Effects of Nap at Laboratory and Aroma at Home on Autonomous Nervous and Endocrine Systems

A thesis submitted in partial fulfillment of the requirements for the degree of

Doctor of Engineering

June, 2019

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Dedicated to all the scientists who have contributed and will continue to do so, for the advancement of the field of Ambient Biomedical Engineering

and thereby

for the betterment of the society

ACKNOWLEDGMENT

I would like to express my sincere gratitude to Shusaku Nomura, my academic advisor, for his high academic standards and sense of responsibility, his encouragement and constant guidance to pursue my Ph.D. and for his unwavering support throughout my doctoral studies.

I would also like to thank Prof. Koichi Yamada, Prof. Akira Shionoya, Associate Prof Tessai Hayama, and Associate Prof. Yuta Nishiyama for sharing their time and incredible expertise with me on reviewing my dissertation and for invaluable instructions and comments on improving my thesis.

It is a pleasure to work with such a dedicated and talented group. My research team members: Mr. Takemi Kobayashi, Mr. Takurou Ichihashi and other members of Ambient Biomedical Engineering Laboratory, Nagaoka University of Technology, is sincerely acknowledged for the for all the support provided for me during my study.

I am grateful for all the students of the Nagaoka University of Technology who agreed to participate in my studies and highly cooperated with us of our experiments.

I am also appreciative of the generous support I have received throughout my doctoral studies: The Japanese Government (MEXT) Scholarship, funded by the Ministry of Education, Culture, Sports, Science and Technology through which I received financial assistance for the last half years, which enabled me to study in Japan.

I want to extend a sincere note of gratitude to my family and parents, for their ongoing encouragement and for being my constant inspiration and strength.

My sincere appreciation also goes to my friends, who are my proud of my life.

ABSTRACT

We conducted a series of experiments to investigate the psycho-physiological effects of nap and aroma on human. A two-hour afternoon nap (Exp.1) and awakening after slow wave sleep (SWS) (Exp.2) were studied in a laboratory and aroma study in a field setting (Exp.3).

In Exp.1, forty-three male university students with normal sleep-wake cycles were assigned to two groups: group 1 slept for 6 hours from 0:00 to 6:00 a.m. and group 2 napped for 2 hours from 13:15 to 15:15. There was no prominent difference in the subjective quality of sleep between groups. This was supported by the lack of any significant difference in sleep efficiency and latency between groups. Additionally, the heart rate (HR) after the afternoon nap was significantly higher than the baseline level before nap (overshoot), while the heart rate in the morning was the same level as recorded the night before. In Exp.2, in a within-subjects experiment design, ten male students participated in two conditions: 1) Awakening in the middle of the slow wave sleep (AMS), i.e. sleep from 0:00 am and wake up at 15min after first time SWS occur and 2) Awakening at the end of the slow wave sleep (AES), i.e. sleep from 0:00 am and wake up at the end of first time SWS. Saliva samples were obtained every 30min during sleep and every 15min for 1 h after awakening. Our results indicated that the HR after awakening was significantly higher in two conditions and was lower in AMS than AES before getting up. Additionally, salivary Immunoglobulin A (IgA) and alpha-amylase (α-AMY) concentration in AES was higher in terms of the post-awakening mean peak levels, while cortisol and dehydroepiandrosterone (DHEA) were no significant increase after awakening from SWS. In Exp.3, twenty male students participated in the study and were exposed to three different odorants (apple, cedarwood, and citrus ginger) or a scentless control sample each night in their home

environment in a counterbalanced order. In this within-subjects study design, all subjects were instructed to sleep at home for 6 hours, from 00:00 to 06:00, and HR was measured using a wristwatch-type HR monitor. Saliva samples were obtained before sleep and after awakening. While subjective sleep quality at awakening did not differ among conditions, tension and anxiety, assessed by the Profile of Mood State questionnaire, was significantly decreased from the night before sleep to the next morning on which citrus ginger exposure was provided. Moreover, HR during sleep and after awakening, and cortisol secretion after awakening were significantly higher in the citrus ginger condition.

Through these researches, following findings were yielded: the relatively longer afternoon nap may be comparable with a night sleep in terms of sleep quality whereas it may enhance autonomic arousal after awakening, the sympathetic-adrenal medullary activity (SAM) was enhanced after AES, and citrus ginger aroma during sleep may enhance sympathetic nervous and endocrine system activity while alleviating psychological tension and anxiety. Future studies testing a wider range of aromas and their compounds are required to clarify the complex psycho-physiological effects of aromatherapy.

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CHAPTER 1
INTRODUCTION

1.1 Background

1.1.1 Sleep and Stress

Sleep is an important part of our daily life and it's a restorative process for the physical psychological and mental health, and insufficient sleep causes a variety of negative influence on our life and work, including stress [1-4], sleepiness[5], quality of life[6,7], school achievement and academic performance[8-10], cognitive, memory and learning capacity[11] and so on.

Furthermore, Insufficient sleep, poor sleep quality are common complaints in modern society. For instance, Wong reported that the prevalence of sleep quality problems among Hong Kong general population was 39.4% [6], other researches pointed out nearly 44% and over 60% of the sample reported the poor quality of sleep [4,12]. In addition, according to the social statistics studies among JAPAN: 2015 National Time Use Survey Conducted by The NHK Broadcasting Culture Research Institute [13] (Fig.1).sleep duration decreased with the years; The report by the Ministry of Health Labor and Welfare about National Health and Nutrition Survey 2017 indicated that Sleep duration very dissatisfied increased with years[14] (Fig 2.).

Moreover, suboptimal sleep and sleep-related disorders may be an indicator of poor health status [3,15]. Sleeping less than 6 h was greater risk of cold compared to sleeping more than 7h [16], sleep disturbances increase the risk of type 2 diabetes [17], insufficient sleep increases mortality risk[18], insomnia and sleep deprivation impairments in neurocognitive and psychomotor performance [19,20], behavior and neurobehavioral function [21], sleep disorders are associated with maladaptive changes in the hypothalamic-pituitary-adrenal (HPA) axis, leading to neuroendocrine deregulation[22] and chronic insomnia is a risk factor for anxiety and

depression[1,23]. In addition, poor sleep quality with low executive functions and reduce labor productivity [18,24] leading to large economic loss(Table 1)[18]. In short, these studies suggest that sleep problems should be carefully taken into consideration.

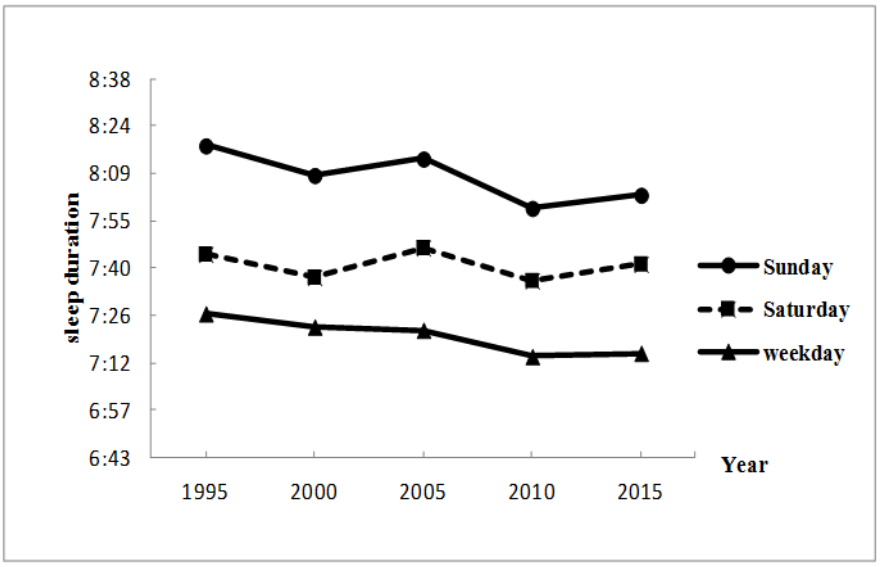

Figure 1: Changes in sleep duration

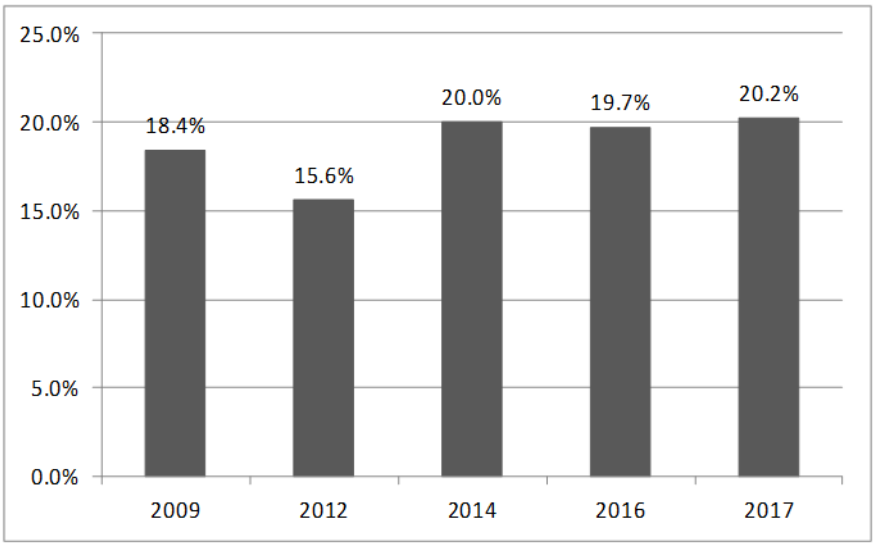

Figure 2: Percentage of sleep dissatisfied with years

Stress and sleep have a long time been known to bidirectional effects with each other [2-4]. Acute and chronic stressors were considered to be the most significant risk factor for sleep

disturbance, sleep architecture and circadian rhythms [2,25]. However, the amount of stress can have cardiovascular disease, sleepiness, fatigue and adversely affect the performance and neuroendocrine[4,7]. Additionally, more and more people under stress, Bhatia et al. studied showed that 87.4% nurses have occupational stress [26], Ministry of Health Labor and Welfare survey results demonstrate that the prevalence of stress in JAPAN was about 50%[27] (Fig3). However, experienced stress is not exclusively a Japan problem, and also concerns other countries [28] such as Greece (59%), Philippines (58%), Tanzania (57%), United States of American (55%) and so on (Table 2). Moreover, it is found that the cost loss of enterprises due to high stress may reach 1.5 million yen per person [29].

Table 1: Economic Costs of Insufficient Sleep across Five OECD Countries

	United States	**Japan**	**Germany**	**United Kingdom**	**Canada**
Economic costs	$411 billion	$138billion	$60billion	$50 billion	$21.4 billion
Percent of GDP	2.28%	2.92%	1.56%	1.86%	1.35%

It is quite clear that HPA-axis sensitivity to subsequent stressors. Moreover, It also plays an important role in the sleep-wake cycle, excessive activation of the HPA axis can dysfunction of the neuroendocrine induce sleep disturbances [2], while sleep loss and chronic stress can lead to metabolic dysfunction[22], suggesting that both sleep and stress are closely linked to the HPA axis[2,30], and a bidirectional interaction between sleep and endocrine activity is well documented, which confirmed the close interrelationship between sleep, stress, and metabolism[22].

Overall, insufficient sleep and stress are a public health problem and co-enact with each other have an effect on psycho-physiological, it’s not only person complaints and become to

social issues with a huge impact on economics. Therefore, it is essential to consider questions such as sleep timing, sleep duration, and sleep efficiency and as well as sleep architecture that can affect the quality of sleep, and it is important to tackle sleep problems early in order to prevent further deterioration, and we also should pay more attention to study the correlation with sleep and stress.

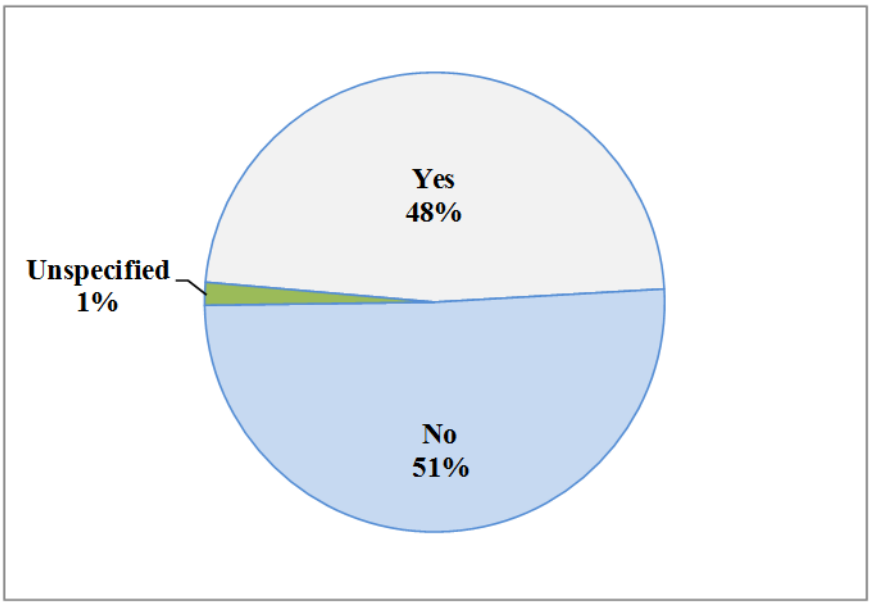

Figure 3: Do you have any stress in your life?

Table 2: Experienced stress a lot of yesterdays

Greece	59%
Philippines	58%
Tanzania	57%
Albania	55%
Iran	55%
Sri Lanka	55%
United States of America	55%
Uganda	53%
Costa Rica	52%
Rwanda	52%
Turkey	52%
Venezuela	52%

1.1.2 Stress reaction pathways and saliva biomarkers

1.1.2.1 Stress reaction pathways

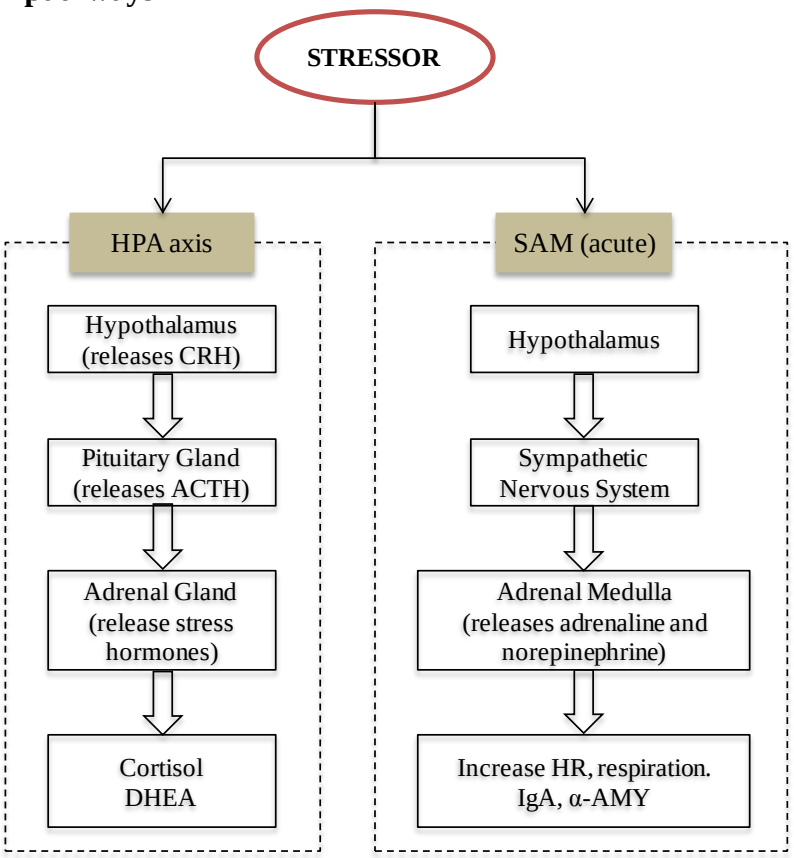

Figure 4: The stress response pathway

Stress is a feeling of being overwhelmed, worried or run-down. There are two main stress responses pathways. The acute stress response known as SAM responds very quickly to immediate danger, the hypothalamus activates sympathetic of the autonomous nervous system (ANS), which secretes adrenaline and norepinephrine, leading to increases sympathetic activity and decreases parasympathetic activity, such as, increasing HR and blood pressure, speeding breathing up. The activation of the HPA axis was considered to be one major physiological response elicited by stress. The hypothalamus releases CRH (corticotropin-releasing hormone) which travels through the bloodstream to the pituitary gland. The pituitary gland releases ACTH (adrenocorticotropic hormone) which travels through the bloodstream to the adrenal cortex which releases corticosteroids. Currently, mainly seven biomarkers have a close relationship

with mental stress [31], for example, cortisol, DHEA, DHEA sulfate (DHEA-s), IgA, α-AMY, testosterone(TE), and secretary chromogranin A(CgA) and so on.

1.1.2.2 Cortisol awakening response (CAR)

With regard to biomarkers, they are secreted into various human secretory substances such as saliva, blood, urine, hair, nail, and breast milk. Plasma can reflect acutely circulating hormone levels [32], however, the blood collection is under a stress by the needle punch and cannot correctly measure and evaluate the true stress state. In a urine test, it is difficult to detect immediate stress reaction, and it is necessary to measure metabolic substances. Hair and nail use to reflect the long term stress [33]. Considering saliva testing has the advantage that it is non-invasive, easy to make multiple samples and stress-free, besides salivary substances considered as a reliable measure for SAM and HPA axis. Amount of research has to apply saliva to an assessment of stress [31, 34-36].

Cortisol, a steroid hormone released from the adrenal cortex, is considered a reliable measure for the HPA axis[37-39], a temporal elevation of cortisol secretion is observed at awakening in the morning: this is termed the “CAR”.Much research has aimed at determining factors that influence the levels of CAR, but the results obtained are contradictory in age, gender, stress, and depression [37-47]. Additionally, the transition from sleep to waking is essential for CAR, with regards to effects of sleep-related factors on the CAR, such as sleep quality, sleep duration, nightly awakenings, and time of awakening results have been inconsistent. A larger cross-sectional study was done by Emily willians[48] group suggesting that the CAR is not associated with sleep quality. Whereas other studies pointed out poor sleep is correlated with decreased morning awakening salivary cortisol[49,50]. Sleep duration and time of awakening unrelated to morning cortisol levels were claimed [37,40]. However, sleep length research

showed that nap 90-min resulted in CAR while shorter naps of 50min duration did not [51], and nightly awakening in the early part of night sleep was no CAR [52]. Considering the role of time of waking for CAR, morningness had higher levels of free cortisol awakening responses than evening-oriented [42,48,53].

Unlike CAR, few studies pay attention to others salivary biomarkers on sleep study and discrepancy was observed, Hucklebridge documented no post-awakening peak of DHEA secretory activity [54]. Yet, one of our LAB studies reported marked elevation in DHEA after awakening [55,56]. The natural secretion of biomarkers has a diurnal change, the highest level is in the morning and the lowest level in the night [37,56,57]. On the other hand, salivary IgA and α-AMY concentration gradually increase while sleep was reported by Hasegawa[58].

Furthermore, sleep is characterized by non-REM sleep (NREMS) and rapid eye movement sleep (REMS), during the early nocturnal period the major portion is SWS [42-44,59]. However, reporting post-awakening cortisol after the early parts of sleep are sparse. One of study repeated nightly awakening during nocturnal, the result show cortisol levels during the first half of the night did not rise significantly after awakening in the night, while the cortisol levels were measured just two times (immediately and 15min after awakening)[52], Tobias Stalder demonstrate that two post-awakening samples cannot be sure to catch the CAR peak or may lead erroneous conclusions[60].

1.1.3 Benefits and discrepancies of Nap

Excessive daytime sleepiness was common, It's known that 41.9% of students who had daytime sleepiness in Hong Kong[25], and approximately 45.7% of adolescent report daytime sleepiness[8], Sleepiness and dip in performance in the afternoon, or "post-lunch dip," occurs regardless of meal consumption [61], thus it is considered to be a part of the biological rhythm

[62]. This circasemidian deterioration of cognitive or physical performance can be a menace to our daily life, e.g. high incidence of sleep-related accidents in the early afternoon [63].

One of the promising countermeasures for the post-lunch dip is a short nap, a nap of less than 30 min [64]. There have been extensive studies reporting the efficacy of a short nap, such as reducing sleepiness [65-70] improving cognitive and behavioral performance [66-71] including evidence from practical activities such as driving [65] and intravenous insertion [72]. Short naps are associated with physiological arousal after awakening, including an increase in the HR toward self-awakening [69], suppressed electroencephalogram (EEG) alpha activity during eyes-open wakefulness [65,66] , prevention of the occurrence of slow eye movement [70], shortening of event-related brain potential (ERP) latency [67].

A relatively longer nap, a nap of 60 min or more, has also been reported to have positive effects, such as ameliorating sleepiness caused by a night shift [73] or sleep loss [74], and improvement of cognitive performance [73-75] and memory [76].

However, while there are a number of studies reporting the behavioral and cognitive efficacy of long naps, reports on the benefit of long naps on physiological functioning are limited and sometimes controversial. There are studies illustrating higher physiological arousal in terms of cardiac [73] and endocrine [74] function following a long nap. Meanwhile, an observational study comparing cardiovascular activation between a long nap (siesta) and a night sleep reported reduced physiological arousal following the long nap compared to the night sleep [77]. With regard to the most recent epidemiological studies, one study has reported the association of hypertension with a midday siesta [78], while another study concluded that an hour of sleep in the daytime is associated with a reduced likelihood of hypertension in men [79].

1.1.4 Benefits and discrepancies of Aromatherapy

Aromatherapy has been widely used in complementary and alternative medicine. According to a survey reported by the Aroma Environment Association of Japan, the aroma-related market in 2015 amounted to 333.7 billion yen (126% increase over 2011), and the aromatherapy market to 60.9 billion yen [80].

Aromatherapy has also been increasingly studied through scientific research. Previous studies showed that lavender aroma contributed to improved sleep quality [81], decreased blood pressure [82,83], and improved task performance [84], and relieved job-related stress [85]. Jasmine odor led to greater sleep efficiency, alertness, mental performance, and reduced sleep movement [86]. The natural essential oil of orange could reduce salivary cortisol and pulse rate [87]. Apple aroma was shown to reduce tension, anxiety, and EEG theta activity [88]. Ginger has led to pain relief [89]. Cedrol inhalation induced an increase in parasympathetic activity and a reduction in sympathetic activity [90].

