±11.2	p < 0.05*
Female	76.9 ±6.4	74.3 ±6.4	p < 0.05*	73.7 ±5.6	p < 0.05*	73.1 ±5.4	p < 0.05*

*Significant difference, paired t-test: p-value < 0.05

Table 4.7: Heart rate of interval-2 for each odor trial and before odor exposure

Gender	Before Odor Exposure (B)	Interval-2					
		After 1-minute Odor Exposure (O1)		After 2-minute Odor Exposure (O2)		After 3-minute Odor Exposure (O3)	
		Mean	Mean	p-value (B-O1)	Mean	p-value (B-O2)	Mean
Male	78.7 ±10.3	77.9 ±10.8	p > 0.05	76.9 ±10.4	p > 0.05	75.5 ±11.1	p < 0.05*
Female	76.9 ±6.4	74.1 ±5.7	p < 0.05*	74.9 ±5.5	p > 0.05	74.9 ±5.2	p > 0.05

*Significant difference, paired t-test: p-value < 0.05

Table 4.8: Heart rate of interval-3 for each odor trial and before odor exposure

Gender	Before Odor Exposure (B)	Interval-3					
		After 1-minute Odor Exposure (O1)		After 2-minute Odor Exposure (O2)		After 3-minute Odor Exposure (O3)	
		Mean	Mean	p-value (B-O1)	Mean	p-value (B-O2)	Mean
Male	78.7 ±10.3	78.1 ±10.5	p > 0.05	76.7 ±10.5	p > 0.05	75.8 ±11.3	p < 0.05*
Female	76.9 ±6.4	74.0 ±5.5	p < 0.05*	74.7 ±5.7	p > 0.05	74.4 ±6.1	p > 0.05

*Significant difference, paired t-test: p-value < 0.05

4.1.1. Heart Rate for “Control-Odor” Sequence

It can deny that a meditation can release not only the stress but also reduce a heart rate. During the meditation, people stop activities and focus their mind. This leads to the decrease of the heart rate. During the experiment, a subject did not move in a

pleasant environment. In this point of view, the experiment was similar to a meditation. Some interference in the effect of the odor stimulation might come from an idle state of subjects. As a consequence, the overall trend in Figure 4.2 might not show the actual effect of the odor stimulation on a heart rate.

To investigate an influence of the jasmine scent, the data were separated into two groups, i.e. a data during the control period and a data during the stimulation period. Individually, the graphs of the heart rate during the control and stimulation (odor) periods were plotted as shown in Figure 4.3 and 4.4, respectively.

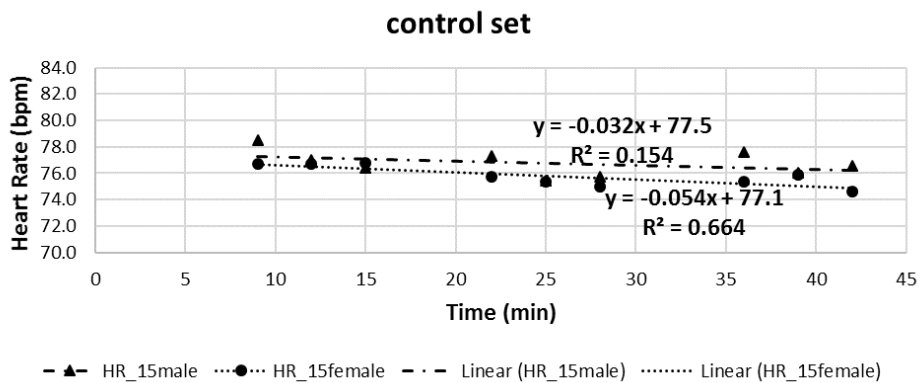


Figure 4.3: Heart rate for control set of control-odor sequence

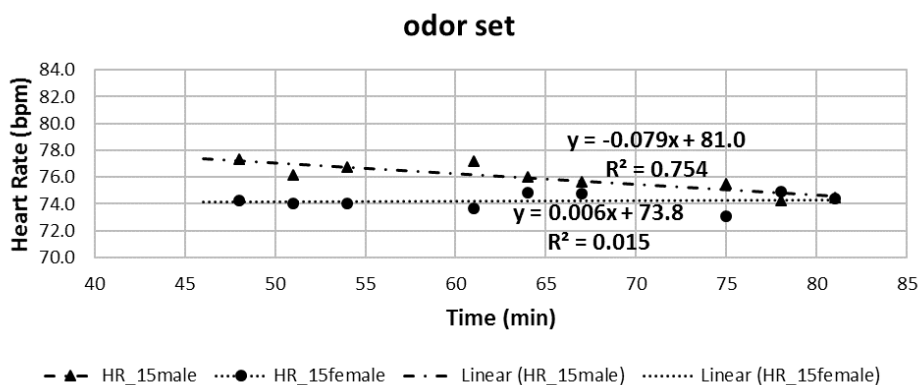


Figure 4.4: Heart rate for odor set of control-odor sequence

This section was devoted to study the effect of the odor stimulation in case that the experiment was undertaken in the sequence of the resting, control and stimulation stages. Clearly, the overall trend in Figure 4.2 could not show an apparent effect of the odor stimulation on the subject but revealed that the heart rate of male subjects was

higher than that of female subjects throughout the experiment. The experimental results agreed with the data reported by T. Matsukawa et al. [46]. This was due to the fact that muscles in males physiologically had more sympathetic-nerve activities than those in females.

As mentioned above, this may be because the heart rate was dominated by the idle state of a subject. Nonetheless, the heart rate of the male subjects decreased at the rate of 0.032 bpm/min during the control period, as shown in Figure 4.3. On the other hand, the decrease rate of the heart rate in the male subject grew to 0.079 bpm/min during the odor stimulation, as shown in Figure 4.4. Under the condition that both control and stimulation periods were the same in the experiment, the effect of the meditation-like factor was suppressed, but the effect of the odor stimulation was magnified. The double decrease of the heart rate reflected the effect of the jasmine scent on the male subject.

In the experiment, the female subjects were exposed to the jasmine scent in the same fashion as the male subjects. The heart rate of the female subjects decreased at the rate of 0.054 bpm/min during the control period. However, the heart rate was approximately constant but swung about 74 bpm. The results might reveal that female subjects were sensitive to physical activities. As noted in chapter 2, the heart rate decreases to indicate the relaxing state of the human body. Their heart rate might reach the steady state after the 45-minute control period. It was obvious that the female subject became insensitive to the jasmine scent after the long period in the control step since their heart accordingly beat at the minimum rate. In this case, the odor did not have a visible effect on the female subjects. However, the jasmine stimulation was likely to maintain the heartbeat at the constant rate than that of the male subject, as the standard deviation of the heart rate in the female was approximately two times less than that of the rate in the male subjects.

4.1.2. Heart Rate Changes for Individual Subjects (control-odor sequence)

Heart rate changes for the control and odor data set of the “control-odor” experiment sequence of the individual 15 male subjects and 15 female subjects are shown in Figure 4.5 and Figure 4.6, respectively. For males, 8 of 15 subjects showed

the decreasing of heart rate in the control data set while 10 of 15 subjects showed the decreasing of heart rate in the odor data set.

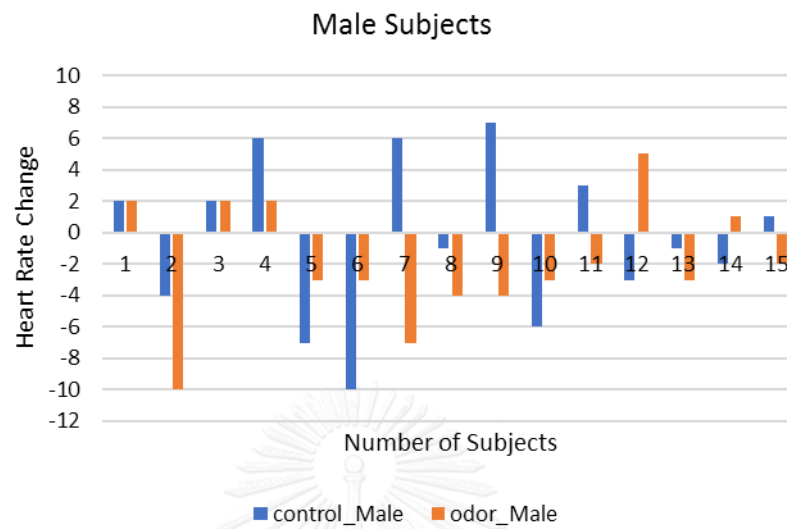


Figure 4.5: Heart rate changes of males for control-odor sequence

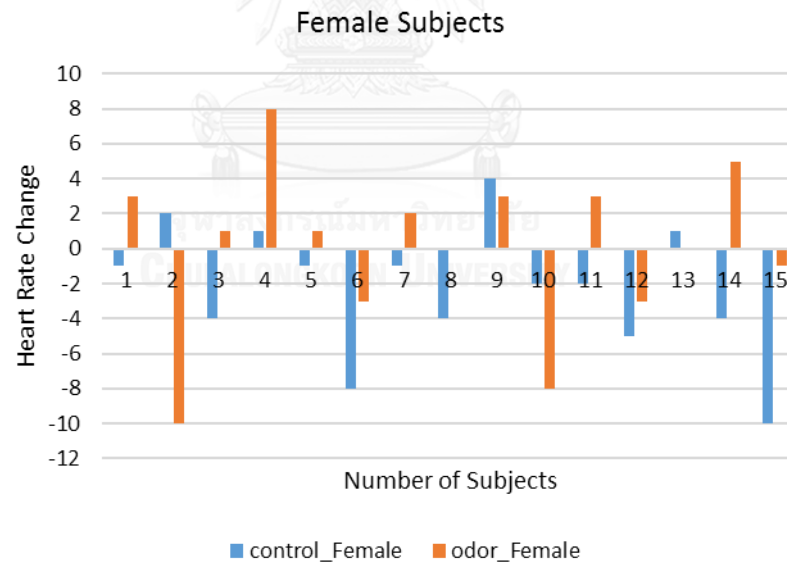


Figure 4.6: Heart rate changes of females for control-odor sequence

Heart rate changes were calculated from the difference between the heart rate of the interval-3 (I3) of the third trial and the first interval-1 (I1) of the first trial. Heart rate changes for odor condition were the difference between the heart rate of the last interval-3 (I3) of third trial and the first interval-1 (I1) of the first trial odor data set.

For control data set: HR changes = $HR_{I3_C3} - HR_{I1_C1}$

where,

HR_{I3_C3} = Heart rate value at the interval_3 of control trial_3

HR_{I1_C1} = Heart rate values at the interval_1 of contrail trial_1

For odor data set: HR changes = $HR_{I3_O3} - HR_{I1_O1}$

where,

HR_{I3_O3} = Heart rate value at the interval_3 of odor exposure trial_3

HR_{I1_O1} = Heart rate value at the interval_1 of odor exposure trial_1

4.1.3. Heart Rate of “Odor-Control” Sequence

It was clear that the odor stimulation showed a great potential for exerting some measurable effects on the heart rate. Nonetheless, the experiment in the sequence of resting, control and stimulation steps could not ensure that the jasmine scent had some effects on decreasing the heart rate because of the inconsistency of the results in the male and female subjects. To suppress the “meditation-like” effect on the heart rate during the prior control step, the sequence in the experiment was changed to resting, stimulation (odor), control steps.

The experimental result showed that the heart rate of the male subjects was higher than that of the female subjects. The overall graph in Figure 4.7 was likely to have a constant decline throughout the experiment. In agreement to the prior experiment, this revealed that the “meditation-like” effect may be dominant in the long-period experiment.

