An Evaluation of the Psychophysiological Effects of Aromas in Alleviating Acute Stress Response

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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

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DECLARATION

I, L. Sugeeswari lekamge, declare that this thesis titled “An Evaluation of the Psychophysiological Effects of Aromas in Alleviating Acute Stress Response” and the work presented in it, are my own. I confirm that:

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ABSTRACT

We conducted a series of experiments to investigate the psychophysiological effects of aromas under a short-term cognitive stressor, in a highly reproducible manner using an olfactometer. Each experiment consisted of a 10-min initial rest period, a 30-min calculation task, and a 15-min recovery period. Within-subject design was employed where male university students (n=19, 6, and 19 in Exp.1,2, and 3 respectively) performed the calculation under each aromatic or control stimulus presented in counter-balanced/ randomized order. Each stimulus was intermittently delivered (first 20s of each 1-min interval) via a cannula placed under the nostrils and connected to a customized olfactometer. Dose-dependent effects of 1 and 20% Orange were investigated in Exp.1 whereas eight different aromas: Chocolate, Strawberry, Green tea, Apple, Citrus ginger, Chamomile, Cedarwood, and Musk were investigated in a preliminary investigation (Exp.2). Based on the findings of Exp.2, Apple and Chamomile were further investigated in Exp.3. Along with a visual analogue scale (VAS) for psychological assessment, cardiac activity on electrocardiograms, and peripheral activity using skin temperature and skin conductance level (SCL) were recorded throughout each experiment. These measures indicated that the calculation task functioned as an acute stressor. Remarkably, the increase/decrease in heart rate (HR) and high-frequency (HF) component of heart rate variability during the task were significantly smaller with 1% Orange (compared to 20% and Control). Suppressed increase/decrease in HR and HF were also observed in Exp.2 with Cedarwood, Strawberry, Green tea, Apple, and Citrus ginger (compared to Control). These results indicated an inhibition of sympathetic nervous system elevation and parasympathetic nervous system suppression. Demonstrating a remarkable suppression in the increase/decrease in HR and HF, Exp.3 verified the efficacy of Apple in alleviating cardiac stress response. However, the psychological measures in terms of VAS demonstrated no significant benefit of the above aromas as demonstrated through cardiac parameters. Discrepancies were also observed between the subjective impressions and the physiological responses. Moreover, the effects demonstrated through peripheral responses were inconsistent with those through cardiac responses, e.g. there were no significant differences in nose temperature between the two doses of Orange, negative effects were observed with Cedarwood and Apple on SCL (Exp.2) and with Apple on skin temperature (Exp.3).

Overcoming the limitations in past aroma studies brought by conventional passive exposure administration methods, the study successfully introduced a proprietary olfactometer

ensuring well-controlled aroma administration. While contemporary studies on discrepant psychophysiological effects are in a preliminary stage and such effects are frequently and merely attributed to the large variation in experimental design and aroma administration method, our study investigated various psychophysiological effects through a series of well-controlled experiments, employing an olfactometer. Investigations on the differences based on age and gender is a direction for prospective research which will enable to overcome the limited generalizability of our findings resulted due to the homogeneous study sample. In conclusion, the study verified the efficacy of mild Orange and Apple aromas in inhibiting cardiac stress response. Further, the presence of a dose-dependent effect and a stimulus-specific nature of aroma which leads to discrepant psychophysiological effects were revealed.

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CHAPTER 1

INTRODUCTION

1.1 Background

1.1.1 Benefits of Aromatherapy

Over the decades, aromatherapy has been used worldwide as a major facet of alternative and complementary medicine. In addition to the use of aromas in various consumer products, the use of ambient aromas in service or living environments is also playing a key role within the recent and emerging concept of ambient relaxing environments. Throughout history, many scholars have investigated the benefits of aroma and the identified benefits can be basically categorized as behavioural, psychological, and physiological benefits.

1.1.1.1 Behavioural Benefits

Lavender which is one of the most commonly used aromas in aromatherapy enhances sleep quality [1] and improves cognitive task performance [2]. Shaded white tea which is a kind of green tea is also efficacious in improving cognitive performance [3, 4]. Strawberry aroma reduces infusion-related nausea and vomiting [5] and ginger is also reported as an effective medicament in reducing nausea among the patients in an acute care setting [6]. In addition to that, massage with aromatic ginger and orange essential oil relieves knee pain among the elderly [7]. Damask rose [8] and sweet marjoram [6] are among the other aromas with reported efficacy in relieving pain.

1.1.1.2 Psychological Benefits

In addition to the behavioural effects discussed above, various psychological benefits are reported so far, associating with a number of aromas. Orange aroma reduces anxiety and stress [9, 10, 11] and improves positive mood [9, 10]. Lavender which is regarded as one of the prominent aromas in olfactory research also reduces anxiety and stress [6, 10, 12], enhances positive mood [10], and improves concentration during cognitive tasks [13]. Apple [14], sweet marjoram [6], and chamomile [12, 15] are the other aromas with reported anxiolytic activity while yuzu [16], chocolate [17], and shaded white tea [3, 4] have demonstrated efficacy in improving mood. Moreover, chocolate [18], shaded white tea [3], bergamot and lavender [19] aromas lead to enhanced relaxation.

1.1.1.3 Physiological Benefits

Through physiological measures including cardiovascular, endocrinological, and neurological parameters, various aromas have demonstrated their beneficial effects. Anxiolytic effects of orange aroma were observed through reduced pulse among children during dental treatment [20]. A combination of aromas including orange, lavender, and chamomile also led to reduced pulse among nursing students practicing intravenous injection for the first time [21]. Demonstrating relaxing effects, bergamot inhalation inhibits cardiac stress response [22]. Inhalation of cedarwood [23] and oil massage with bergamot blended with lavender [19] reduce blood pressure, and heart rate/pulse. Reductions in blood pressure could also be observed among prehypertensive and hypertensive subjects following inhalation of a mix of lavender, ylang-ylang, and marjoram aromas [24]. A combination of chamomile and lavender also was effective in stabilizing blood pressure among percutaneous coronary intervention patients [12].

The effects of aroma in reducing endocrinological stress response are evident through a number of reports on reduced stress-related hormones. Orange aroma reduces salivary alpha amylase [11] and salivary cortisol among children during dental treatment [20]. Green tea resulted in reduced salivary chromogranin A (CgA) levels among the students submitted to a mental task stress load [4]. Inhalation of yuzu also resulted in CgA reductions among women during the follicular phase of menstrual cycle [16]. Musk is also reported to have reduced salivary cortisol [25] and demonstrated antidepressant-like effects in an animal model of depression through reductions in serum corticosterone [26]. Moreover, lavender functions as an effective treatment for neurological disorders [27].

1.1.2 Discrepant Results reported in the Literature

Despite the above mentioned beneficial effects of aroma on human behavior, psychology, and physiology, a considerable number of studies have failed to demonstrate any significant effect of aroma where some studies have even led to negative effects. A study demonstrated the effects of orange aroma in reducing anxiety and improving mood among patients waiting dental treatments [10]. Contrasting with these findings, another study failed to show any significant effect of orange aroma on anxiety or mood experienced by dental patients [28]. Anxiolytic effects of orange aroma were evident in two experimental studies [9, 11], but a field study investigating the effects of orange aroma on anxiety among pregnant women during labour resulted in no such beneficial effect [29]. Physiological benefits of orange were reported through reduced salivary α-amylase [11], cortisol, and pulse [20], however the results

reported with several other studies were inconsistent with the above findings demonstrating no significant effect of orange aroma in terms of HR, EMG [9], blood pressure, respiration, or pulse [29]. In addition to that, no specific effect of orange could be seen in a study using orange ice lollies in preventing nausea and vomiting related to dimethyl sulfoxide (DMSO) among patients undergoing stem cell transplantation [30]. A similar study investigating the effects of orange interventions on symptoms associated with DMSO during stem cell reinfusions showed positive results but only with the fruit and not with the aroma [31]. Most importantly this study reported an increased heart rate following orange aroma inhalation contrasting with the inhibitory effects of orange reported previously. Bergamot aroma inhalation inhibited cardiac stress [22] and massage with bergamot blended with lavender and almond oils demonstrated relaxant effects with reduced blood pressure and pulse [19] among healthy participants. Yet, bergamot aroma was not effective in treating anxiety, nausea or pain perceived by pediatric patients undergoing stem cell infusion [32]. Similarly, inhalation of bergamot mixed with lavender and cedarwood oils resulted in no significant effect on anxiety or depression among patients undergoing radiotherapy [33].

Lavender which is one of the prominent aromas reputed for its sedative effects [6, 10, 12, 19, 24], also reported no significant psychological or physiological effects after coronary artery bypass surgery [34]. Resulting in an intriguing finding, one of our previous studies indicated an enhancement of autonomic stress response by lavender aroma inhalation during a short-term calculation task [13].

Inconsistent findings are reported with apple aroma as well. Despite the demonstrated efficacy in reducing tension and anxiety [14], apple aroma failed to reduce anxiety or enhance mood among the patients waiting for scheduled dental treatments [28]. Anxiolytic effects of chamomile were seen in several studies involving percutaneous coronary intervention patients [12] and outpatients with mild to moderate generalized anxiety disorder [15]. However, a combination including chamomile, lavender, and orange aromas did not show any significant effect on anxiety among nursing students practicing the intravenous injection for the first time [21]. Consistent with the idea of relaxant effects of cedarwood, reduced sympathetic nervous system (SNS) activity and increased parasympathetic nervous system (PNS) activity were observed during inhalation of Cedrol which is extracted from cedarwood [23]. Despite such beneficial effects, cedarwood showed no significant effect in reducing anxiety or depression among patients undergoing radiotherapy [33].

Table 1: Summary of past aroma studies (Exp.1)

Study	Aroma	Subjects (stress load)	Study design	Method of stimulation	Dose-variation	Psychological effects	Physiological effects
F. Rashidi-Fakari et al. [29]	orange	pregnant women during labour n=100(F)	between-subject	P.E (napkin)	No	anxiety n.s	SBP, DBP, RSP, Pulse n.s
S. Gonella et al. [30]	orange	patients undergoing SCR n=69	between-subject	O.C (ice lollipop: orange and non-citrus)	No	-	nausea and vomiting↓ [with ice lollipops but not limited to orange]
M. Jafarzadeh et al. [20]	orange	dental patients n=30	within-subject	P.E (diffuser)	No	-	Δcortisol↓, Δpulse↓
Y. Nagata et al. [11]	sweet orange, peppermint	healthy undergraduate students (no stressor) n=79	between-subject	P.E (tissue paper)	No	anxiety↓[sweet orange]	salivary α-amylase↓[sweet orange]
T. C. Goes et al. [9]	sweet orange, tea tree	healthy graduate students (SCWT) n=40(M)	between-subject	P.E (surgical mask)	Yes	anxiety and stress↓, positive mood↑[sweet orange]	HR, EMG n.s
P. Potter et al. [31]	Orange (aroma, fruit)	patients undergoing SCR n=60	between-subject	P.E (aromatherapy sampler), O.C (fruit)	No	symptom intensity, symptom relief n.s [aroma], symptom intensity↓, symptom relief ↑[fruit]	HR↑[aroma], HR, blood pressure n.s[fruit]
C. B. Faturi et al. [35]	orange, tea tree	Wistar rats (EPM followed by LDP) n=20(M)	between-subject	P. E (cotton)	Yes	-	anxiolytic behaviour↑[orange]
A. Toet et al. [28]	apple, orange	dental patients n=117(M)+ 102(F)	between-subject	P.E. (dispenser)	No	anxiety, mood, or perceived pain n.s	-
Y.B. Yip et al. [7]	mixed aroma (ginger, orange)	older persons n=59	between-subject	Oil massage	No	quality of life n.s	knee pain↓
J. Lehrner et al. [10]	lavender, orange	dental patients n=200	between-subject	P.E. (dispenser)	No	anxiety and stress↓, positive mood↑[lavender, orange]	-
S. G. Gray et al. [36]	lavender, sweet orange, tea tree	Residential-care residents with dementia and behavioural challenges n=13	within-subject	P.E	No		resistive behaviour n.s
Y. Sugawara et al. [37]	ylang yang, orange, geranium, cypress,	heathy adults (Kraepelin test/ stepping up and down)	unk.	inhaler	No	Favourablenes s ↓ [orange (after physical work), orange,	-

	bergamot, spearmint, juniper	n=17-27 in each group				geranium (after mental work)], Favourablenes s ↑ [cypress (after physical work), juniper (after mental work)]	
T. Hongratanaworaki [19]	mixed aroma (bergamot, lavender, almond)	healthy volunteers n=40	between -subject	Oil massage	No	relaxation↑	SBP, DBP, Pulse↓
T. Matsumoto et al. [16]	yuzu	women during follicular phase of menstrual cycle n=20(F)	within-subject	P.E (diffuser)	No	positive mood↑	salivary CgA↓
D. H. Ndao et al. [32]	bergamot	pediatric patients undergoing SCR and their parents n=37	between -subject	P.E (diffuser)	No	anxiety n.s	nausea, pain n.s
S. -M. Peng et al. [22]	bergamot	healthy undergraduate students (no stressor) n=114	between -subject	P.E (ultrasonic atomizer)	No	-	LF/HF↓
P.H. Graham et al. [33]	mixed aroma (cedarwood, lavender, bergamot)	patients undergoing radiotherapy n=313	between -subject	P.E. (bibs)	No	anxiety and depression n.s	-
A. Bikmoradi et al. [8]	damask rose	patients with burn wounds n=50	between -subject	P.E (gauze)	No	-	pain↓
I. H. Kim [24]	mixed aroma (lavender, ylang-ylang, marjoram, neroli)	prehypertensiv e and hypertensive subjects n=83	between -subject	P.E (necklace, aroma stone)	No	-	BP↓, decreased salivary cortisol

P.E., passive exposure; O.C., oral consumption; ↑increase, ↓decrease; n.s, not significant; unk., unknown; M, male; F, female; EMG, electromyogram; CgA, chromogranin A; HR, heart rate; HRV, heart rate variability; LF, low-frequency component of HRV; HF, high-frequency component of HRV; BP, blood pressure; SBP, systolic blood pressure; DBP, diastolic blood pressure; RSP, respiration; SCR, stem cell reinfusion; SCWT, Stroop color-word test; EPM, elevated plus-maze test; LDP, light/dark paradigm

Table 2: Summary of past aroma studies (Exp.2,3)