However, despite some studies indicating the positive effects of aromatherapy on the body and mind, several studies also reported inconsistent effects. One study reported that orange aroma reduced anxiety and improved mood in patients waiting for dental treatment [91]. Meanwhile, when the study was repeated in a dental clinic, no anti-anxiety or mood improvement effect of the orange aroma was found [92]. Apple aroma reduced tension and anxiety in one study [88], whereas another study reported no significant effects of the aroma on anxiety or mood [92]. Some studies reached inconsistent conclusions on the effects on the body and the mind. Cedarwood significantly inhibited the physiological stress response. However, this was not accompanied by any positive mood or sedative effects [93,94]. In a previous study, we found that mild orange essential oil aroma inhibits the cardiac stress response against a

short-term acute stressor, while it does not have a significant effect on subjective stress [95]. In contrast, lavender lowered the subjective stress and enhanced the physiological stress response against the same acute stressor [96].

Various reasons can cause the inconsistencies in reported aroma efficacies, such as differences in aroma administration (aroma diffuser, aroma infiltrated mask or olfactometer), the aroma intensity (e.g. dose, duration, repetition), and the study population (e.g. age, gender, health status). In particular, the sleep environment is likely to have a profound effect. An aroma study conducted in a clinical or laboratory setting induces more stress on the participants than a study conducted at home or in a familiar environment. A study evaluating the effects of aroma massage with lavender oil on sleep in children with autism attending a residential school failed to find any beneficial effect on sleep patterns [97].

1.2 Motivation

For longer afternoon nap, the studies investigating the physiological effects, inconsistent was found not only among psycho-behavioural experimental studies but also in the more recent large scale field studies. Additionally, there are few studies directly comparing the physiological impact of sleep and awakening between the normal nocturnal sleep and the long nap in the afternoon. Moreover, most research has focused on the HPA axis, while stressing hormones related to the ANS is less well known and only a small number of sleep studies with four stress biomarkers simultaneously. Particularly, the profile of saliva hormone levels after the awakening of SWS during nocturnal sleep has not been tested. Therefore, it should be important to evaluate the effects of acute partial deprivation of nocturnal sleep with 1h post-awakening saliva collection. Although a wide variety of physiologically positive effects of aroma have been reported by clinical or laboratory studies as stated above, the outcomes are frequently

inconsistent, and the physiological effect of aroma use at home has not been thoroughly investigated. Therefore, it should be important to evaluate the efficacy of aroma in terms of physiological effects in the home environment.

1.3 Research Framework and Objectives

1.3.1 Research Framework

Based on a comprehensive review of literature on sleep and stress-related substances, motivated by these issues and identified limitations, to investigate the effects on psycho-physiological of short nap and aroma on human, we did a series of experiments (Fig 5). A 2h afternoon nap (Exp.1) and early night SWS (Exp.2) were scientific laboratory studies with polysomnography(PSG) and electrocardiogram (ECG) recording throughout the experiment sessions and another one is a field study of aroma on sleep (Exp.3) in applied science. Considering the physiological measures that are accessible within the home environment with minimum intervention, a wristwatch-type (Exp.3) HR measurement device was employed to monitor the HR during sleep and after awakening. Salivary secretion was assessed to evaluate physiological (endocrine) arousal, before sleep and after awakening, naturally secreted saliva accumulated during a 3-min period(Exp.2, Exp.3), and during sleep, saliva was collecting continuously by using the peristaltic pump system(Exp.2).

1.3.2 Objectives

For nap sleep, to investigate the physiological impact of the long nap, of 120 min, in the afternoon on sleep architecture, cardiac autonomous nervous system, and subjective quality of sleep and mood.

For slow wave sleep, to further clarify the physiological impact of SWS on the ANS, HPA axis and SAM activation with four salivary biomarkers, which salivary cortisol, DHEA,

IgA, and amylase were assayed as indexes of the HPA, and SAM system, respectively.

For aroma study, to evaluate the physiological effect of aroma at home with three different aromas: apple, cedarwood, and citrus ginger.

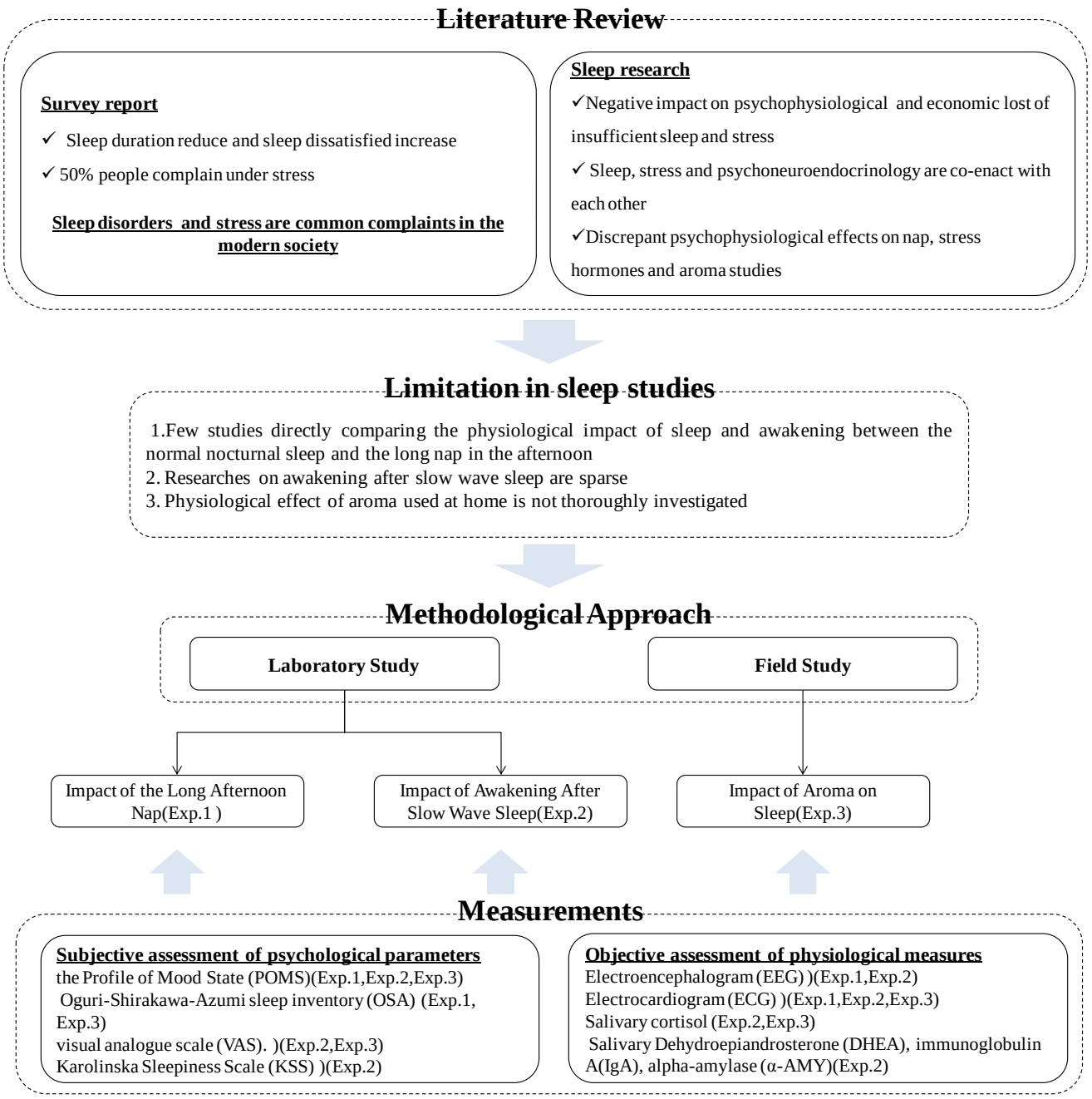

Figure 5: Research Framework

1.4 Chapter Summary

In this chapter, based on scientific evidence from the survey and literature, the issues on sleep and mental health have always been pressing. On the other hand, both sleep and stress are closely linked to the HPA axis and confirming the close interrelationship between sleep, stress, and metabolism. While inconsistent and limitations were found on nap studies and aroma on psycho-physiological effects. Addressing the limitations, and to investigate the psycho-physiological effects of nap and aroma on human three experiments were did. Two laboratory studies: A two-hour afternoon nap (Exp.1) and awakening after SWS (Exp.2) with four salivary biomarkers (cortisol, DHEA, IgA, and amylase) were assayed as indexes of the HPA and SAM system and aroma study in a field with three different aromas, apple, cedarwood, and citrus ginger (Exp.3).

CHAPTER 2
IMPACT OF THE LONG AFTERNOON NAP

2.1 Subjects

Forty-three university male students (mean age of 21.6 ± 1.10 years and mean body mass index of 20.8 ± 2.45 kg/m^2) participated in the study. All participants were healthy with normal sleep-wake cycles, and no history of sleep-related disorders. Specifically, none of the participants in this study was suspected of obstructive sleep apnea. The study was conducted in accordance with the ethical principles of the Helsinki Declaration and informed consent was obtained from all participants. The study was approved by the ethics committee of the Nagaoka University of Technology.

2.2 Methods

2.2.1 Experimental Procedure

In a between-subjects experiment design, participants were assigned to two groups: 1) Night Sleep (NS) group in which participants underwent 6 hours of nocturnal sleep (n = 17) and 2) Long Nap group (LN) in which participants underwent a 2-hour nap in the afternoon (n = 26). There was no significant difference in the age or BMI ($p > 0.05$) between groups. Fig. 6 and Fig. 7 show the protocol and layout of the experiment in this study respectively.

All participants underwent sleep control at their home at least 7 days before the experiment, where they were instructed to go to bed before midnight and to get up after 6:00 a.m. in the morning. Daytime napping was not allowed during the sleep control period. No sleep deficit was introduced for the night before the experiment in either group. In the days before the experiment, all participants underwent overnight PSG recordings in the laboratory room to adapt the laboratory setting to avoid the first-night effect (unfamiliar environment of a sleep laboratory

and sleep disorder). The subjects kept sleep diaries and wore wristwatch-type wearable device to identify sleep-wake pattern during sleep control.

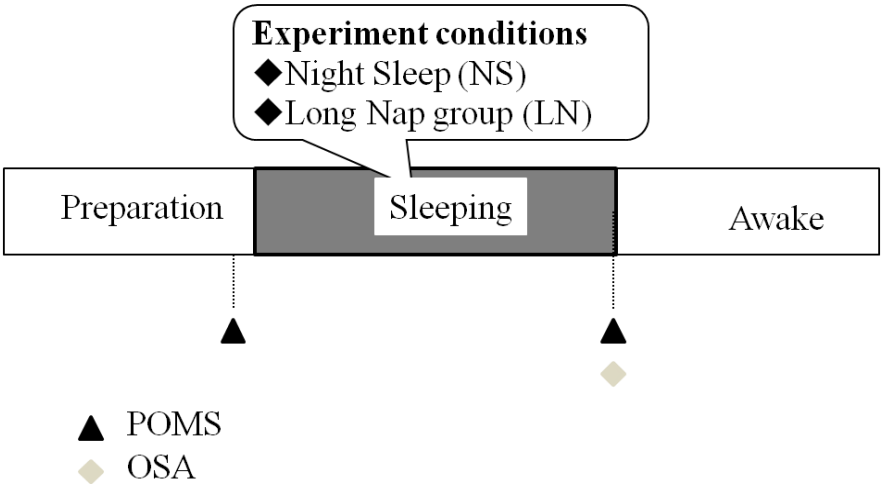

Figure 6: Schema of the experimental design (Exp.1)

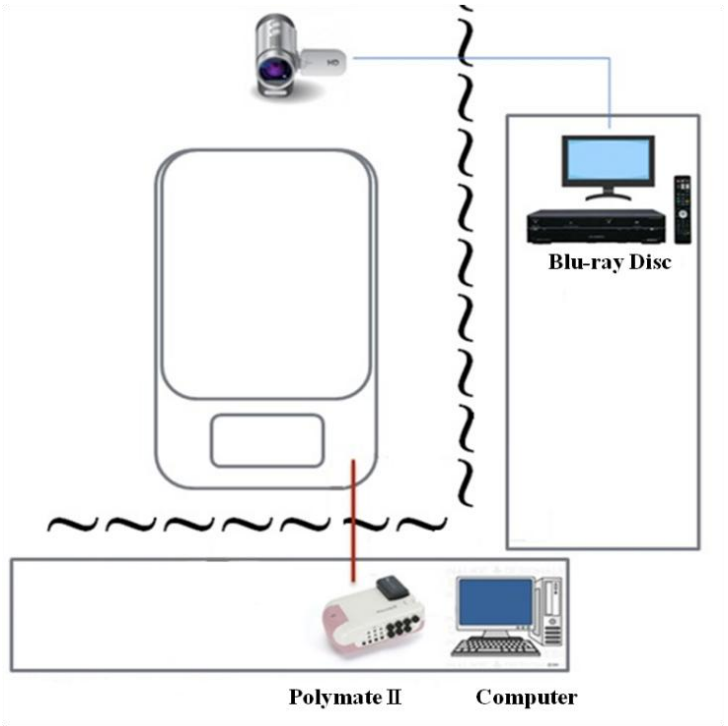

Figure 7: Experimental layout (Exp.1)

On the day of the experiment, participants in the NS group visited the laboratory room at

22:30. After the preparation for PSG and ECG recording, participants were instructed to take a full night sleep from 0:00 am. At 6:00 a.m. the following morning, participants were awakened by an experimenter. In the LN group, participants visited the laboratory room at 12:30, and after PSG and ECG recording preparation, they were instructed to take a 2-hour nap from13:15 to 15:15. Participants were instructed to stay lying on the bed even if they awoke before 15:15, and they were awakened at 15:15 if they kept sleeping beyond the time. The experiment was carried out daily (one participant a day) in a soundproof and environment-controlled room (mean temperature and humidity is 25℃ and 53%).

2.2.2 Measurements

2.2.2.1 Subjective assessment of psychological parameters

Participants were asked to complete the profile of mood state (POMS) as the measure of psychological mood state before sleeping and after awakening. POMS is a measure of 6 identified mood factors, i.e. tension-anxiety (T-A), depression-dejection (D), anger-hostility (A-H), vigor (V), fatigue (F), and confusion (C) [98]. Subjective sleep quality was assessed after awakening using the Oguri-Shirakawa-Azumi sleep inventory (OSA) [99]. OSA contains five mood factors, for example, sleepiness on rising (Factor I), initiation and maintenance of sleep (Factor II), frequent dreaming (Factor III), refreshing (Factor IV), and sleep length (Factor V).

2.2.2.2 Objective assessment of physiological measures

In order to record PSG and ECG throughout the experiment sessions, i.e. 6-h night sleep in the NS group and 2-h afternoon sleep in the LN group, Polymate II(Polymate II, TEAC company, Tama, Tokyo) is used in this experiment. Throughout the study, international 10-20 systems (The 10-20 system refers to the distances between adjacent electrodes are either 10% or 20% base on the standard landmarks of the skull, which are the nasion, inion, and the left and right

preauricular point) [100] for EEG were continuously recorded from electrode positions C3, C4, O1 and O2, ECG, electrooculogram (EOG) and electromyograms (EMG) were also recorded. The electrodes were affixed to the participants as below in Fig.10.

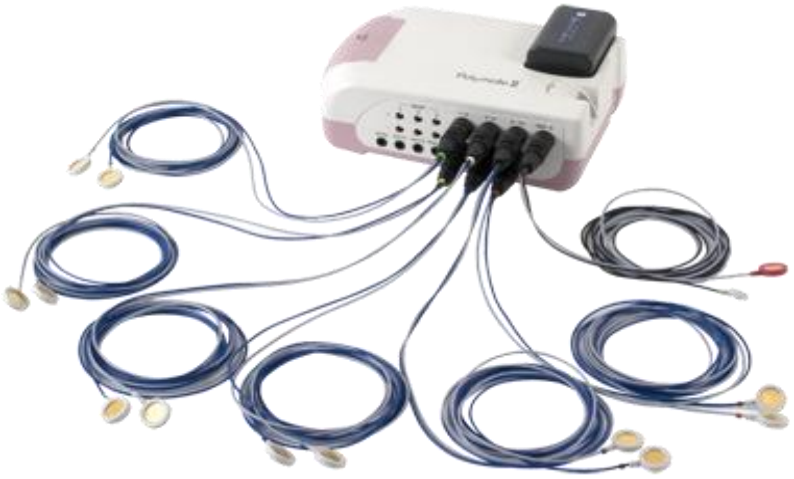

Figure 8: Polymate II

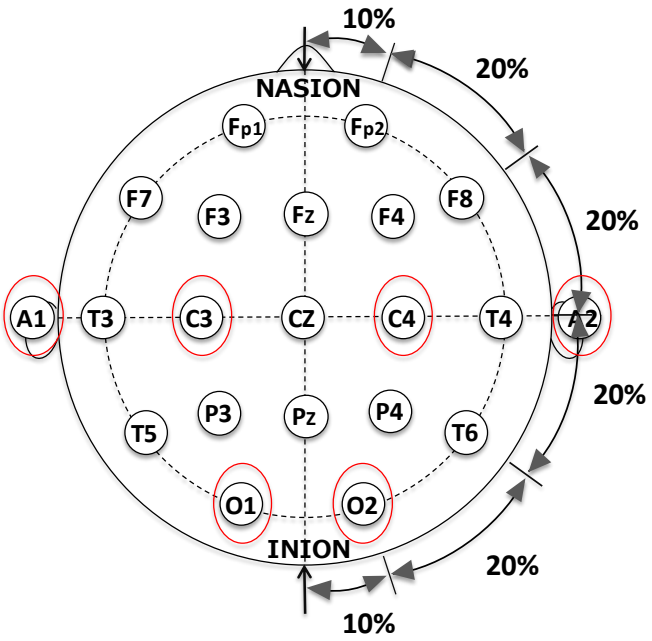

Figure 9: 10-20 electrode system

The sleep stages were manually scored at consecutive 20-s intervals from the PSG

recordings with AP view software according to Rechtschaffen and Kales' criteria [101-103]. In the R&K scoring manual, NREMS was divided into sleep stages 1, 2, 3, and 4 and rapid eye movement (REM) sleep. Stages 3 and 4 are combined into stage 3(deep sleep) according to the American Academy of Sleep Medicine (AASM) Manual. Some of EEG patterns important for sleep staging are listed in Fig.11-17 and Table 3 [103], ex: Alpha waves, Sleep spindle, K complex, Vertex sharp, Wave Slow waves and so on. EOG is for eye movement, there are reading eye movement (Fig.11), and slow eye movement (Fig.12), REM (Fig.16) and eye blinks, the detail was depicted in Table 4.

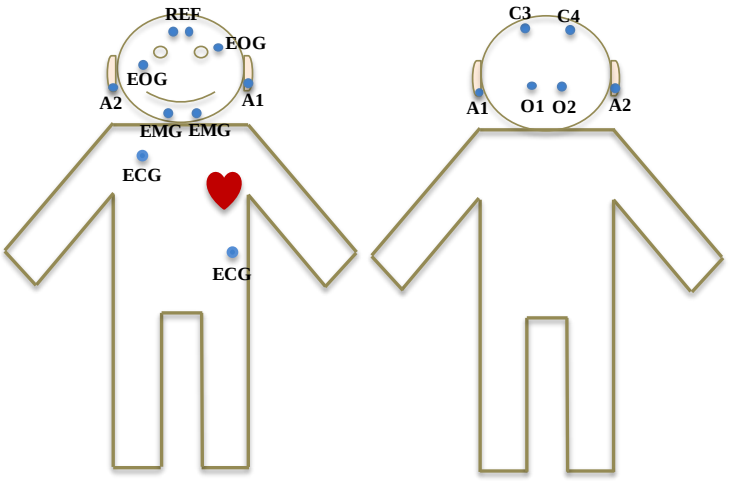

Figure 10: Electrode placement

Table 3: Summary of important waveform characteristics

	Alpha Rhythm	Sleep Spindle	K-Complex	Vertex Sharp Wave	Slow Wave Activity	Saw-Tooth Waves
Frequency (Hz)	8–13	11–16	N/A	N/A	0.5–2	2–6
Amplitude/ shape	Oscillation	Spindle-shaped oscillation	High amplitude (usually >100 µV)	sharp wave	High-amplitude broad wave >75 µV peak to peak	Triangular, serrated
Duration	Variable	≥0.5	>0.5 sec	<500 msec	0.5–2 sec	Variable
Associated sleep stages/ events	Stage W Stage N1 Stage R Arousals	Stage N2 Stage N3	Stage N2 Stage N3	Stage N1	Stage N2 Stage N3	Stage R

EEG = electroencephalogram; N/A = not applicable.