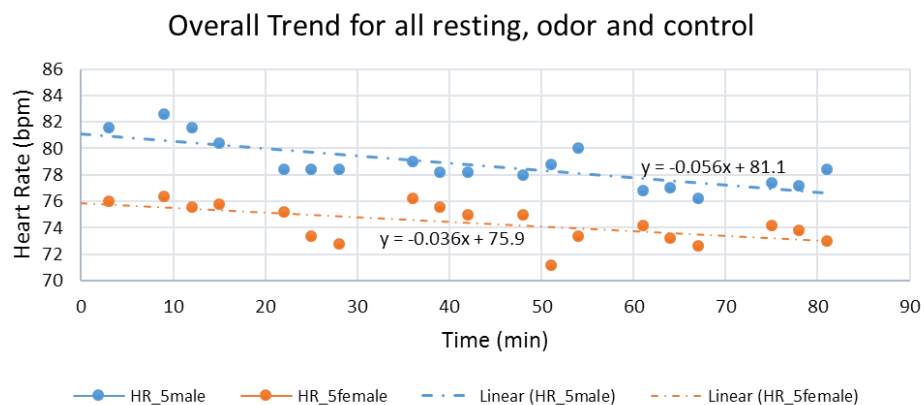
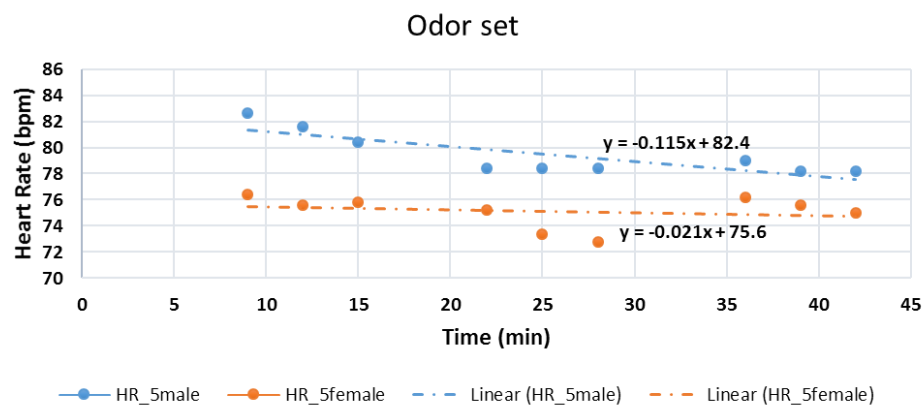
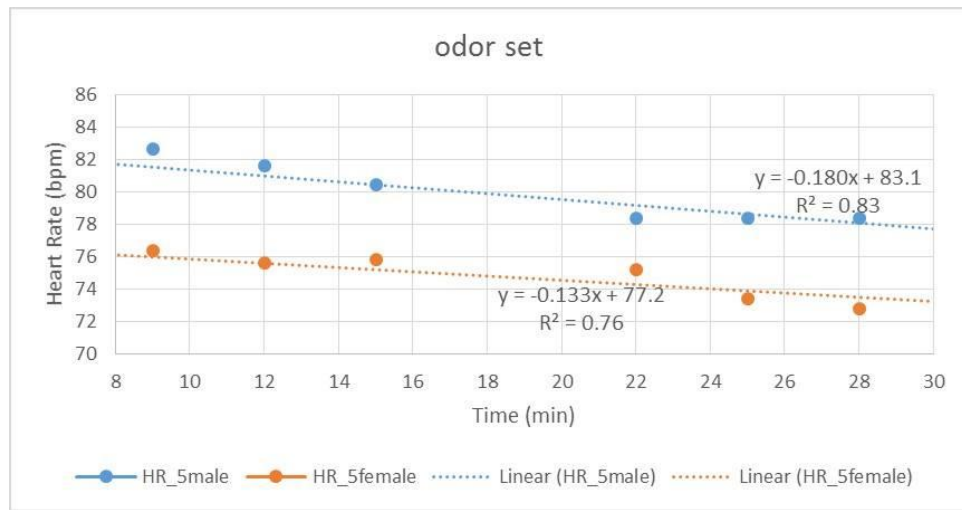


Figure 4.7: Heart rate for odor-control sequence

Figure 4.8a shows that a 45-minute plot was not appropriate to demonstrate the effect of the jasmine stimulation in both male and female subjects. In this experiment, the odor stage was undertaken after the resting period. It is undeniable that the “meditation-like” effect remained the background influence on the decrease of the heart rate. The heart rate thus declined and entered the steady-state faster than that in the previous experiment. To avoid any misinterpretation, the period of the graph was reduced to 30-minute. Before the heart rate reached the minimum at the saturation state, the graph in Figure 4.8b showed that the heart rate of the male subject declined at the rate of 0.180 bpm/min. It was approximately 2.3-time higher that the rate during the stimulation period in the previous experiment. This confirmed that the odor stimulation could exert the significantly stronger effect on the heart rate when the subject was exposed to a good aroma in the non-saturated.

The graph in Figure 4.8a could not indicate any influence of the jasmine stimulation on the female subjects. As discussed above in connection with the “meditation-like” effect, the heart rate reached the minimum faster than that in the prior experiment. In agreement with the result from the male subjects, the heart rate of the females reached to the minimum value faster than in the control period (without odor exposure) in the previous experiment. The heart rate of the female subjects decreased steadily at the rate of 0.133 bpm/min, as shown in Figure 4.8b. It is evident that the odor effect could be detected in this experiment because the subject was exposed to the odor in the non-saturated state. Also, this may be an explanation why some massages with the application of some aroma, such as jasmine scent, could relieve the stress significantly more effectively than those without any pleasant odor.

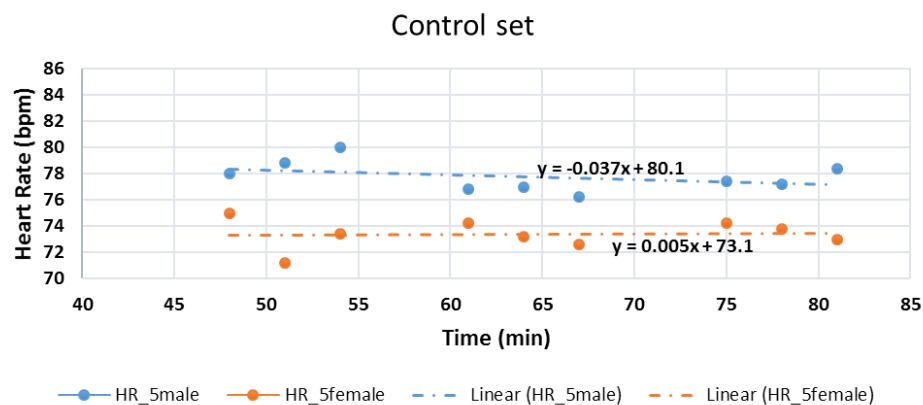




(b)

Figure 4.8: Heart rate for odor set of odor-control sequence. (a) The response in the 45-minute odor-stimulation period. (b) The response in the 30-minute odor-stimulation period

Figure 4.9a and 4.9b showed the responses of the subjects during the control period. Clearly, the graphs in both figures exhibited a similar trend with a much less declination slope of the heart rate, when compared to the graph during the odor stimulation in Figure 4.7b. The graphs, however, had an R^2 value less than 0.5. The very low R^2 value of the graph reflected that the equation might not be able to represent the relation of the data. Instead, the slope in the equation might be the result of an alias from the random fluctuation in the saturated state of the subject, since the data were likely to swing randomly about a certain heart rate in both male and female subjects.



(a)



Figure 4.9: Heart rate for control set of odor-control sequence. (a) The response in the 45-minute control period. (b) The response in the previous odor3 period and 45-minute control period

4.1.4. Heart Rate Changes of Individual Subjects (odor-control sequence)

Heart rate changes of the individual 5 male subjects and 5 female subjects for the “odor-control” sequence are shown in Figure 4.10 and Figure 4.11. Calculation for the heart rate change was the same calculation as “control-odor” sequence. Therefore, the heart rate change for the odor exposure condition was the difference between the heart rate of the interval-3 (I3) of odor exposure trial-3 and the first interval-1 (I1) of odor exposure trial-1. Heart rate change for control condition was the difference between the heart rate for the first interval-1(I1) of control trial-1 and the last interval-3 (I3) of control trial-3. A decreasing in heart rate change was still found in all five male subjects while only two female subjects showed more decreased heart rate change in the odor set than in the control set.

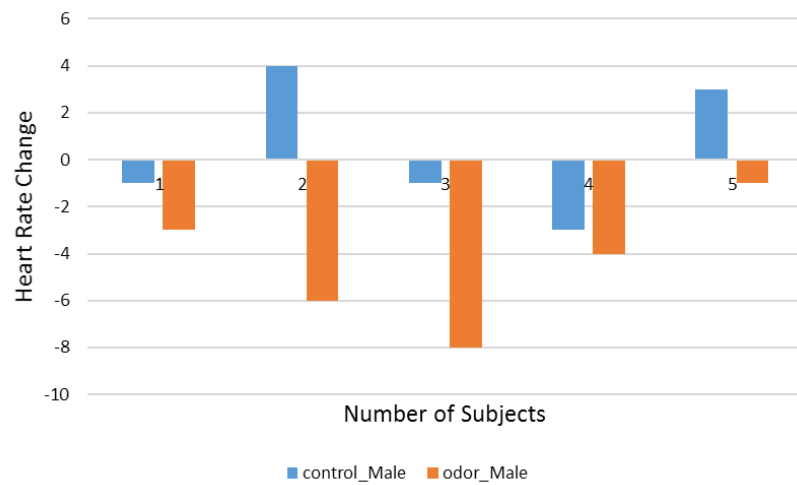


Figure 4.10: Heart rate changes of males for odor-control sequence

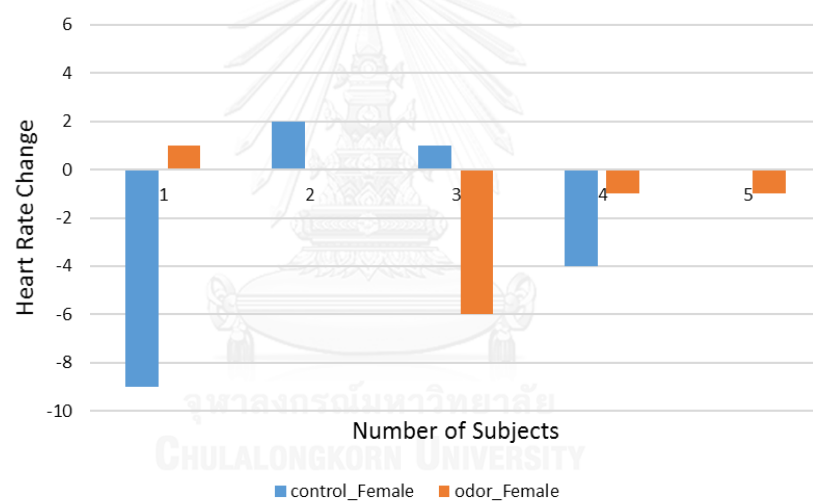


Figure 4.11: Heart rate changes of females for odor-control sequence

It could be summarized all the heart rate results for this section as follows;

1. Heart rates of males were higher than females for all conditions (resting, control and odor data set)
2. Heart rates of males tended to be more decreased when male subjects were stimulated by jasmine odor.
3. No difference could not be observed between 'control-odor' and 'odor-control' sequence. This implies that there is no effect of the measurement sequence on the odor response.

4.2. SDNN Parameter

An average SDNN of 15 males and 15 females for the “control-odor” sequence is shown in the following Figure 4.12. A SDNN represents the magnitude of deviation from the average value. An increased in SDNN was found in male subjects ($y = 0.144x + 43.0$). However, a slightly increased SDNN was found in female subjects ($y = 0.003x + 41.1$). In both control and odor set, an increasing in SDNN was found in male subjects than in female subjects. An average baseline SDNN value of male subjects was found to be slightly higher than female subjects. The results showed that the same trend as reported by P. Pavithran et al. [61]. They also found that SDNN values of male subjects were higher than the same aged female subjects. This higher value of SDNN of male than female indicates that males have higher variation in R-R interval than females.

A SDNN was not so much difference between control and odor set in both males and females. It increased in both control and odor set of male subjects as shown in Figure 4.13. Although it was slightly increased in control set of females, it had an almost stable trend in odor set of those female subjects as shown in Figure 4.14.