Study	Aroma	Subjects (stress load)	Study design	Method of stimulation	Psychological effects	Physiological effects
G.N. Martin [18]	chocolate, spearmint, strawberry, almond, vegetable, garlic onion, cumin	healthy university students and volunteers (no stressor) n=21	within-subject	P.E. (paper strips)	relaxation↑[chocolate, spearmint]	EEG (θ)↓[chocolate, spearmint]
M.P. Pase et al. [17]	chocolate	healthy middle-aged participants (cognitive test battery having 10 tasks) n=72	between-subject	O.C.	positive mood↑	-
E. Ozdemir et al. [5]	strawberry	patients undergoing cryopreserved PBSC infusion n=158	between-subject	O.C. (lollipop)	-	nausea and vomiting↓
A. Yoto et al. [4]	green tea, shaded white tea	healthy volunteers (Kraepelin test) n=9(M)+9(F)	within-subject	O.C.	task performance↑, mood disturbance↓	salivary CgA↓
S. Murao et al. [3]	green tea, shaded white tea	healthy volunteers (arithmetic mental task, auditory oddball target detection task) n=6(M)+6(F)	within-subject	P.E. (tea cup)	task performance↑, positive mood↑, relaxation↑	EEG(α, β)↑
T. S. Lorig et al. [14]	spiced apple, eucalyptus, lavender	healthy volunteers (mental task series) n=4(M)+5(F)	within-subject	P.E. (vials)	anxiety and tension↓[spiced apple]	EEG (θ)↓[spiced apple]
A. Toet et al. [28]	apple, orange	dental patients n=117(M)+ 102(F)	between-subject	P.E. (dispenser)	anxiety, mood, or perceived pain n.s	-
Y.B. Yip et al. [7]	mixed aroma (ginger, orange)	older persons n=59	between-subject	oil massage	quality of life n.s	knee pain↓
J.R. Johnson et al. [6]	ginger, lavender, mandarin, marjoram	acute care patients n=9389	single arm	P.E.	anxiety↓[lavender, marjoram]	pain↓[marjoram], nausea↓[ginger]
M.-Y. Cho et al. [12]	mixed aroma (roman chamomile, lavender, neroli)	PCI patients n=56	between-subject	P.E (aroma stone)	anxiety↓, sleep quality↑	stabilized blood pressure
J.D. Amsterdam et al. [15]	chamomile	outpatients with mild-moderate GAD n=61	between-subject	O.C. (capsule)	anxiety↓	-

M. Kim et al. [21]	mixed aroma (chamomile, lavender, sweet orange)	nursing students practicing intravenous injection for the 1st time n=55	between-subject	P.E. (aroma lamp)	anxiety n.s	pulse, Δpulse, and ΔSBP↓
P.H. Graham et al. [33]	mixed aroma (cedarwood, lavender, bergamot)	patients undergoing radiotherapy n=313	between-subject	P.E. (bibs)	anxiety and depression n.s	-
S. Dayawansa et al. [23]	cedarwood	healthy volunteers (no stressor) n=26 (cedarwood) n=9 (control)	unk.	Olfactometer	-	HR, SBP, DBP↓, HF↑
N.N. Ayuob [26]	musk	Swiss albino mice (CUMS) 40(M)	between-subject	P.E. (cotton)	-	serum corticosterone↓
H. Fukui et al. [25]	musk, rose, floral	healthy university students (no stressor) n=8(M)+8(F)	within-subject	P.E. (filter paper)	-	cortisol↓[musk, rose, floral]
S. Nomura et al. [13]	lavender, jasmine	healthy university students (Kraepelin test) n=17(M)	within-subject	Olfactometer	concentration↑[lavender]	nose temperature and HF↓[lavender], HR↑[lavender and jasmine]

P.E., passive exposure; O.C., oral consumption; ↑increase, ↓decrease; n.s, not significant; unk., unknown; M, male; F, female; EEG, electroencephalogram; CgA, chromogranin A; HR, heart rate; HF, high-frequency component of heart rate variability; SBP, systolic blood pressure, DBP, diastolic blood pressure; PBSC, peripheral blood stem cell; PCI, percutaneous coronary intervention; GAD, generalized anxiety disorder; CUMS, chronic unpredictable mild stress

1.1.3 Limitations in Aroma Research

1.1.3.1 Limitations due to the Adoption of Conventional Methods of Aroma Administration

It can be seen that the inconsistent findings frequently reported in aroma research as discussed in section 1.1.2 are frequently attributed to the limitations brought by conventional methods of aroma administration. Passive exposure administration which is quite common in aroma research causes difficulties in strictly controlling the duration and concentration of aroma administration. Besides, the outcomes of such studies might be affected by the olfactory fatigue.

Among the common practices used in passive exposure administration are, impregnated masks [9] or other materials [8, 24, 29, 33] worn by the participants. Some other studies have used impregnated tissue papers [11] or perfumer's paper trips [18] placed in a constant distant from the subjects. Inhalation of aroma using inhalers [37], aromatherapy samplers [31], cups [3] or vials [14] containing the odourants have also been frequently used in previous research.

Meanwhile, aroma stones [12, 24], aroma lamps [21], dispensers [10, 28], ultrasonic atomizers [22], and aroma diffusers [16, 20, 32] are used in diffusing the odours in the ambient environment. While there is immense evidence on the use of various passive exposure administration techniques as discussed above, only a very few studies are reported so far which introduced well-controlled methods of aroma administration, e.g. olfactometers [13, 23].

1.1.3.2 Variation in the Method of Aroma Administration and Experimental Design

In addition to the limitations brought by employing conventional exposure administration methods, the considerable variation in these administration methods across studies (as reflected in Table 1 and 2) introduces another major limitation in aroma research. Further, the current literature highlights a wide variation in the experimental design, limiting the ability to make direct comparisons among their findings.

1.1.3.3 Limitations due to the Absence of a Load-test

A number of field experiments have been conducted investigating the effects of aroma within various clinical settings. Psychophysiological effects of aroma on patients undergoing stem cell reinfusion (SCR) [5, 30, 31, 32], or radiotherapy [33], patients treated in intensive care units after percutaneous coronary intervention [12], and pregnant women during labour [29] are reported previously. Effects of olfactory stimulation on prehypertensive and hypertensive subjects [24], outpatients with mild-moderate generalized anxiety disorder [15], and residential-care residents with dementia and behavioural challenges [36] are also reported. Moreover, the effects of aroma are investigated the subjects being patients waiting for dental treatments [10, 20, 28], patients with burn wounds [8], and nursing students practicing intravenous injection for the first time [21].

In addition to the above-mentioned field studies, laboratory experiments investigating the efficacy of aroma under different stress load tasks are reported. Various cognitive stressors including Kraepelin calculation task [4, 13, 37], Stroop colour-word test [9], and other cognitive test batteries [3, 14, 17] have been employed in previous studies. Physical stressors are also employed [37], but seldom.

While there are reports on studies that have successfully employed stress load tasks in the laboratory environment, simple exposure studies with no any acute stressor are reported quite frequently [11, 18, 23, 25]. The absence of a stress load task forms another limitation in

aroma research since the outcomes of such experiments might be strongly deteriorated by the initial condition of the participants.

1.1.3.4 Disregarding the Dose-dependent Effects of Aroma

A considerable amount of research has been carried out in the past to investigate the psychophysiological effects of orange and various other citrus fragrances as summarized in Table 1. With a remarkable number of studies failing to verify any beneficial effect and some studies resulting in negative effects, the reported results on the effects of orange are highly inconsistent among the studies as detailed in section 1.1.2. In addition to the common limitations in aroma research discussed previously, the poor attention paid on controlling the concentration of aroma can be identified as a potential reason that may lead to discrepant results. Among the many studies concerning the psychophysiological effects associated with orange aroma, only a very few have incorporated the dose variation in the experimental design. Goes et al. (2012) investigated the dose-dependent effects of orange aroma, but their results showing no physiological benefit should be interpreted cautiously given the small sample size (n=8) as for a between-subject design [9]. An animal study employing Wistar rats has demonstrated dose-dependent effects of orange aroma, where the highest anxiolytic effect was observed with the highest dose [35].

1.2 Motivation

Among the studies investigating the psychophysiological effects of various olfactory stimuli, a considerable number of studies have reported findings which are inconsistent with each other. The outcomes of aroma research are frequently limited due to several reasons as identified and discussed in detail in section 1.1.3. These limitations include the utilization of conventional passive exposure administration methods which impose difficulties in controlling the duration and concentration of aroma administration, the large variation among aroma studies in terms of experimental design and method of aroma administration, and the absence of a load test. While the inconsistency among the findings persistent in aroma research is frequently and merely attributed to the wide variation among the studies, less effort is made so far in introducing sound experimental design which overcomes the above limitations. Taken together, there is a need to investigate the psychophysiological effects of aroma in a well-controlled experimental set-up using a well-controlled method of aroma administration under the presence of a stress load. Orange aroma being a major example with reported inconsistent findings, further encompasses a lack of attention on its dose-dependent effects. Among other

aromas which resulted in inconsistent findings are Chocolate, Strawberry, Green tea, Apple, Citrus ginger, Chamomile, Cedarwood, and Musk. Further, the available scientific evidence to verify the efficacy of these aromas in alleviating psychophysiological stress is insufficient. Having identified eight different aromas, a preliminary investigation needs to be carried out as the first step in order to identify the aromas with inhibitory potentials. Upon identifying the prominent aromas from among the eight aromas, thorough investigations are required to be carried out to verify their efficacy in alleviating psychophysiological stress.

1.3 Research Framework and Objectives

1.3.1 Research Framework

Based on a comprehensive review of literature on olfactory psychophysiology, major drawbacks (detailed in section 1.1.2) and the underlying limitations in aroma research (detailed in 1.1.3) have been identified. Overcoming the identified limitations, the study has introduced an approach for experimenting psychophysiological effects of olfactory stimuli in alleviating acute stress response, which utilizes a customized olfactometer for aroma administration in a well-controlled experimental setting under the presence of a cognitive load test. Addressing the research gaps associated with identified aromas: Orange and eight other aromas (Chocolate, Strawberry, Green tea, Apple, Citrus ginger, Chamomile, Cedarwood, and Musk), the study comprised of a series of three experiments (Figure 1) of which the specific objectives are stated in 1.3.2.2.

1.3.2 Objectives

1.3.2.1 Main Objective

To investigate the psychophysiological effects of olfactory stimuli under a short-term cognitive stressor, in a well-controlled experimental setting using an olfactometer for precisely controlling the duration and concentration of aroma administration.

1.3.2.2 Specific Objectives

- To investigate the dose-dependent effects of 1 and 20% Orange on alleviating acute stress response
- To identify the aromas having inhibitory potentials from among eight different aromas: Chocolate, Strawberry, Green tea, Apple, Citrus ginger, Chamomile, Cedarwood, and Musk, so that the identified aromas could be investigated further for their efficacy in alleviating psychophysiological stress

- To investigate the efficacy of two identified aromas: Apple and Chamomile in alleviating psychophysiological stress

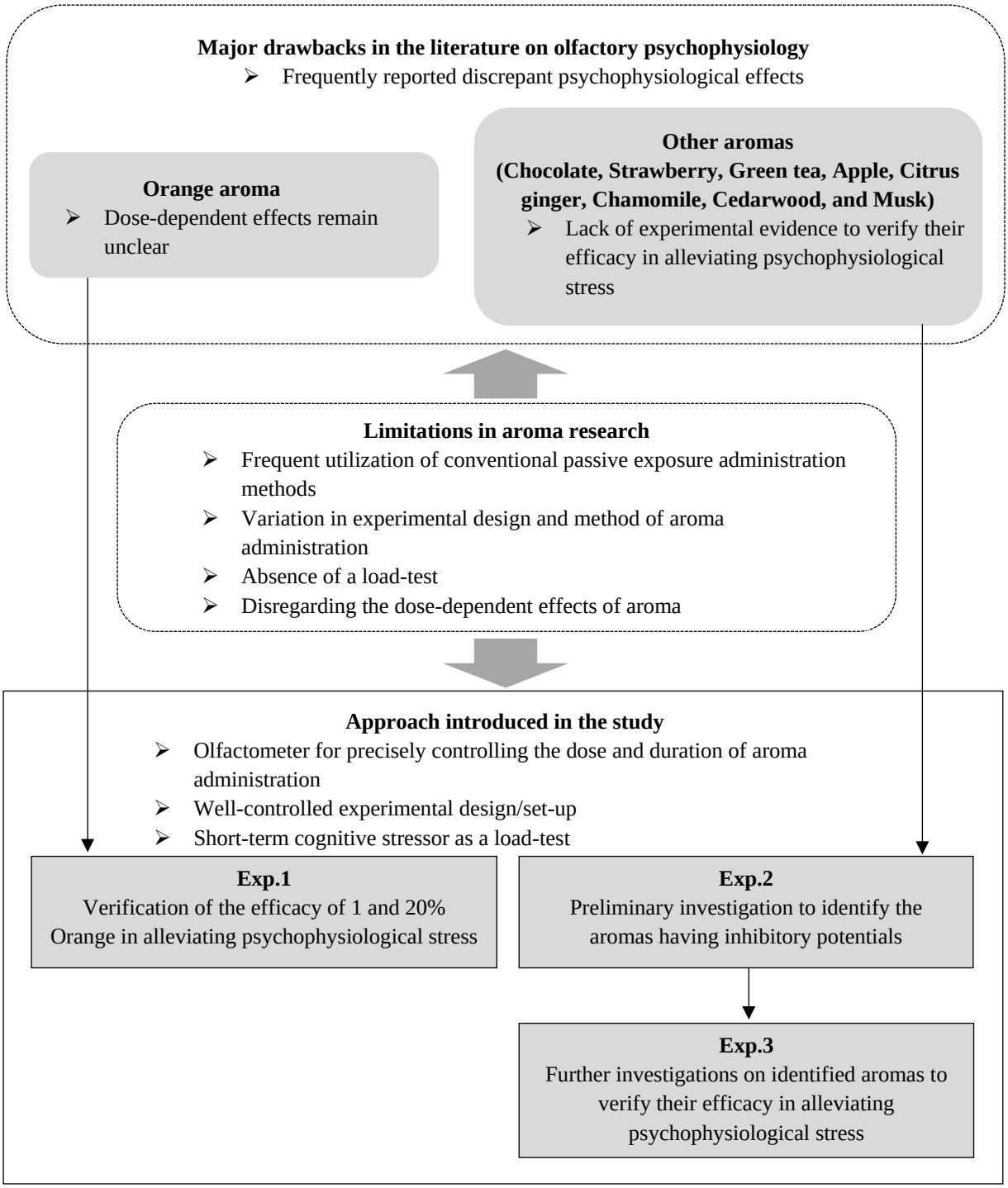


Figure 1: Research framework

1.4 Chapter Summary

Based on scientific evidence from the literature, various health benefits of aroma including behavioural, psychological, and physiological benefits are discussed within this chapter. Discrepant psychophysiological effects of aroma frequently reported in the literature has been identified as a major drawback in aroma research. A number of examples have been provided for such discrepancies reported in the past with orange, other citrus fragrances, and the eight aromas concerned in the present study. Use of conventional methods of aroma administration, large variation in the method of aroma administration and experimental design, absence of a load-test, and poor attention paid on the dose-dependent effects of aroma have been identified and discussed as the major limitations in aroma research that usually lead to above mentioned discrepant effects.