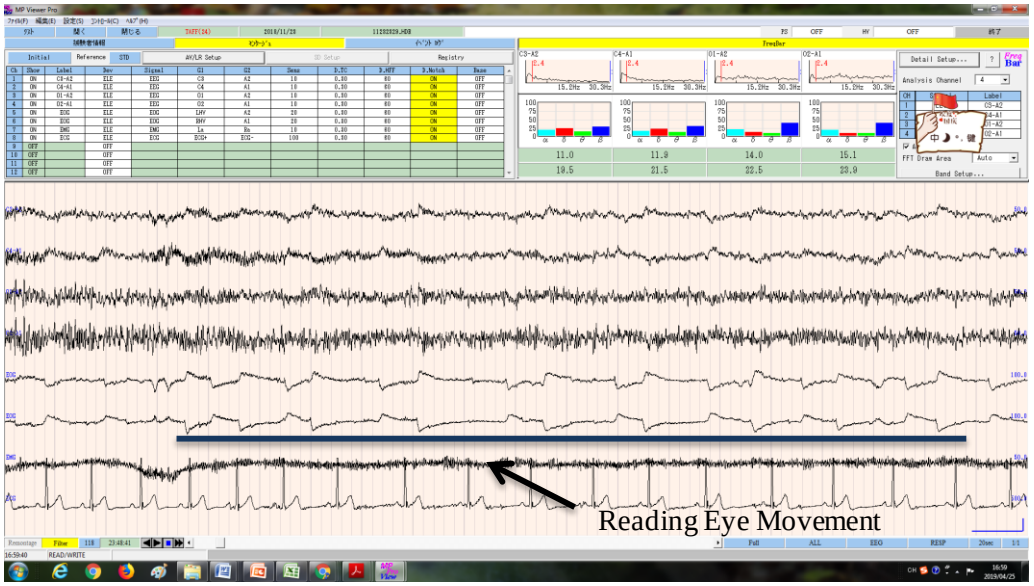

Figure 11: Reading eye movement

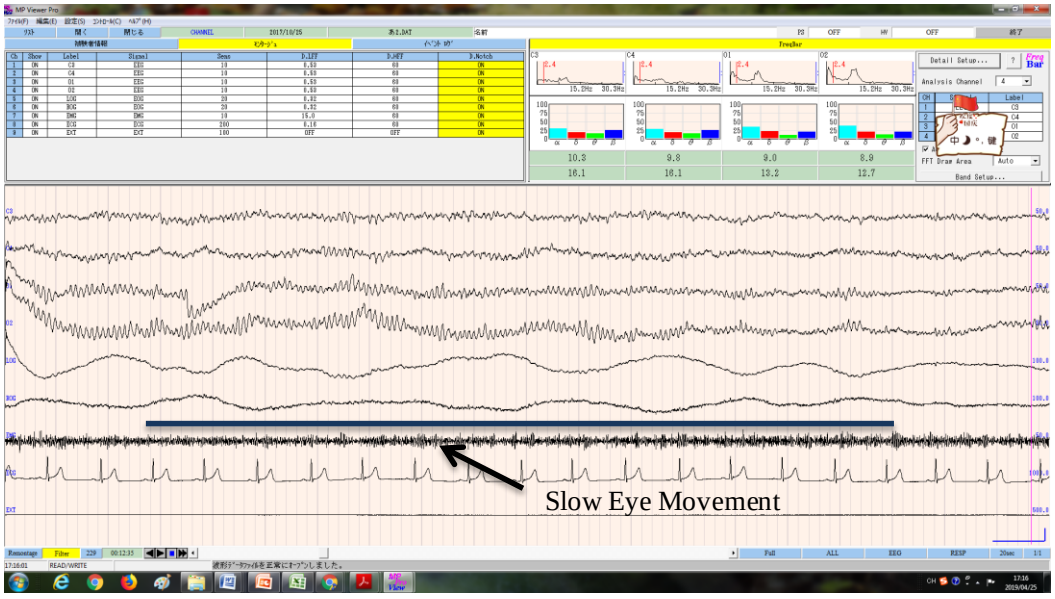

Figure 12: Slow eye movement

Table 4: Eye Movements Pattern Definitions

Slow eye movements	Rapid eye movements (REMs)	Reading eye movements	Eye blinks
Conjugate, fairly regular, sinusoidal eye movements lasting > **500 msec.**	Irregular sharply peaked eye movements lasting < 500 msec.	Trains of conjugate eye movements consisting of a slow phase followed by a rapid phase in the opposite direction during reads.	Conjugate vertical eye movements at a frequency of 0.5–2 Hz, in wakefulness with the eyes open or closed.

International criteria for sleep stage scoring [101-103] and percent of each stage [104]. The sleep stages were manually scored at consecutive 20-s sequential epochs if in one epoch come with two or more stages, the greatest portion was scored.

1. Wake (Fig.13) generally constitutes approximately less than 5% of sleep.

   A) Subjects whose central alpha rhythm was more than 50% of the epoch when the eyes are closed.

   B) If without visually alpha rhythm:

   - Eye blinks
   - Reading eye movements
   - Irregular conjugate REM-associated with normal or high chin muscle tone

2. Stage 1(Fig.14) generally accounts for approximately 2% to 5% of total sleep.

   A) Subjects whose central alpha rhythm was less than 50% of the epoch when the eyes are closed.

   B) If without visually alpha rhythm:

   - Low-amplitude mixed-frequency (LAMF) activity (range of 4 to7 Hz) compared to stage W

- Vertex sharp waves
- Slow eye movements

3. Stage 2(Fig.15) generally accounts for approximately 50% of total sleep.

    - One or more K complexes
    - One or more of sleep spindles
    - Low EMG

4. SWS (Fig.16) generally account for approximately 20% of sleep and predominate in the first third of the early night sleep.

    - More than 20% slow wave
    - Low EMG

5. REM sleep (Fig.17) generally accounts for approximately 25% of sleep and predominates in the last third of night sleep.

    - LAMF activity
    - Low EMG
    - REM

6. Body Movement (Fig.18) is judged with higher muscle tones movement and over 50% of the epoch was obscured by EMG artifact.

A) If alpha rhythm in the epoch, judge as stage W

B) If no alpha rhythm precedes and follows the epoch judge as stage W, the epoch was also scored as stage W.

C) Otherwise, the epoch was judged at the same stage as follows epoch.

Scoring was conducted by two well-trained experimenters separately and was double checked afterward. Sleep architecture variables, which include minutes per stage, percentages of each stage and body movement, sleep latency (SL) which is the duration of time from lights out to the onset of sleep (wake to stage 1), time in bed (TIB) which means the duration of time from lights out to get up, total sleep time (TST) which is the amount of sleep time (stage 1, stage 2, SWS, and REM) and sleep efficiency (SE), were calculated. SE is a percentage reflecting the amount of time spent asleep relative to the total time in bed.

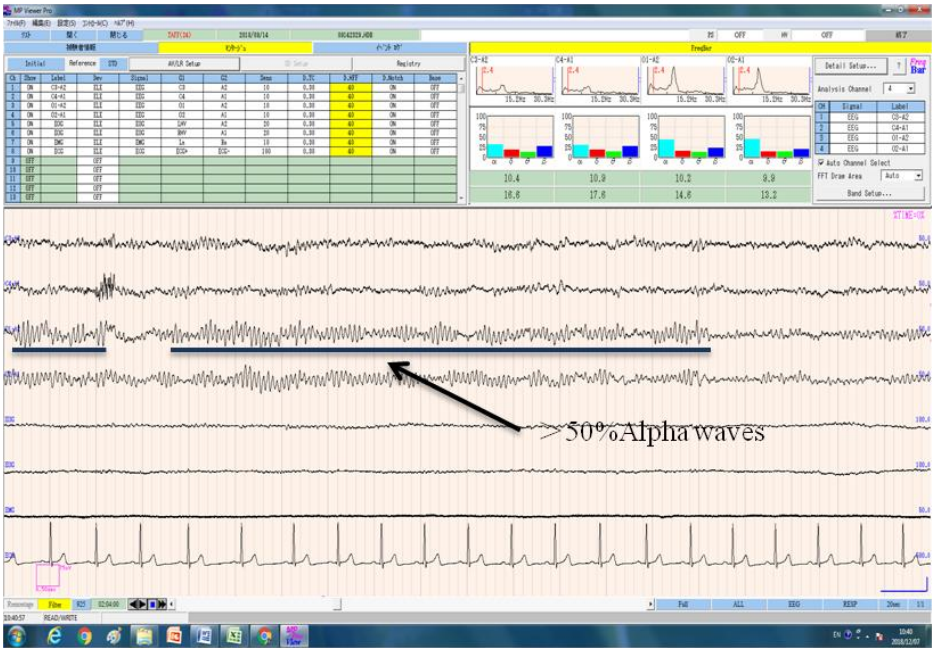

Figure 13: Wake

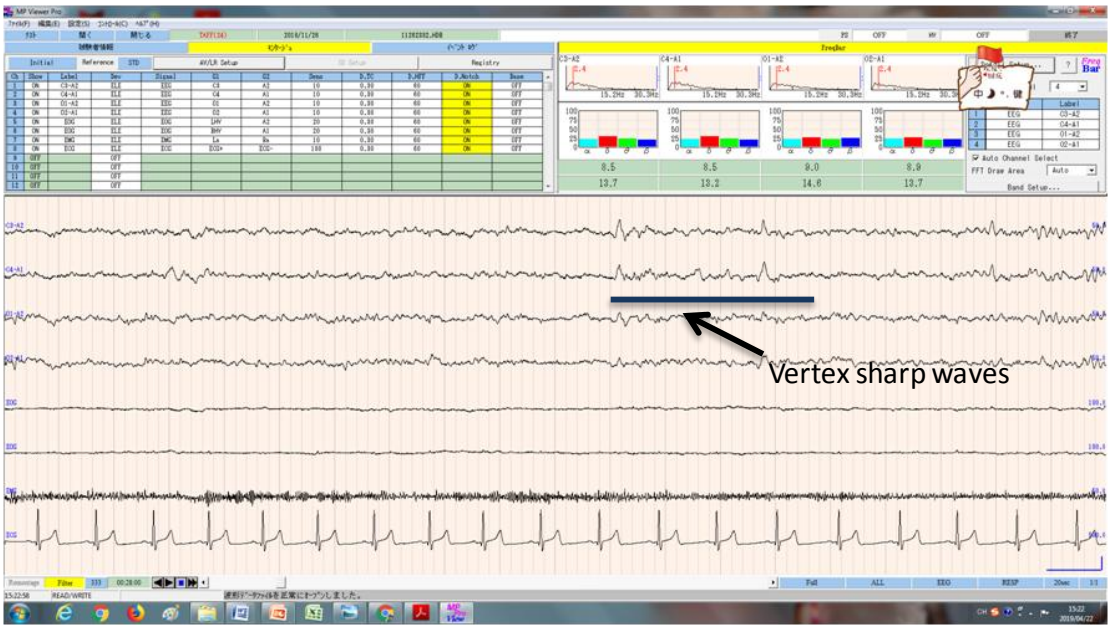

Figure 14: Stage 1

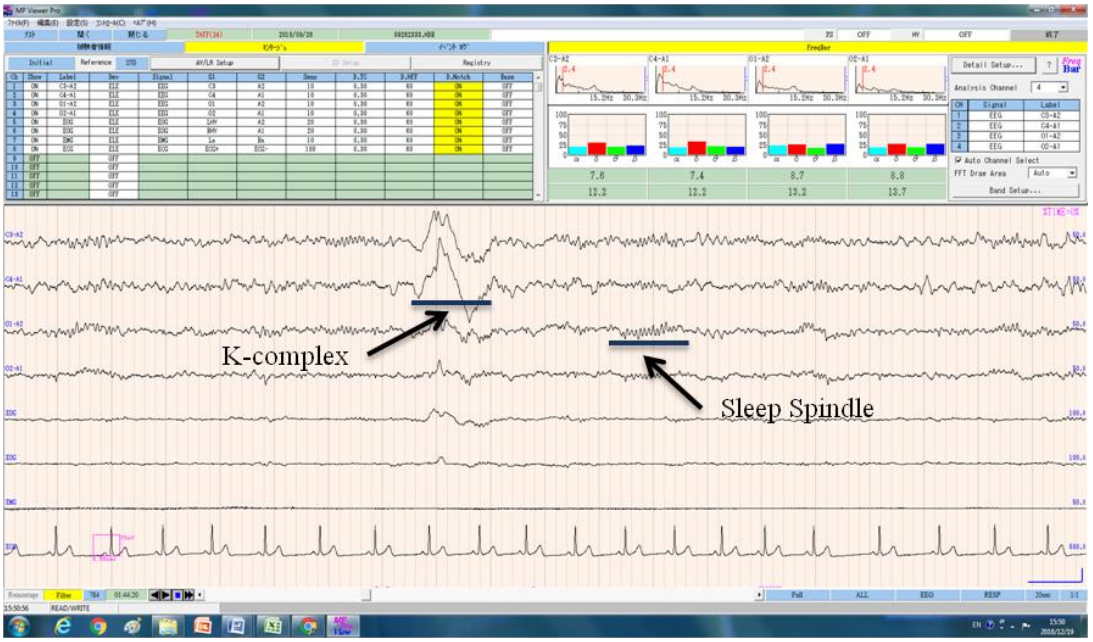

Figure 15: Stage 2

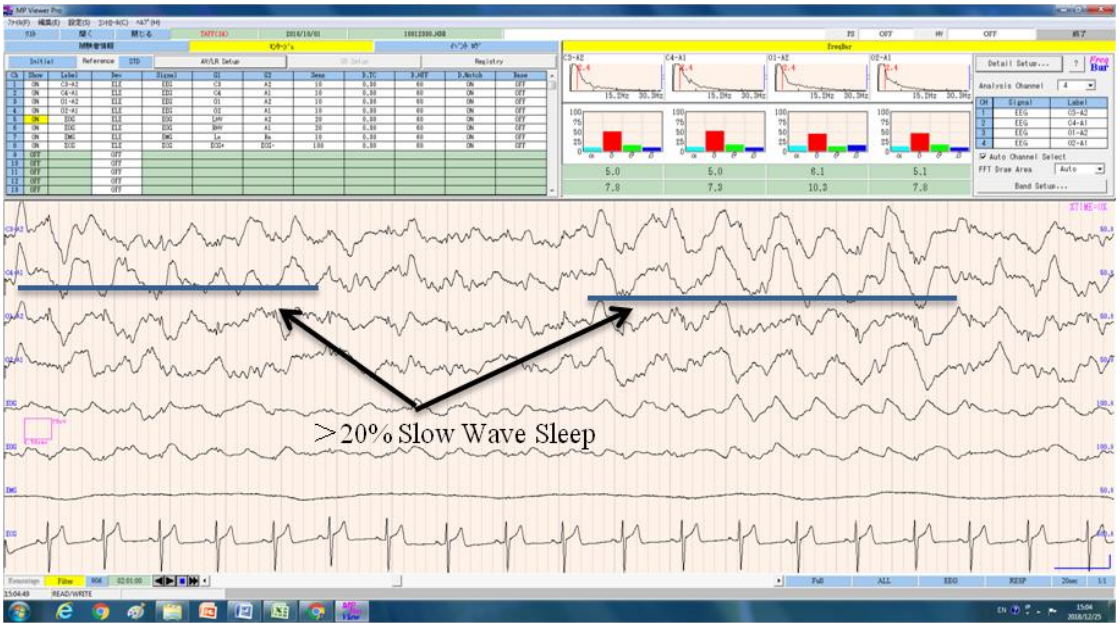

Figure 16: Slow wave sleep

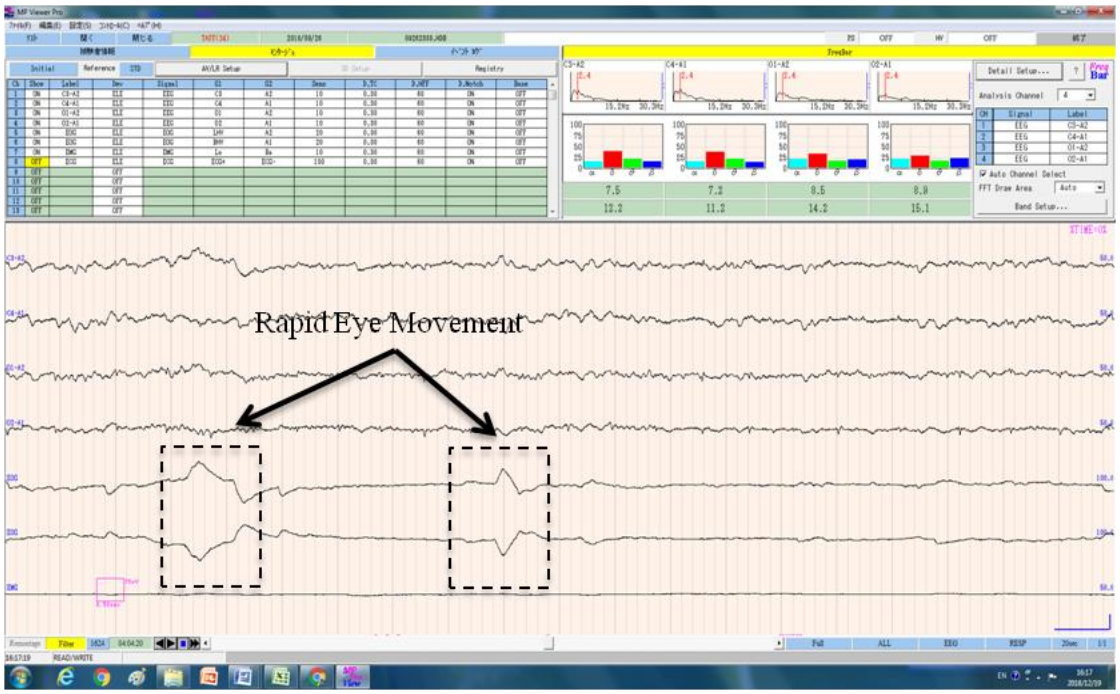

Figure 17: Rapid eye movement

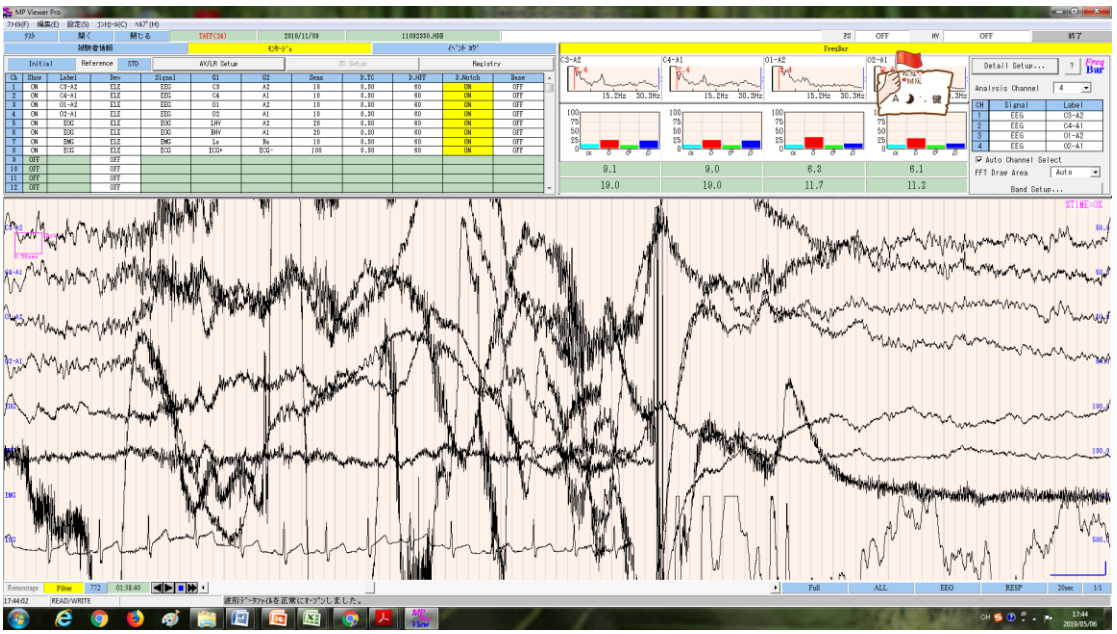

Figure 18: Body movement

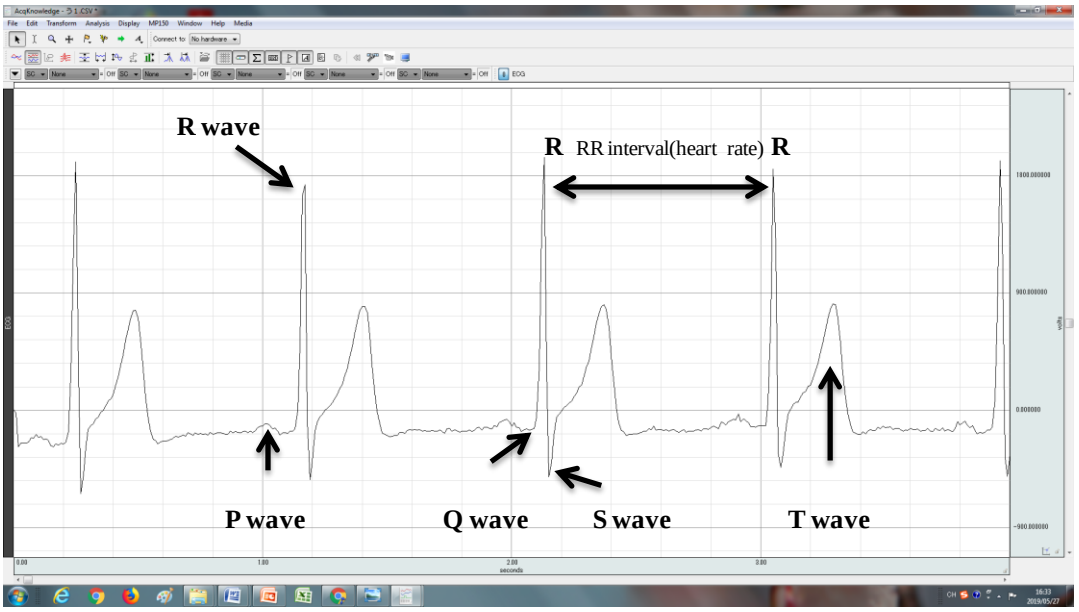

Figure 19: The normal ECG with component waves labeled

Using the ECG data, the HR and heart rate variability (HRV) which is a frequency domain of the heartbeat in a time series were analyzed. HR is measured in beats of the heart per minute (BPM). HRV is a variation in the time interval between hearts beat to beat. The most widely used methods are time-domain and frequency-domain. HRV two main spectral components: a high-frequency (HF) component (0.15-0.40 Hz), and a low-frequency (LF)

component (0.04-0.15 Hz), respectively considered markers of parasympathetic and sympathetic [105]. HR and the HF component of HR variability were recorded at 1-min intervals.

2.2.3 Statistics

Comparing within- and between-conditions, paired and independent t-test was performed. P value below 0.05 was assumed to indicate statistical significance.

2.3 Results

2.3.1 Sleep architecture

Table 5 shows sleep variables in the NS and LN groups. Mean TSTs were 319.8 min in the NS group and 91.8 min in the LN group. The percentage of time in the wake and stage 1 period was significantly larger in the LN group than in the NS group ($p < 0.01$-0.001). The percentage of time in the SWS, REM sleep and move periods were significantly smaller in the LN group than in the NS group ($p < 0.001$). Although these findings illustrate neurologically shallow sleep in the LN group, there was no significant difference in the percentage of stage 2, SE, and SL between the groups.

2.3.2 Psychological measures

Table 6 shows scores of OSA following awakening in the NS and the LN group. There were significant differences in factor II (initiation and maintenance of sleep; $p < 0.01$) and in factor III (frequent dreaming; $p < 0.05$). No significant difference was observed in other factors between groups.

Table 7 shows the change in POMS scores from before sleep and after awakening in the NS and the LN group. There were no significant differences found among factors between groups.

Table 5: Results [mean (SD)] of sleep architecture in NS and LN group

	NS	**LS**	***p*-value**

	min	%	min	%	
Wake	30.6 (29.4)	8.5 (8.2)	28.3 (25.7)	23.5 (21.4)	0.003
stage1	30.3 (20.7)	8.4 (5.7)	33.0 (18.6)	27.4 (15.4)	0.000
stage2	157.2 (33.7)	43.7 (9.4)	47.1 (26.0)	39.3 (21.7)	n.s
SWS	61.7 (26.8)	9.7 (4.1)	4.2 (9.5)	3.5 (7.9)	0.000
REM	70.7 (18.5)	17.1 (7.5)	7.5 (9.9)	6.3 (8.2)	0.000
Move	9.6 (8.9)	2.7 (2.5)	0.0 (0.1)	0.0 (0.1)	0.001
Total	360.0	100	120.1	100.0	
Total sleep time (TST)	319.8 (30.3)		91.8 (25.6)		
Sleep efficiency (SE)		90.8 (5.9)		82.7 (21.2)	n.s.
Sleep latency (SL)	12.1 (13.8)		9.5 (8.7)		n.s.