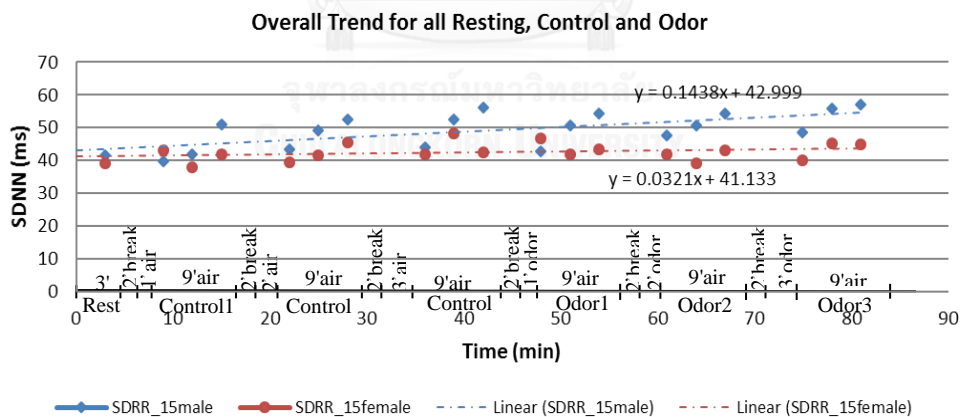


Figure 4.12: SDNN of overall measurement

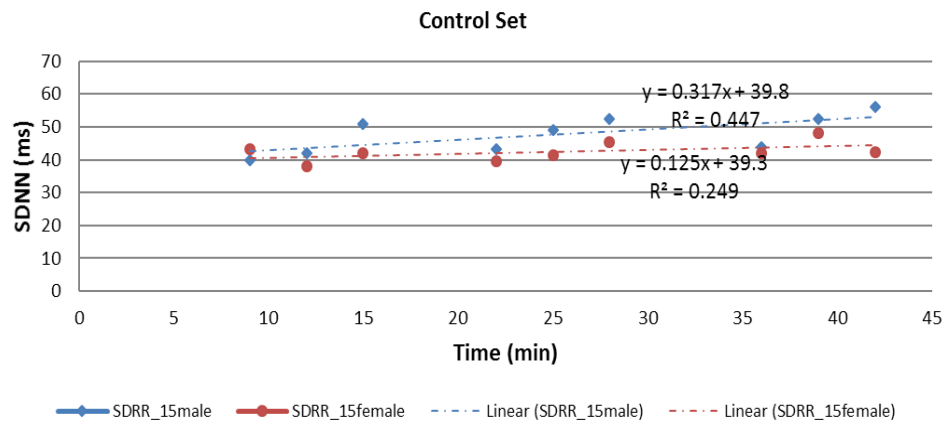


Figure 4.13: SDNN of control set

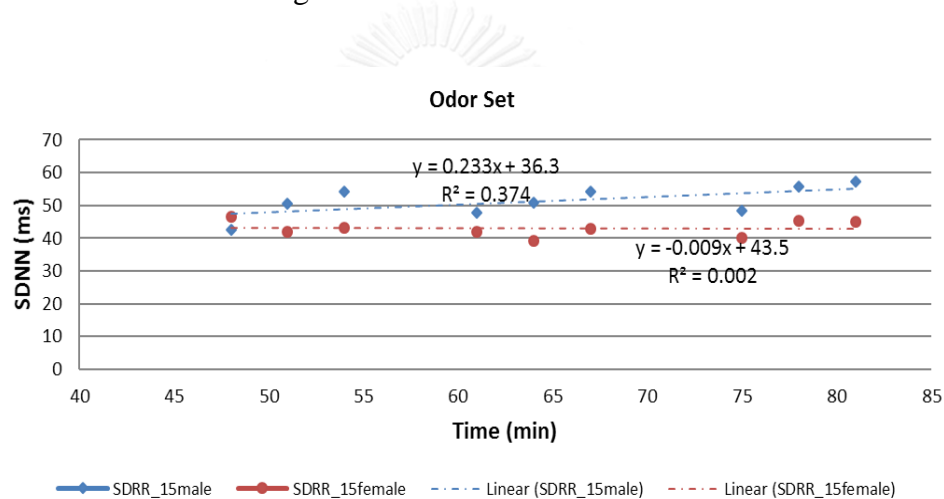


Figure 4.14: SDNN of odor set

4.3. RMSSD Parameter

Average RMSSD values plot of 15 males and 15 females for overall resting, control and odor exposure is shown in Figure 4.15. RMSSD represents the variation of consecutive R-R interval difference. The lowering in RMSSD means the variation of consecutive R-R interval seems to be not so much changed and every heartbeat rhythm can be the same timing. In this experiment, the “control-odor” sequence was done. It was observed that RMSSD in male subjects were more fluctuating than in female subjects for the overall measurement. RMSSD was slightly increased in male subjects ($y = 0.066x + 33.2$) while it was slightly decreased in female subjects ($y = -0.011x + 34.0$) during the measurement. Data sets were separated into control set and odor set as shown in Figure 4.16 and Figure 4.17.

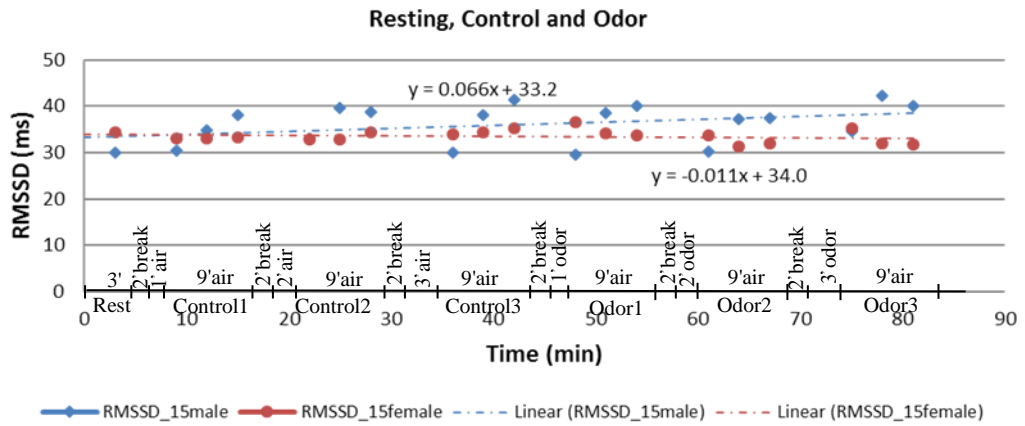


Figure 4.15: RMSSD of overall measurement

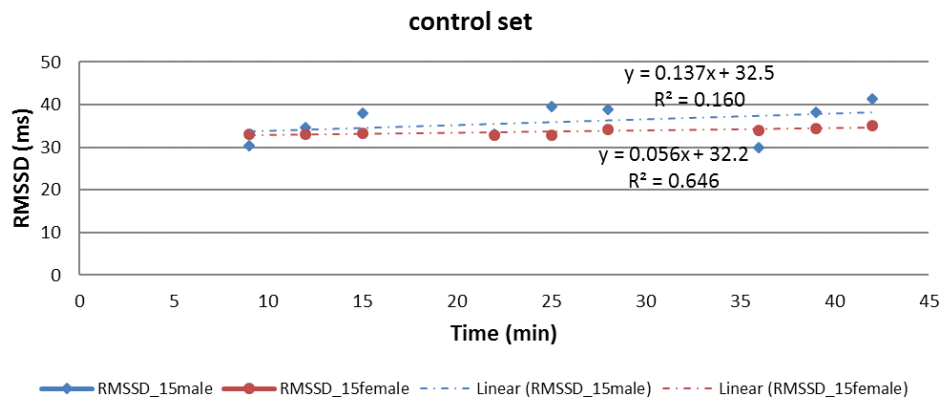


Figure 4.16: RMSSD of control set

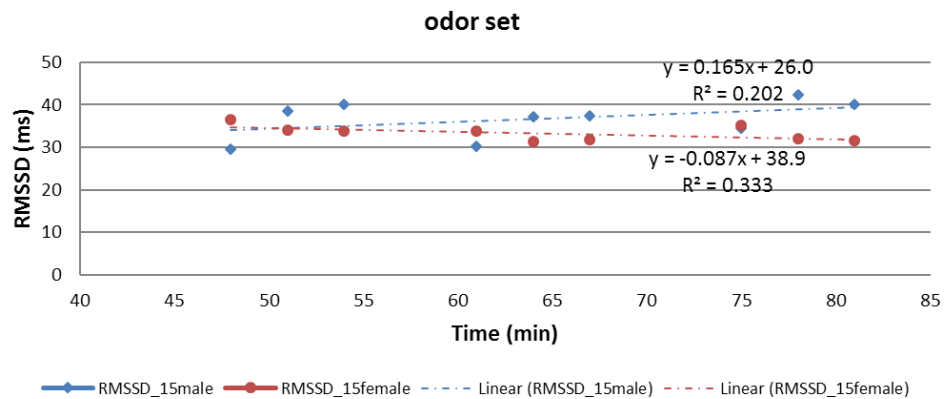


Figure 4.17: RMSSD of odor set

For the control set, RMSSD increased in male subjects ($y = 0.137x + 32.5$) and it slightly increased in female subjects ($y = 0.056x + 32.2$). For the odor set, RMSSD values in male subjects slightly increased ($y = 0.165x + 26.0$) more than the overall control set ($y = 0.137x + 32.5$). For the odor set, slightly decreased in female subjects ($y = -0.087x + 38.9$) was observed. However, no statistical significant was found in RMSSD.

4.4. Normalized Low-Frequency to High-Frequency Ratio

Average values of normalized low-frequency to high-frequency ratios for both male and female were shown in Figure 4.18. The overall trend of nLF/nHF ratio for all measurement (resting, control and odor set) was not so much changed. However, the nLF/nHF ratios of the female subjects were found significantly lower (paired t-test: $p < 0.05$) compared to that of male subjects in most experiments. Comparison of nLF/nHF ratio between male and female for all experiment condition was listed in Table 4.9. Our experiment was performed in sitting position. P. Pavithran et al. [61] also found that normalized low-frequency power, as well as LF/HF ratio, were significantly lower in female compared to male in the supine position. This indicates that there is no difference in nLF/nHF ratio between the different positions during measurement.

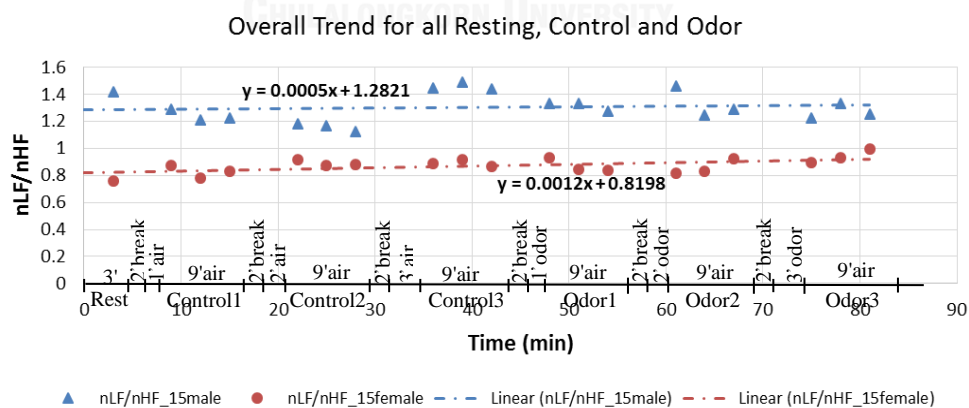


Figure 4.18: nLF/nHF of overall measurement

Table 4.9: The nLF/nHF ratio of both 15-male and 15-female for all experiments

Experiment condition	Gender		Paired t-test (p- value) between male and female
	Male (mean±sd)	Female (mean±sd)	
Resting	1.41±0.62	0.76±0.26	p = 0.001*
Control1-I1	1.29±0.59	0.87±0.30	p = 0.012*
Control1-I2	1.21±0.54	0.78±0.18	p = 0.005*
Control1-I3	1.22±0.49	0.83±0.22	p = 0.006*
Control2-I1	1.18±0.47	0.92±0.29	p = 0.038*
Control2-I2	1.16±0.45	0.87±0.25	p = 0.020*
Control2-I3	1.12±0.53	0.88±0.35	p = 0.073
Control3-I1	1.45±0.86	0.89±0.42	p = 0.017*
Control3-I2	1.49±0.91	0.92±0.42	p = 0.019*
Control3-I3	1.44±1.12	0.87±0.27	p = 0.035*
Odor1-I1	1.33±0.53	0.93±0.43	p = 0.015*
Odor1-I2	1.33±0.64	0.84±0.37	p = 0.009*
Odor1-I3	1.27±0.56	0.84±0.38	p = 0.010*
Odor2-I1	1.46±0.72	0.81±0.29	p = 0.002*
Odor2-I2	1.25±0.57	0.83±0.25	p = 0.009*
Odor2-I3	1.29±0.62	0.92±0.33	p = 0.027*
Odor3-I1	1.22±0.49	0.90±0.44	p = 0.032*
Odor3-I2	1.33±0.81	0.93±0.37	p = 0.048*
Odor3-I3	1.25±0.54	1.00±0.32	p = 0.065

*Significant difference, paired t-test: p-value < 0.05

Experiment sequence, in this case, was the “control-odor” sequence. Measured data sets were separated into the control and odor set. For male subjects, the nLF/nHF ratio of the control set was slightly increased ($y = 0.008x + 1.1$) while the odor set was slightly decreased ($y = -0.002x + 1.4$). The nLF/nHF ratio was found not so much changed between the control set ($y = 0.002x + 0.8$) and that of odor set ($y = 0.003x + 0.7$) in female subjects as shown in Figure 4.19 and Figure 4.20.