Addressing the identified limitations, the study has introduced an approach that investigates the phsychophysioloigcal effects of aroma under a short-term cognitive stressor, using a proprietary olfactometer for well-controlled aroma administration, in a well-controlled experimental setting. Addressing the limitations observed particularly with Orange and eight other aromas (Chocolate, Strawberry, Green tea, Apple, Citrus ginger, Chamomile, Cedarwood, and Musk), a series of experiments were designed, the specific aims being: identifying the dose dependent effects of Orange, preliminarily investigating the eight aromas for identifying aromas with inhibitory potentials, and further investigating two identified aromas: Apple and Chamomile for their efficacy in alleviating stress.

CHAPTER 2

MATERIALS AND METHODS

2.1 Subjects

Male university students who were healthy with normal olfactory function were employed in the experimental series. The number of subjects employed in each experiment along with the mean (±SD) age and body mass index are stated in Table 3.

Table 3: Number of subjects, age, and body mass index

Experiment	No. of Subjects	Age (years) mean (±SD)	Body mass index (kg/m^2) mean (±SD)
Exp.1	19	22.1 ± 1.03	22.3 ± 5.05
Exp.2	6	21.7 ± 1.51	21.3 ± 2.91
Exp.3	19	22.0 ± 0.84	21.1 ± 2.53

The experiments were carried out in accordance with the ethical principles of the Helsinki Declaration and informed consent was obtained from each subject. The experiments were approved by the Ethics Committee of Nagaoka University of Technology.

2.2 Experimental Procedure

2.2.1 Experimental Protocol

The experiments were conducted following a similar experimental protocol as of our previous study which investigated the psychophysiological effects of lavender and jasmine aromas [13]. Each experiment consisted of a 10-min initial rest period as the initialization period (denoted as R1), a 30-min calculation task to induce cognitive stress (denoted as T), and a subsequent 15-min recovery period (denoted as R2), as shown in Figure 2.

2.2.2 Experimental Design

Within-subject experimental design was employed throughout the experimental series. Each subject performed the calculation task under each of the aroma conditions on separate days. In order to avoid the order effect due to the execution order of conditions in within-subject experiments, aroma presentation was counter-balanced in Exp.1 and Exp.3. Since the sample size was too small as for counter-balancing, the order of aroma presentation was randomized in the preliminary investigation (Exp.2). Table 4 shows the counter-balancing of the aroma conditions in Exp.1.

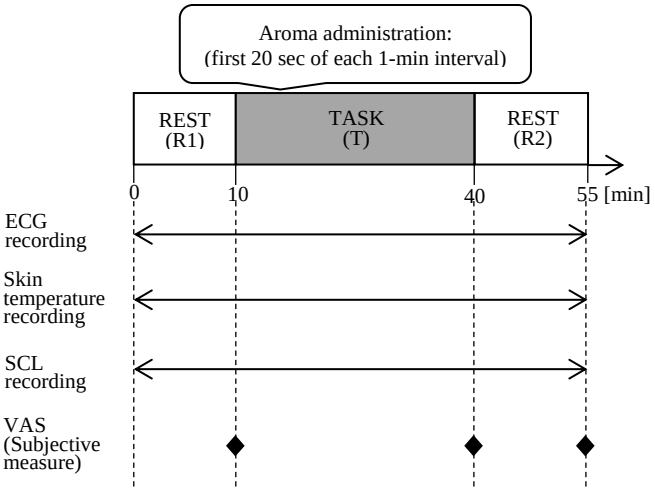


Figure 2: Schematic presentation of the protocol

Table 4: Counter-balancing (Exp.1)

Subject	Aroma condition		
A	Ctl	1%	20%
B	Ctl	1%	20%
C	Ctl	1%	20%
D	Ctl	1%	20%
E	Ctl	20%	1%
F	Ctl	20%	1%
G	Ctl	20%	1%
H	1%	Ctl	20%
I	1%	Ctl	20%
J	1%	Ctl	20%
K	1%	20%	Ctl
L	1%	20%	Ctl
M	1%	20%	Ctl
N	20%	Ctl	1%
O	20%	Ctl	1%
P	20%	Ctl	1%
Q	20%	1%	Ctl
R	20%	1%	Ctl
S	20%	1%	Ctl

All experiments were conducted under well-controlled environmental conditions in an air-controlled laboratory. The mean (± SD) temperature and humidity of the laboratory were 21.9 ± 1.2°C and 20.2 ± 4.5% (Exp.1), 19.8 ± 2.0°C and 30.6 ± 10.3%, (Exp.2), and 23.3 ± 2.5°C and 42.0 ± 11.4% (Exp.3).

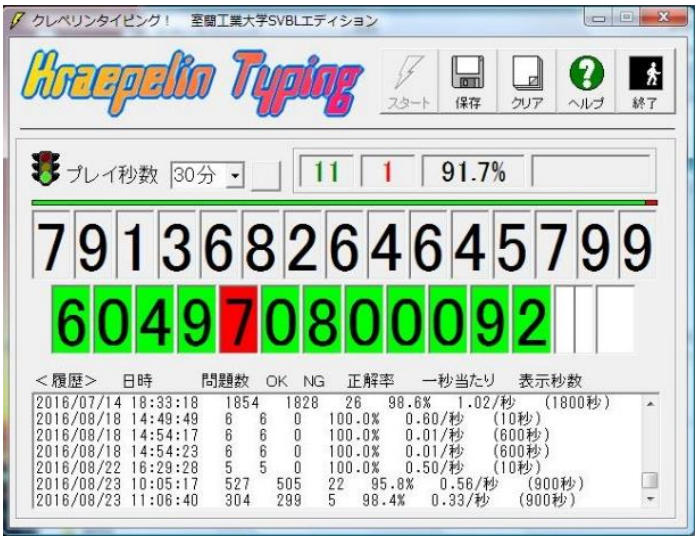


Figure 3: Kraepelin Test interface

2.2.3 Short-term Cognitive Stressor

Kraepelin test was employed as the short-term cognitive stressor, where the subjects were required to continuously add single digit numbers displayed on a computer screen (Figure 3). All the subjects were instructed to perform the calculation as quickly and accurately as possible. Despite being simple, the Kraepelin calculation requires sustained concentration and attention. Due to this reason, it has been frequently used by a number of previous studies to induce acute cognitive stress [13, 38, 39, 40].

2.2.4 Olfactory Stimuli

For Exp.1, Orange essential oil (Takasago International Corporation, Kanagawa, Japan), composed mainly of limonene (97–98%), citral (0.5–0.8%), and geraniol (0.02–0.04%), was prepared at 1 wt% (for 1%) and 20 wt% (for 20%) with the odourless solvent triethyl citrate (TEC). The concentrations 1 and 20% were selected based on the findings of a pre-screening test using 80 participants. In the pre-screening test, both 1 and 20% Orange were rated as favourable, with no statistically significant difference between the preferences for the two doses. However, a significant difference was observed between the two doses in terms of perceived strength. The questionnaire used in the pre-screening test is attached as Appendix 5. TEC was also used as the Control stimulus in Exp.1.

In Exp.2, the eight aromas: Chocolate, Strawberry, Green tea, Apple, Citrus ginger, Chamomile, Cedarwood, and Musk (Takasago International Corporation, Kanagawa, Japan) were used as the olfactory stimuli. The key compounds of the above aromas used in Exp.2 are listed in Table 5. Dipropylene glycol (DPG) was used as the Control stimulus.

Table 5: Key compounds of aromas used in Exp.2

Sample	Odour	Key Compounds
Chocolate	Sweet chocolate	Vanillin, Ethyl maltol
Strawberry	Sour strawberry	2-Methyl butyric acid, Ethyl maltol
Green Tea	Fresh green tea	β-Ionone, Lemon oil
Apple	Green apple	Allyl heptoate, Butyl acetate
Citrus ginger	Lemon and ginger	Lemon oil, α-Pinene
Chamomile	Chamomile	Limonene, Hexyl acetate
Cedarwood	Cedarwood	Cedarwood oil, Acetyl cedrene
Musk	Musk	L-Muscone, Galaxolide

In Exp.3, Apple and Chamomile (Takasago International Corporation, Kanagawa, Japan) were used as the olfactory stimuli. DPG was used as the Control stimulus.

2.2.5 Aroma Administration

2.2.5.1 Multi-channel Olfactometer

A multi-channel olfactometer (Tatsumi Kagaku Co.,Ltd., Kanazawa, Japan) was used in this study through which the flow rate and the timing of aroma presentation could be precisely controlled via a computer programme.

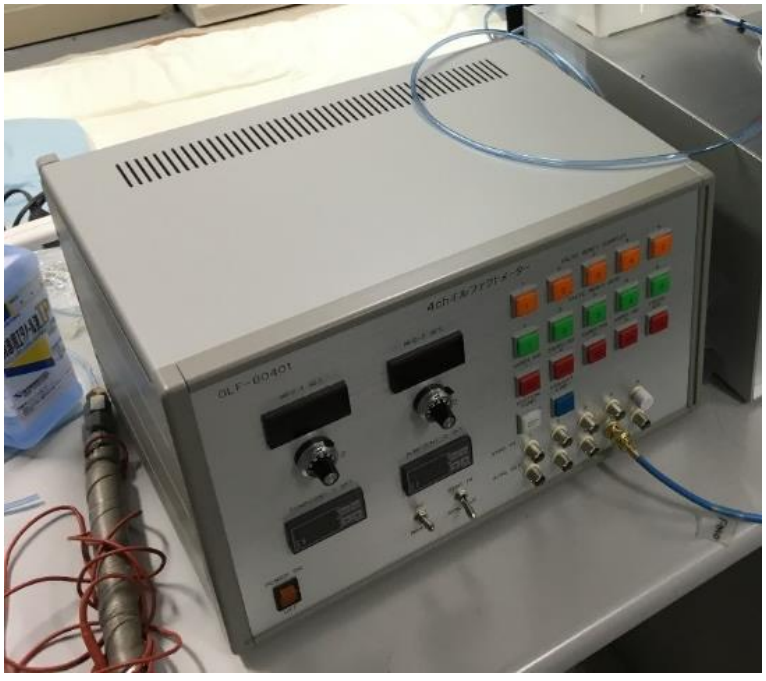


Figure 4: Multi-channel olfactometer

Figure 5: A subject wearing a cannula

2.2.5.2 Method of Intervention

During the task, each of the aromatic stimuli or the Control stimulus was intermittently delivered (for the first 20 s of each 1 min interval) via a cannula placed under the nostrils and connected to the customized olfactometer. Olfactory fatigue which is a common limitation associated with conventional exposure administration methods could be prevented through intermittent delivery.

2.3 Measurements and Instrumentation

2.3.1 Behavioural Measures

2.3.1.1 Task Performance

Performance of the calculation task was measured in terms of the accuracy and speed of the calculation.

2.3.2 Psychological Measures

2.3.2.1 Subjective Impression

Subjective impressions were collected using a 7-point Likert scale ranging from 1 to 7. The subjects were asked to rate their impressions on perceived strength of, preference for, and familiarity with each aroma, and the comfort and relaxation they experienced during aroma inhalation (Aroma Evaluation Questionnaire used in obtaining the subjective impressions is attached as Appendix 1). From among the above items, strength and preference which demonstrated significant differences have been discussed within this research work.

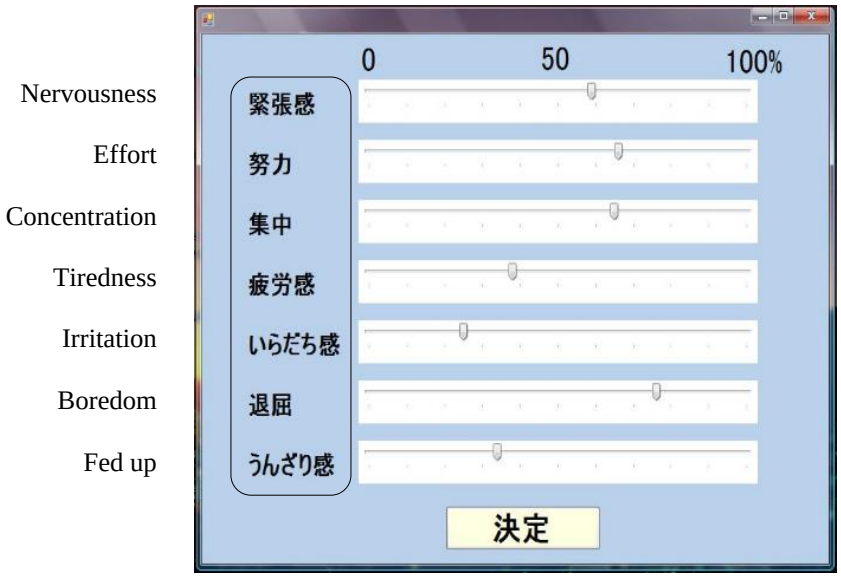


Figure 6: Visual analog scale interface

Figure 7: Visual analog scale output

2.3.2.2 VAS Scores

The subjects were asked to complete a visual analog scale (VAS) comprising of seven items: frustration, tension, concentration, monotonous, effort, fatigue, and boredom at the end of R1, at the end of T, and at the end of R2 (Figure 2). The VAS was a calibrated line having two end points: 0 and 100% on which the subjects were required to mark their perception on each of the above items. The time required for a single marking was less than 10 s. The interface of the Japanese version of the VAS employed in the study and its corresponding output are shown in Figure 6 and 7 respectively.

To compensate the large variation in VAS scores, each item was normalized as 0.0 to 1.0. The VAS scores for the seven items were considered in Exp.1 and Exp.3, whereas the VAS scores for five selected items were summarized into one averaged value in Exp.2 in order to make the interpretation simpler. The five selected items were frustration, concentration, effort, fatigue, and boredom which were found to increase with a stressor in our previous study [13].

2.3.3 Physiological Measures

2.3.3.1 Heart Rate and Heart Rate Variability

Throughout each experiment (R1-T-R2), the electrocardiogram (ECG) recordings were obtained using a bio-amplifier (MP150, BIOPAC Systems Inc., Goleta, CA, USA) at a sampling rate of 200 Hz with 16-bit resolution. Using the ECG data, the heart rate (HR) and heart rate variability (HRV) which is a frequency domain of the heartbeat in a time series were analyzed. The high-frequency (HF) component (0.15 to 0.40 Hz of HRV) represents cardiac parasympathetic nervous system activity [41].