Table 6: Results [mean (SD)] of OSA sleep inventory after awakening in NS and LN

OSA	**NS**		**LS**		***p* value**
Factor I (sleepiness on rising)	17.70	(5.34)	17.25	(5.53)	n.s.
Factor II (initiation and maintenance of sleep)	17.76	(3.37)	12.60	(6.91)	0.005
Factor III (frequent dreaming)	25.07	(5.68)	20.20	(7.59)	0.033
Factor IV (refreshing)	16.98	(4.76)	17.36	(7.58)	n.s
Factor V (sleep length)	15.43	(5.27)	13.72	(7.94)	n.s

Table 7: Results [mean (SD)] of the change in POMS from before sleep to after awakening in NS and LN group.

POMS	NS		LS		*p* value
T-A (Tension-Anxiety)	-0.71	(2.42)	-0.54	(2.25)	n.s
D (Depression-Dejection)	-0.47	(1.62)	0.42	(1.59)	n.s
A-H (Anger-Hostility)	-0.06	(1.85)	0.33	(0.76)	n.s
F (Fatigue-Inertia)	-0.41	(3.08)	-0.50	(3.01)	n.s
V (Vigor-Activity)	0.12	(2.00)	0.38	(2.65)	n.s
C (Confusion)	0.06	(1.64)	0.08	(1.41)	n.s

2.3.3 Physiological measures

2.3.3.1 HR

Fig. 20 shows the change in HR from sleep to awakening in the NS and the LN group. Note that the horizontal axis (time) is depicted with a different time scale for the NS (lower axis) and the LN (upper axis) groups in order to coordinate the timing of sleep onset and awakening; it is simply made to illustrate the profile of HR in the time scale should not affect the statistical analysis and interpretation of this manuscript. Each value in the NS and the LN represent the averaged HR for 10 and 3 min, respectively.

Comparing within conditions, there was a significant decline in HR from the onset of sleep to the stable state during sleep ($p < 0.01$ where HR at the sleep onset vs mean HR from 30 min after the sleep onset to before awakening) and a significant increase in HR from stable sleep state to after awakening ($p < 0.01$ where mean HR from 30 min after the sleep onset to before awakening vs HR at awakening) in both conditions. Additionally, it is noteworthy that the prominent increase of HR after awakening in the LN group ($p < 0.01$ where mean HR for 30 after awakening vs HR at the sleep onset) reached approximately 10 bpm above the baseline level (overshoot) at its peak and this level was maintained for at least 30 min, whereas the increase of

HR in the morning in the NS group did not significantly exceed the HR of the night before ($p > 0.05$ where mean HR for 30 after awakening vs HR at the sleep onset). Comparing between conditions, there was a significantly larger decrease in HR before sleep to during sleep in the NS group than in the LN group ($p < 0.01$ where the decline in HR from the sleep onset to the stable state during sleep [30-120 min after the sleep onset] in NS group vs that in LN group).

2.3.3.2 HF component of HR variability

Fig. 21 shows the change in the HF component of HR variability from sleep to awakening in the NS and the LN groups. Note that the horizontal axis (time) is depicted with a different time scale for the NS (lower axis) and the LN (upper axis) groups in order to coordinate the timing of sleep onset and awakening. Each HF the sleep onset to before awakening) and HF during sleep to after awakening ($p < 0.01$ where mean HF from 30 min after the sleep onset to before awakening vs HF at awakening) in both conditions. Comparing conditions, no significant differences were found in the profile of HF.

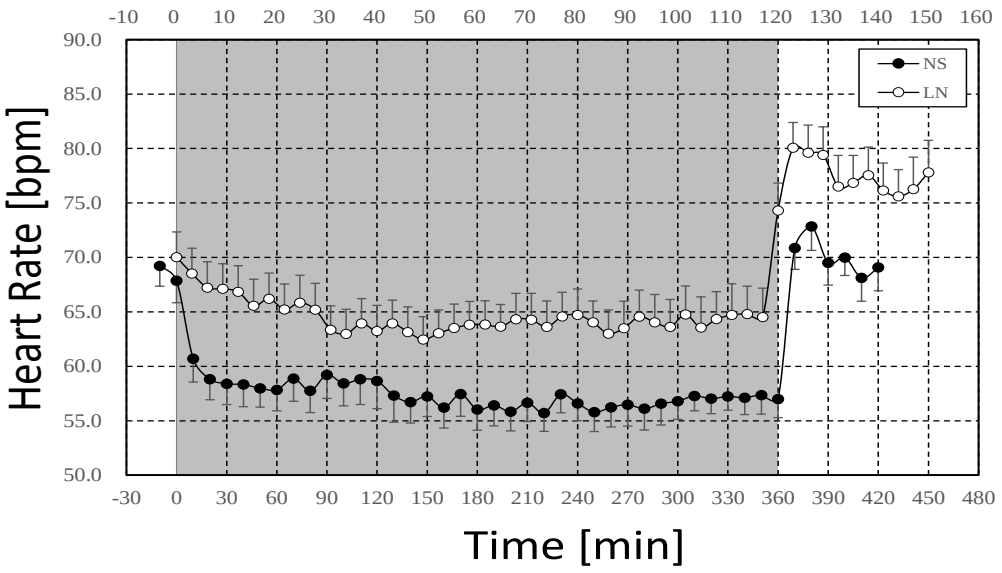

Figure 20: Averaged change in the HR during sleep to after awakening in NS and LN group

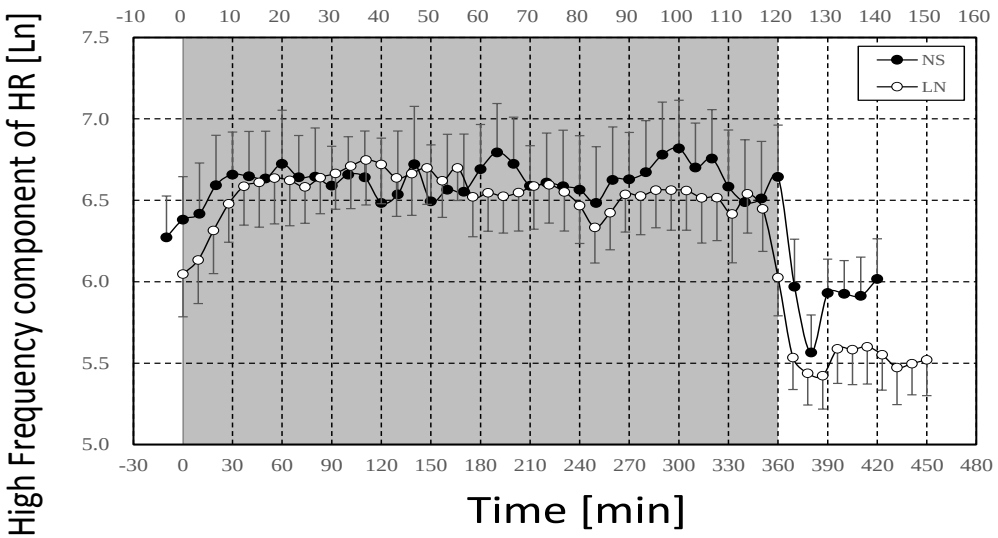

Figure 21: Averaged change in the HF component during sleep to after awakening in NS and LN group.

2.4 Discussion

In this study, we investigated the physiological impact of a long nap of 2-h in the afternoon on sleep architecture, cardiac autonomous nervous system activity, and subjective quality of sleep and mood.

Subjective sleep quality of a 2-h nap in the afternoon was comparable with those of a 6-h nocturnal sleep according to (supposed) decisive factors for subjective sleep quality, such as OSA Factor I (sleepiness on rising), Factor IV (refreshing), and Tension-Anxiety (T-A), Fatigue (F), and Vigor (V) of POMS. Using these factors, no significant differences were observed among groups. These results are supported by the sleep architecture as there was no difference in the SE or SL between the NS and the LN groups. Although the composition of the sleep architecture differed between the NS group and the LN group, the SWS and REM were contained during a 2-h nap in the afternoon. A relatively longer nap including non-REM and REM sleep cycle minimizes the severity of sleep inertia [106]and even improves performance on a visual perceptual task [75] and relational memory[76]. Thus, holistically, a 2-h nap in the afternoon may be comparable with

6-h night sleep in terms of subjective and objective sleep qualities.

We observed a prominent increase to above baseline, or "overshoot," of HR following awakening from a 2-h nap. To our knowledge, only one study has directly demonstrated such a phenomenon. Though this study was dated and included technical limitations, such as a small number of subjects, no control of sleep habit, alcohol intake before taking a nap, no PSG recording, etc., it reported a similar trend of HR increase after a 2-h nap compared with that in a no-nap condition [107]. Though it does not measure HR, Vgontzas et al. (2007) reported a cortisol "overshoot" following a 2-h nap in the afternoon followed by a night of sleep deprivation [74]. Cortisol is a hormone representing the hypothalamus-pituitary-adrenal (HPA) activity of humans. CAR is observed at awakening in the morning [37]. This morning surge of HPA hormones is found in hormones other than cortisol [56]. Therefore, HR and cortisol overshoot after awakening from a 2-h nap might imply the same or even greater physiological arousal following a long nap than on awakening in the morning.

HR overshoot and temporal cortisol increase are frequently recorded following exercise [108,109]. These responses occur to meet the requirement to balance the greater oxygen cost of fat catabolism during the early recovery period following exercise. There must be a gap between exercise and afternoon nap, the overshoot of HR and cortisol found following a longer afternoon nap may responsible for the higher mortality frequently reported in prospective cohort studies [110] via oxygen debt at awakening. However, the association between mortality and a siesta habit is still an issue of discussion. Naska et al. (2007) estimated that individuals who routinely take a siesta had 37% lower mortality than non-siesta takers when the possible confounds were strictly controlled in their prospective study [111]. Further laboratory studies targeting the physiological overshoot after awakening are needed to increase our understanding of the

significance of long naps.

2.5 Chapter Summary

This section we investigated how sleep architecture and the autonomous nervous activity differs between normal nocturnal sleep and a long nap in the afternoon. In results, there was no prominent difference in the subjective quality of sleep between groups. This was supported by the lack of any significant difference in sleep efficiency and latency, between groups. Additionally, the heart rate after the afternoon nap was significantly higher than the baseline level before nap (overshoot), whereas the decrease in the heart rate during sleep was significantly larger in the night sleep than that in the long nap in the afternoon. These results suggest that a relatively longer afternoon nap may be comparable with 6 hours of night sleep in terms of subjective and objective sleep qualities, whereas it may enhance greater autonomic arousal after awakening than that in the morning.

CHAPTER 3
IMPACT OF AWAKENING AFTER SLOW WAVE SLEEP

3.1 Subjects

Ten male university students (mean age of 22.9 ±0.88years and mean body mass index of 23.0 ± 3.15 kg/m2) participated in the study. All subjects were healthy with normal sleep-wake cycles, and no personal endocrine illness or history of sleep-related disorders. Specifically, none of the participants in this study was suspected of obstructive sleep apnea.

3.2 Methods

3.2.1 Experimental Procedure

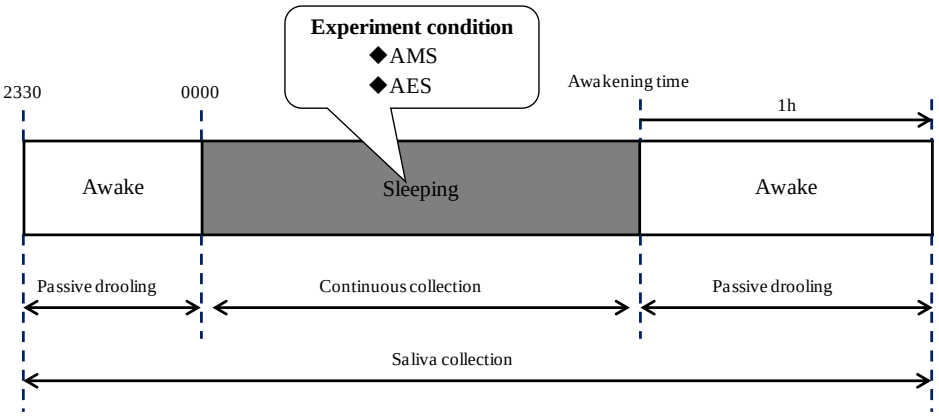

Figure 22: Schema of the experimental design (Exp.2)

In Exp.2, in a within-subjects experiment design, ten male students participated in two conditions: 1) AMS, i.e. sleep from 0:00 am and wake up at 15min after first time SWS occur and 2) AES, i.e. sleep from 0:00 am and wake up at the end of first time SWS. According to our lab sleep experiments data analysis, the duration of the first times SWS occurrence takes about 30mins. Thus, when the first time the SWS was found, the subject was to wake up after 15min or end of SWS. The transition from SWS to other sleep stages continues two minutes has judged the end of a period of SWS.

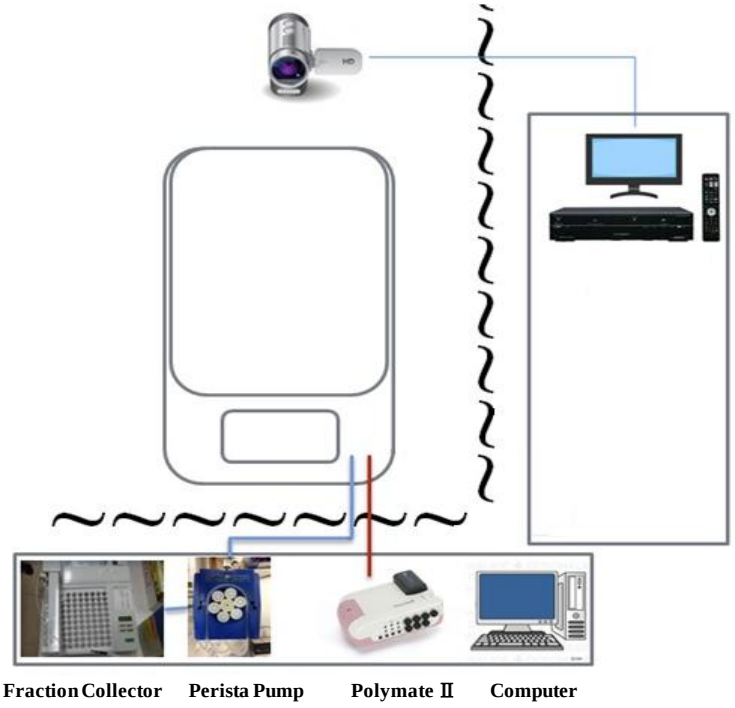

Figure 23: Experimental layout (Exp.2)

On the day of the experiment, participants visited the laboratory room at 22:30. After the preparation for PSG and ECG recording, participants were instructed to take a night sleep from 0:00 am and awakened up by the experimenter according to PSG recording at AMS or AES, respectively. The sleep stages were manually scored the same with Exp.1.

All participants underwent sleep control at their home at least 7 days before the experiment same with Exp.1 and first night sleep (FNS) before the experiment to adapt the laboratory setting, and a regular sleep-wake pattern will reduced first-night effect. The experiment was carried out daily (one participant a day) in a soundproof and environment-controlled room, and the subjects were prohibited from drinking alcohol beginning the day before the study and from eating, drinking, smoking, or performing the rigorous exercise from 1 h before study initiation to after study completion.

Saliva samples were obtained every 30min during sleep and every 15min for 1 h after

awakening. Before sleep and after awakening, naturally secreted saliva accumulated during a 3-min period[112] was collected using a straw to take saliva into a small container (1.5 ml polypropylene cylindrical container, so-called Eppendorf tube), saliva samples were collected at 30, and 15min before getting into the bed, and immediately after waking up, 15, 30, 45, and 60min. During sleep, saliva was collected continuously by using the peristaltic pump (a peristaltic high-flow pump; SJ-1211H Peristapump®, ATTO, Tokyo, Japan), connecting to mouthpiece (The Doctor's® NightGuard™ Advanced Comfort™ Dental Protector, Prestige Brands Holdings, Inc., Tarrytown, NY, USA) and saliva suction tube (SP-2, SENKO Medical Instrument Manufacturing Co. Ltd., Tokyo, Japan). Using this system, saliva collection could be under a non-invasive and non-stressful environment without disturbing sleep. Therefore, the changes in hormone concentrations during sleep can be studied. The saliva samples were kept in the bio-freezer in -25 Celsius by the day of biochemical analysis.

Figure 24: Passive drooling

Figure 25: Saliva collection

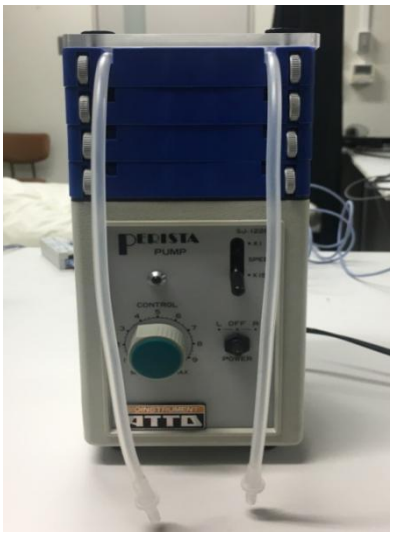

Figure 26: SJ-1211H Peristapump

3.2.2 Measurements

3.2.2.1 Subjective assessment of psychological parameters

Participants were asked to complete the POMS [98]and Visual Analogue Scale (VAS)[113] as the measure of psychological mood state before sleeping and after awakening. VAS is usually a horizontal line, 100 mm in length. Subjects rated their sleepiness with the Karolinska Sleepiness Scale (KSS) [114].

3.2.2.2 Objective assessment of physiological measures

PSG and ECG recordings were conducted throughout the experiment sessions, and HR and the high-frequency (0.15-0.40 Hz) component of HR variability were recorded at 1-min intervals same with Exp.1.

The biomarkers (cortisol, DHEA, IgA, and α-AMY) concentration before and after awakening was measured by Enzyme-linked immunosorbent assay (ELISA; High Sensitivity cortisol enzyme immunoassay kit, Sal metrics LLC, USA). ELISA is nowadays one of the major molecular determination techniques. The simple principle of ELISA is based on the antigen-antibody reaction which means, antigens from the sample are attached to a surface. Then, a matching antibody is applied over the surface to bind antigen. And antibody is linked to the enzyme reaction which is for detecting the mass of a target substance. The subsequent reaction produces a detectable signal, the enzyme via optical density of reaction to make a color change.

The brief description of ELISA for cortisol is as follows: 1) A 96-well microtiter plate is coated with a capture antibody; 2) saliva samples are added as references, and any antigen present binds to capture antibody; 3) Add a constant amount of antibody (enzyme conjugate) which is the detect biomarker (antigen) 4) Add tetramethylbenzidine (TMB) solution to induce enzyme reaction with enzyme conjugate which is captured by antigen 5) stop solution is added, and is converted enzyme to detectable form.

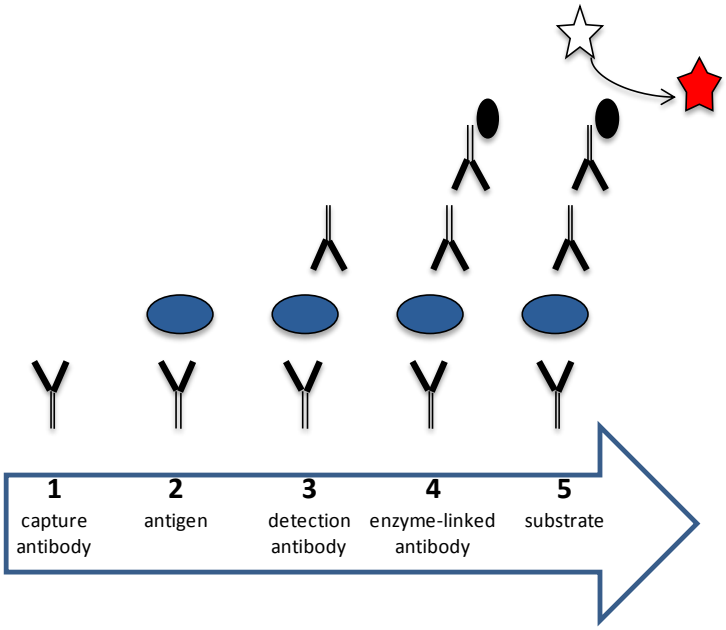

Figure 27: A sandwich ELISA

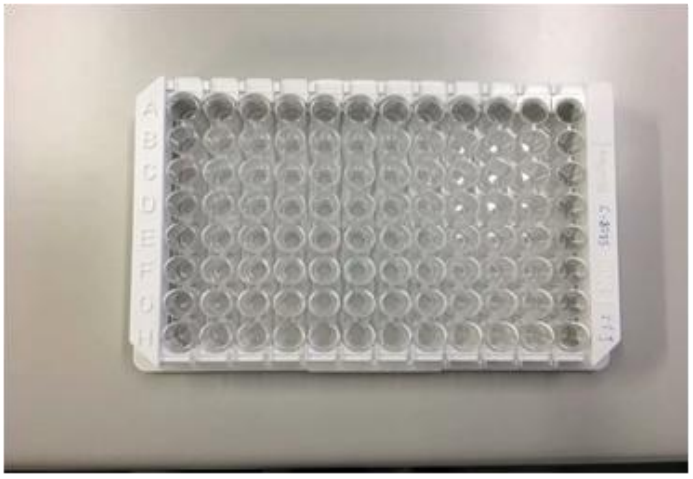

Figure 28: A 96-well microtiter plate being used for ELISA

3.2.3 Statistics

Comparing within-(FNS, AMS and AES) and between-conditions (AMS vs AES), a pair-wise, two-tailed t-test was performed. P value below 0.05 was assumed to indicate statistical significance

3.3 Results

3.3.1 Physical condition and sleep time

No subjects were suffering from any disease during any of the experiment days. The average sleep time on 7days sleep control were 7.9 ± 0.19h (FNS), 8.1 ± 0.23h (AMS), and 8.4 ± 0.35h (AES), respectively. There is no significant difference between AMS and AES conditions.

3.3.2 Psychological measures

Table 8 shows the change in POMS scores from before sleep to after awakening in the FNS, AMS and AES. There were significant differences for tension-anxiety (T-A) ($p<0.05$) in the FNS and Vigor (V) ($p<0.01$) in AMS condition. Comparing between conditions, a significant difference was observed for Anger-Hostility (A-H) ($p<0.05$) in AMS vs AES.