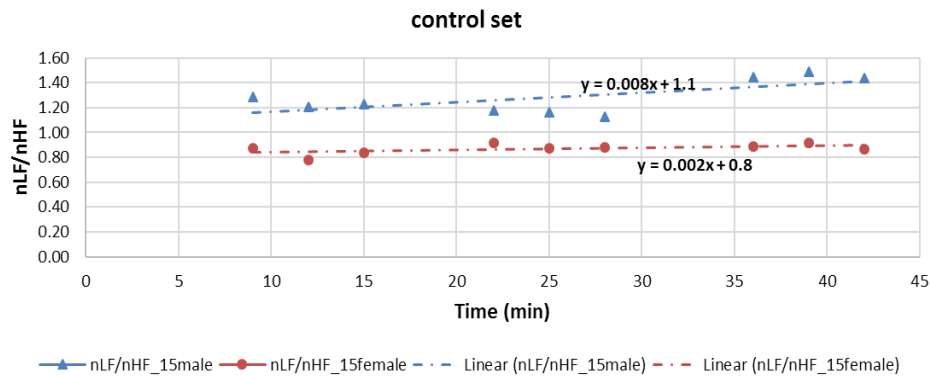


Figure 4.19: nLF/nHF of control set

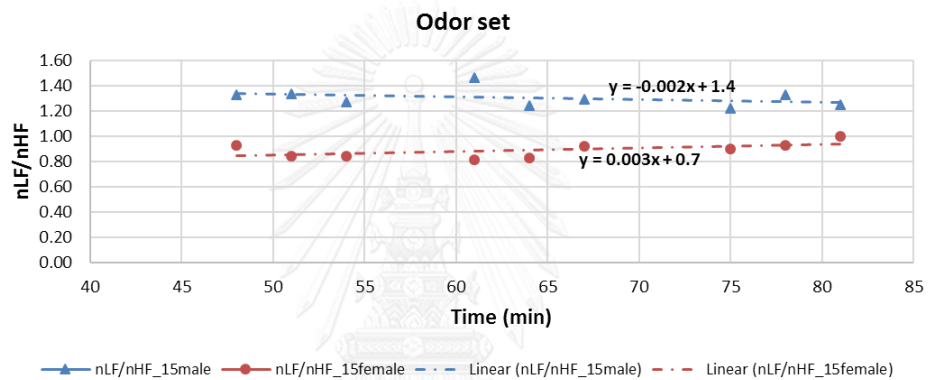


Figure 4.20: nLF/nHF of odor set

4.5. Stress Response Scale Changes

18 items of stress response scale were used to evaluate the psychological condition of the subjects. Firstly, subjects were asked before starting the measurement to evaluate the resting condition. After sniffing odor for 1-minute and measurement for 9-minute, subjects were asked again to evaluate the psychology condition for the first odor exposure. The subjects were asked again after finishing the second trial of odor set with 2-minute sniffing. Lastly, after the third trial of odor set, the subjects were asked again to evaluate the final condition. SRS-18 scores for one representative subject at each condition are tabulated in Table 4.10, 4.11, 4.12, and 4.13. The higher rating scores that were chosen by the subjects represent the higher level of stress. Mean value of the SRS-18 for the individual subjects was calculated for each condition.

Table 4.10: Representative SRS-18 data before breathing the odor

No.	Items	Before Breathing the odor				
		Definitely Not/No	Slightly Not/No	Moderately	Rather True/Yes	Definitely True/Yes
1	Get angry easily	1	2	3	4	5
2	Feeling sad	1	2	3	4	5
3	Worry about something	1	2	3	4	5
4	Feeling angry	1	2	3	4	5
5	Crying feeling	1	2	3	4	5
6	Cannot control feeling	1	2	3	4	5
7	Mortifying	1	2	3	4	5
8	Unhappy	1	2	3	4	5
9	Feeling down	1	2	3	4	5
10	Irritate nervous	1	2	3	4	5
11	Cannot believe in many things	1	2	3	4	5
12	Don't like everything	1	2	3	4	5
13	Thinking bad things	1	2	3	4	5
14	Cannot conclude on talking	1	2	3	4	5
15	Need someone made me comfort	1	2	3	4	5
16	No patience	1	2	3	4	5
17	Not to be alone	1	2	3	4	5
18	Cannot concentrate on anything	1	2	3	4	5
	Overall Mean	1.39				

Table 4.11: Representative SRS-18 data after the first trial of odor set with 1-minute breathing

No.	Items	For 1-minute Odor Exposure				
		Definitely Not/No	Slightly Not/No	Moderately	Rather True/Yes	Definitely True/Yes
1	Get angry easily	1	2	3	4	5
2	Feeling sad	1	2	3	4	5
3	Worry about something	1	2	3	4	5
4	Feeling angry	1	2	3	4	5
5	Crying feeling	1	2	3	4	5
6	Cannot control feeling	1	2	3	4	5
7	Mortifying	1	2	3	4	5
8	Unhappy	1	2	3	4	5
9	Feeling down	1	2	3	4	5
10	Irritate nervous	1	2	3	4	5
11	Cannot believe in many things	1	2	3	4	5
12	Don't like everything	1	2	3	4	5
13	Thinking bad things	1	2	3	4	5
14	Cannot conclude on talking	1	2	3	4	5
15	Need someone made me comfort	1	2	3	4	5
16	No patience	1	2	3	4	5
17	Not to be alone	1	2	3	4	5
18	Cannot concentrate on anything	1	2	3	4	5
	Overall Mean	1.33				

Table 4.12: Representative SRS-18 data after the second trial of odor set with 2-minute breathing

No.	Items	For 2-minute Odor Exposure				
		Definitely Not/No	Slightly Not/No	Moderately	Rather True/Yes	Definitely True/Yes
1	Get angry easily	1	2	3	4	5
2	Feeling sad	1	2	3	4	5
3	Worry about something	1	2	3	4	5
4	Feeling angry	1	2	3	4	5
5	Crying feeling	1	2	3	4	5
6	Cannot control feeling	1	2	3	4	5
7	Mortifying	1	2	3	4	5
8	Unhappy	1	2	3	4	5
9	Feeling down	1	2	3	4	5
10	Irritate nervous	1	2	3	4	5
11	Cannot believe in many things	1	2	3	4	5
12	Don't like everything	1	2	3	4	5
13	Thinking bad things	1	2	3	4	5
14	Cannot conclude on talking	1	2	3	4	5
15	Need someone made me comfort	1	2	3	4	5
16	No patience	1	2	3	4	5
17	Not to be alone	1	2	3	4	5
18	Cannot concentrate on anything	1	2	3	4	5
	Overall Mean	1.17				

Table 4.13: Representative SRS-18 data after the third trial of odor set with 3-minute breathing

No.	Items	For 3-minute Odor Exposure				
		Definitely Not/No	Slightly Not/No	Moderately	Rather True/Yes	Definitely True/Yes
1	Get angry easily	1	2	3	4	5
2	Feeling sad	1	2	3	4	5
3	Worry about something	1	2	3	4	5
4	Feeling angry	1	2	3	4	5
5	Crying feeling	1	2	3	4	5
6	Cannot control feeling	1	2	3	4	5
7	Mortifying	1	2	3	4	5
8	Unhappy	1	2	3	4	5
9	Feeling down	1	2	3	4	5
10	Irritate nervous	1	2	3	4	5
11	Cannot believe in many things	1	2	3	4	5
12	Don't like everything	1	2	3	4	5
13	Thinking bad things	1	2	3	4	5
14	Cannot conclude on talking	1	2	3	4	5
15	Need someone made me comfort	1	2	3	4	5
16	No patience	1	2	3	4	5
17	Not to be alone	1	2	3	4	5
18	Cannot concentrate on anything	1	2	3	4	5
	Overall Mean	1.17				

Mean scores for each condition are also listed in Table 4.14 for all 15 male subjects. For all 15 female subjects, mean scores are tabulated in in Table 4.15. And, it is summarized in Table 4.16 for both male and female subjects.

Table 4.14: Mean scores of the SRS-18 scale for all 15 male subjects

Subject (male)	Before Odor Exposure	For 1-minute odor exposure	For 2-minute odor exposure	For 3-minute odor exposure
1.	1.39	1.33	1.17	1.17
2.	2.11	2	2.11	2.06
3.	1.78	1.89	1.89	2.5
4.	1.56	1.33	1.06	1
5.	2.67	2.61	2.17	2.06
6.	1.39	1	1	1
7.	1.61	1.5	1.28	1.06
8.	2.17	2.11	1.94	1.94
9.	2.39	1.9	1.72	1.61
10.	2.56	2.67	2.33	2.61
11.	2.17	2.67	2.78	2.89
12.	2.28	2.22	2.33	2.11
13.	2.11	1.28	1.17	1.11
14.	2.22	1.67	1.72	2.17
15.	2.44	2.33	2.33	2.28
Mean	2.06	1.90	1.80	1.84
SD	±0.41	±0.54	±0.56	±0.64

Table 4.15: Mean scores of the SRS-18 scale for all 15 female subjects

Subject (female)	Before Odor Exposure	For 1-minute odor exposure	For 2-minute odor exposure	For 3-minute odor exposure
1.	1.61	1.00	1.11	1.00
2.	2.22	2.00	1.79	1.78
3.	2.00	1.50	1.39	1.44
4.	2.72	2.39	2.22	2.17
5.	2.28	1.22	1.11	1.06
6.	2.17	2.00	2.00	2
7.	2.06	1.22	1.28	1.22
8.	2.06	1.31	1.06	1.06
9.	2.67	2.39	2.06	1.94
10.	1.61	1.39	1.28	1.28
11.	4.72	3.33	2.78	2.94
12.	2.28	1.22	1.11	1.06
13.	3.00	2.67	2.89	2.37
14.	2.44	2.06	1.83	1.78
15.	1.39	1.20	1.11	1.11
Mean	2.35	1.79	1.67	1.61
SD	±0.79	±0.68	±0.62	±0.59

Table 4.16: Summary of SRS-18 scale for both males and females

Gender	Before Odor Exposure (B)	For 1-minute Odor Exposure (O1)		For 2-minute Odor Exposure (O2)		For 3-minute Odor Exposure (O3)	
	Mean ±SD	Mean ±SD	p-value (B-O1)	Mean ±SD	p-value (B-O2)	Mean ±SD	p-value (B-O3)
Male	2.06 ±0.41	1.90 ±0.54	p < 0.05*	1.80 ±0.56	p < 0.05*	1.84 ±0.64	p < 0.05*
Female	2.35 ±0.79	1.79 ±0.68	p < 0.05*	1.67 ±0.62	p < 0.05*	1.61 ±0.59	p < 0.05*

*Significant difference, paired t-test; p-value < 0.05

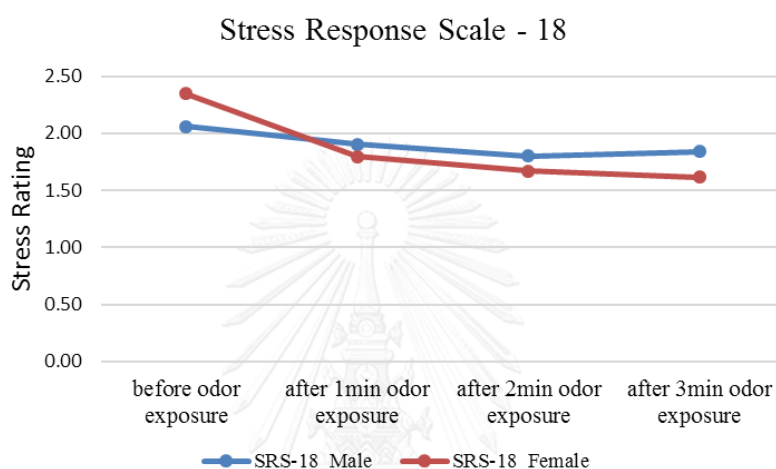


Figure 4.21: Stress response scale (SRS-18) score

In the psychology test for the stress condition, SRS-18 scores were significantly decreased in both male and female subjects. By means of the questionnaire results in male subjects, SRS-18 scores were significantly decreased after jasmine odor stimulation for 1-minute (paired t-test: $p < 0.05$), 2-minute ($p < 0.05$) and 3-minute ($p < 0.05$) compared to the scores before odor stimulation. In female subjects, SRS-18 scores were also significantly decreased after jasmine odor stimulation for 1-minute (paired t-test: $p < 0.05$), 2-minute ($p < 0.05$) and 3-minute ($p < 0.05$) compared to the scores before odor stimulation.