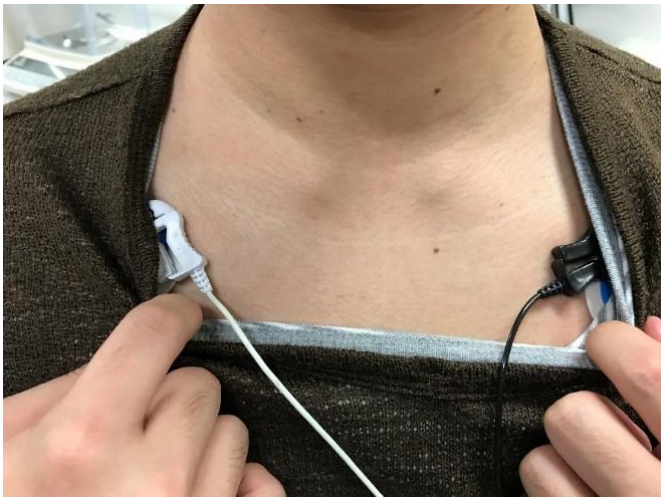


Figure 8: Electrocardiogram measurement

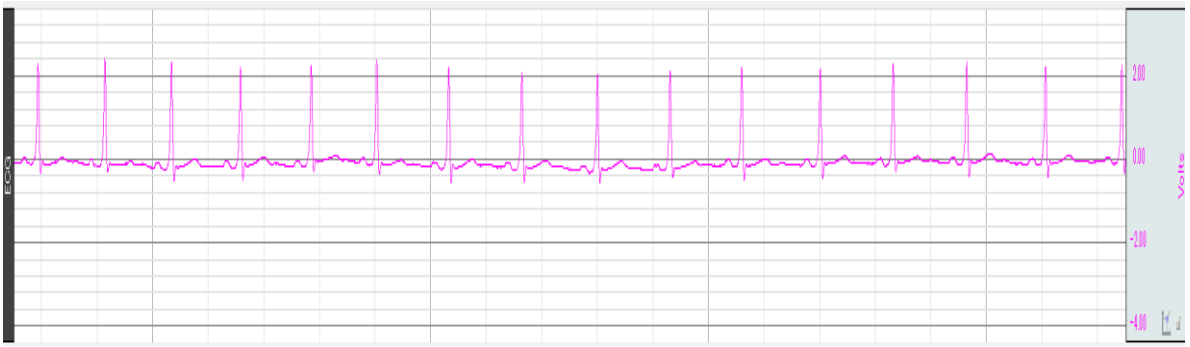


Figure 9: Electrocardiogram on BIOPAC AcqKnowledge

Figure 10: BIOPAC Bio-amplifier

2.3.3.2 Skin Temperature

Throughout R1-T-R2, the temperature at the tip of the nose, dorsal hand, and that at the forehead (as a reference) were obtained using a thermistor probe. The temperature data was recorded at a sampling rate of 1.0 Hz, using a high precision eight-channel data logger (ITP082-25, 24 NIKKISO-THERM Co., Ltd., Tokyo, Japan).

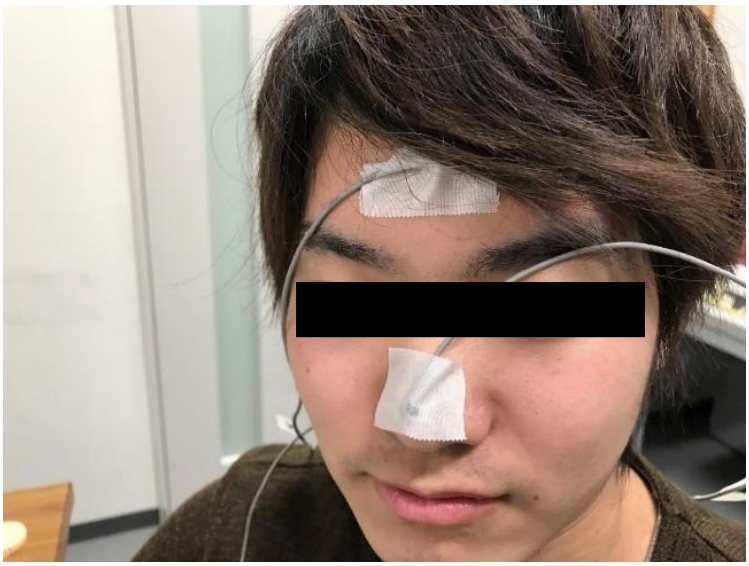


Figure 11: Skin temperature recording (nose/forehead)

Figure 12: Skin temperature recording (dorsal hand)

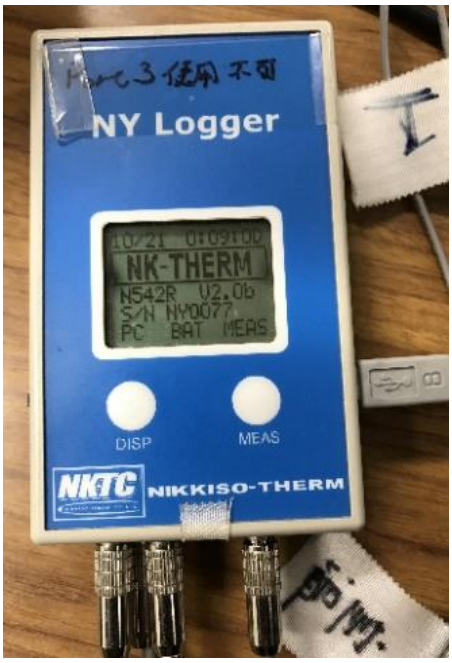


Figure 13: High precision 8-channel data logger

2.3.3.3 Skin Conductance Level

Throughout R1-T-R2, the skin conductance level (SCL) was recorded using the BIOPAC bio-amplifier at a sampling rate of 200 Hz with 16-bit resolution.

An overview of the experiment including the method of aroma administration and the psychophysiological measurements is shown in Figure 15.

Figure 14: Skin conductance level recording

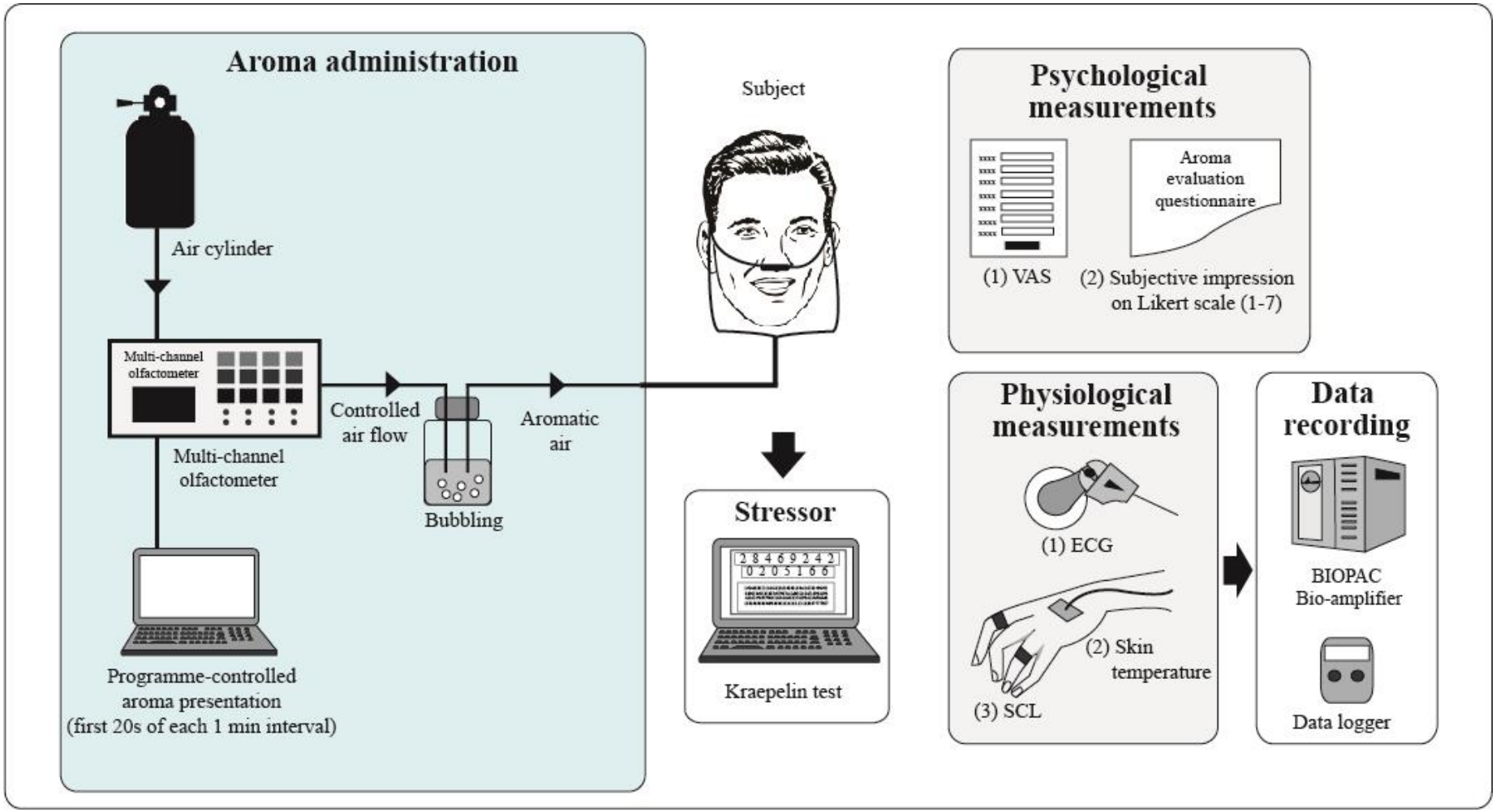


Figure 15: Overview of the experiment

2.4 Analysis

2.4.1 Biomedical Data Analysis

2.4.1.1 HR Analysis

Software

- AcqKnowledge 4.1: Output RR Interval data
- Excel: (BIOPACK_RR-Check_ver02.xlsx, BIOPACK_RR-Check-HRVCal.xlsx)
- HrvCalc: Heart rate variability analysis software