Table 8: Scores (S.D.s) in POMS

POMS	**∆T-A** (Tension-Anxiety)	**∆D** (Depression-Dejection)	**∆A-H** (Anger-Hostility)	**∆F** (Fatigue)	**∆V** (Vigor)	**∆C** (Confusion)
FNS	-2.40 (2.84) *	0.20 (1.23)	0.10(0.57)	0.60 (2.84)	-0.10(4.25)	-0.40 (1.65)
AMS	-0.80(1.14)	-0.90(1.73)	-0.50 (0.97)	-1.10 (4.23)	-1.60 (1.26) **	-0.30 (1.77)
AES	-0.80 (2.15)	-0.30(1.42)	0.50 (1.08)	0.10 (3.96)	-0.70 (2.45)	-0.50 (1.65)

*$p<0.05$, **$p<0.01$ comparison within conditions

Table 9: Scores(S.D.s) in the KSS

	FNS	**AMS**	**AES**
∆KSS	-1.80(2.10) *	-0.70 (2.83)	-0.90(2.51)

Table 9 shows scores the difference of KSS from the night before sleep to awakening. The difference was showed in the FNS ($p<0.05$), there was no significant difference found between AMS and AES conditions.

3.3.3 Physiological measure

3.3.3.1 HR

Fig.29 shows the change in HR from sleep to awakening. There was a significant increase in HR from sleep to awakening in FNS and two conditions ($p < 0.01$ where HR for -15～0 min before awakening vs HR for 0～60min after awakening). A comparison of the AMS and AES, for15min before awakening significant difference was observed between AMS and AES ($p < 0.05$), where HR in AES is larger than AMS condition.

3.3.3.2 HF component of HR variability

Fig.30 shows the change in the HF component of HRV from sleep to awakening. There was a decline in HF from sleep to awakening in AMS ($p < 0.01$ where HR for -15～0 min before awakening vs HR for 0～60min after awakening), in FNS and AES ($p < 0.05$).

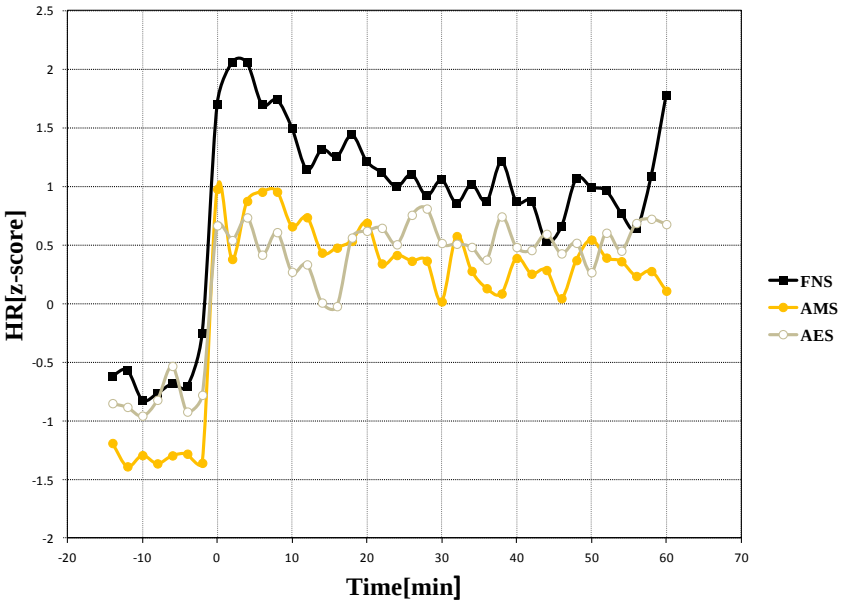

Figure 29: Changes in HR during sleep and after awakening (mean±S.E.)

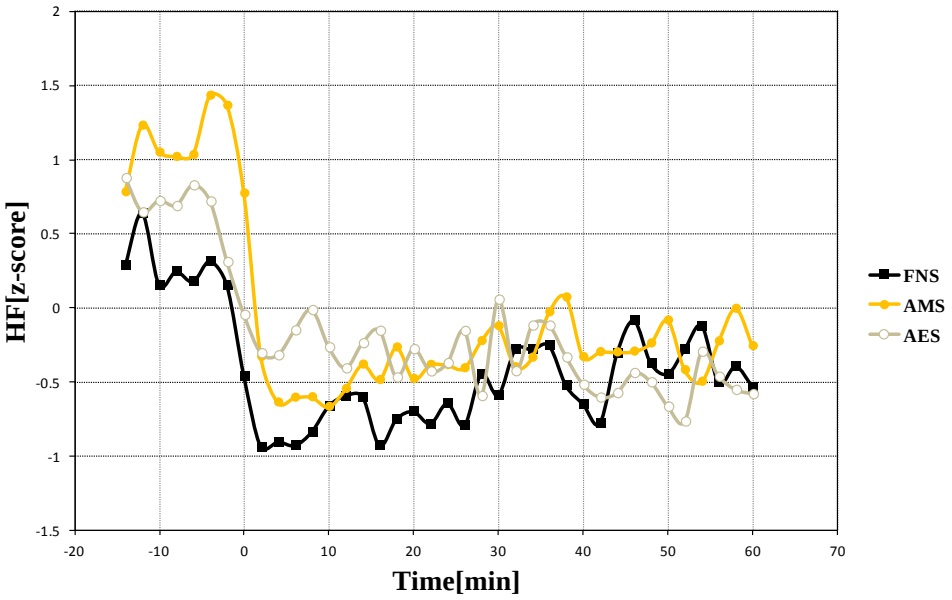

Figure 30: Changes in HF during sleep and after awakening (mean±S.E.)

3.3.3.3 Bio-markers

Fig.31 shows changes in cortisol concentration before getting up and after awakening. Cortisol concentration increased after awakening in FNS, which is considered as CAR[37-39] ($p<0.01$). Comparison between two conditions, the mean cortisol level in the AES condition 1h after awakening was significantly greater($p<0.01$).

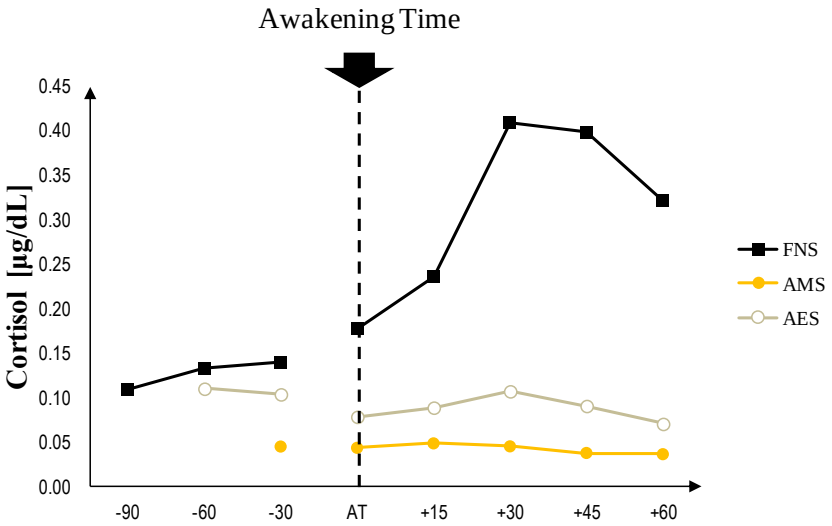

Figure 31: Salivary cortisol concentration before sleep and after awakening (0,15,30,45) (mean±S.E).

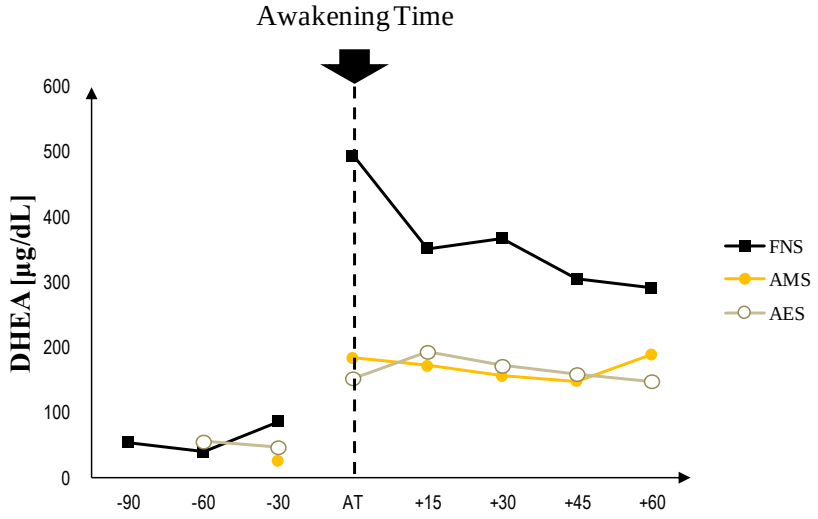

Figure 32: Salivary DHEA concentration before sleep and after awakening (0,15,30,45) (mean±S.E).

Fig.32 shows changes in DHEA concentration before getting up and after awakening. The DHEA level significant increase ($p<0.01$where DHEA before awakening vs DHEA for 1h after awakening) after awakening in FNS considered as DAR [55,56], AMS and in AES. Comparison between two conditions, no significant was found between AMS and AES.

Fig.33 shows changes in IgA concentration before getting up and after awakening. IgA concentration decreased sharply after awakening in FNS ($p<0.01$ where IgA immediately upon

awakening vs 15, 30, 45 and 60 minutes post awakening) and AES($p<0.05$). No significant was found between AMS and AES.

Fig.34 shows changes in α-AMY concentration getting up and after awakening. α-AMY concentration decreased significantly after awakening in FNS ($p<0.05$ where IgA immediately upon awakening vs 15, 30, 45 and 60 minutes post awakening), AMS and AES. No significant was found between AMS and AES.

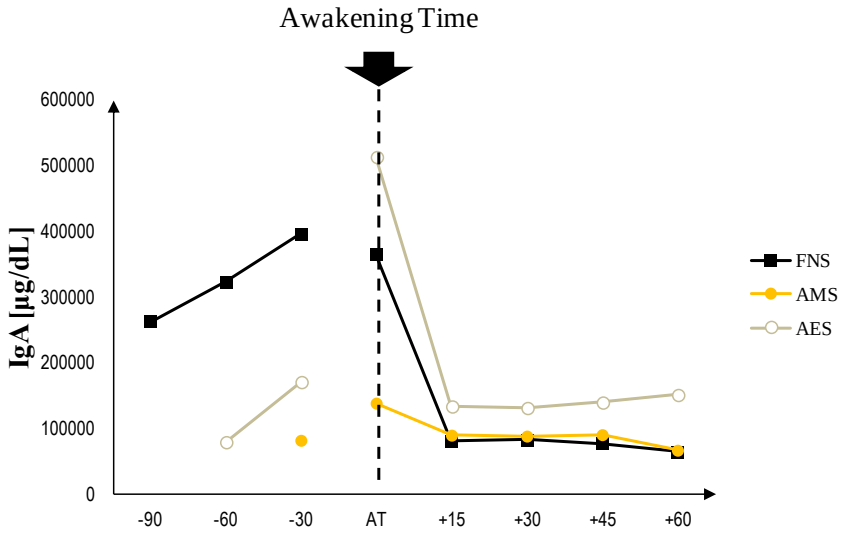

Figure 33: Salivary IgA concentration before sleep and after awakening (0,15,30,45) (mean±S.E).

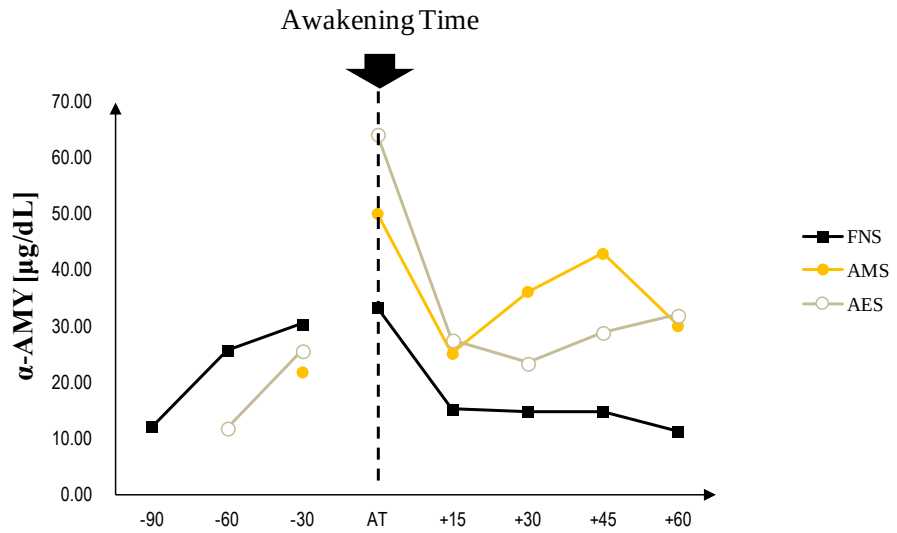

Figure 34: Salivary α-AMY concentration before sleep and after awakening (0,15,30,45) (mean±S.E)

3.4 Discussion

In this study, we investigated the impact of slow wave sleep on the autonomous nervous system and two stress reaction pathways by using four salivary biomarkers, which are cortisol, DHEA, IgA, and α-AMY.

Although the composition of the sleep architecture was differed between the AMS and AES, significant different in Vigor (V) ($p<0.01$) of POMS was found in AMS. A relatively longer nap including SWS tend to exacerbate sleep inertia which is a filling of grogginess after waking [106] and these symptoms often occur in deep sleep in the first part of the night or when sleep duration is insufficient.

With respect to physiological parameters, the HR after awakening was significantly ($p<0.01$) higher in each condition.This implies that ANS is associated with physiological arousal after awakening and sensitive to sleep system[115,116].Cheryl c reported that sympathetic activity is negative with depth of sleep[117], and HR is lowest in the SWS[118], Our results showed that before getting up HR is lower in AMS than AES ($P<0.05$). In AES condition, SWS ends and prepares to transit to other sleep stages with HR arousal.

Four salivary biomarkers (cortisol, DHEA, IgA, and α-AMY) were assayed as an index of the HPA and SAM system, respectively. The previous study indicated that salivary amylase level was a more significant increase than cortisol[119]. Our results showed salivary IgA and α-AMY concentrations showed a similar profile in the time series, increase to the peak right after awakening and decrease after awakening, while cortisol and DHEA no awakening response after slow wave sleep. A rapid increase in cortisol levels upon awakening peaking at around 30-40min after awakening in the morning, which is considered as circadian rhythm[37-39] and controlled by the suprachiasmatic nuclei (SCN) was known as an internal circadian clock

[120,121]. Ines Wilhelm research suggested that cortisol response to morning awakening is different from the circadian rise in HPA axis [122,123]. Thus, CAR is modulated by SCN, endocrine, and autonomic activity. After awakening from an early deep sleep, no increase in cortisol and DHEA, IgA and α-AMY concentration showed a significant increase that possibly demonstrates that salivary substances major influenced by SAM and HPA axis activation after an early night deep sleep. The SAM system was enhanced after AES, although there is no significant different between two conditions on IgA and α-AMY concentrations after awakening, it's noteworthy that the peak levels of IgA and α-AMY are lager in AES (IgA: 137819.16μg/dL / 513910.87μg/dL; α-AMY 50.09μg/dL / 64.26μg/dL). Additionally, there is significant different in AES on IgA and α-AMY compare peak with mean levels of post awakening.

After awakening from SWS, the levels of cortisol and DHEA on significant change, there may be some reasons as below: first, normally cortisol was lowest in the early part of sleep[37,38] and SWS drives inhibition of HPA during early nocturnal sleep[124,125]; Second, sleep duration and time of awakening seems to impact on its magnitude. Such as: get up early had higher levels of free cortisol awakening responses than evening-oriented[42,48,53], nap more than 90-min resulted in CAR while shorter naps of 50min duration did not[51], and nightly awakening in the early part of night sleep was no CAR[52] suggesting that waking up per se is insufficient for adrenocortical stimulation[53] and sleep length too short to general CAR (in AMS 40mins and 90mins in AES); Third, Sleep disturb influences on cortisol, sleep debt and disruption has a harmful impact on endocrine function[126] leading to the time of the cortisol rhythm influenced by sudden changes in the sleep-wake schedule[127] and delays the recovery of the HPA[128].

3.5 Chapter Summary

In this chapter, we conducted a laboratory study focusing on the effects of SWS on the ANS, the HPA axis and SAM activation with four salivary biomarkers, which are salivary cortisol, DHEA, IgA, and amylase. Saliva collecting and ELISA analysis were briefly described. Our results indicated that the HR after awakening was significantly higher in each condition, and before getting up HR is lower in AMS than AES, salivary IgA and α-AMY concentration showed more higher at AES, while the levels of cortisol and DHEA were no significant increase after SWS. These results suggest that the SAM system was enhanced after awakening from the AES.

CHAPTER 4
IMPACT OF AROMA ON SLEEP - A FIELD STUDY

4.1 Subjects

Twenty healthy male university students (mean age of 20.9 ± 0.74 years and mean body mass index of 21.0± 2.36 kg/m2) participated in the study. No subject had any olfactory dysfunction. Besides, all subjects were confirmed to have a normal olfactory function with a simple smell test (Open Essence, FUJIFILM Wako Pure Chemical, Ltd., Japan).

Figure 35: Smell Test

4.2 Methods

4.2.1 Experimental Procedure

Considering the difference in the home environment of each subject [Subjects-subject] and to avoid a group effect, the experiment was conducted in a within-subject manner with randomized order. Fig. 36 shows the schema of the experiment in this study.

Before starting the experiment, the subjects underwent sleep control for a week same with laboratory study. They were asked to maintain a regular sleep habit: going to bed at around 00:00 and waking up at around 07:00. Subjects were asked to follow the sleep schedule during

sleep control days as closely as possible. They were asked to not take a daytime nap during the period of the study. Their sleep habit during the control days was confirmed by self-reported data (the mean sleep time of the 7 days was 7.5 ± 0.91 h).

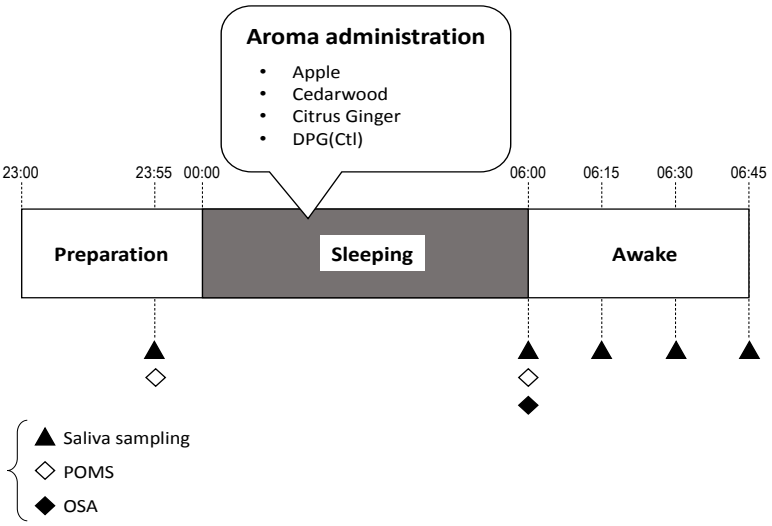

Figure 36: Schema of the experimental design(Exp.3)

The subjects were prohibited from using perfumes or fragrances of their own during the whole study period. Furthermore, considering the intervention to the secretory hormone within saliva, they were prohibited from drinking alcohol beginning the previous day of the beginning of the study and from eating, drinking, smoking, or performing rigorous exercise from 1 h before (22:00) the study initiation to after study completion (07:00 the following day).

On the day of the study, the subjects were instructed to sleep at 00:00 and wake up at 06:00 in their home. During the sleep, the subjects were exposed to apple, cedarwood, or citrus ginger aroma or a scentless sample (control). All subjects went through all three aroma conditions and the control condition at different nights in a counter-balanced order. There was an interval of more than two days between each experiment day.

Three aroma samples, apple, cedarwood, and citrus ginger, were used in the study

(Takasago International Corp., Japan). The main components of apple are allyl heptanoate and butylacetate; for cedarwood, they are cedarwood oil and acetyl cedrene; and for citrus ginger, they are lemon oil and α-pinene. A scentless solvent, dipropylene glycol (DPG), was used as the control stimulus. These aroma samples in liquid form were infiltrated into a granular material (calcium silicate, 4 mm in diameter, Sanwa insecticide Co., Ltd., Japan) and then kept in a polypropylene cup with a lid. To keep the fragrance of each sample, they were prepared one by one on the day of the experiment. Each subject was asked to place the aroma cup at the head of their bed and uncover it at the time getting into bed at the start of the experiment.

Table 10: The main components of the aroma

Sample	**Odor**	**Key compounds**
Apple	Green Apple	Allyl heptoate, Butyl acetate
Citrus Ginger	Lemon & Ginger	Lemon oil, α-Pinene
Cedarwood	Cedarwood	Cedarwood oil, Acetyl cedrene

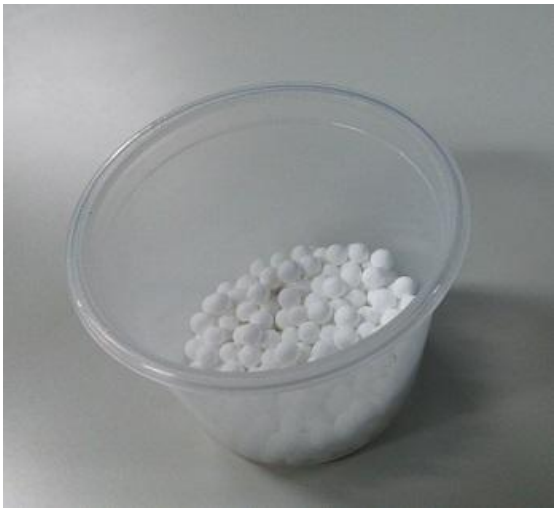

Figure 37: Aroma sample

4.2.2 Measurements

4.2.2.1 Subjective assessment of psychological parameters

To investigate the subjective impression of each aroma, subjects were asked to rate their impressions in advance by means of a VAS [113]. The four categories were “pleasant-unpleasant”, “weak-strong”, “sedative-excitative” and “rousable-not rousable”.

On the day of the experiment, subjects were asked to complete the POMS to determine the psychological mood state before sleep and after awakening [98]. The subjects were also asked to complete the OSA [99] immediately after awakening to record their subjective sleep quality.