Changes of SRS-18 scores for both male and female were shown in Figure 4.21. SRS-18 scores were found more significantly decreased in female than in male subjects after jasmine odor stimulation compared to before odor stimulation. The results suggest that jasmine odor stimulation can reduce the stress of both male and female. Moreover, the more sniffing time, the more reduction of stress was observed.

4.5.1. Psychological Stress and Heart Rate Changes

Subjects were asked for the psychological stress using the SRS-18 questionnaires in the following four conditions as mentioned in the previous section:

- (i) before odor exposure in the resting condition
- (ii) after 1-minute odor exposure and measurement for 9-minute
- (iii) after 2-minute odor exposure and measurement for 9-minute
- (iv) after 3-minute odor exposure and measurement for 9-minute.

A 9-minute measurement interval was also separated into three intervals: interval-1, interval-2, and interval-3 as it was mentioned before. Therefore, the average heart rate was calculated for every 3-minute. Heart rate for the resting condition was measured for 3-minute before odor exposure. Heart rate for this resting condition was used as the baseline to compare with the heart rate of every interval after odor exposure. Heart rate at interval-1 and SRS-18 score for each odor exposure condition were shown in Figure 4.22. Relations of heart rate and SRS-18 at the interval-2 and interval-3 are shown in Figure 4.23 and 4.23, respectively. The same SRS-18 score was used for every interval 1, 2 and 3 because subjects were asked only one time after 9 min measurement for each condition.

In every interval 1, 2, and 3, heart rate values were decreased as well as SRS-18 rating after odor exposure for 1-minute, 2-minute, and 3-minute compared to before odor exposure condition in both males and females. SRS-18 scores were found more decreased in female subjects than male subjects after each odor exposure compared to before odor exposure condition.

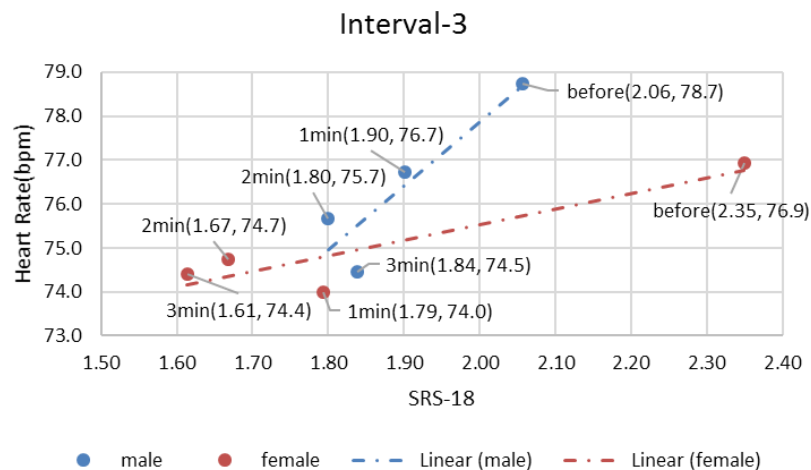


Figure 4.22: SRS-18 and heart rate values for interval-3

4.5.2. Psychological Stress and nLF/nHF Ratio

SRS-18 score and nLF/nHF ratio at interval-1 before and odor exposure were shown in Figure 4.23. For the interval-2 and 3, the results were shown in Figure 4.24 and Figure 4.25.

Comparison of the mean nLF/nHF ratio between before odor exposure and after odor exposure for 1-minute, 2-minute, and 3-minute at interval-1 are listed in Table 4.17. For interval-2 and interval-3, the results are summarized in Table 4.18 and 4.19. It was found that stresses of male and female were reduced after exposing to odor. However, it should be noted that the relation between SRS-18 and nLF/nHF ratio is different in males and females. It was found that after exposing to odor, the nLF/nHF ratio decreased in males while it was slightly increased in females. Decreasing the nLF/nHF ratio might link to physical relaxation in males. Slightly increased in females might be other factors in physical response because some females in the experiment do not like the jasmine. However, statistical analysis using paired t-test did not show any significant change in nLF/nHF ratio before and after odor exposure.

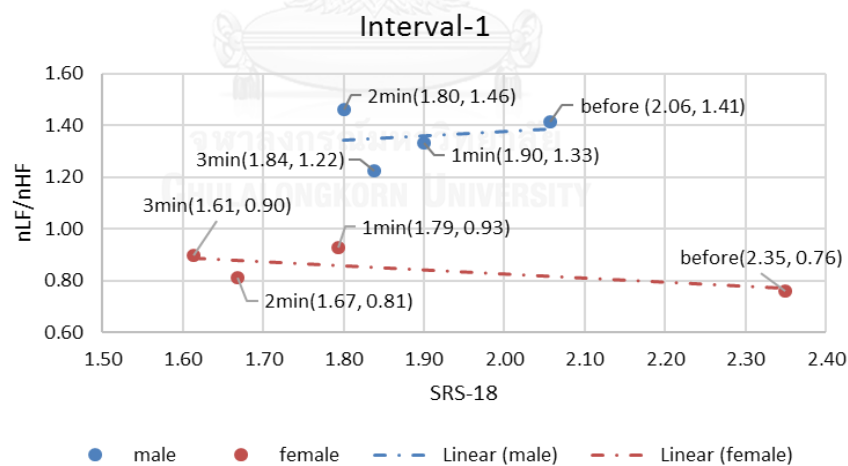


Figure 4.23: SRS-18 and nLF/nHF ratio for interval-1

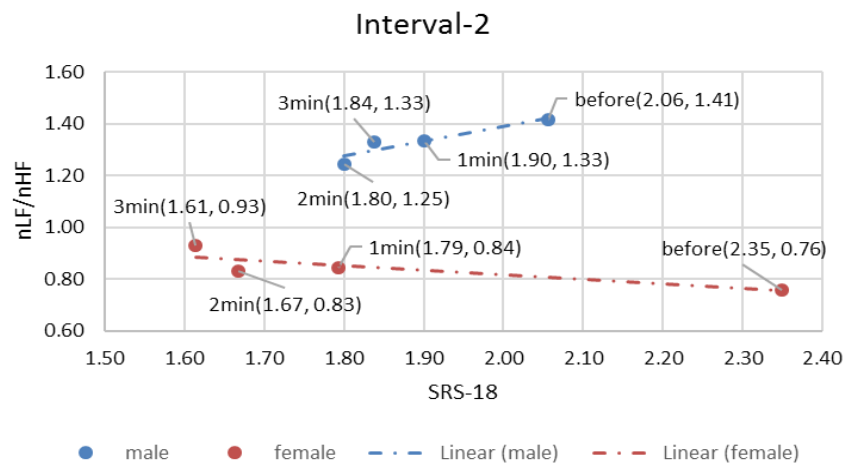


Figure 4.24: SRS-18 and nLF/nHF ratio for interval-2

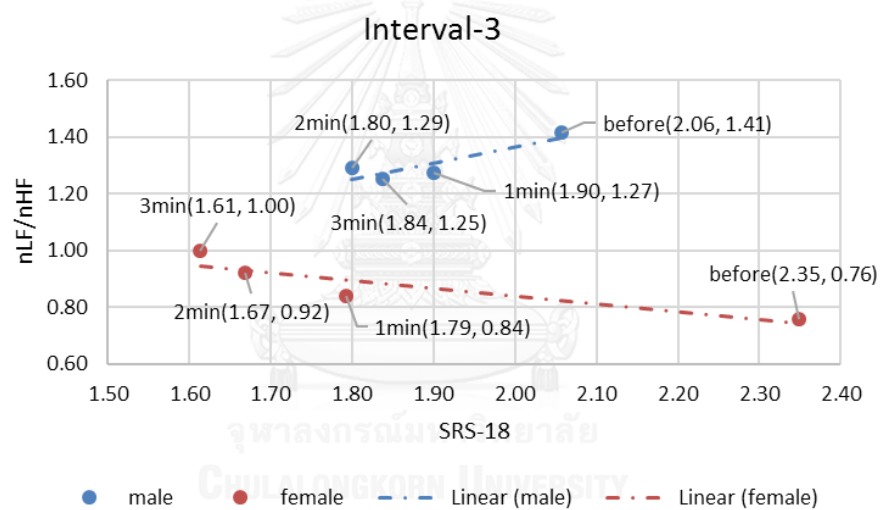


Figure 4.25: SRS-18 and nLF/nHF ratio for interval-3

Table 4.17: Mean nLF/nHF at interval-1 before and after odor exposure

Gender	Before Odor Exposure (B)	Interval-1					
		After 1-minute Odor Exposure (O1)		After 2-minute Odor Exposure (O2)		After 3-minute Odor Exposure (O3)	
	Mean	Mean	p-value (B-O1)	Mean	p-value (B-O2)	Mean	p-value (B-O3)
Male	1.41 ±0.62	1.33 ±0.53	p > 0.05	1.46 ±0.42	p > 0.05	1.22 ±0.49	p > 0.05
Female	0.76 ±0.26	0.93 ±0.43	p > 0.05	0.81 ±0.29	p > 0.05	0.90 ±0.44	p > 0.05

Table 4.18: Mean nLF/nHF at interval-2 before and after odor exposure

Gender	Before Odor Exposure (B)	Interval-2					
		After 1-minute Odor Exposure (O1)		After 2-minute Odor Exposure (O2)		After 3-minute Odor Exposure (O3)	
		Mean	p-value (B-O1)	Mean	p-value (B-O2)	Mean	p-value (B-O3)
Male	1.41 ±0.62	1.33 ±0.64	p > 0.05	1.25 ±0.57	p > 0.05	1.33 ±0.81	p > 0.05
Female	0.76 ±0.26	0.84 ±0.37	p > 0.05	0.83 ±0.25	p > 0.05	0.93 ±0.37	p > 0.05

Table 4.19: Mean nLF/nHF at interval-3 before and after odor exposure

Gender	Before Odor Exposure (B)	Interval-3					
		After 1-minute Odor Exposure (O1)		After 2-minute Odor Exposure (O2)		After 3-minute Odor Exposure (O3)	
		Mean	p-value (B-O1)	Mean	p-value (B-O2)	Mean	p-value (B-O3)
Male	1.41 ±0.62	1.27 ±0.56	p > 0.05	1.29 ±0.62	p > 0.05	1.25 ±0.54	p > 0.05
Female	0.76 ±0.26	0.84 ±0.38	p > 0.05	0.92 ±0.33	p > 0.05	1.00 ±0.32	p < 0.05*

*Significant difference, paired t-test: p-value < 0.05

4.6. R-S Amplitude Results Analysis

R-peak and S-peak were also extracted from ECG signal using moving average threshold method to find the R-S amplitude as shown in Figure 4.26. Firstly, the amplitude of all the R-S pairs was calculated then the average of all R-S amplitude was calculated for each data set. Average values of the R-S amplitude for all measurement (resting, control, and odor exposure) are shown in Figure 4.27. R-S amplitude values plot for the control and odor set are shown in Figure 4.28 and 4.29, respectively. It was found that average values of R-S amplitude for female were found lower than male subjects (paired t-test; p < 0.05 for resting between 15-male and 15-female). However, the change of R-S amplitude could not be observed the differences in resting, control or odor data set.