STEP 1
Obtain RR Interval data using AcqKnowledge 4.1

I. Open 【Graph(.acq)】

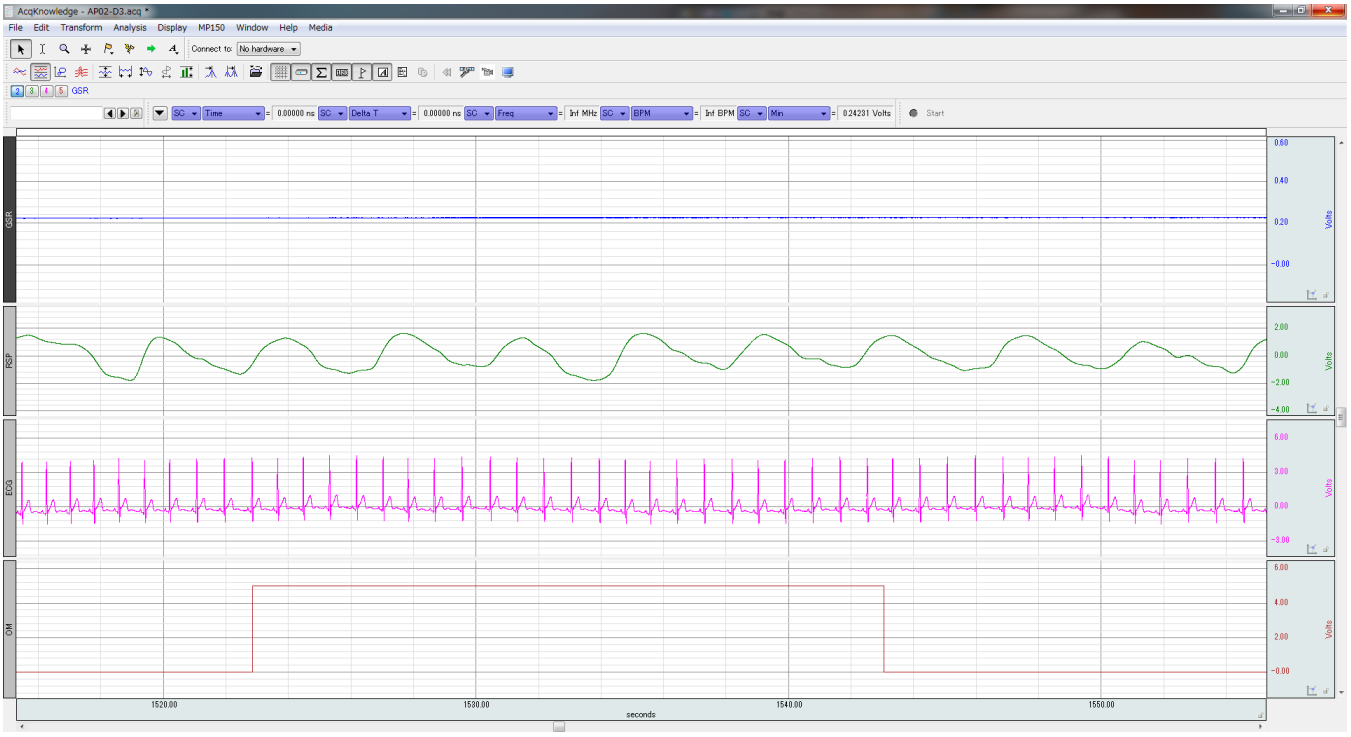


Figure 16: 【Graph(.acq)】

II. Tab「Analysis－Heart Rate Variability」→Window「Analysis – Heart Rate Variability」

III. Tab「RR Intervals」→change Minimum BPM to 30, Maximum BPM to 135

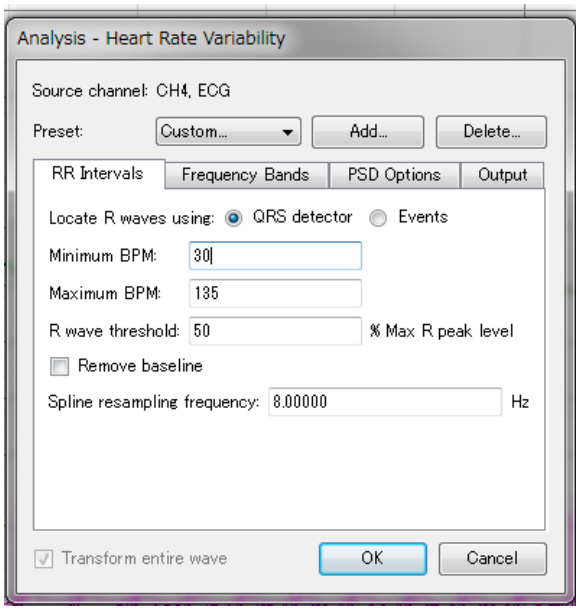


Figure 17: Tab「RR Intervals」

IV. Tab「Output」→Display RR interval table 「✓」 →Push「OK」→Open Window 「HRV Analysis Results」

V. Push「Copy to Clipboard」→Push「OK」

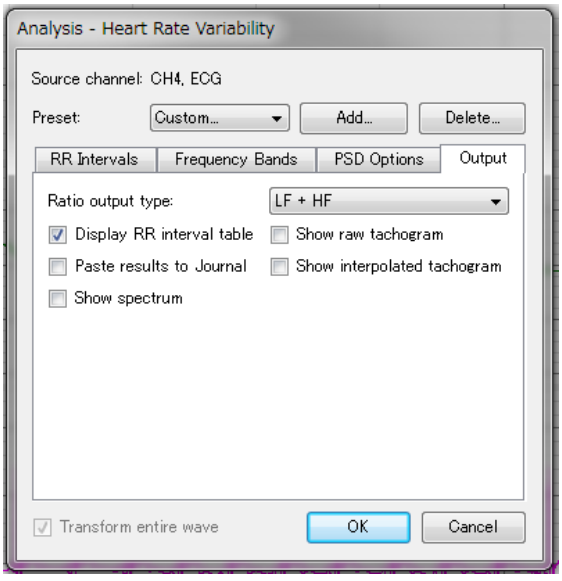


Figure 18: Tab「Output」

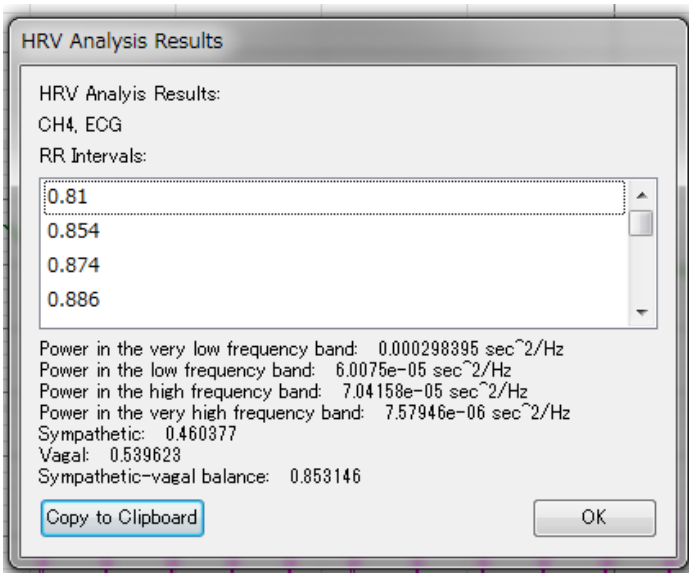


Figure 19: Window「HRV Analysis Results」

STEP 2
Noise removal of RR Interval data

I. Open the Excel file「BIOPACK_RR-Check_ver02.xlsx」
II. Paste the RR interval data acquired in STEP 1
III. Check whether there are outliers

IV. Identify the point (time in seconds) the outlier occurred and correct the value of 「BIOPACK_RR-Check_ver02.xlsx」

- Fill with yellow colour before correction, and fill with green colour after correction
- 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.

V. Repeat IV until there are no outliers

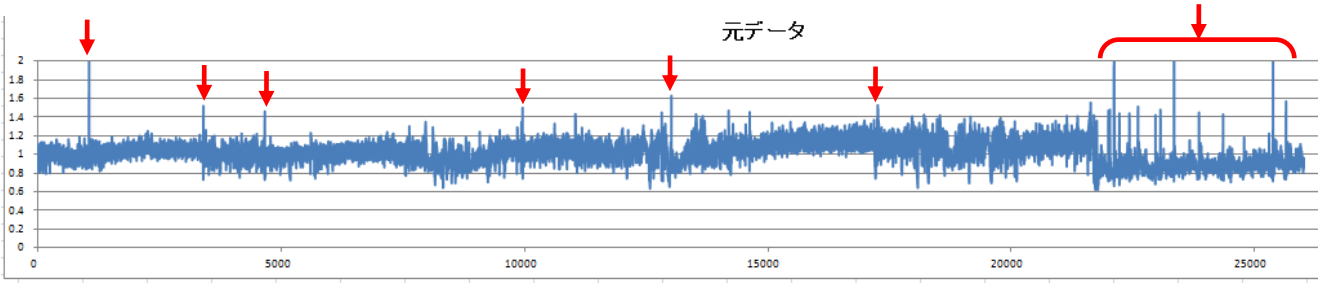


Figure 20: Before the removal of outliers

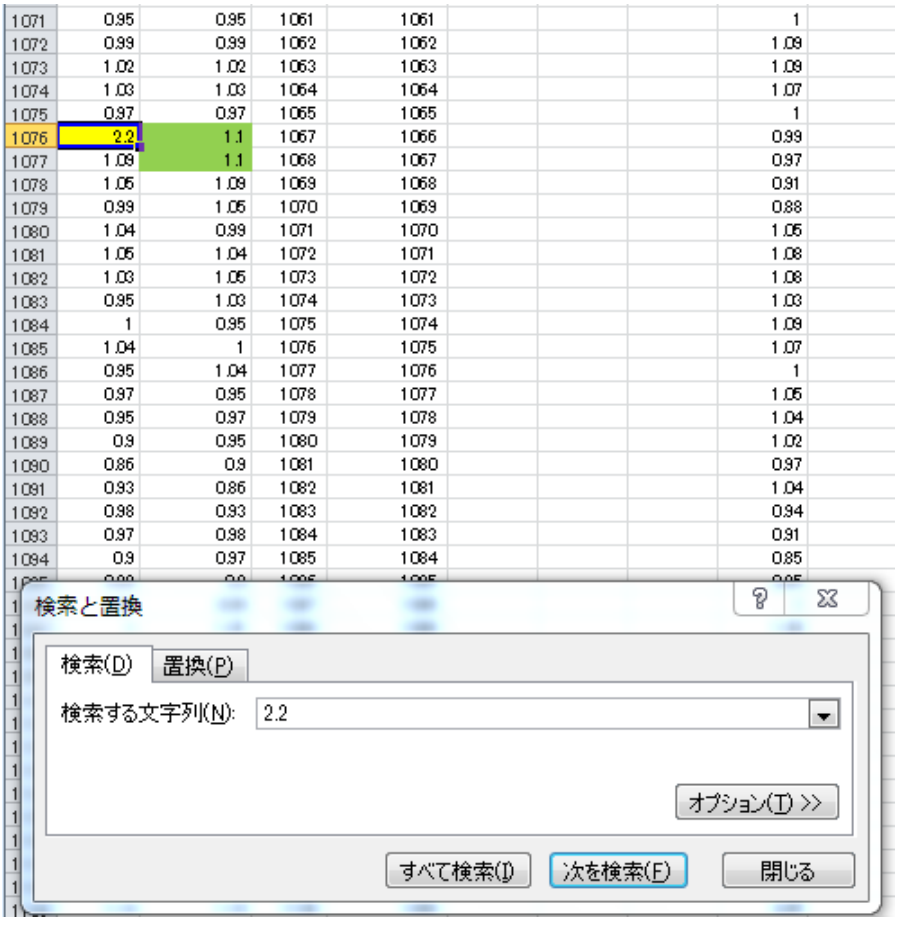


Figure 21: Removing outliers

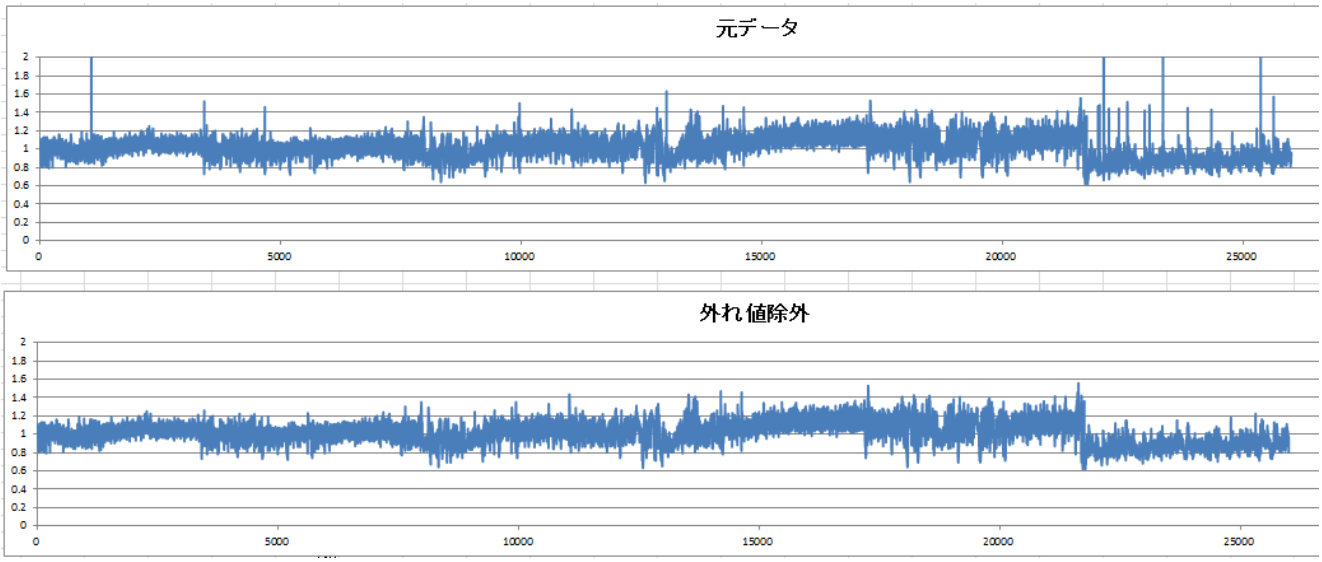
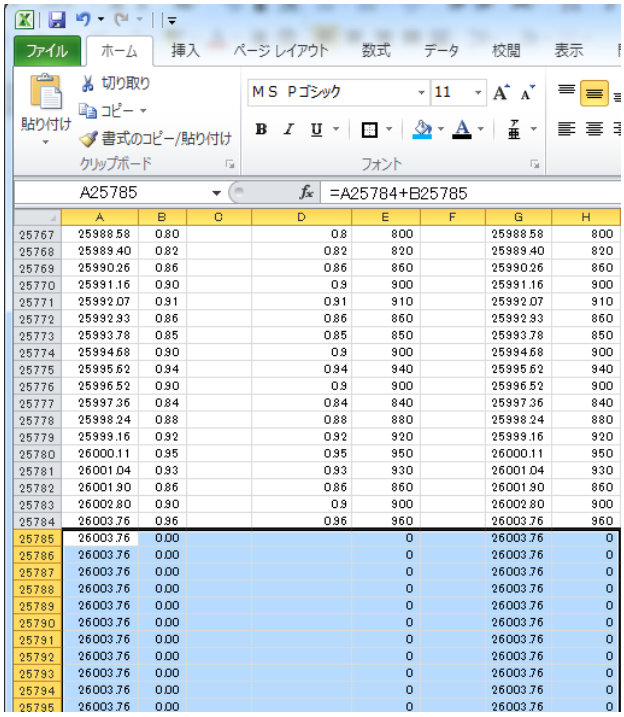


Figure 22: After the removal of outliers

STEP 3
Analysis of heart rate variability

I. Copy the outlier removed data obtained in STEP 2 and paste in the Excel file "BIOPACK_RR - Check - HRVCal.xlsx"
II. Erase additional data



	A	B	C	D	E	F	G	H
25767	25988.58	0.80		0.8	800		25988.58	800
25768	25989.40	0.82		0.82	820		25989.40	820
25769	25990.26	0.86		0.86	860		25990.26	860
25770	25991.16	0.90		0.9	900		25991.16	900
25771	25992.07	0.91		0.91	910		25992.07	910
25772	25992.93	0.86		0.86	860		25992.93	860
25773	25993.78	0.85		0.85	850		25993.78	850
25774	25994.68	0.90		0.9	900		25994.68	900
25775	25995.62	0.94		0.94	940		25995.62	940
25776	25996.52	0.90		0.9	900		25996.52	900
25777	25997.36	0.84		0.84	840		25997.36	840
25778	25998.24	0.88		0.88	880		25998.24	880
25779	25999.16	0.92		0.92	920		25999.16	920
25780	26000.11	0.95		0.95	950		26000.11	950
25781	26001.04	0.93		0.93	930		26001.04	930
25782	26001.90	0.86		0.86	860		26001.90	860
25783	26002.80	0.90		0.9	900		26002.80	900
25784	26003.76	0.96		0.96	960		26003.76	960
25785	26003.76	0.00			0		26003.76	0
25786	26003.76	0.00			0		26003.76	0
25787	26003.76	0.00			0		26003.76	0
25788	26003.76	0.00			0		26003.76	0
25789	26003.76	0.00			0		26003.76	0
25790	26003.76	0.00			0		26003.76	0
25791	26003.76	0.00			0		26003.76	0
25792	26003.76	0.00			0		26003.76	0
25793	26003.76	0.00			0		26003.76	0
25794	26003.76	0.00			0		26003.76	0
25795	26003.76	0.00			0		26003.76	0