4.2.2.2 Objective assessment of physiological measures

Considering the nature of a field sleep study at home, it is important to minimize the burden accompanied by physiological measurement as much as possible. We used heart rate as a measure recorded by a wristwatch-type wearable device and salivary cortisol level as physiological measures.

The HR was obtained using a wristwatch-type wearable HR recording device (Fitbit Charge HR, Fitbit Inc, USA, size: 20 x 7 x 7 cm, weight: 141 g). This state-of-the-art device records HR every 5 min. Based on an optical architecture sensing the pulse wave, and low battery consumption, it allows to record HR continuously for about 5 days. Subjects were asked to wear the device from an hour before sleep until 90 minutes after awakening.

Salivary cortisol level was measured as an indicator for physiological arousal at awakening and reflects the activation of the HPA system, which is a major physiological stress reaction pathway [129]. Therefore, cortisol is considered an objective measure of human mental

stress [30,130]. In particular, the remarkable elevation of cortisol secretion in the morning, reaching its daily peak at around 45 min after awakening, is frequently referred to as a feasible stress biomarker [37,131,132]

Figure 38: Fitbit device

Subjects were asked to collect saliva before sleep and after awakening in a passive drool manner [112]. They were asked to keep their saliva samples in a freezer of their own and bring them to our laboratory later with a refrigerant to keep them frozen. To measure the cortisol concentration at night and to estimate CAR in the morning, subjects were instructed to collect their saliva just before getting into the bed, at awakening, and 15, 30, and 45 minutes after awakening, as described in Fig. 36. The saliva samples were kept in a bio-freezer at -25°C until the day of the biochemical analysis. The cortisol concentration was determined by ELISA. The fulfillment of the saliva collection was checked via e-mail and a self-check sheet.

4.2.3 Statistics

For the comparison between the aroma conditions, a pair-wise, two-tailed t-test with Bonferroni correction was used, in which p values calculated from paired comparisons were multiplied by the possible combinations of t-test repetitions. For the comparison of the within

conditions for the psychological measures, a pair-wise, two-tailed t-test was used. P values below 0.05 were considered to indicate statistical significance. The data set for one subject, who did not follow the instructions of the experiment, was omitted from the analysis. The cortisol data for another subject was omitted since he experienced a canker sore, which possibly interfered with the saliva collection.

4.3 Results

4.3.1 Sleep behavior

The mean ± S.D. sleep duration for each condition, as confirmed by the self-report sheet, was 6.0 ± 0.33 h in the control condition, 6.0 ± 0.26 h in the apple aroma condition, 5.9 ± 0.30 h in the cedarwood aroma condition, and 5.9 ± 0.25 h in the citrus ginger aroma condition. There were no subjects generally followed the instructions for the sleep schedule at home.

4.3.2 Psychological measures

Table 11 shows the subjective ratings for each aroma. As the table shows, each aroma sample, i.e., apple, cedarwood, and citrus ginger was strong enough to be perceived. Only citrus ginger was judged as “excitative” compared to the DPG sample (control).

Table 12 shows the difference in the scores of POMS factors from the night before sleep to the next morning. There was a significant decrease in tension-anxiety (T-A) for the citrus ginger condition ($p < 0.01$) and a decrease in depression (D) ($p < 0.05$) in the apple condition. There were no significant differences among conditions.

Table 13 shows scores of OSA factors at the time of awakening. There were no significant differences among conditions.

Table 11: The scores [mean (S.D.)] on the VAS

VAS	DPG (Ctl)	Apple	Cedarwood	Citrus ginger
Pleasant (-50) – Unpleasant (+50)	-10.0 (13.3)	-22.9(18.1)	5.28(25.2)	-6.53(28.6)
Weak (-50) – Strong (+50)	-28.9 (20.0)	6.25 (24.0)**	10.7 (26.7) **	12.6 (27.4) **
Sedative (-50) – Excitative (+50)	-10.6 (12.6)	-8.44 (13.6)	5.14 (23.5)	6.94 (19.4) *
Rousable (-50) – Not rousable (+50)	6.11 (22.1)	-9.58 (20.9)	-9.17 (22.4)	-5.00 (20.1)

Table 12: Change in the Profile of POMS scores from before to after sleep [mean (S.D.)]

POMS	DPG (Ctl)	Apple	Cedarwood	Citrus ginger
ΔT-A (Tension-Anxiety)	-0.93 (2.12)	0.47 (2.27)	-1.11 (2.27)	-1.17 (1.54) **
ΔD (Depression)	-0.47 (1.36)	-0.65 (1.11) *	-0.44 (1.15)	-0.33 (1.08)
ΔA-H (Anger-Hostility)	0.07 (1.22)	-0.18 (1.13)	0.06 (1.00)	-0.28 (1.32)
ΔF (Fatigue)	-0.87 (3.78)	-0.76 (3.29)	-0.72 (2.70)	-0.72 (2.35)
ΔV (Vigour)	0.27 (2.49)	0.65 (2.29)	-0.72 (2.44)	0.56 (2.23)
ΔC (Confusion)	0.27 (1.83)	-0.47 (1.66)	0.06 (1.66)	-0.28 (1.18)

Table 13: Evaluation of the OSA sleep inventory scores [mean (S.D.)]

OSA	DPG (Ctl)	Apple	Cedarwood	Citrus ginger
Factor I	15.3 (4.8)	13.7 (6.8)	14.7 (6.7)	12.5 (5.2)
Factor II	16.6 (5.7)	17.9 (6.8)	17.9 (5.2)	15.8 (7.3)
Factor III	22.3 (7.0)	20.6 (8.2)	23.5 (7.8)	20.1 (7.6)
Factor IV	16.9 (6.0)	17.5 (4.7)	15.8 (3.9)	14.8 (5.8)
Factor V	14.6 (6.2)	15.3 (8.6)	14.9 (6.9)	12.4 (5.8)

4.3.3 Physiological measures

4.3.3.1 HR

Fig. 39 shows the changes in HR after going to bed and during sleep. Note that HR values were baseline-corrected by the mean value during the 10 min before the start of the experiment. This was done because subjects did not have restrictions on their behavior (e.g. reading the book, watching TV) until 10 min prior to going to bed. Regardless of the condition, HR began to drop gradually after going to bed and was maintained at a lower rate thereafter. This reflects the typical HR profile after sleep onset to sleep state. The HR decrease in the citrus ginger condition was smaller than for the other conditions. As a result, the mean HR during the whole sleep period in the citrus ginger condition was significantly greater than the control ($p < 0.01$), apple ($p < 0.05$), and cedarwood ($p < 0.05$) conditions as shown in Fig. 40.

Fig. 41 shows the changes in HR from 30 min before to an hour after awakening. Note that the same baseline-correction was applied to each HR value. Regardless of the conditions, HR immediately surged at awakening, which is typical. A comparison of the conditions showed that HR in the citrus ginger condition stayed at a higher level after awakening than in the other conditions. As a result, the mean HR during an hour after awakening in the citrus ginger condition was significantly higher than in the control condition ($p < 0.05$) and in the apple condition ($p < 0.05$) as shown in Fig. 42.

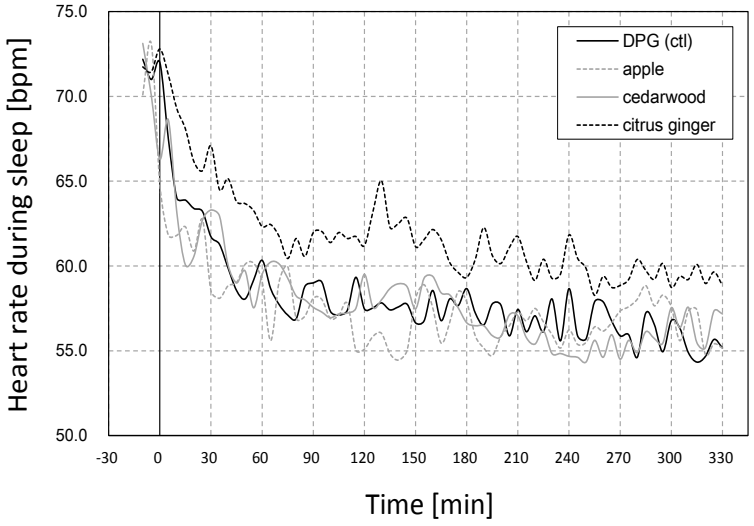

Figure 39: Changes in the HR during sleep. Zero denotes the time of going to bed

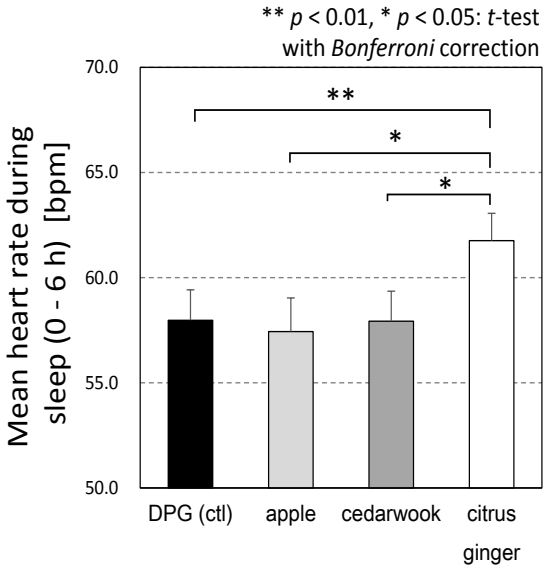

Figure 40: Mean heart rate during sleep (0–6 h). Error bars denote standard error of the mean (S.E)

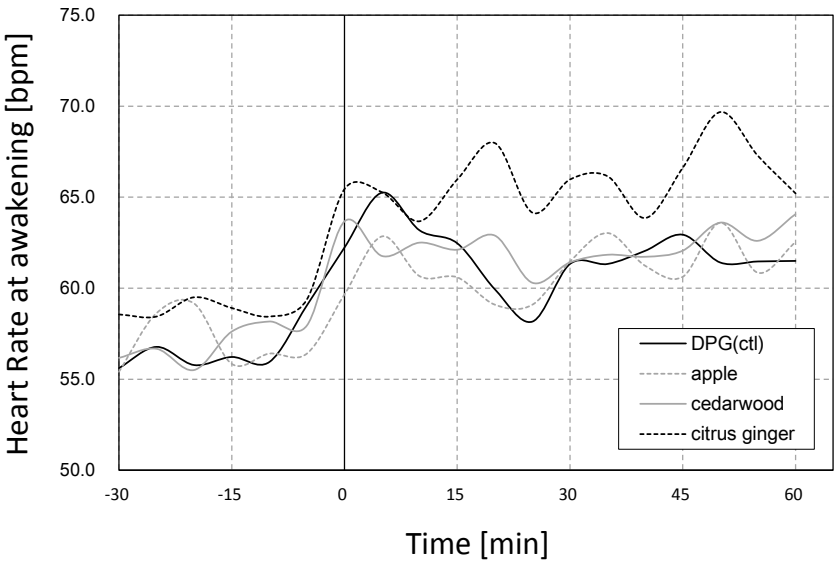

Figure 41: Changes in HR before and after awakening.

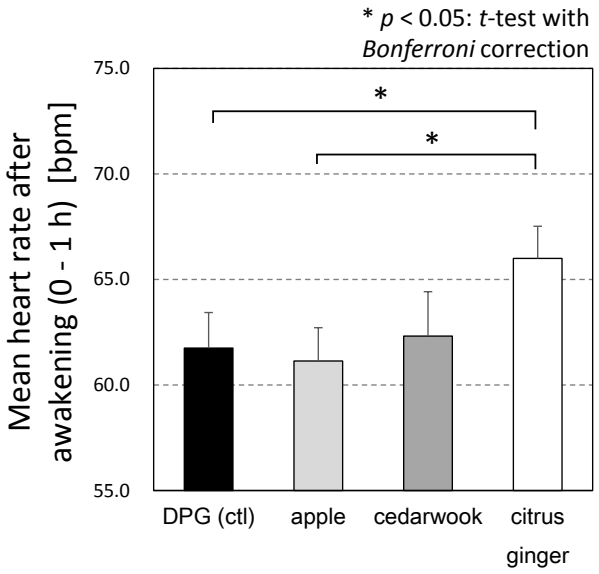

Figure 42: Mean heart rate after awakening (0–1 h). Error bars denote S.E

4.3.3.2 Cortisol concentration

Fig. 43 shows changes in the cortisol concentration before sleep and after awakening. Regardless of the condition, the cortisol level increased after awakening. This reflects a typical cortisol surge after awakening, the so-called CAR[37,131,132]. There were no statistically significant differences among the conditions observed after multiple comparisons. However, the

increase in cortisol in the citrus ginger condition was relatively higher than that for the other conditions. Pair-wise comparison with the control condition showed that the mean cortisol level in the citrus ginger condition an hour after awakening was significantly greater as shown in Fig. 44.

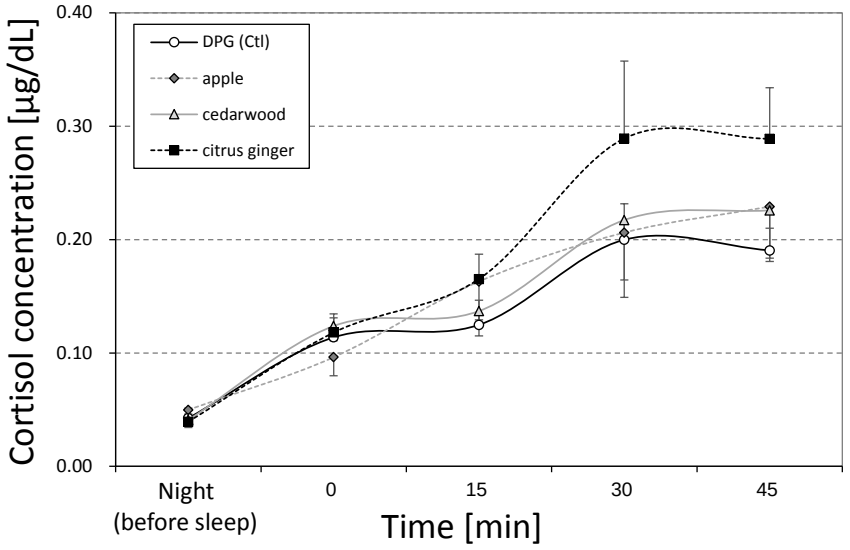

Figure 43: Changes in the cortisol level after awakening. Error bars denote S.E.

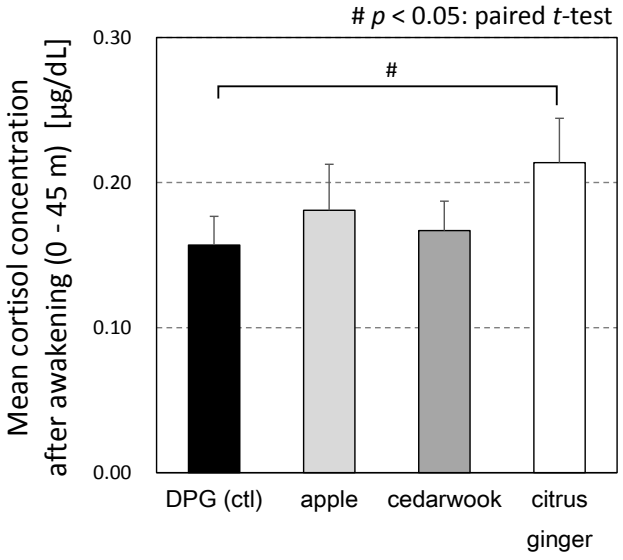

Figure 44: Mean cortisol level after awakening (0–1 h). Error bars denote S.E.

4.4 Discussion

In this study, we conducted a field sleep study to evaluate the physiological effect of

aroma at home with three different aromas—apple, cedarwood, and citrus ginger.

Although the aromas employed in this study are frequently used in aromatherapy, the subjective quality of sleep in terms of OSA was not different among conditions as shown in Table 3. However, it should be noted that the sleep period in this study was limited to six hours, which was relatively shorter than usual for all subjects; hence, the possibility that the aromas did improve the subjective sleep quality cannot be ruled out. In POMS, the tension-anxiety and depression-dejection ratings were significantly reduced from the night before to the morning for citrus ginger aroma ($p < 0.01$) and for apple aroma ($p < 0.05$). Past daytime aroma studies showed that the citrus aroma improved mood [133] and ginger reduced anxiety [134]. Another daytime aroma study with endemic citrus fruit, "Yuzu" (Citrus junos Sieb.), reported the decrease of tension–anxiety, depression, anger-hostility, and confusion in terms of POMS [135]. Our results suggested that such a positive effect of citrus ginger aroma can also be expected after night-time use.

With respect to physiological parameters, the heart rate during sleep and after awakening was significantly higher ($p < 0.01$ and $p < 0.05$, respectively) in the citrus ginger condition than those in the control condition. This implies that the citrus ginger aroma enhanced the cardiac autonomous nervous system activity, during sleep and after awakening. This effect of citrus ginger on physiological arousal was corroborated by the enhancement in the activity of the endocrine system at awakening, as reflected by a significantly greater CAR ($p < 0.05$).

Inhalation of citrus ginger aroma during sleep may enhance the sympathetic nervous system and endocrine system activity in addition to alleviating psychological tension and anxiety. Moreover, this effect of citrus ginger does not seem limited to a well-controlled laboratory setting but was also shown to be effective during home use in the present study. To the best of our

knowledge, such an enhancement of physiological parameters during sleep and after awakening due to citrus ginger aroma has not been reported previously. While the key component of citrus ginger aroma is lemon which considered as a stimulating scent has an effect on increase HR [136,137].

On the other hand, our previous daytime aroma study with citrus ginger revealed that the inhalation of citrus ginger alleviated the stress-induced cardiac response, i.e. the inhibition of a heart rate increase under an acute stressful situation and the inhibition of a decrease of a high-frequency component of heart rate variability [93]. Thus, the aroma works like a sort of sedative against a psychological stressor during daytime and as a stimulant during and after sleep as shown in this study. We observed a similar effect for mild orange aroma. The orange aroma showed a significant alleviating effect on cardiac autonomous nervous activity under an acute stressor in a daytime [95] and enhanced cardiac functioning during sleep [138]. As these aromas contain limonene as the main component, further studies of the physiological effect of this typical component of citrus aromas during sleep are desirable. Further, apple and cedarwood aroma, which did not show a physiological effect on the cardiac or endocrine system during sleep, had significant alleviating effects on the cardiac autonomous system under an acute stressful situation in a previous daytime study [93]. This is the reason why we selected these aromas for the present study. Therefore, there might be specific aromas that have different or even antagonistic effects on the human body during day-time and night-time. Attention should be paid to the night-time effect when selecting aromas for aromatherapy.

It is of great interest that the citrus ginger aroma enhanced the CAR, which has been frequently related to chronic stress such as work overload and job strain [139-142]. However, because we used a within-subject approach with a counter-balanced order of the aroma inhalation,

which might have canceled the effect of long-term stress if any, the enhancement of CAR by citrus ginger should be genuinely a pharmacological effect on the endocrine system. There are still very few studies focusing on the variation in CAR due to a particular aroma. One study reported the enhancement of CAR by the lavender aroma, which is one of the most common sedative aromas [143]. In contrast, another study reported the suppression of CAR by Thesaron, which is an alerting odorant [144]. Additionally, the citrus ginger aroma was considered to be excitative in this study. Further discussion requires additional aroma studies focusing on CAR, but it is likely that there might be specific aromas that enhance or suppress the endocrine system at awakening. CAR is abnormally decreased in patients with post-traumatic stress disorder [138]. It hampers the normal physiological arousal at awakening. Therefore, if citrus ginger can increase CAR, this aroma might be used as an alternative medicine for normalizing CAR level and morning physiological arousal.

4.5 Chapter Summary

In this part, we conducted a field sleep study to evaluate the physiological effects of apple, cedarwood, and citrus ginger aroma. Tension-anxiety and depression-dejection were significantly reduced in the morning after exposure to citrus ginger ($p < 0.01$) and apple aroma ($p < 0.05$). With regard to physiological parameters, heart rate during sleep and after awakening, and cortisol level at awakening were significantly higher for citrus ginger aroma than those of the control condition (ps < 0.01–0.05). In conclusion, inhalation of citrus ginger aroma during sleep may enhance sympathetic nervous and endocrine system activity in addition to alleviating psychological tension and anxiety.

CHAPTER 5
GENERAL DISCUSSION

5.1 Major Contribution of the Study

Nap and aroma have a positive effect on relieving fatigue and sleepiness. Our result also suggested that the relatively longer afternoon nap may be comparable with a night sleep in terms of sleep quality. We use four stress biomarkers simultaneously as an index during early night short sleep; further, clarify the physiological impact of SWS on HPA axis and SAM activation. Our results SAM activity was enhanced after awakening at the end of SWS. Moreover, the saliva was collected using the peristaltic pump system so that the physiological impact of SWS could be evaluated under a non-invasive and non- stressful environment without disturbing sleep. For aroma at home, this is a field study with monitoring HR overnight, we can check physiological information in the experimental study. So, the experimental design of "field study + physiological measurement (HR and CAR)" forms an advantage of our study. We found that the inhalation of citrus ginger aroma during sleep may enhance sympathetic nervous and endocrine system activity while alleviating psychological tension and anxiety. It is well known that olfaction has a powerful influence on physiological and psychological, the discrepancy between psychological and physiological measure is frequently observed, citrus ginger has a negative impact on the body but not on mind. When one chooses the aroma for therapeutic purpose, not only with preference, but physiological effects should be taken into consideration. In this regard, the findings of the present study would be very important.

5.2 Limitations and Directions for Future Research

This study has several limitations which should be accommodated for in the future study.

The homogenous sample of male university students may make it difficult to generalize the results of this study.

For laboratory study of day and night nap, Even though we do control sleep a week before the experiment starts. However, as the different character of human, sleep time was uncontrolled and awakening time not the same. Participants were asked to get up the only the first time we found SWS, and further study we should also record next SWS, and investigate the relationship or correlation among bio-markers. The assessment of cognitive and behavioral performance should have been carried out in a manner that allowed for comparison with previous nap studies. Assessment of endocrine or inflammatory biomarkers should have been carried out to expand the findings regarding the physiological impact at awakening after the long nap in the afternoon.

For field study of aroma**:** First, the subjects' room size at home was not uniform. Although subjects were instructed to place the aroma sample near the pillow, the concentration of the odor could not be controlled. The effect of scent acclimatization was not controlled in the same sense. Second, the sleep duration was limited to six hours in order to estimate the physiological impact of awakening in a well-controlled manner. Third, the interval between the experiment days is two days at the shortest. As a matter of logic, it is supposable that some physiological effect of an odour lasts more than two days; there is no order effect found in this study, though. Forth, a single measure design. The aroma administration in this study is one time in each aroma condition. While repeated administration is desirable in principle to assess the reproducibility of the results, it makes the sleep habit control period much longer and eventually places a greater burden on the participants, e.g., for at least 29 days in a case of two-time repeated measures employed in this study. For this reason, a single measure design was employed in this study. Future studies testing a wider range of aromas and their compounds in a similar

experimental setting are required to clarify the complex psycho-physiological effects of aromatherapy.