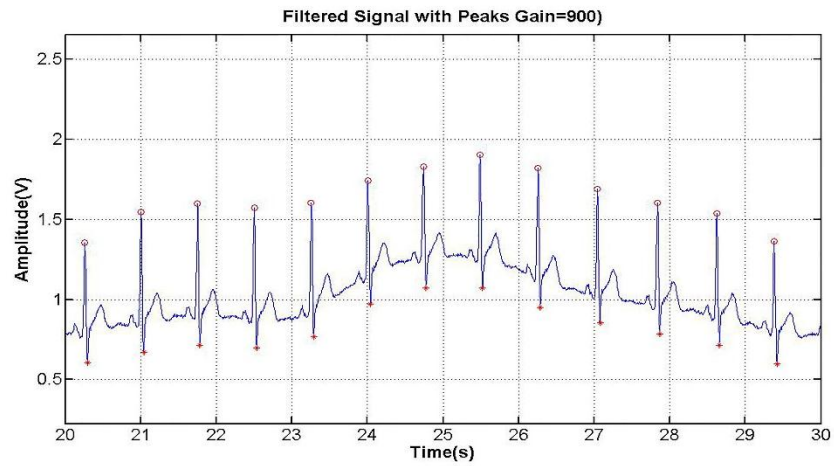


Figure 4.26: R-S peak detection

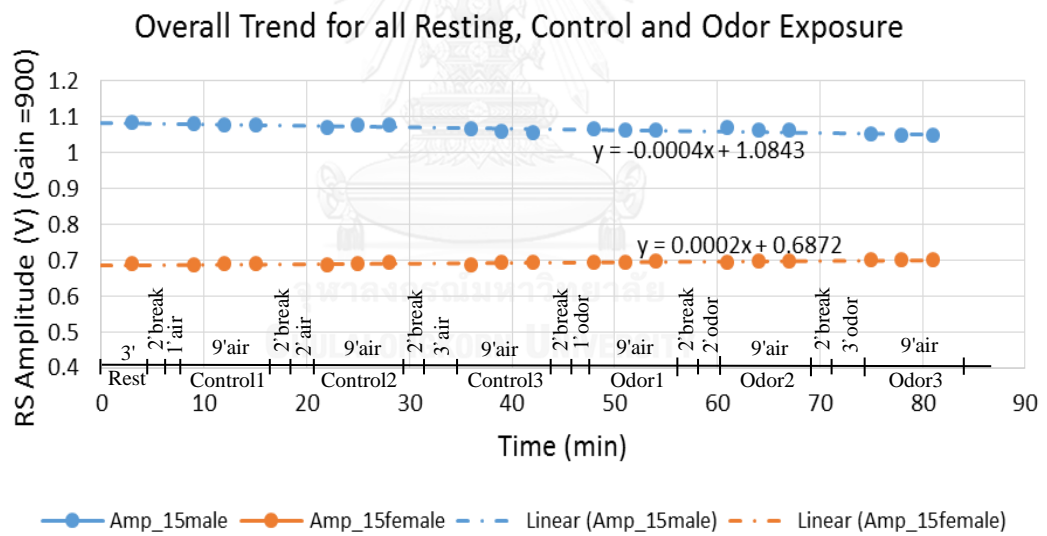


Figure 4.27: R-S amplitude plot for all measurements

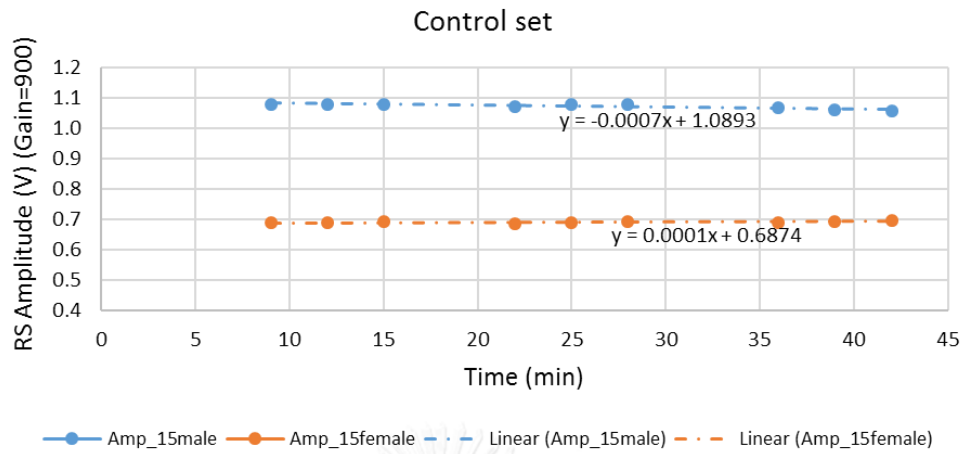


Figure 4.28: R-S amplitude of control set

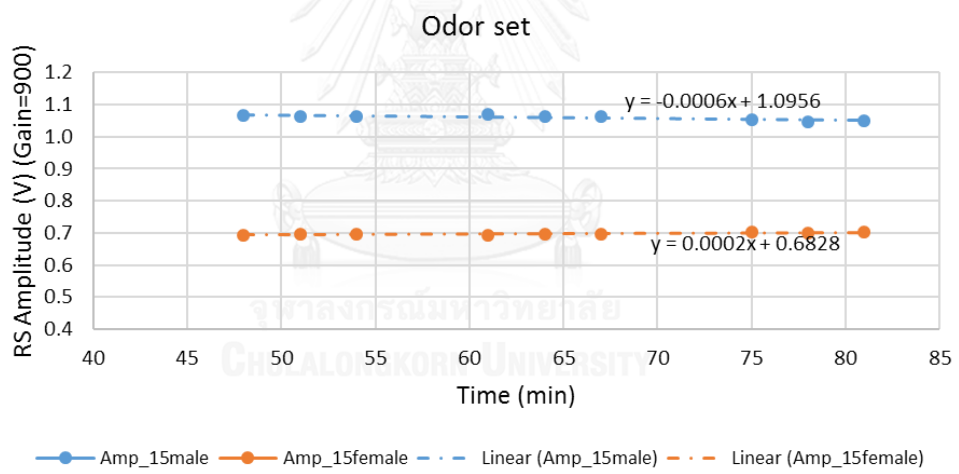


Figure 4.29: R-S amplitude of odor set

CHAPTER 5

CONCLUSION AND FUTURE WORK

It was considered to address the stressful society and stress-related heart problem in accordance with the relaxing effect of a pleasant odor. Based on the effect of odor perception in an emotional and nervous system, there was an expected outcome that there will be the psychological changes and physiological changes such as HRV. In this dissertation, correlations between HRV parameters and psychological stress due to jasmine odor inhalation were investigated in different gender.

To implement the experimental system and the research analysis, the following works were done in step by step approach.

(i) 15-male (26.3 ± 3.7 years, 67.2 ± 10.3 kg) and 15-female (30.1 ± 3.4 years, 58.8 ± 10.4 kg) recruited in the study.

(ii) Stress test using SRS-18 was used to evaluate the stress of the subjects before and after subjects were exposed to the jasmine odor.

(iii) An ECG data acquisition system was constructed.

(iv) Lead-II ECG data was measured in accordance with the experimental procedure.

(v) HRV analysis was used as a non-invasive method to evaluate the psychophysiological changes.

(vi) Detection of R-peaks and calculation of all consecutive RR intervals were basic steps to find the RR tachogram for the HRV analysis.

(vii) Heart rate, SDNN, and RMSSD parameters were calculated for HRV time domain analysis.

(viii) A Low-frequency component and a high-frequency component were extracted from HRV spectral analysis and the ratio of normalized low frequency to high frequency was calculated.

According to the results of the present study, it can be concluded as follow;

(i) A faster heart rate decreasing trend was found in male subjects for the odor data sets compared to control data sets of males and females as well. This means that a pleasant effect of linalool contained in jasmine might be more effective and impose in male subjects. It was found that the stress of both male and female groups was also

decreased after odor stimulation. Relaxing effect of jasmine odor can support in decreasing heart rate. The results suggest that a physiological response in terms of heart rate was more active in male subjects than in those female subjects.

(ii) Average heart rates of male subjects were also found higher than that of female subjects in almost all measurement intervals. However, the relation between heart rate and odor stimulation could not be observed.

(iii) Average SDNN values that represent the overall HRV were slightly increased in male subjects for the odor data sets although it was not statistically significant ($p > 0.05$). In the meanwhile, SDNN values for both control and odor data sets were not so much changed in female subjects.

(iv) SDNN values in the average of all male subjects were slightly higher than that of all female subjects in most of the measurement intervals.

(v) Average RMSSD values that represent the short-term HRV were slightly increased in male subjects for the odor data sets although it was not statistically significant ($p > 0.05$). However, RMSSD was not so much changed in female subjects for the odor data sets as well as control data sets.

(vi) Average nLF/nHF ratios of male subjects were higher than that of female subjects in almost all measurement intervals (resting, control and odor data sets). This can be an intrinsic character of males and females.

(vii) Changes of nLF/nHF ratios for male subjects had a slightly increased trend in the control data sets but it had a slightly decreased trend in the odor data sets for the male subjects. In males, low-frequency spectrum seems to be suppressed by odor stimulation as the nLF/nHF ratio slightly decreased. For female subjects, nLF/nHF ratios were almost constant in both control and odor data sets even a slightly increased was observed. However, changes of nLF/nHF ratios were not significant between control and odor exposure in both male and female subjects

(viii) For the psychological stress condition, according to the results of questionnaire, there was significantly decreased in stress response scale in both males and females after each odor exposure compared to before odor exposure. Changes range was more significant in female than in male subjects. Those findings suggested that psychological response was more active in female than male subjects after jasmine odor stimulation.

From the above results, it can be concluded that the stimulation by jasmine odor will be as follows.

1. For the qualitative evaluation using SRS-18, a reduction of the stress in both male and female subjects was observed. The stress reduction in female was more than male.

2. For the quantitative evaluation using ECG information, lowering in the heart activities such as heart rate, low-frequency spectrum, and normalized low-frequency to high-frequency ratio were observed in males than females after odor stimulation.

3. Changes of SDNN and RMSSD were not so much after odor stimulation, therefore fluctuation of heart rate might not depend on the odor effect.

4. In different gender, the effect of odor might be a different way for the nLF/nHF ratio response. It seems that ECG changes in males were more changed but the changes were very little in females in response to jasmine odor. However, the verbal report suggested that females were more sensitive to odor than males.

In this research, the only pleasant odor was used and the data was measured after breathing the odor, however it didn't measure during breathing the odor. Therefore, it is interesting to study the effect of pleasant and unpleasant odor during breathing for the future work. Moreover, qualitative and quantitative study about the relaxing effect of the pleasant odor on the people who have depression symptom and mental health problem will also be an interesting future topic.

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APPENDIX



จุฬาลงกรณ์มหาวิทยาลัย
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Studying about Menstruation Period Effect in Females

Refer to Table 4.2: Status of the participated female subjects in the experiment, females were separated into two groups according to their menstruation period as shown in Table A.1 and A.2. The first group (Group A) included the females whose menstruation period was about one-week closer to the previous or next menstruation period. The second group (Group B) included the females whose menstruation period was within the period of one-week after previous menstruation and one-week before next menstruation. Figure A.1 shows the menstruation period related to each group.