Figure 23: Outlier removed data

III. Copy data and paste into “Notepad”. Enter the number of lines and 2 in the first line of Notepad.

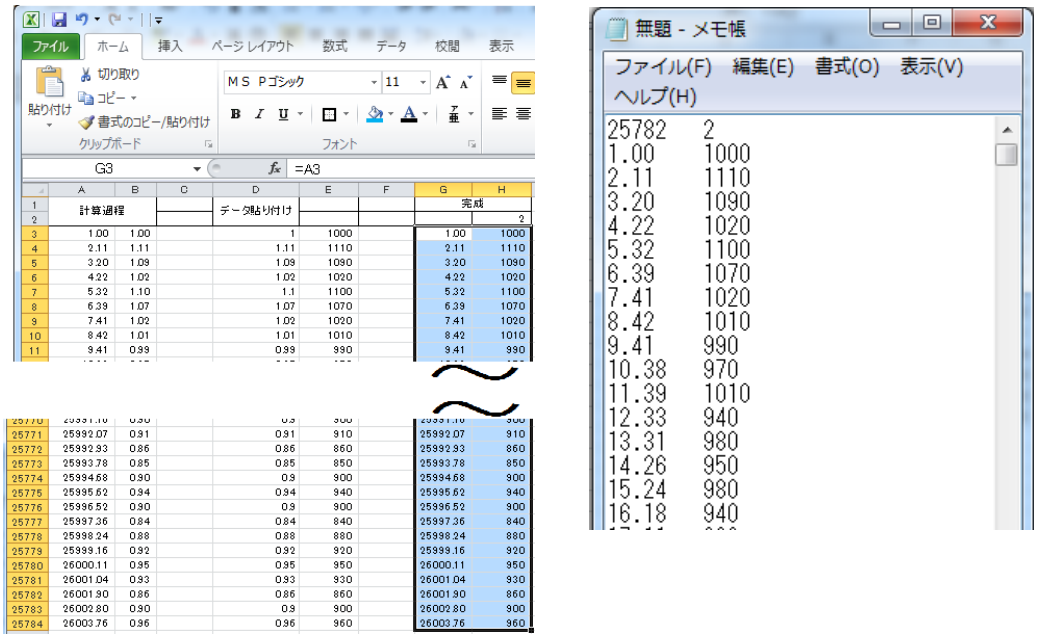


Figure 24: Copying data to Notepad

IV. Open「HrvCalc.exe」

V. 1. Select the data file「.txt」

2. Enter the same time duration

3.「LF 下限」is 0.05

4. Change the analysis interval to 3 seconds

VI. Analysis result is output to the folder where the 「.txt」is saved

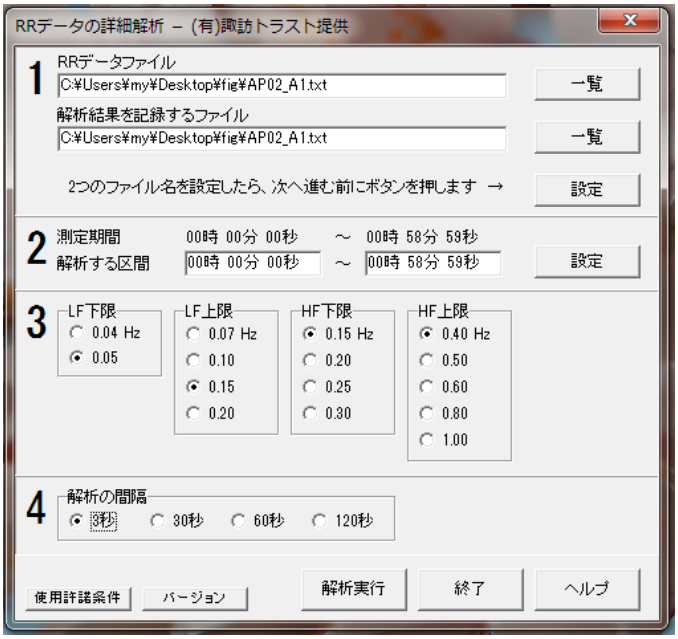


Figure 25: Setting the analysis software

2.4.2 Statistics

Paired t-tests were performed for the statistical analysis of physiological measures and the VAS scores whereas Wilcoxon signed-rank test was performed for the analysis of subjective impressions. Bonferroni corrections were applied to control for multiple comparisons among conditions. Since Exp.2 aimed to identify some prominent aromas with the best and worst potentials in alleviating stress, each aroma condition used in Exp.2 was compared only against the Control condition. The level of statistical significance (p) was set at 0.05.

2.5 Chapter Summary

Healthy male university students were employed in the study, the number of subjects being nineteen in Exp.1 and Exp.3, and six in Exp.2 which was a preliminary investigation.

Each experiment comprised of a 10-min initial rest period, a 30-min calculation task period Kraepelin test being the short-term cognitive stressor, and a subsequent 10-min recovery period. Olfactory stimuli: 1 and 20% Orange, and TEC (Control) in Exp.1; Chocolate, Strawberry, Green tea, Apple, Citrus ginger, Chamomile, Cedarwood, Musk, and DPG (Control) in Exp.2; and Apple, Chamomile, and DPG (Control) in Exp.3 were intermittently delivered during the task period with the use of a cannula placed under the nostrils and connected to a customized olfactometer. Within-subject experimental design was employed, the order of aroma presentation being counter-balanced (Exp.1, Exp.3) or randomized (Exp.2).

Psychological measures included the subjective impressions on each aroma condition and the VAS scores which were obtained at the end of the rest period, at the end of the task period, and at the end of the recovery period. Throughout each experiment, the heart rate and heart rate variability on electrocardiograms, the skin temperature, and the skin conductance level were measured to identify the effect on cardiac and peripheral autonomic nervous system activity.

BIOPAC AcqKnowledge software was used in the analysis of ECG and SCL data. Paired t-tests and the Wilcoxon signed-rank tests were performed in statistical analyses. The level of statistical significance (p) was set at 0.05.

CHAPTER 3

RESULTS AND DISCUSSION

3.1 Results and Discussion (Exp.1)

3.1.1 Results

3.1.1.1 Task Performance

No significant differences were observed in task performance for any of the aroma conditions.

3.1.1.2 Subjective Impressions and VAS Scores

Subjective impressions on the preference and strength of each aroma condition are listed in Table 6. Both 1 and 20% Orange were perceived as stronger compared to the Control condition ($p < 0.01$). However, no significant differences were observed between the two doses of Orange either in terms of preference or strength ($p > 0.05$).

Table 6: Results [mean (SD)] of subjective impression on 7-point Likert scale (Exp.1)

Strength		
TEC (Ctl)	2.4	(0.9)
O1%	4.2	(0.9) ##
O20%	4.2	(1.1) ##
Preference		
TEC (Ctl)	3.8	(0.7)
O1%	4.1	(0.6)
O20%	4.1	(1.2)

$p < 0.01$ by comparison between condition with regard to TEC (Ctl)

Changes in VAS scores from R1 to T to R2 are shown in Table 7. Under the Control condition, the VAS scores for frustration, concentration, effort, fatigue, and boredom were significantly higher at T compared to those at R1 ($p < 0.001$-$p < 0.05$) and the scores for frustration, tension, concentration, effort, and fatigue were significantly lower at R2 compared to those at T ($p < 0.001$-$p < 0.05$). These results indicated that the Kraepelin calculation employed in the study functioned as an acute stressor as has been evident in previous studies [13, 38, 39, 40]. A similar trend was observed under 1 and 20% Orange which demonstrated significantly higher VAS scores at T than at R1 for frustration, tension, concentration, effort, and fatigue ($p < 0.001$-$p < 0.05$). However, no significant differences could be observed between any of the aroma conditions.

Table 7: Results [mean (SD)] of VAS scores (Exp.1)

	Δ（R1-T）			Δ（T-R2）		
Frustration						
Ctl	23.8	(25.6)	***	-15.0	(26.7)	*
O1%	11.1	(17.8)	*	-10.4	(19.5)	*
O20%	17.0	(22.2)	**	-5.2	(29.0)	
Tension						
Ctl	3.7	(30.4)		-18.4	(19.5)	***
O1%	17.6	(24.6)	**	-21.1	(24.7)	**
O20%	16.2	(23.4)	**	-15.6	(21.8)	**
Concentration						
Ctl	16.7	(26.9)	*	-21.3	(30.1)	**
O1%	24.2	(23.9)	***	-33.1	(26.2)	***
O20%	17.2	(29.2)	*	-28.3	(32.6)	**
Monotonous						
Ctl	-11.4	(28.2)		15.6	(22.7)	**
O1%	-17.7	(34.4)	*	18.4	(25.6)	**
O20%	-16.5	(27.6)	*	18.6	(25.6)	**
Effort						
Ctl	40.1	(21.8)	***	-33.4	(19.2)	***
O1%	32.9	(20.4)	***	-38.0	(20.7)	***
O20%	29.7	(27.8)	***	-32.3	(28.1)	***
Fatigue						
Ctl	26.0	(28.9)	***	-18.4	(23.7)	**
O1%	23.0	(22.1)	***	-13.1	(23.0)	*
O20%	32.7	(25.2)	***	-13.8	(16.4)	**
Boredom						
Ctl	12.3	(21.5)	*	-4.6	(17.2)	
O1%	4.1	(25.8)		-2.4	(19.1)	
O20%	12.7	(25.0)	*	-6.1	(22.8)	

*** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$ by comparison within condition

3.1.1.3 Heart Rate and Heart Rate Variability

The changes in the HR and the HF component of HRV from R1 to T to R2 for the three aroma conditions are shown in Figure 26 and 27. Raw values are standardized (so-called z-score, as the mean and standard deviation for the population are transformed to 0.0 and 1.0) with respect to each subject and condition. The baseline value for each condition (mean value at R1) was equalized among the conditions as 0.0 due to the large individual variations. Raw values (mean per 2.5 mins) of each of the physiological measures are attached as Appendix 7.

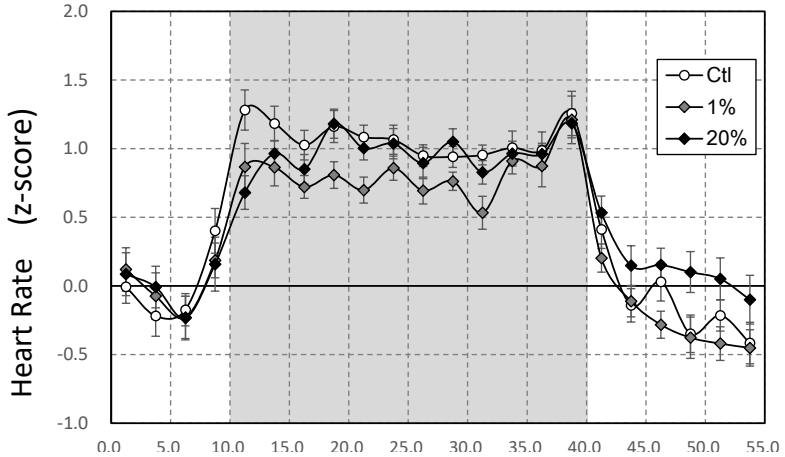


Figure 26: Changes in heart rate (mean ± SEM per 2.5 min)

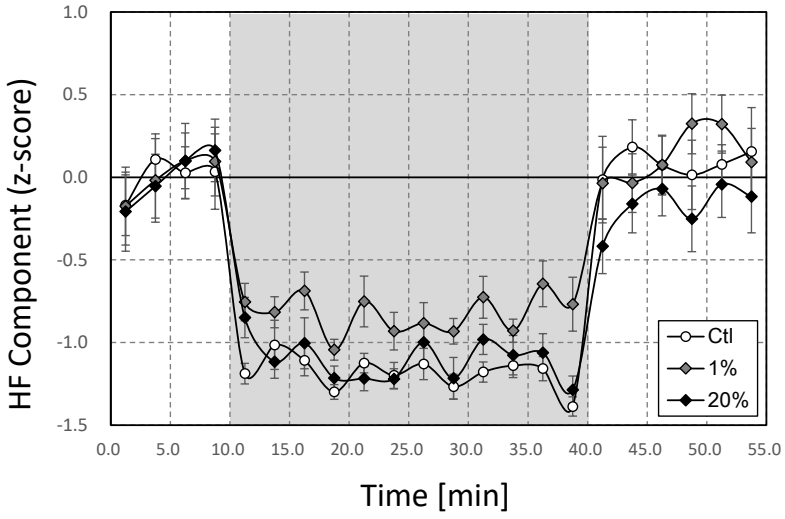


Figure 27: Changes in high-frequency component of heart rate variability (mean ± SEM per 2.5 min)

With all aroma conditions, the HR was significantly higher at T than at R1 ($p < 0.001$), and returned to baseline at R2 ($p < 0.001$). The HF component of HRV was significantly lower at T than at R1 ($p < 0.01$), and returned to baseline at R2 ($p < 0.001$-$p < 0.01$). These results indicate a typical acute stress response as previously reported [39].

However, the average change (increase) in HR during the task was significantly smaller with 1% Orange than with 20% Orange ($p < 0.01$) and the Control condition ($p < 0.01$), as shown in Figure 28. This indicates that 1% Orange inhibits the stress-induced cardiac SNS elevation. Also, the average change (decrease) in the HF component of HRV during the task was significantly smaller with 1% Orange than with 20% Orange ($p < 0.01$) and the Control

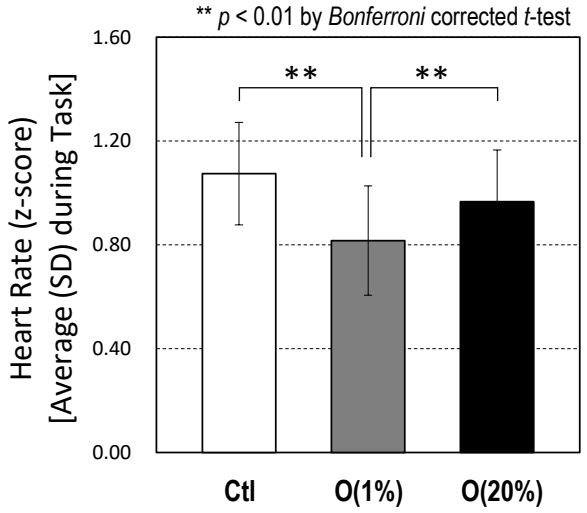


Figure 28: Average changes in heart rate during the task (mean ± SD)

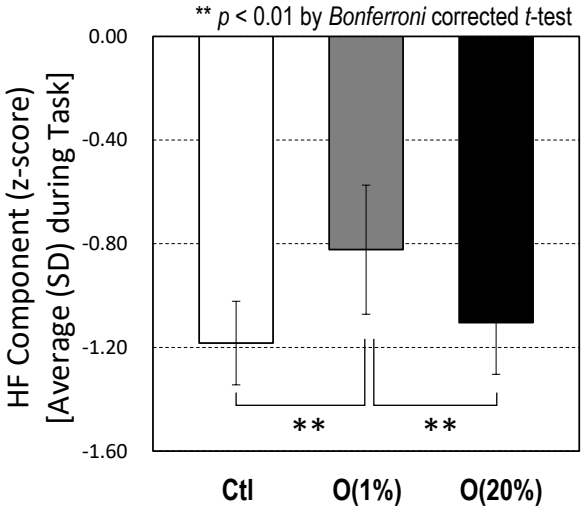


Figure 29: Average changes in the HF component during the task (mean ± SD)

condition ($p < 0.01$), as shown in Figure 29. This indicates that 1% Orange inhibits the stress-induced cardiac PNS suppression.

3.1.1.4 Nose Tip Temperature

The changes in the relative temperature at the tip of the nose using the temperature of the forehead as a reference (i.e. the difference between the nose and forehead temperatures) are shown in Figure 30. The same standardization and baseline correction procedure used for HR and HF was applied here.