CHAPTER 6
CONCLUSION

6.1 Conclusion

With more and more people issues on sleep and mental health, it's become global problems with a huge impact on economics, more attention should be paid to evaluate, manage and relieve the mental stresses, preventing the development of stress-related disease. To investigate the effects of nap and aroma on autonomous nervous activity, sleep architecture, sleep quality and salivary biomarkers level related to stress, we conducted three experiments, day and early night nap which did in the laboratory and a field study of aroma on normal sleep. A two-hour nap showed that there was no prominent different in the subjective quality of sleep between the group. Autonomous arousal after awakening was found in three studies. The HR after the afternoon nap was significant higher than the baseline level before nap (overshoot), HR was lower in the middle of slow wave sleep, whereas significant higher after awakening than during sleep and HR was significantly higher in inhalation of citrus ginger aroma during sleep and after awakening than other conditions. Additionally, the peak levelof IgA, and α-amylase were larger in AES condition, while the levels cortisol and DHEA were no significant increase after awakening from SWS. These results suggest that a relatively longer afternoon nap may be comparable with 6 hours of night sleep in terms of subjective and objective sleep qualities, whereas it may enhance greater autonomic arousal after awakening than that in the morning, SAM system was enhanced after awakening from the end of SWS, and inhalation of citrus ginger aroma during sleep may enhance sympathetic nervous and endocrine system activity in addition to alleviating psychological tension and anxiety.

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RESEARCH PUBLICATIONS

Journal Publications

1. Jiang F, Kobayashi T, Ichihashi T, et al. The effect of citrus ginger aroma on heart rate and salivary cortisol level during sleep at home[J]. IEEJ Transactions on Electrical and Electronic Engineering, 2018.11.28.
2. Jiang F, Kobayashi T, Ichihashi T, et al. Effect of a relatively long afternoon nap on autonomous nervous activity, sleep architecture, and subjective sleep quality[J]. IEEJ Transactions on Electrical and Electronic Engineering, 2018.5.11.

Conference Proceedings

1. Jiang F, Kobayashi T, Ichihashi T, et al. “Effect of a relatively long afternoon nap on autonomous nervous activity, sleep architecture, and subjective sleep quality. ,” Biological Engineering Symposium, Nagano Prefecture, Japan, 2017.9.16.

APPENDICES

Appendix 1: checklist

チェックシート(checklist)

ID:_____

	夜(Night)		朝(Morning)
実験日 (Date)	就寝時間 (Time to sleep)	Fitbit	起床時間 (Wake up time)
例日目（XX/XX）	24時　0分		6時　0分
1日目（　/　）	時　分		時　分
2日目（　/　）	時　分		時　分
3日目（　/　）	時　分		時　分
4日目（　/　）	時　分		時　分
5日目（　/　）	時　分		時　分
6日目（　/　）	時　分		時　分
7日目（　/　）	時　分		時　分

備考. (remark)

※体調不良、急な飲み会があったなど、　何か想定外の事が発生した場合など、日時を明確にしてその旨を記入して下さい。

If there is an unexpected event such as bad physical conditions, drinking, and so on, please fill out in the remark with the date and time.

Appendix 2: Investigation before experiment

1. あなたの個人情報について、記入して下さい。

氏名（ふりがな）　＿＿＿＿＿＿（＿＿＿＿＿＿＿＿＿＿）

学科・学年・学籍番号　＿＿＿＿＿＿＿＿＿＿＿＿＿＿

大学アドレス　＿＿＿＿＿＿＿＿＿＿＿＿＿＿＿＿

携帯アドレス　＿＿＿＿＿＿＿＿＿＿＿＿＿＿＿＿

連絡用電話番号　＿＿＿＿＿＿＿＿＿＿＿＿＿＿

身長（　　）cm　　体重（　　）kg

年齢（　　）歳　　生年月日（　　年　　月　　日）

性別　　男　・　女

2. 普段のあなたの生活習慣などについてお聞きします。

2－1　あなたはどの位の頻度でアルコールを摂取しますか。

一ヶ月に（　　）回

2－2　あなたは一日に何本のタバコを吸いますか。

（　　）本

2－3　あなたはどの位の頻度で運動をしますか。

一ヶ月に（　　）回

2－4 あなたの部屋はおよそ何畳ですか？

およそ（　　）畳

2－5 あなたは普段、香水や部屋の芳香剤を使っていますか？

はい・いいえ

使っている場合、どんなもの（香りの系統など）を使っているか、可能であれば書いて下さい（　　）

2－6　普段使用している薬物（アレルギー薬、サプリメント、ピルなども含む）はありますか？

あり　・　なし

⇒ あ り の 場 合 、 薬 名 に つ い て も 簡 単 に ご 記 入 く だ さ い 。

（　　）

2－7　あなたは今までに何か大きな病気にかかったことがありますか？あるいはかかっていますか？

あり　・　なし

⇒ あ り の 場 合 、 病 名 に つ い て も 簡 単 に ご 記 入 く だ さ い 。

（　　）

Appendix 3: Experimental preparation

機器類

- □ ペリスタポンプ
- □ フラクションコレクター
- □ Polymate Ⅱ(本体、コード)
- □ ブルーレイレコーダー
  (本体、モニター出力ケーブル)
- □ ビデオカメラ
  (本体、電源ケーブル、外部出力ケーブル)
- □ モニター(ビデオカメラ用)
- □ 呼吸計測機器
  (電源、回路、バンド)
- □ PC×3(脳波用、オルファクトメーター用、報告書記録用【ネット利用可:要申請】)
- □ 電気スタンド×4
- □ 電源タップ×5
- □ 電子天秤
- □ バッテリー充電器
- □ バッテリー×2(Polymate Ⅱ用)
- □ 加湿器(本体、フィルター)
- □ 温度・湿度計
- □ 時計

脳波関連の小物類

- □ アルコール(1-2 本/1 週間)
- □ 脳波ペースト
- □ 脳波用研磨剤
- □ 国際 10-20 法のゴムテープ
- □ ブルーセンサー(120 個/1 ヶ月)
- □ メッシュテープ(C3,C4,O1,O2 用テープ)
- □ キープシルク(肌用テープ)
- □ 3M テープ(マウスピースにメラチューブを固定する用、口の端用テープ)
- □ 耳栓
- □ ヘアピン×4
- □ カット綿(1 袋/1 週間)
- □ 密閉容器(カット綿用)

唾液採取装置関連の小物類

- □ ピペット×2(1000μ、200μ)
- □ チップ×2(1000μ 用、200μ 用)
- □ PP チューブ(360 本/1 ヶ月)
- □ ストロー(3cm にカット)
- □ エッペン(570 本/1 ヶ月)
- □ ラック×2(PP チューブ、エッペン用)
- □ Tygon チューブ(ペリスタポンプ用)
- □ チューブコネクター×2
- □ 脱イオン水
- □ ビーカー
- □ ラップ
- □ 発泡スチロール製の箱(簡易冷蔵庫)
- □ 保冷剤
- □ カット綿(メラチューブ用【薄い】)
- □ メラチューブ(1 本/1 人の被験者)
- □ マウスピース(1 個/1 人の被験者)
- □ 針
- □ 糸

全般的に関連する小物類

- □ 机×4(ベッド周辺×3、報告書記録用×1)
- □ マジックペン(赤、青、黒を各 2 本)
- □ ハサミ×2
- □ 洗濯バサミ×6
- □ (カーテンの端、カーテン同士固定用)
- □ コロコロ
- □ ファブリーズ
- □ 布団
- □ 掛け布団
- □ 枕
- □ シーツ(布団、枕)
- □ ナビロール
- □ アイワイプ×2
- □ アルミホイル
- □ ジップロック×2(大、小)
- □ 被験者キット収納ケース
- □ 質問紙

Appendix 4: Experiment check sheet

被験者が来るまでに(～23 時 20 分)
□ 保冷剤・冷凍ボックス・脱イオン水の用意
□ 空調を行う
□ 電源を入れる（PC[脳波用]、PC[報告書用]、ビデオカメラ・モニター、ブルーレイレコーダー）
□ 波バッテリー、メモリーカードの確認(駆動可能時間、カード内のデータが空か)
□ 実験報告書を準備（PC で記録）
□ 唾液採取装置の準備(加工済みマウスピース、メラチューブを組み合わせたもの)
　□ 初回被験者の場合はマウスピースの加工
　□ お湯を用意し加工前のマウスピースを 2 分程度過熱
　□ 水に一回通して被験者に口の奥までいれてもらい 1 分程度噛んでもらう
□ フラクションコレクター設定確認(wait0、time30min、本数 4)
□ PP・エッペンチューブ(ラベリング、重さ計測)の準備
□ ペリスタポンプのシリコンチューブのセッティング確認(傷の有無、吸引できるか実際に確認)
□ ペリスタポンプ速度確認(×1、CONTROL4.75)
□ 脳波のコードを左右で分ける
□ 生理計測用のテープ・ブルーセンサー等を用意する
□ ビデオ録画準備

23 時 30 分～0 時 00 分
□ 被験者に対し、尿意やのどが渇いていないかの確認を行う
□ 唾液 N1 採取し時間を記録(23 時 30 分)
□ 質問紙（就寝前）を記入してもらう、時間を記録
□ 計測機器取り付け順序(呼吸計測機器→ECG 電極→耳栓→リファレンス→脳波基準電極の耳→眼電位→アゴ→脳波)
□ 抵抗値の確認(10kΩ以下なら OK)
□ 脳波計ケーブルの固定（右肩前面にまとめてテープで固定）
□ 頭ネット（耳まで覆うようにかぶせる）
□ 脳波・筋電・眼電位波形・呼吸波形の確認
□ カーテンを閉める
□ 唾液 N2 採取(23 時 45 分)
□ 脳波記録開始、開始時間を記録
□ 温度・湿度確認
□ ナイトガードをくわえる（舌の下にメラチューブの先があたっているか確認）
□ メラチューブを顔にテープで固定（他のテープの上に重ねない）
□ 口の端をテープで止める(唾液量が少ない人限定)
□ 被験者の携帯の電源を切る(実験者はマナーモードに)
□ 被験者に横になってもらう
□ 呼吸波形の再確認

実験開始直後
□ 消灯のトリガー（F1）を押す
□ 睡眠許可(閉眼)のトリガー（F2）を押し睡眠許可の時間を記録
□ ポンプ起動

睡眠中
□ 唾液の滴下を確認して時間を記録しフラクションコレクターStart
□ 睡眠中の行動（体動、いびきなど）と時間を記録

起床後（睡眠許可 2 時間後）
□ 点灯、起床トリガー（F3）を押し起床時間を記録
□ ナイトガード回収
□ M0 を採取し時間を記録(X 時 X 分)
□ 質問紙（起床後）を記入してもらう、時間を記録
□ M1 を採取(X 時 X+15 分)
□ M2 を採取(X 時 X+30 分)
□ M3 を採取(X 時 X+45 分)
□ M4 を採取(X+1 時 X 分)
□ 滴下終了を確認し時間を記録
□ 確認後ポンプ停止

☐　M4 採取後センターを取り外し、完了時間を記録

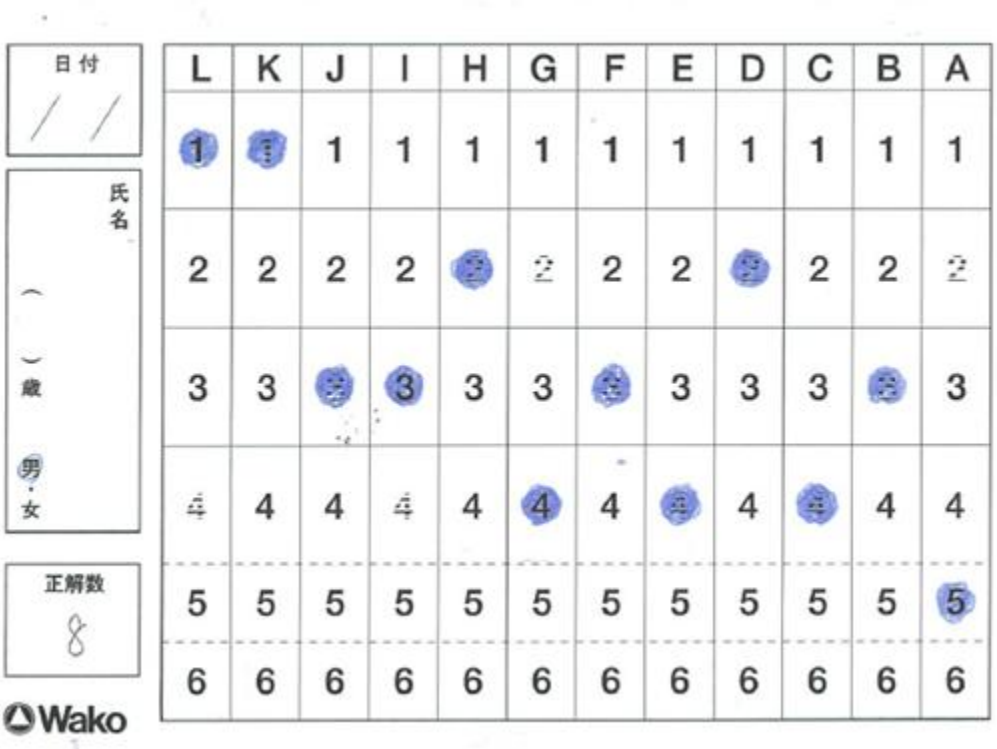
被験者退出後

☐　APMoniter よりポリメイト切断し終了
☐　脳波記録をリネームし記録用 PC にデータを移動
☐　ポリメイトの電源 OFF
☐　バッテリー交換
☐　PC[脳波用]をシャットダウン
☐　睡眠中サンプル１，２を PP からエッペンに分注
☐　エッペンの測定
☐　報告書入力（エッペンの重さ、サンプルの重さ、睡眠中の行動などを記録）
☐　PC[報告書用]をシャットダウン
☐　ビデオ録画のリネーム、ファイナライズ行う
☐　ディスクに実験コード、被験者 ID、日付を記入（SE０９　A　15/6/15）
☐　ビデオも電源 OFF
☐　ポンプの清掃（可能であればナイトガード回収時に低速で行う）
☐　センサーの清掃
☐　ベッドの清掃
☐　空調、換気扇を確認し消灯
☐　サンプルを実験コードと被験者 ID を書いたジップロックにいれ研究室で保管

※　詳細は４実験手順を確認
※　実験時必ず持参しチェックを行う。複数人の場合は進捗を確認しながら行う
※　作業順は必要に応じて変更可、同時進行で行ってもよい。
※　青マーカーの時間は目安

Appendix 5: Smell test

	1	2	3	4	5	6
A	いおう	墨汁	ニス	畳	分からない	無臭
B	腐敗臭	皮革	材木	畳	分からない	無臭
C	線香	はちみつ	コーヒー	香水	分からない	無臭
D	線香	メントール	かび	畳	分からない	無臭
E	バナナ	りんご	ピーナッツ	みかん	分からない	無臭
F	コーヒー	パイナップル	カレー	バター	分からない	無臭
G	消毒液	家庭用ガス	いおう	蒸れた靴下・汗臭い	分からない	無臭
H	草	ばら	りんご	干しぶどう	分からない	無臭
I	いおう	線香	畳	ひのき	分からない	無臭
J	家庭用のガス	皮革	蒸れた靴下・汗臭い	みそ	分からない	無臭
K	練乳（コンデスミルク）	チョコレート	シナモン（ニッキ）	ピーナッツ	分からない	無臭
L	材木	コーヒー	わさび	炒めたにんにく	分からない	無臭



Appendix 6: POMS

Profile of Mood States

Subject's Initials

Birth date
Date
Subject Code No.

Directions: Describe HOW YOU FEEL RIGHT NOW
by circling the most appropriate number after each of the words listed below:

FEELING	Not at all	A little	Moderate	Quite a bit	Extremely
1. Friendly	1	2	3	4	5
2. Tense	1	2	3	4	5
3. Angry	1	2	3	4	5
4. Worn Out	1	2	3	4	5
5. Unhappy	1	2	3	4	5
6. Clear-headed	1	2	3	4	5
7. Lively	1	2	3	4	5
8. Confused	1	2	3	4	5
9. Sorry for things done	1	2	3	4	5
10. Shaky	1	2	3	4	5
11. Listless	1	2	3	4	5
12. Peeved	1	2	3	4	5
13. Considerate	1	2	3	4	5
14. Sad	1	2	3	4	5
15. Active	1	2	3	4	5
16. On edge	1	2	3	4	5
17. Grouchy	1	2	3	4	5
18. Blue	1	2	3	4	5
19. Energetic	1	2	3	4	5
20. Panicky	1	2	3	4	5
21. Hopeless	1	2	3	4	5
22. Relaxed	1	2	3	4	5
23. Unworthy	1	2	3	4	5
24. Spiteful	1	2	3	4	5
25. Sympathetic	1	2	3	4	5
26. Uneasy	1	2	3	4	5
27. Restless	1	2	3	4	5
28. Unable to	1	2	3	4	5
29. Fatigued	1	2	3	4	5

30. Helpful	1	2	3	4	5
31. Annoyed	1	2	3	4	5
32. Discouraged	1	2	3	4	5
33. Resentful	1	2	3	4	5
34. Nervous	1	2	3	4	5
35. Lonely	1	2	3	4	5
36. Miserable	1	2	3	4	5
37. Muddled	1	2	3	4	5
38. Cheerful	1	2	3	4	5
39. Bitter	1	2	3	4	5
40. Exhausted	1	2	3	4	5
41. Anxious	1	2	3	4	5
42. Ready to fight	1	2	3	4	5
43. Good-natured	1	2	3	4	5
44. Gloomy	1	2	3	4	5
45. Desperate	1	2	3	4	5
46. Sluggish	1	2	3	4	5
47. Rebellious	1	2	3	4	5
48. Helpless	1	2	3	4	5
49. Weary	1	2	3	4	5
50. Bewildered	1	2	3	4	5
51. Alert	1	2	3	4	5
52. Deceived	1	2	3	4	5
53. Furious	1	2	3	4	5
54. Effacious	1	2	3	4	5
55. Trusting	1	2	3	4	5
56. Full of pep	1	2	3	4	5
57. Bad-tempered	1	2	3	4	5
58. Worthless	1	2	3	4	5
59. Forgetful	1	2	3	4	5
60. Carefree	1	2	3	4	5
61. Terrified	1	2	3	4	5
62. Guilty	1	2	3	4	5
63. Vigorous	1	2	3	4	5
64. Uncertain about things	1	2	3	4	5
65. Bushed	1	2	3	4	5

Appendix 7: VAS

INFORMATION POINT:

Visual Analogue Scale (VAS)

A Visual Analogue Scale (VAS) is a measurement instrument that tries to measure a characteristic or attitude that is believed to range across a continuum of values and cannot easily be directly measured. For example, the amount of pain that a patient feels ranges across a continuum from none to an extreme amount of pain. From the patient's perspective this spectrum appears continuous – their pain does not take discrete jumps, as a categorization of none, mild, moderate and severe would suggest. It was to capture this idea of an underlying continuum that the VAS was devised.

Operationally a VAS is usually a horizontal line, 100 mm in length, anchored by word descriptors at each end, as illustrated in Fig. 1. The patient marks on the line the point that they feel represents their perception of their current state. The VAS score is determined by measuring in millimetres from the left hand end of the line to the point that the patient marks.

How severe is your pain today? Place a vertical mark on the line below to indicate how bad you feel your pain is today.

No pain |_____| Very severe pain

Figure 1 Effects of the interpersonal, technical and communication skills of the nurse on the effectiveness of treatment.

There are many other ways in which VAS have been presented, including vertical lines and lines with extra descriptors. Wewers & Lowe (1990) provide an informative discussion of the benefits and shortcomings of different styles of VAS.

As such an assessment is clearly highly subjective, these scales are of most value when looking at change within individuals, and are of less value for comparing across a group of individuals at one time point. It could be argued that a VAS is trying to produce interval/ratio data out of subjective values that are at best ordinal. Thus, some caution is required in handling such data. Many researchers prefer to use a method of analysis that is based on the rank ordering of scores rather than their exact values, to avoid reading too much into the precise VAS score.

Further reading

Wewers M.E. & Lowe N.K. (1990) A critical review of visual analogue scales in the measurement of clinical phenomena. ***Research in Nursing and Health*** **13**, 227–236.