Table A.1: Status of the females (Group A) whose menstruation periods were about one-week closer to the previous or next menstruation

No.	Age (years)	Height (cm)	Weight (kg)	Blood pressure (mmHg) (Sys./Dia.)	Sleep hour (one night before exp.)	Menstruation finished state
1.	25	165	57	103/69	7 hrs	3 weeks ago
2.	29	159	46	107/77	6 hrs 30-minute	3 weeks ago
3.	29	158	67	110/62	7 hrs	3 weeks ago
4.	32	160	57	114/72	7 hrs 30-minute	1 day ago
5.	27	153	43	106/73	7 hrs	3 weeks ago
6.	38	150	61	115/73	5 hrs	2 days ago
7.	29	158	64	112/62	7 hrs	3 weeks ago
8.	29	158	44	106/64	6 hrs	3 weeks ago

Table A.2: Status of the females (Group B) whose menstruation periods were within the period of one-week after and one-week before menstruation

No.	Age (years)	Height (cm)	Weight (kg)	Blood pressure (mmHg) (Sys./Dia.)	Sleep hour (one night before exp.)	Menstruation finished state
1.	33	160	56	95/52	8 hrs	1 week ago
2.	27	155	53	108/72	7 hrs	2 weeks ago
3.	31	160	73	110/75	7 hrs	2 weeks ago
4.	30	168	70	104/69	6 hrs	1 week ago
5.	33	164	58	117/72	6 hrs 30-minute	2 weeks ago
6.	26	168	78	114/63	7 hrs	2 weeks ago
7.	34	150	53	110/68	6 hrs 30-minute	2 weeks ago

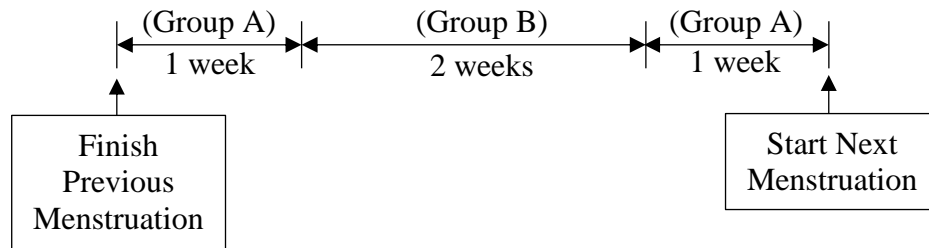


Figure A.1: Menstruation period related to each female group

Heart rate, SDNN, RMSSD, nLF/nHF ratio parameter and psychological stress response of the two female groups (about one-week closer to the menstruation period, Group A and about one-week far from menstruation period, Group B) will be discussed in this Appendix section.

Figure A.2 shows the heart rate of the two female groups for all measurements (resting, control and odor set). The data sets were separated into control set and odor set as shown in Figure A.3 and A.4. Heart rates of the female group (about one-week closer to menstruation period, Group A) were slightly higher than the female group (about one-week far from menstruation period, Group B). This might be stated that females who were closer to menstruation period have higher stress than females who were far from menstruation period.

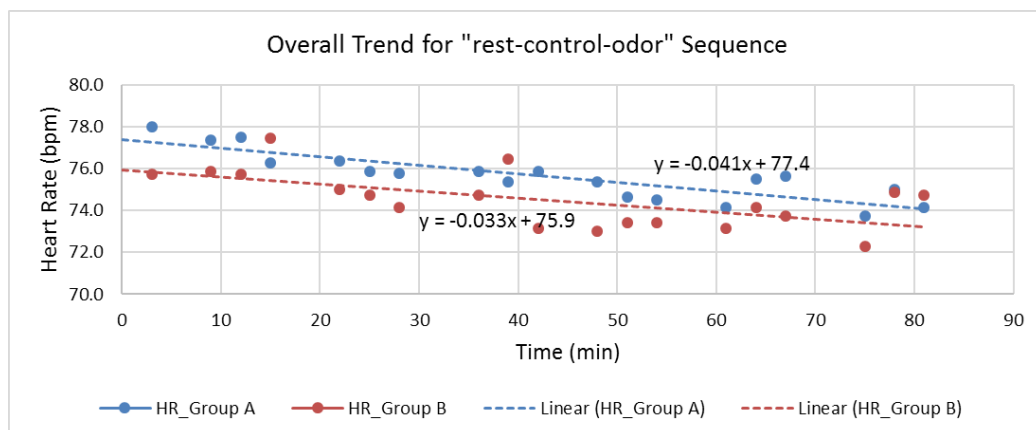


Figure A.2: Heart rate of the two female groups for all measurements

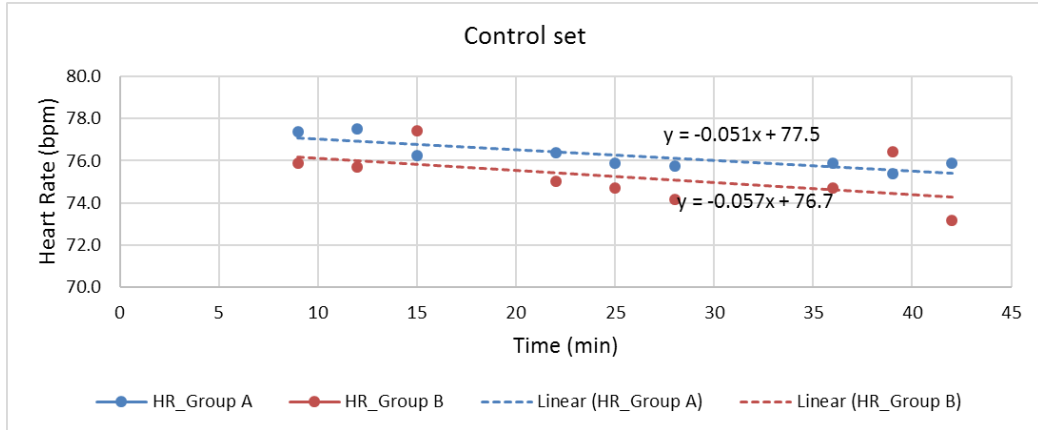


Figure A.3: Heart rate of the two female groups for control set

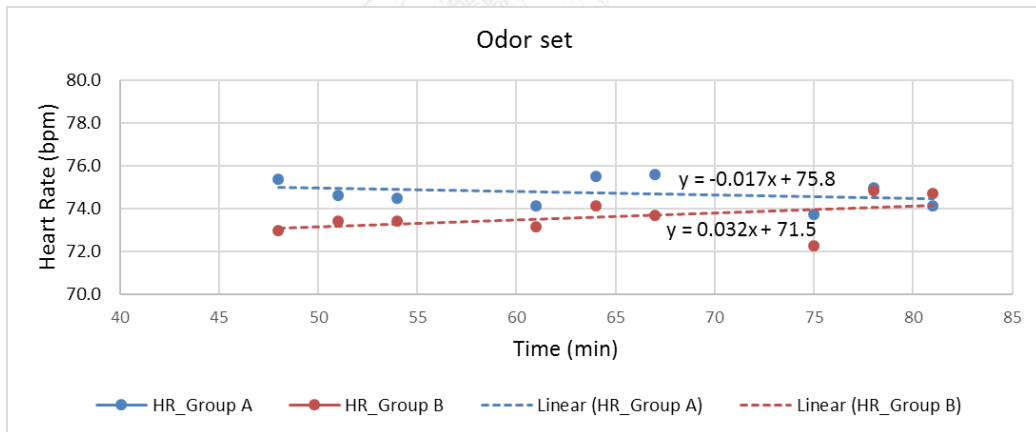


Figure A.4: Heart rate of the two female groups for odor set

Figure A.5, A.6, and A.7 show the SDNN of the two female groups for all measurements (resting, control, and odor exposure), respectively. Figure A.8, A.9, and A.10 show the RMSSD of the two female groups for all measurements (resting, control and odor exposure), respectively. Although the SDNN and RMSSD were not so much significant difference between the two female groups (Group A and Group B), SDNN and RMSSD of Group A (about one-week closer to the menstruation period) were slightly higher than that of Group B (about one-week far from the menstruation period). The result might be expressed that heartbeats were more fluctuated in Group A than Group B and fluctuating in heartbeat might be stated that higher stress in this Group A than Group B.