The nose tip temperature was significantly lower at T than at R1 ($p < 0.001$), and returned to baseline at R2 ($p < 0.001$-$p < 0.01$) with all aroma conditions, indicating a typical

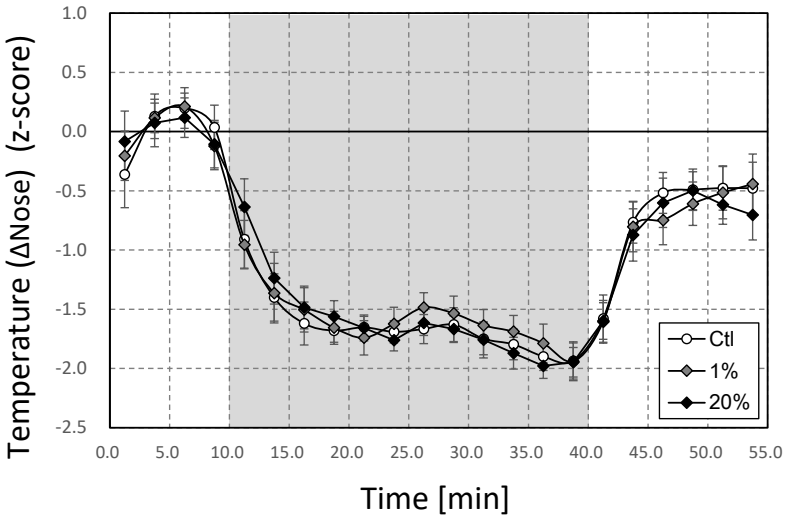


Figure 30: Changes in nose temperature (mean ± SEM per 2.5 min)

acute stress response [38, 39]. However, the between-condition comparisons revealed no any significant difference in nose tip temperature ($p > 0.05$).

3.1.2 Discussion

3.1.2.1 Interpretation of the Results and Discrepancies within the Psychophysiological Effects

In Exp.1, we investigated the effects of two different concentrations: 1 and 20% of Orange aroma on peripheral and cardiac autonomic nervous system activity under a short-term cognitive stressor, in a highly reproducible manner using an olfactometer.

With all aroma conditions, the peripheral and cardiac responses during the task indicated that the calculation task employed in the study functioned as an acute stressor. During the task, there was an increase in HR which indicated enhanced cardiac SNS activity; a decrease in the HF component of HRV which indicated suppressed cardiac PNS activity; and a decrease in nose tip temperature representing a decrease in blood flow which indicated enhanced peripheral SNS activity. Such hemodynamics or re-allocation of blood in the body is frequently observed as part of the physiological stress response to acute cognitive stressors, as described by Nomura et al. (2016) [13]. The increase in HR and the decrease in the HF component of HRV during the task were significantly smaller with 1% Orange administration. This result suggested an inhibition of cardiac SNS elevation and an inhibition of cardiac PNS suppression following 1% Orange administration. However, no any significant difference in the nose tip temperature was observed between the conditions. The subjective impressions on strength and preference indicated that the subjects were unable to perceive any difference in the strength of 1 and 20%

Orange nor they preferred one aroma condition over the other. Contrasting with the results of the preliminary test, the inability of the subjects to discriminate between the two doses might be due to the fact that the experiments were conducted on separate days for each aroma condition. Further, the VAS scores demonstrated that there was no significant psychological effect of Orange aroma administration during the task. Based on the above findings, it can be claimed that the results associated with 1% Orange in this study might be solely due to its physiological functioning. The results suggested the presence of discrepancies between the psychological and physiological responses, as well as between the peripheral and cardiac responses.

3.1.2.2 Consistencies and Discrepancies with the Findings of Past Research

Due to the limited number of studies that have considered the dose-variation of orange aroma in the past, it is difficult to make a direct comparison of the findings of Exp.1 with those of the past studies. However, consistent as well as discrepant psychophysiological effects can be observed in Exp.1 when compared with the findings of previous studies concerning orange and other citrus fragrances.

Consistent with the findings of Exp.1 which demonstrated an alleviation of autonomic arousal following orange aroma inhalation, Jafarzadeh et al. (2013) reports inhibitory effects of orange aroma reflected through a reduction in salivary cortisol and pulse rate among dental patients [20]. Also, Nagata et al. (2013) observed a reduction in salivary alpha-amylase activity among healthy undergraduate students administered orange aroma [11].

In addition, several studies investigating the effects of other citrus fragrances, such as bergamot and yuzu, also report inhibitory effects on physiological stress response indicating that the members of the citrus family might share a similar functioning. Bergamot is reported to have enhanced the cardiac autonomic balance when used in inhalation aromatherapy [22] and when blended with lavender oil in a sweet almond carrier oil for massage [19]. Another citrus fruit Yuzu has reduced salivary Chromogranin-A among women during the follicular phase of menstrual cycle [16]. Limonene which is a major component found in these citrus fragrances might be contributing significantly in the physiological stress alleviation associated with these fragrances.

However, contrasting with our findings of Exp.1, a significant number of studies have failed to find any physiological benefit of orange aroma inhalation. A clinical trial observing 100 pregnant women during childbirth failed to identify any significant effect of orange aroma

inhalation, as reflected through systolic and diastolic blood pressure, respiration, and pulse rate [29]. Similarly, HR and electromyogram data of individuals undergoing a cognitive stressor reported no physiological benefit of orange [9]. Moreover, opposing with our findings, orange aroma inhalation resulted in an increased HR among cancer patients undergoing stem cell reinfusion [31].

However, it should be noted that the findings of these studies might be limited by the specific characteristics of the study populations, i.e. pregnant women [29] and cancer patients undergoing SCR [31]. Furthermore, they have used conventional passive exposure techniques such as aromatherapy samplers [31], or masks [9] or materials [29] impregnated with aroma. These methods impose difficulties in controlling the dose and duration of aroma administration and to our knowledge, no previous study has employed a precise method in controlling the dose and duration of aroma administration.

Among the two studies which are reported to have considered the dose variation of orange aroma, the results reported by Goes et al. (2012) showing no physiological benefit should be interpreted cautiously, given the small sample size (n=8) as for a between-subject design [9]. Only one animal study using Wistar rats has demonstrated the dose-dependent effects of orange aroma, in which the highest anxiolytic effect was observed at the highest dose [35].

3.2 Results and Discussion (Exp.2)

3.2.1 Results

3.2.1.1 Subjective Impressions and VAS Scores

Subjective impressions on the preference and strength of each aroma condition are summarized in Table 8. Strawberry and Apple were perceived as stronger compared to the Control condition ($p < 0.05$). Preference was higher for Chamomile and Strawberry ($p < 0.05$) whereas it was lesser for Citrus ginger ($p < 0.05$), compared to the Control condition.

Table 8: Results [mean (SD)] of subjective impression on 7-point Likert scale (Exp.2)

	DPG (Ctl)	Cedarwood	Chamomile	Chocolate	Strawberry	Green tea	Apple	Citrus ginger	Musk
Strength	3.2	4.2	4.5	5.0	5.0 #	3.2	4.8 #	4.7	2.2
	(1.3)	(1.6)	(1.1)	(0.6)	(0.8)	(1.1)	(1.2)	(0.9)	(0.4)
Preference	4.3	4.0	5.2 #	2.8	6.5 #	4.2	5.3	2.8 #	4.5
	(0.7)	(1.5)	(1.1)	(1.8)	(0.8)	(1.5)	(1.2)	(1.1)	(1.0)

# $p < 0.05$ by comparison between condition with regard to DPG (Ctl)

Changes in VAS score from R1 to T to R2 are summarized in Table 9. Overall, the VAS score was higher at T than at R1 ($p < 0.01$-$p < 0.10$) and lower at R2 than at T ($p < 0.01$-$p < 0.10$), indicating that the Kraepelin calculation employed in the study functioned as an acute stressor. However, the comparisons of the VAS score of each aroma with that of the Control condition demonstrated no any significant differences.

Table 9: Results [mean (SD)] of VAS score (Exp.2)

	DPG (Ctl)	Cedarwood	Chamomile	Chocolate	Strawberry	Green tea	Apple	Citrus ginger	Musk
VAS									
Δ(R1-T)	0.19 †	0.17 *	0.20 *	0.27 **	0.03 *	0.17 †	0.23 *	0.24 **	0.18 **
	(0.21)	(0.16)	(0.16)	(0.16)	(0.41)	(0.18)	(0.19)	(0.13)	(0.11)
Δ(T-R2)	-0.15 *	-0.07 **	-0.15 *	-0.18 *	-0.09	-0.16 *	-0.15 *	-0.14 *	-0.09 †
	(0.11)	(0.04)	(0.12)	(0.13)	(0.13)	(0.11)	(0.11)	(0.11)	(0.11)

** $p < 0.01$, * $p < 0.05$, † $p < 0.10$ by comparison within condition

3.2.1.2 Heart Rate and Heart Rate Variability

The changes in HR from R1 to T to R2 with two distinctive aromas: Apple (which showed a significant positive effect on HR during the task), Cedarwood (which showed no significant effect on HR during the task), and the Control condition are shown in Figure 31. The raw values are standardized (z-score) and baseline corrected to compensate the large individual variations [13]. Changes in HR for all aroma conditions are summarized in Figure 32.

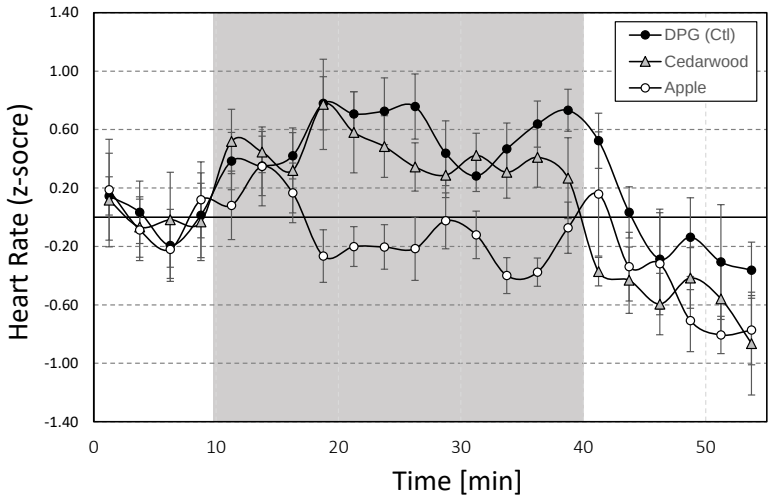


Figure 31: Changes in heart rate (mean±SE) with DPG, Apple, and Cedarwood

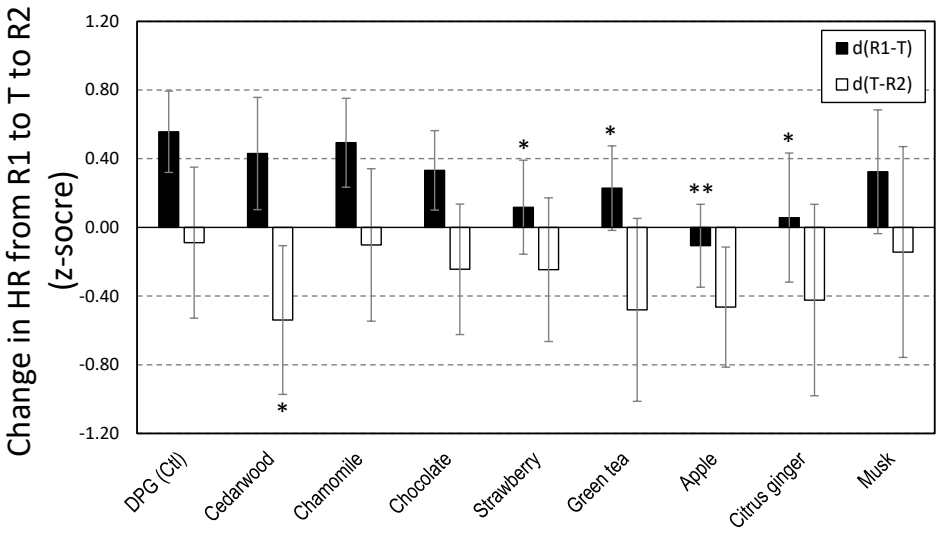


Figure 32: Changes in heart rate (mean±SD) with all aroma conditions

The increase in HR from R1 to T due to the stressor was significantly smaller with Strawberry ($p < 0.05$), Green tea ($p < 0.05$), Apple ($p < 0.01$), and Citrus ginger ($p < 0.05$), compared to the Control condition. This indicates that the aromas: Strawberry, Green tea, Apple, and Citrus ginger inhibit the stress-induced cardiac SNS elevation.

The changes in the HF component of HRV from R1 to T to R2 with two distinctive aromas: Apple and Cedarwood (which showed a significant positive effect on HF during the task) and the Control condition are shown in Figure 33. Changes in HF for all aroma conditions are summarized in Figure 34. The decrease in the HF component from R1 to T due to the stressor was significantly smaller with Cedarwood ($p < 0.001$), Strawberry ($p < 0.01$), Green tea ($p < 0.05$), Apple ($p < 0.001$), and Citrus ginger ($p < 0.05$), compared to the Control

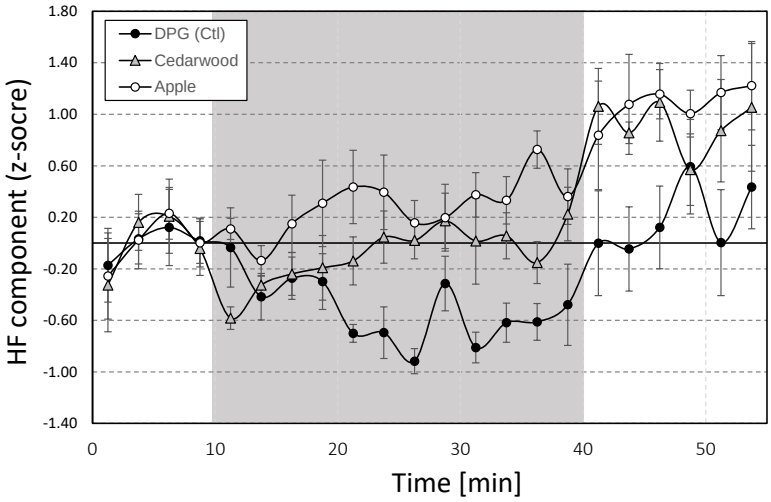


Figure 33: Changes in high-frequency component of heart rate variability (mean±SE) with DPG, Apple, and Cedarwood

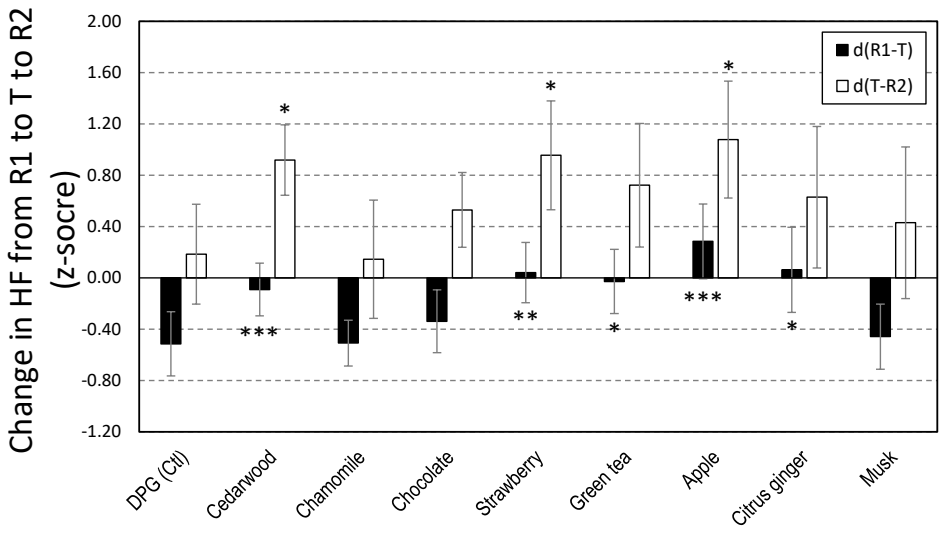


Figure 34: Changes in high-frequency component of heart rate variability (mean±SD) with all aroma conditions

condition. This indicates that the aromas: Cedarwood, Strawberry, Green tea, Apple, and Citrus ginger inhibit the stress-induced cardiac PNS suppression.