NICOLA CRICHTON

Appendix 8: KSS

Karolinska Sleepiness Scale (KSS)

Extremely alert	1
Very alert	2
Alert	3
Rather alert	4
Neither alert nor sleepy	5
Some signs of sleepiness	6
Sleepy, but no effort to keep awake	7
Sleepy, but some effort to keep awake	8
Very sleepy, great effort to keep awake, fighting sleep	9
Extremely sleepy, can't keep awake	10

Appendix 9: OSA

起床時睡眠感調査票（MA版）

朝，目覚めたらすぐ記入してください

記入時刻　午前･午後　　時　　分

この調査票は，あなたの睡眠の状態についてお聞きするものです。
睡眠の時刻等について記入してください。午前・午後はどちらかを○で囲んでください。

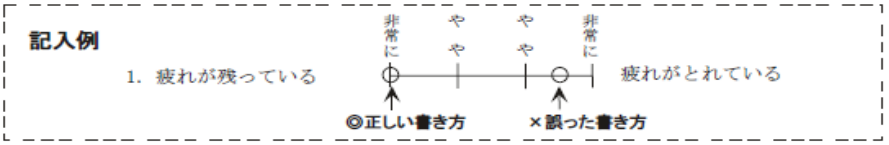
① 昨夜，おやすみになった時刻（午前・午後）　　時　　分

② 今朝，目覚めた時刻　　（午前・午後）　　時　　分

③ 昨夜の睡眠時間　　およそ　　時間　　分

昨夜の睡眠の状態や現在の心身の状態についてお聞きします。４箇所の縦線は各質問項目の状態の程度を示しています。記入例を参考に，あなたの状態にあてはまる**線上**に○印で囲んでください。

記入例



	非常に	やや	やや	非常に	
1.					疲れがとれている
2.					集中力がない
3.					ぐっすり眠れなかった
4.					ストレスを感じる
5.					身体がシャキッとしている
6.					食欲がない
7.					寝つくまでにウトウトしていた状態が少なかった
8.					頭がボーとしている
9.					悪夢はみなかった
10.					寝付きが悪かった
11.					さわやかな気分である
12.					夢をみなかった
13.					睡眠中に目が覚めなかった
14.					答えるのは，めんどうである
15.					睡眠時間が短かった
16.					眠りが深かった

Appendix 10: HR Analysis

➢ Software

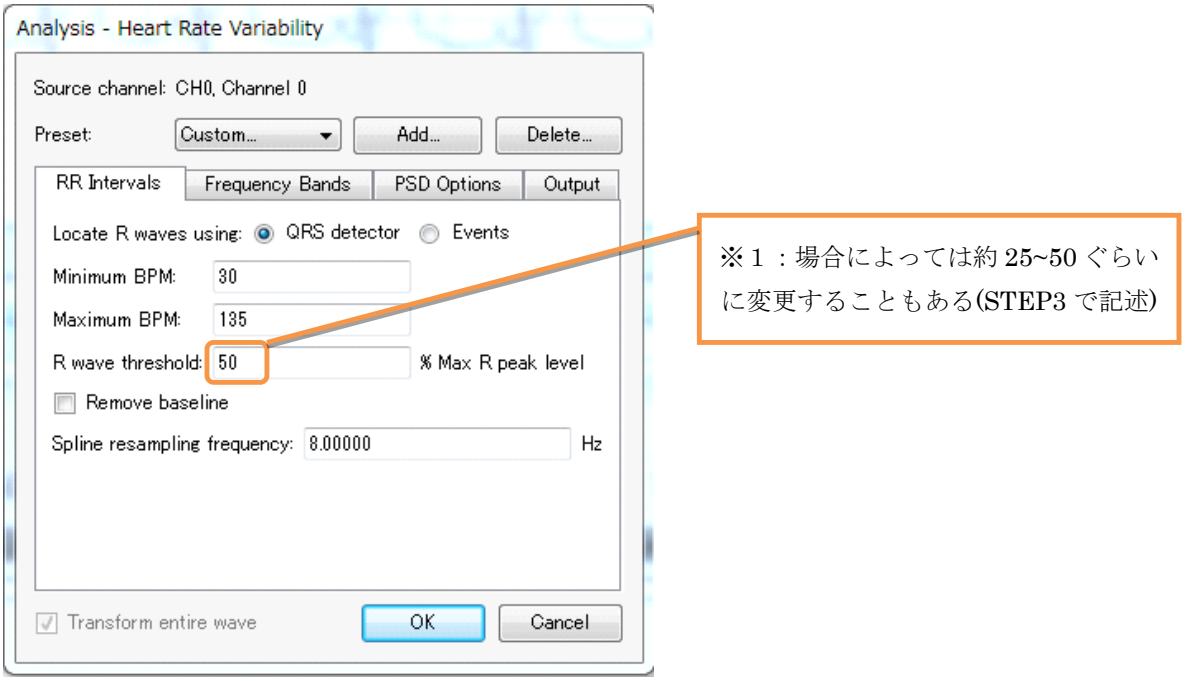
・AcqKnowledge 4.1：Output RR Interval from CSV file

・Excel： (BIOPACK_RR-Check_ver02.xlsx、BIOPACK_RR-Check-HRVCal.xlsx)

・HrvCalc：Heart rate variation analysis software

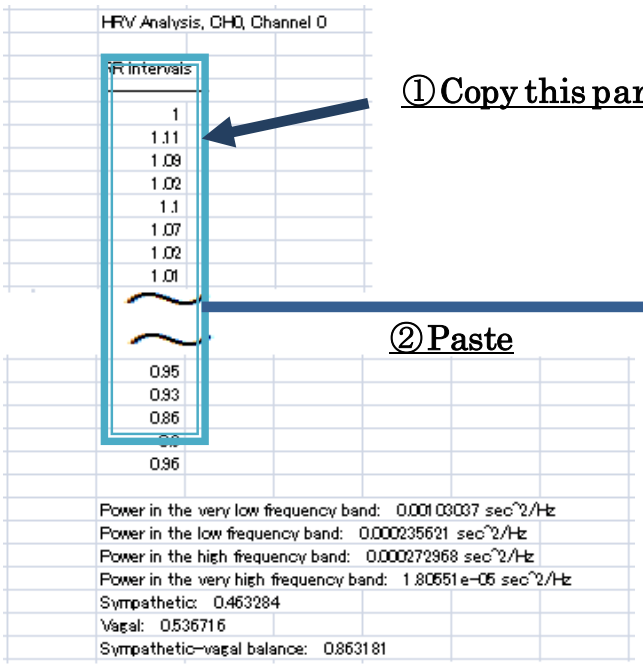
□STEP 1 - Read CSV with AcqKnowledge4.1 and get RR interval

- Process 1- Open「AcqKnowledge4.1」
- Process 2- Tab「File－Open－拡張子【Graph(*.acq)】
- Process 3- Tab「Analysis－Heart Rate Variability」→Window「Analysis – Heart Rate Variability」
- Process 4- Tab「RR Intervals」→change Minimum BPM to 30、Maximum BPM to 135
- Process 5- Tab「Output」→Display RR interval table 「✔」 →Push「OK」→Open the Window 「HRV Analysis Results」
- Process 6- Push「Copy to Clipboard」→Push「OK」



□STEP2- Noise removal of RR interval data

- Process 1 - Open the Excel file「BIOPACK_RR-Check_ver02.xlsx」
- Process 2 - Paste the RR interval data acquired in STEP 1 in an appropriate place



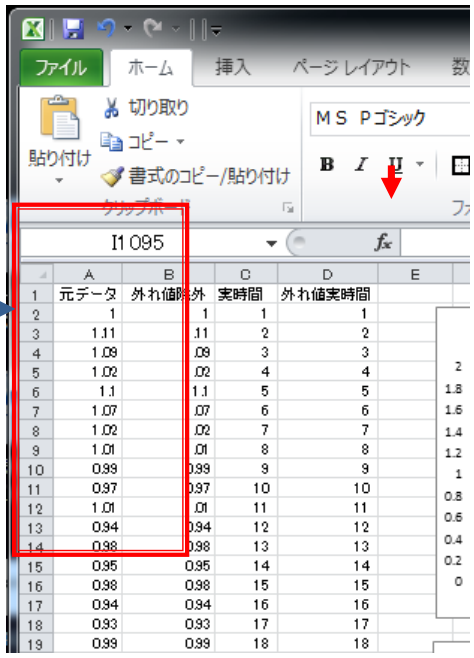


図１．Process 2　　図２．Process 3

- Process 3- Copy the part surrounded by the blue frame in Figure 1 and paste it in surrounded by a red frame
- Process 4- Confirm the graph "original data" and check whether there are outliers (noises).

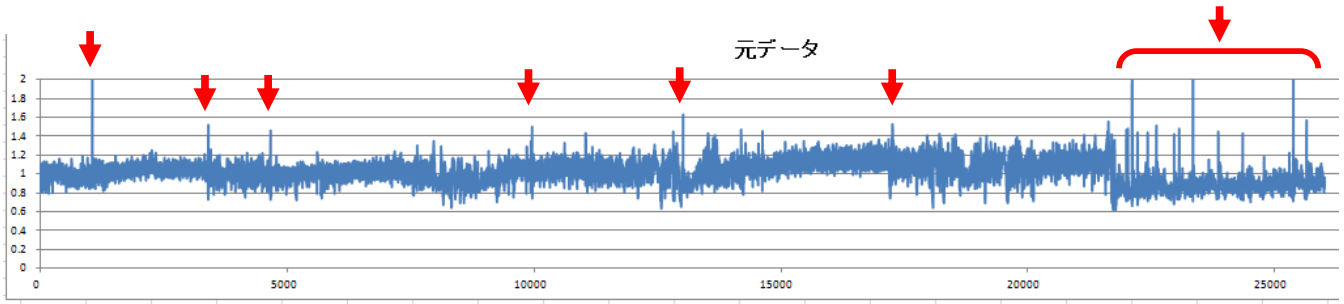


図３．Process 4

- Process 5- Remember the number of seconds

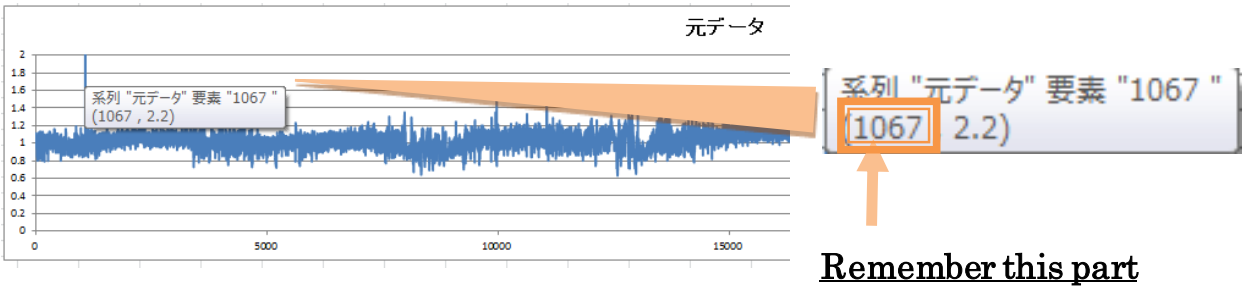


図 4．Process 5 - Read number of seconds outliers

- Process6 – Correct the value of 「BIOPACK_RR-Check_ver02.xlsx」
- Process7- Like Process 5, read the value of outlier

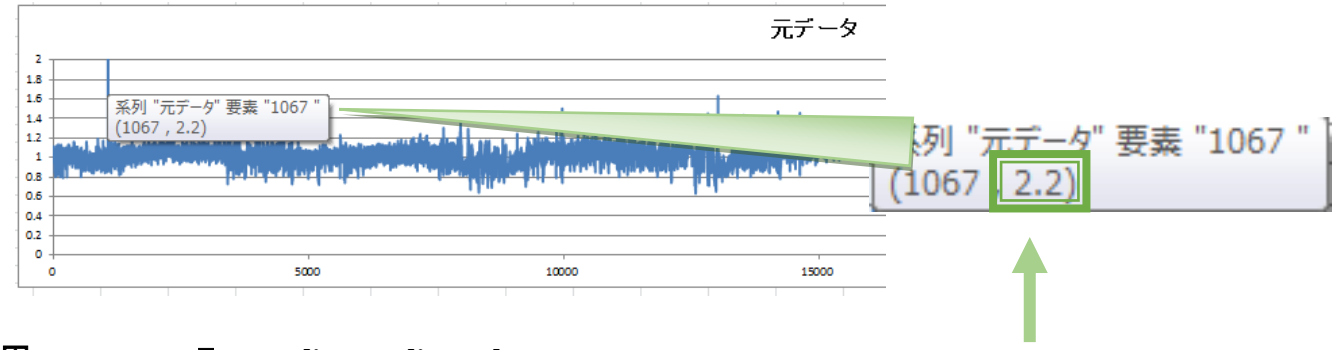


図 5．Process 7 - Reading outlier value

Remember this part

- Process8- Search for outlier values with Ctrl + F and make corrections

※1 ：Fill with yellow before modification, and fill with green after correction

※2 ：If two beats are counted as one beat, it is divided into two, and in the opposite case, it is put together into one. Sometimes 3 or 4 beats are counted as one beat.

- Process9- Repeat Process 5 to 8 and correct until there are no outliers

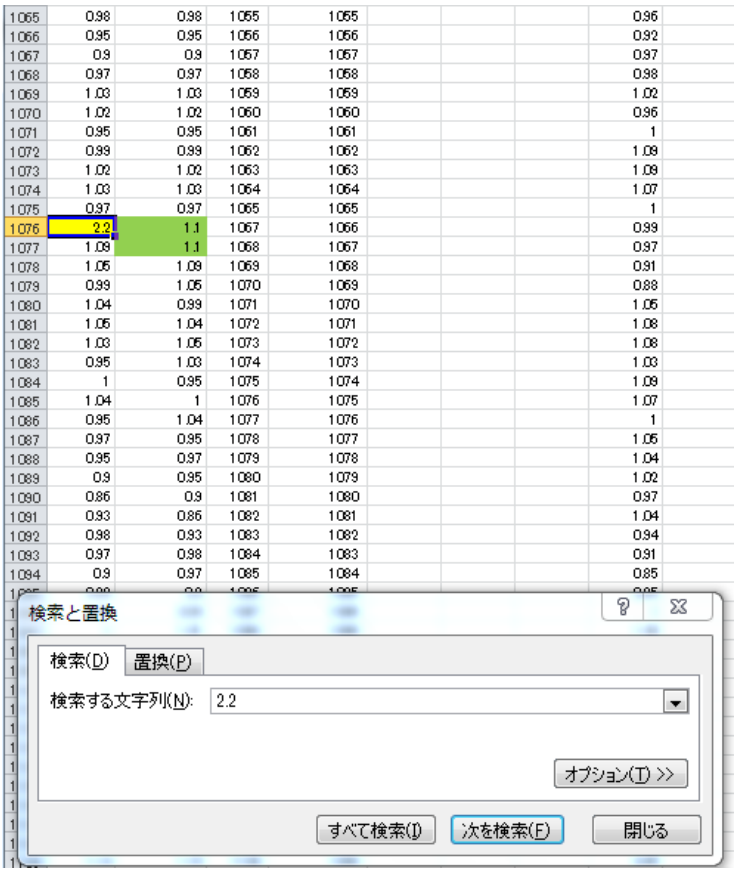


図 6．Process8- Fix outlier

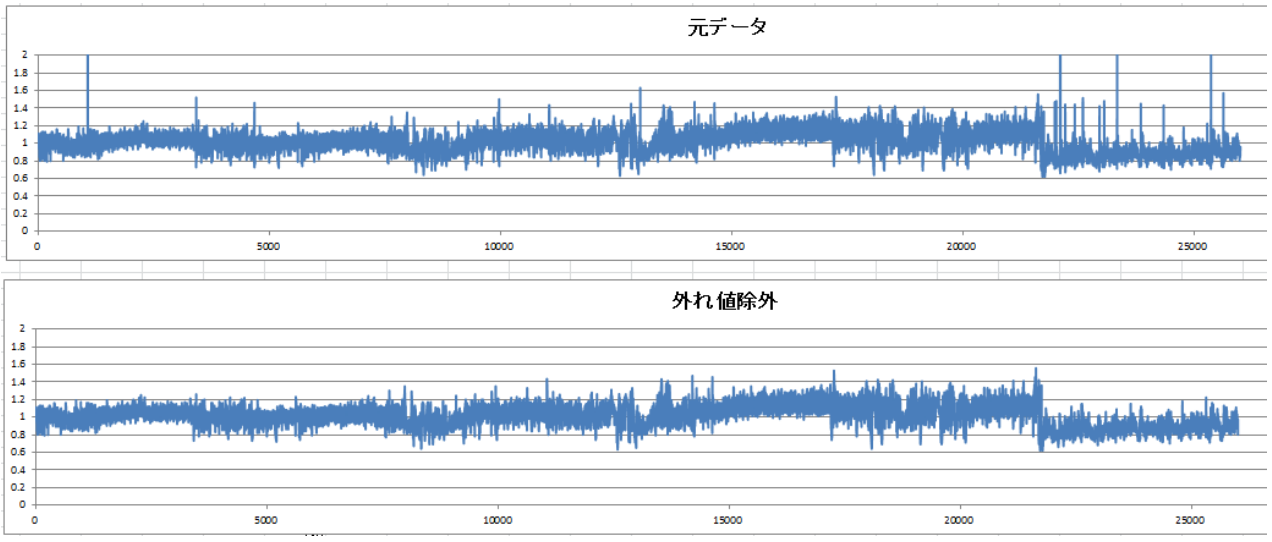


図 7．Process9- Completion of correction of outliers

□STEP3- Analysis of heart rate variability

- Process 1- Copy the outlier exclusion that you corrected in STEP 2 - Process 9 and paste it in the Excel file "BIOPACK_RR - Check - HRVCal.xlsx". Erase extra data under Excel

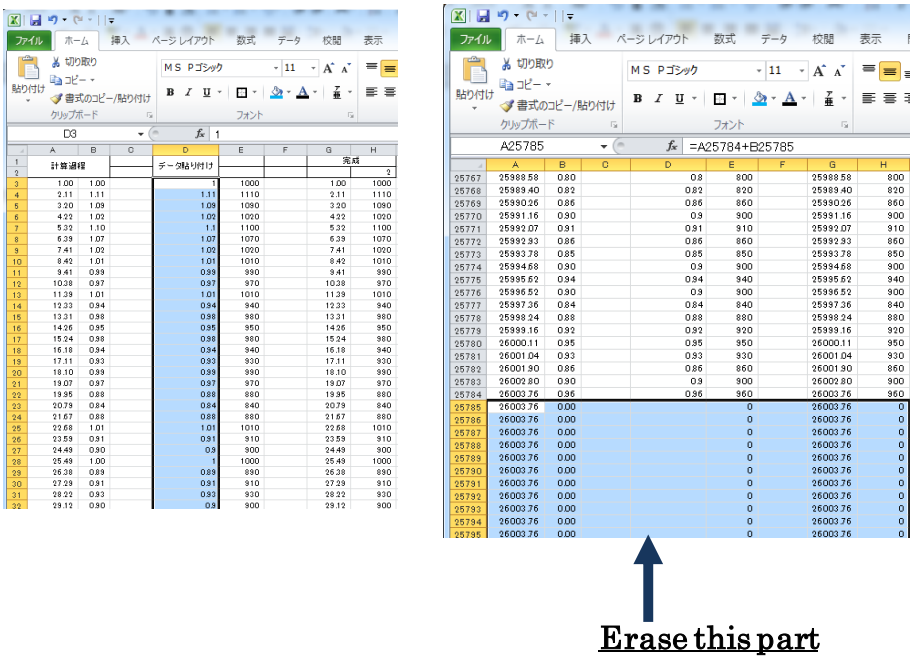


図１．Process１- Pasting outliers exclusion

- Process2- Copy the completed column data and paste it into the software "Notepad".
  Enter the number of lines and 2 in the first line of Notepad.
  Save it.

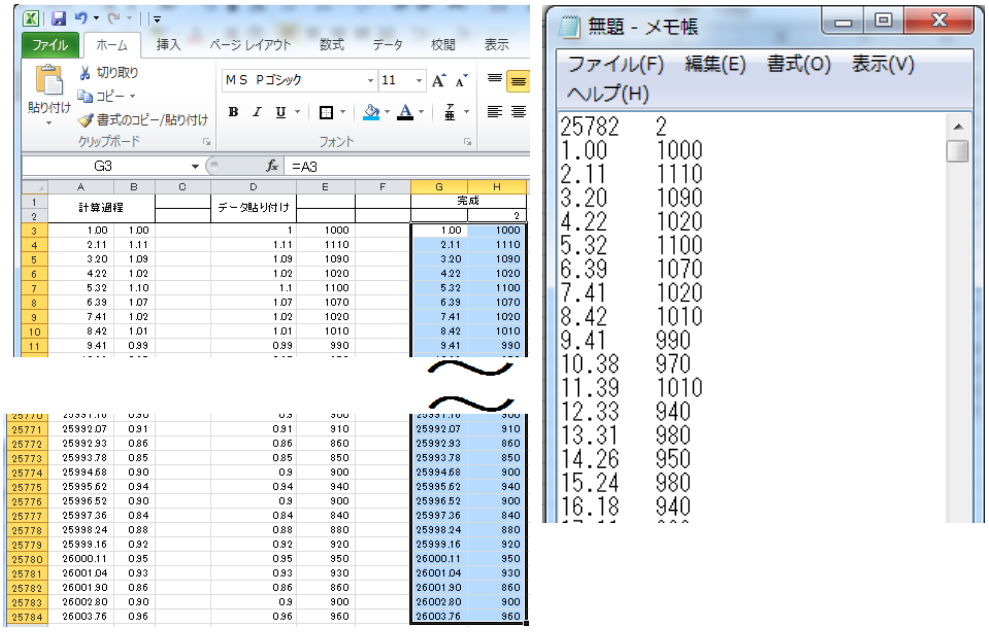


図２．Process２-Paste into notepad

- Process 3- Open「HrvCalc.exe」
- Process4- 1. Select the 「**.txt」
- After setting two files, press 「設定」
- Process5- 2. Enter the same number.
- Process 6- 3.「LF 下限」is 0.05
- Process7- 4. Change the analysis interval to 3 seconds (in the case of scent experiment), Press「解析実行」
- Process 8- The analysis result is output to the folder where the text is saved

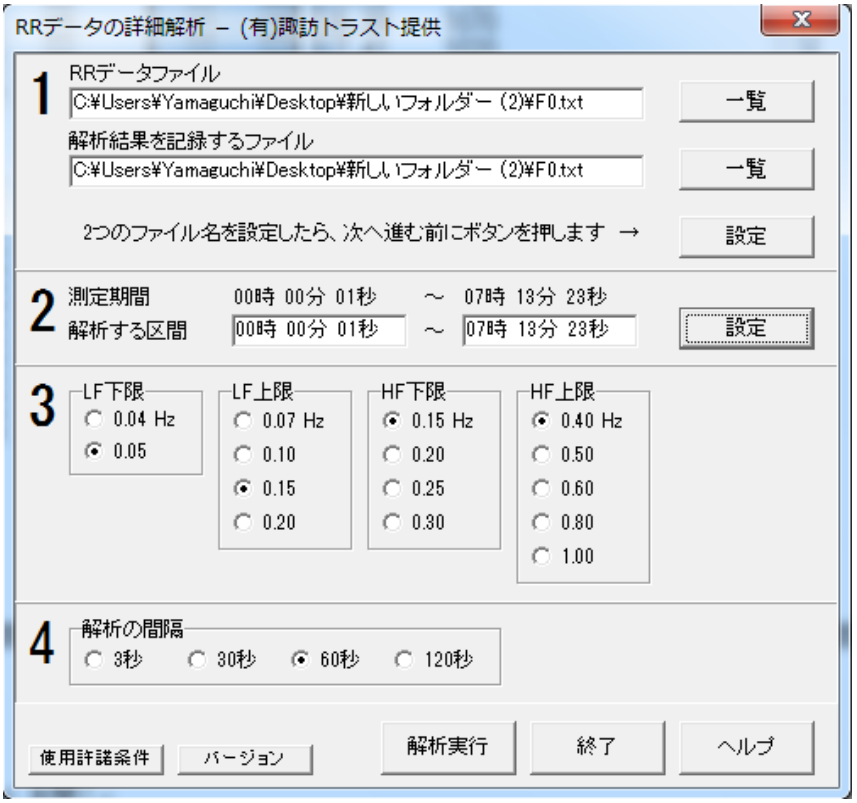


図３．Process４～７- Setting analysis software