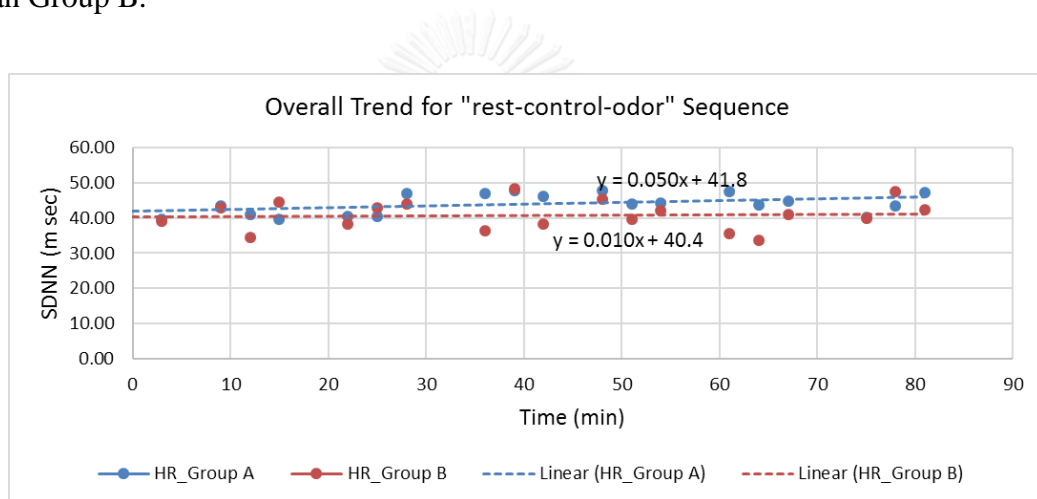


Figure A.5: SDNN of the two female groups for all measurements

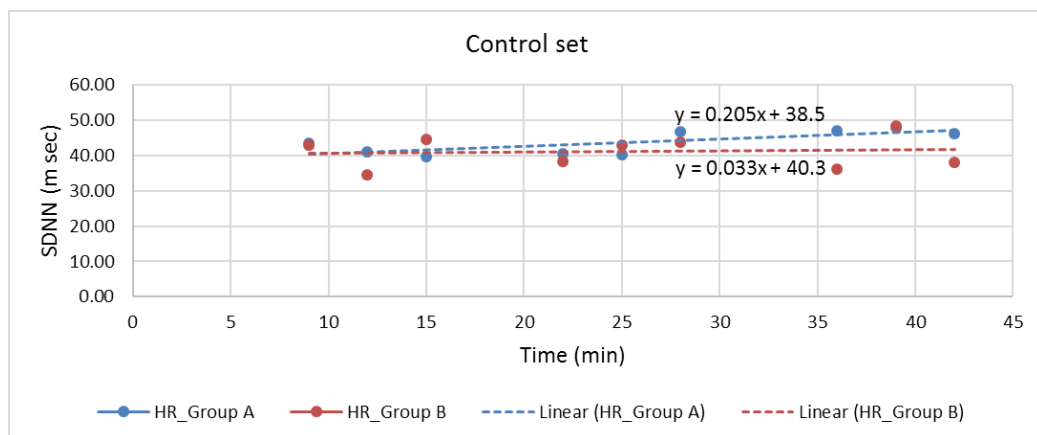


Figure A.6: SDNN of the two female groups for control set

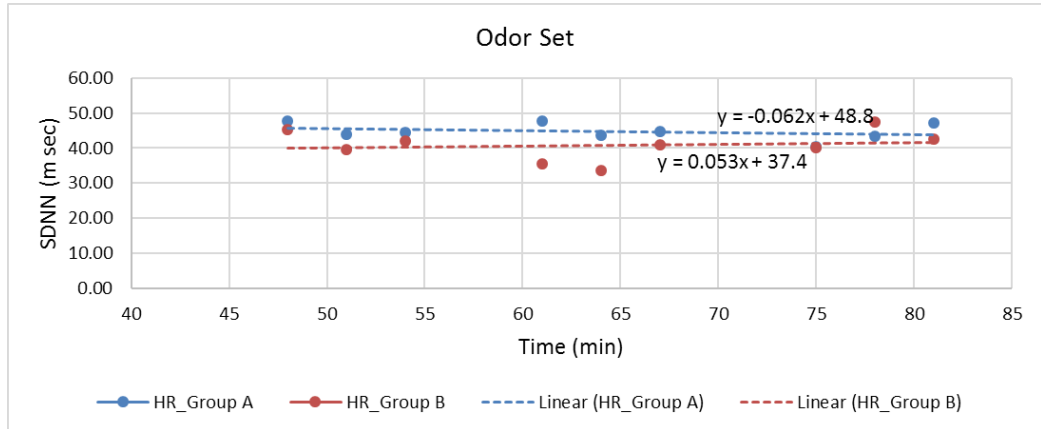


Figure A.7: SDNN of the two female groups for odor set

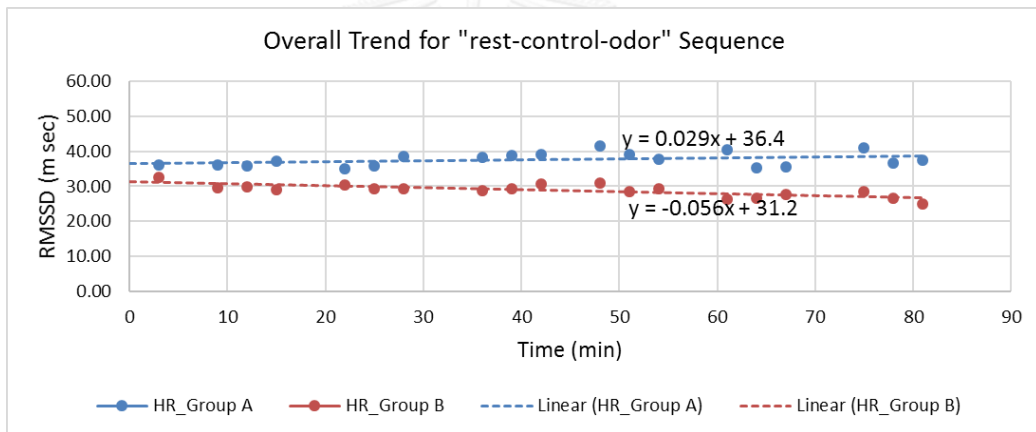


Figure A.8: RMSSD of the two female groups for all measurements

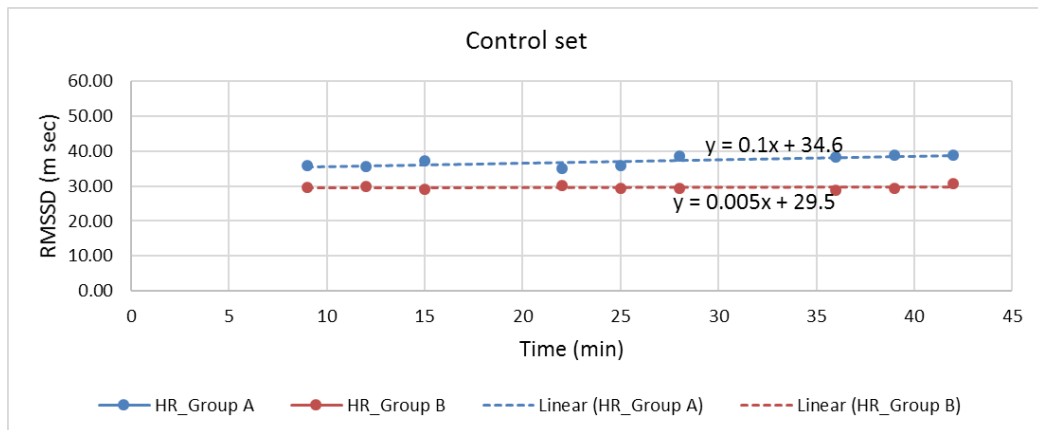


Figure A.9: RMSSD of the two female groups for control set

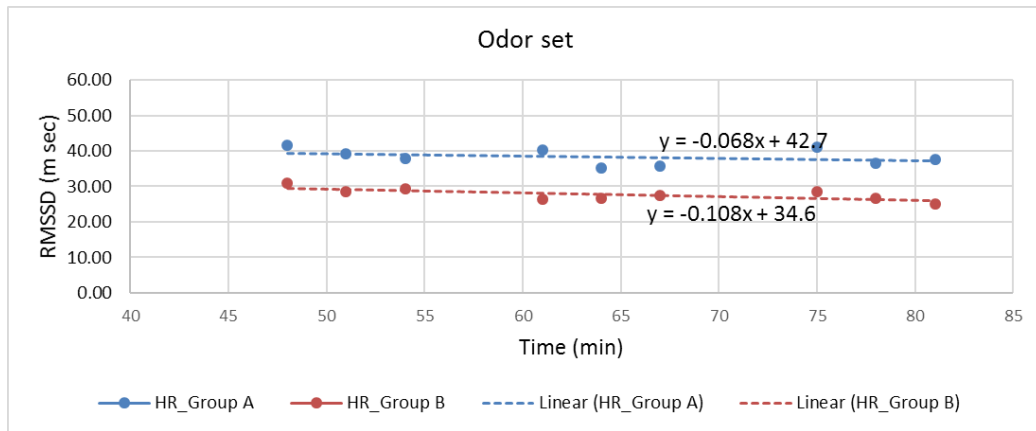


Figure A.10: RMSSD of the two female groups for odor set

Figure A.11 shows the nLF/nHF of the two female groups (Group A and Group B) for all measurements. The nLF/nHF data sets of the two female groups were also separated into control set and odor set as shown in Figure A.12 and A.13. In the control data set, the nLF/nHF were found almost the same between the two female groups. In the odor data set, the nLF/nHF of Group B were slightly higher than Group A. This might be stated that parasympathetic nervous system was activated in Group B and this means that decreasing the heart rate and reducing the stress as well.

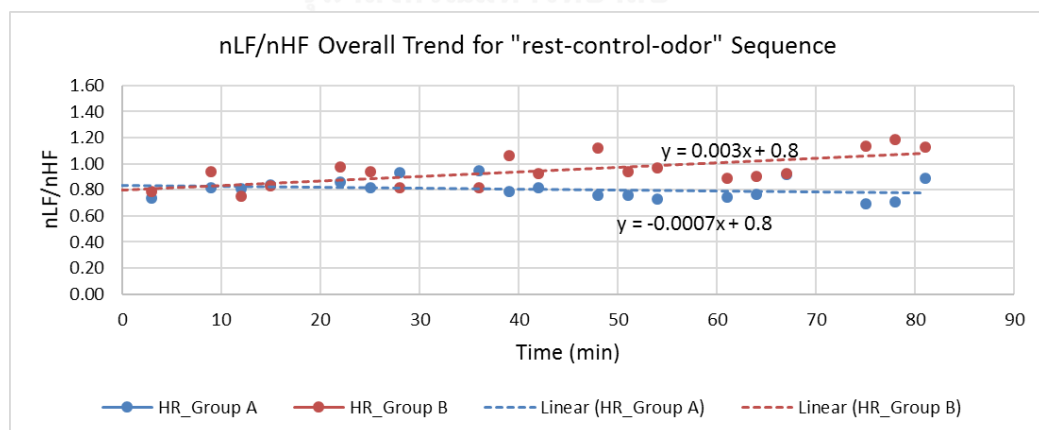


Figure A.11: nLF/nHF of the two female groups for all measurements

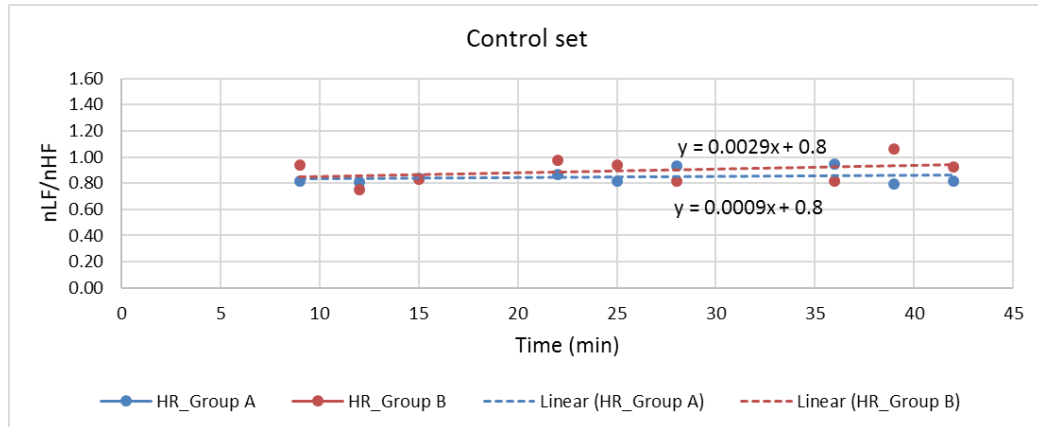


Figure A.12: nLF/nHF of the two female groups for control set

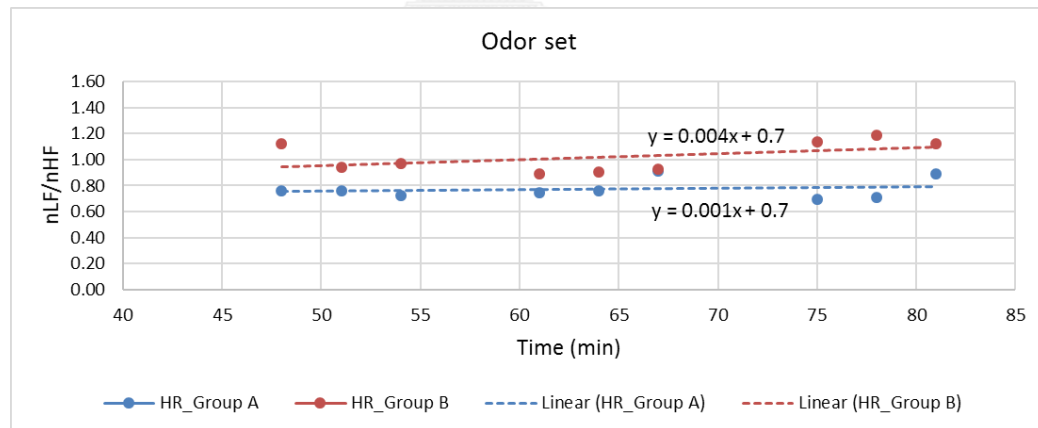


Figure A.13: nLF/nHF of the two female groups for odor set

A qualitative study on psychological stress condition using SRS-18 results also expressed that stress condition of Group A (about one-week closer to the menstruation period) was higher than the stress response of Group B (about one-week far from the menstruation period) as shown in Figure A.14. Although the stress condition of Group A was slightly decreased after odor exposure for 1-minute, 2-minute, and 3-minute, respectively. It was found that stress condition of Group A was still higher than that of Group B in each measurement condition (before odor exposure, after 1-minute odor exposure, after 2-minute odor exposure, and after 3-minute odor exposure).

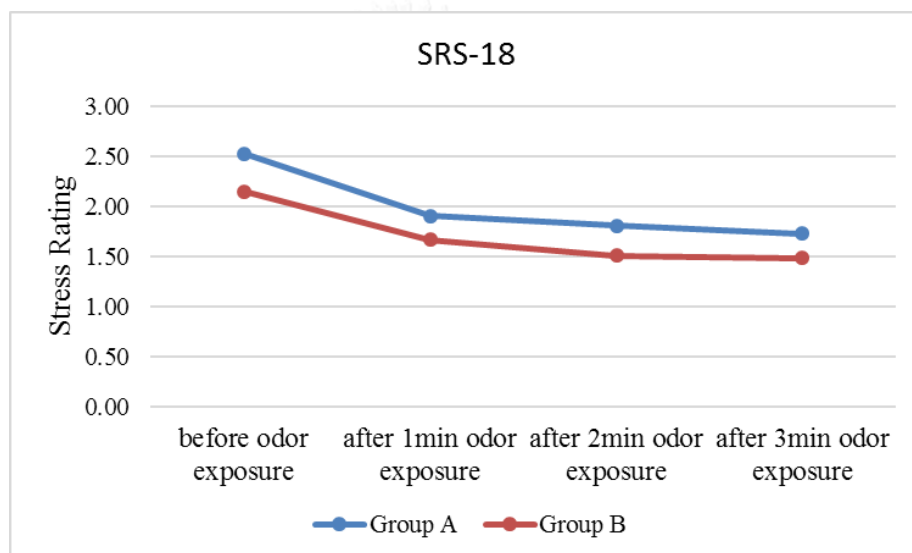


Figure A.14: SRS-18 score for the two female groups

VITA

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