3.2.1.3 Skin Conductance Level

The changes in the SCL from R1 to T to R2 with three distinctive aromas: Musk (which showed a significant positive effect on SCL during the task), Cedarwood and Apple (which showed a significant negative effect on SCL during the task), and the Control condition are shown in Figure 35. Changes in SCL for all aroma conditions are summarized in Figure 36. The increase in SCL due to the stressor was significantly smaller with Musk ($p < 0.05$) whereas it was significantly higher with Cedarwood ($p < 0.01$) and Apple ($p < 0.05$) compared to the

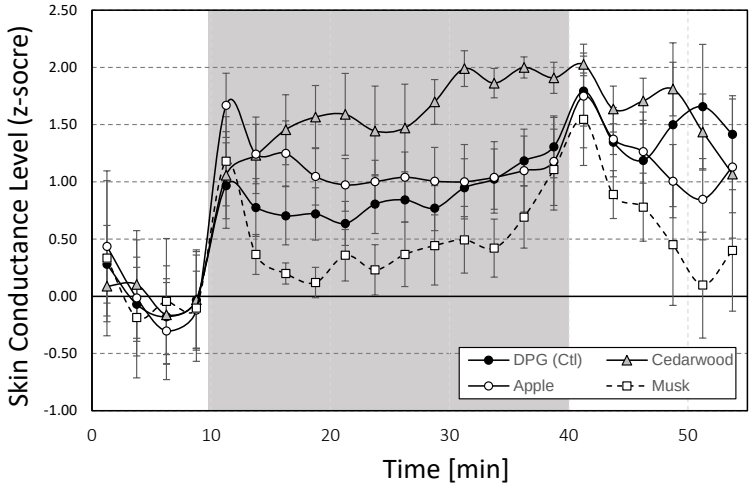


Figure 35: Changes in skin conductance level (mean±SE) with DPG, Apple, Cedarwood, and Musk

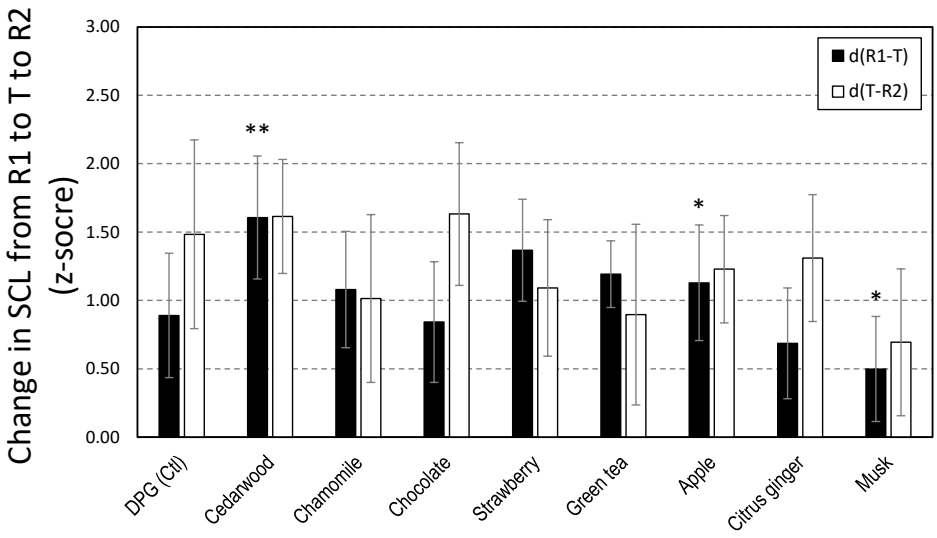


Figure 36: Changes in skin conductance level (mean±SD) with all aroma conditions

Control condition. Based on the results, Musk was the only aroma which inhibited the stress-induced peripheral SNS elevation whereas the stress-induced peripheral SNS elevation was further augmented by Cedarwood and Apple.

Physiological responses including HR, HF component of HRV, and SCL during R1 to T to R2 are summarized in Table 10.

Table 10: Summary of physiological responses during R1 to T to R2

	DPG (Ctl)	Cedarwood	Chamomile	Chocolate	Strawberry	Green tea	Apple	Citrus ginger	Musk
Δ(R1-T)									
HR	N/A	ns	ns	ns	+	+	++	+	ns
HF	N/A	+++	ns	ns	++	+	+++	+	ns
SCL	N/A	--	ns	ns	ns	ns	-	ns	+
Δ(T-R2)									
HR	N/A	+	ns	ns	ns	ns	ns	ns	ns
HF	N/A	+	ns	ns	+	ns	+	ns	ns
SCL	N/A	ns	ns	ns	ns	ns	ns	ns	ns

"+", "++", and "+++" represents the positive effect comparing with DPG (Ctl) in the level of $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively

"-" and "- -" represents the negative effect comparing with DPG (Ctl) in the level of $p < 0.05$ and $p < 0.01$, respectively

3.2.2 Discussion

3.2.2.1 Interpretation of the Results and Discrepancies within the Psychophysiological Effects

In Exp.2, we investigated the effects of Chocolate, Strawberry, Green tea, Apple, Citrus ginger, Chamomile, Cedarwood, and Musk on peripheral and cardiac autonomic nervous system activity under a short-term cognitive stressor, in a highly reproducible manner using an olfactometer. Even though this is a preliminary investigation with a limited number of subjects, eight different aromas were investigated in a single well-controlled within-subject experiment through conducting nine trials for each subject on separate days.

With almost all the aroma conditions, the VAS score during the task indicated that the calculation task employed in the study functioned as an acute stressor. The increase in HR during the task was significantly smaller with Strawberry, Green tea, Apple, and Citrus ginger and the decrease in the HF component of HRV during the task was significantly smaller with Cedarwood, Strawberry, Green tea, Apple, and Citrus ginger. These results suggested an inhibition of cardiac SNS elevation and an inhibition of cardiac PNS suppression by these aromas. The increase in SCL during the task was significantly smaller with Musk whereas it was significantly higher with Cedarwood and Apple indicating an inhibition and augmentation of peripheral SNS elevation.

When comparing the results summarized in Table 8: Subjective impressions, Table 9: VAS score, and Table 10: Summary of physiological responses, discrepancies can be observed between the psychological and physiological responses to aroma administration. Despite the fact that Citrus ginger was significantly less preferred by the subjects, it showed significantly positive effects on cardiac ANS response. On the other hand, Chamomile which was one of the two aromas which were highly preferred by the subjects, demonstrated no significant effect either on cardiac or peripheral ANS response. Remarkably, none of the aromas showed a significant psychological effect in terms of the VAS score, despite the various physiological effects demonstrated by them. In addition to such discrepancies between the psychological and physiological responses, discrepancies between the cardiac and peripheral ANS responses were also observed in the study. Apple showed a highly positive effect on cardiac ANS response whereas it had a negative effect on peripheral ANS response. Cedarwood had a highly negative effect on peripheral ANS response, while imposing highly positive or no effect on cardiac ANS

response. Musk was the only aroma which had a positive effect on peripheral ANS response, but it had no significant effect on cardiac ANS response.

3.2.2.2 Consistencies and Discrepancies with the Findings of Past Research

Findings of Exp.2 demonstrated some consistencies as well as discrepancies with those of the previous studies.

None of the aromas investigated in Exp2. demonstrated a significant psychological effect measured in terms of VAS score representing frustration, concentration, effort, fatigue, and boredom. A similar functioning is reported with some of the aromas in several previous studies. Mixed aroma of ginger and orange showed no significant effect on quality of life [7], and apple showed no significant effect on anxiety or mood [28]. Besides, chamomile [21] and cedarwood [33] also had no significant effect on anxiety.

Inhibiting the cardiac SNS elevation and/or cardiac PNS suppression, Cedarwood, Strawberry, Green tea, Apple, and Citrus ginger demonstrated positive physiological effects. Comparable with these findings, several previous studies also report beneficial effects of these aromas based on cardiovascular as well as endocrinological responses. Inhibitory effects of cedarwood inhalation is evident in a previous study, resulting in an increase in PNS activity and a reduction in SNS activity [23]. A study concerning green tea demonstrate that green tea inhibits the increase of salivary CgA [4]. Further, reduced knee pain [7] and reduced nausea [6] with Ginger, and reduced nausea and vomiting with Strawberry [5] are reported previously. The physiological background associated with ginger and strawberry aromas as demonstrated in our experiment might be strongly involved in such positive behavioral effects observed in previous studies.

Despite such evidence from the past which are consistent with the findings of Exp.2, several other studies report inconsistent psychophysiological effects. While the psychological effects measured in terms of VAS score were not significant for any aroma in our experiment, reduced anxiety is reported with apple [14], and chamomile [12, 15], and improved relaxation and/or positive mood is reported with chocolate [17, 18], and green tea [3, 4] in previous studies. Chamomile which demonstrated no any significant physiological effect in our experiment either in terms of cardiac or peripheral responses, is reported to have stabilized blood pressure [12] and reduced pulse [21]. Furthermore, Musk which showed no significant effect on cardiac ANS response in our experiment has resulted in positive effects demonstrated through reductions in

stress-related hormones: decreased cortisol [25] and inhibited increase of corticosterone in an animal model of depression [26].

From among the above, several studies have used oral administration method [4, 15, 17], whereas all the others have used conventional passive exposure administration techniques [3, 12, 14, 18, 21, 25]. Further, the findings of certain studies might be limited due to the specific characteristics of the study populations considered, e.g. PCI patients in ICU [12], outpatients with mild to moderate generalized anxiety disorder [15], and nursing students practicing intravenous injection [21].

To our knowledge only one study is reported in the literature associated with the eight aromas considered in Exp.2, which has employed an olfactometer for controlling the aroma administration [23]. However, this study is also limited by the absence of an acute stressor as for investigating the effects of aroma on healthy individuals. Moreover, ambiguities are present regarding its study design and the sample size.

3.3 Results and Discussion (Exp.3)

3.3.1 Results

3.3.1.2 Subjective Impressions and VAS Scores

Subjective impressions on the preference and strength of each aroma condition are listed in Table 11. Both aromas Apple (p <0.001) and Chamomile (p <0.05) were perceived as stronger compared to the Control condition. Also, Apple ($p < 0.01$) and Chamomile ($p < 0.05$) were significantly preferred by the subjects compared to the Control condition. No significant differences in the subjective impressions were observed between the two aromas Apple and Chamomile.

Table 11: Results [mean (SD)] of subjective impression on 7-point Likert scale (Exp.3)

Strength			
DPG (Ctl)	2.52	(1.23)	
Apple	3.72	(1.40)	###
Chamomile	3.40	(1.47)	#
Preference			
DPG (Ctl)	4.28	(0.98)	
Apple	5.36	(0.99)	##
Chamomile	4.80	(0.87)	#

$p < 0.001$, ## $p < 0.01$, # $p < 0.05$ by comparison between condition with regard to DPG (Ctl)

Table 12: Results [mean (SD)] of VAS scores (Exp.3)

	Δ（R1-T)		Δ（T-R2）	
Nervousness				
Ctl	1.60	(20.69)	-5.12	(14.34)
Chamomile	5.60	(21.53)	-12.04	(14.89) ***
Apple	7.64	(17.72) *	-13.36	(15.47) *** #
Effort				
Ctl	15.32	(21.28) **	-16.68	(18.24) ***
Chamomile	13.76	(19.63) **	-17.44	(16.19) ***
Apple	17.72	(19.40) ***	-19.16	(17.24) ***
Concentration				
Ctl	9.88	(30.04)	-17.68	(24.00) **
Chamomile	7.48	(23.76)	-13.16	(22.70) **
Apple	9.36	(24.79)	-23.36	(17.12) ***
Tiredness				
Ctl	8.72	(24.69)	-0.08	(23.25)
Chamomile	6.88	(23.00)	-3.96	(17.79)
Apple	10.04	(26.94)	-1.80	(15.03)
Irritation				
Ctl	7.48	(14.94) *	-2.96	(17.74)
Chamomile	10.16	(23.65) *	-9.20	(17.92) *
Apple	13.72	(23.00) **	-7.96	(20.29)
Boredom				
Ctl	6.00	(26.51)	1.80	(15.50)
Chamomile	-10.12	(22.44) * #	8.04	(26.94)
Apple	-3.96	(23.31) #	5.28	(27.39)
Fed up				
Ctl	4.80	(16.97)	-5.60	(15.68)
Chamomile	5.32	(17.11)	-0.68	(16.69)
Apple	9.28	(19.76) *	-5.44	(16.07)

*** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$ by comparison within condition
# $p < 0.05$ by comparison between condition with regard to DPG (Ctl)

Changes in VAS scores from R1 to T to R2 are shown in Table 12. With all aroma conditions, the VAS scores for effort and irritation were significantly higher at T compared to those at R1 ($p < 0.001$-$p < 0.05$) and the scores for effort and concentration were significantly lower at R2 compared to those at T ($p < 0.001$-$p < 0.01$). These results indicated that the Kraepelin calculation employed in the study functioned as an acute stressor. With respect to the Control condition, Apple and Chamomile showed a significant reduction in the VAS score for

boredom during the task ($p < 0.05$). Also, Apple showed a significant reduction in the VAS score for nervousness during Rest2, compared to the Control condition ($p < 0.05$).

3.3.1.3 Heart Rate and Heart Rate Variability

The changes in the HR and the HF component of HRV from R1 to T to R2 with the three aroma conditions are shown in Figure 37 and 38. The raw values are standardized (z-score) and baseline corrected to compensate the large individual variation.

The average change (increase) in HR due to the task was significantly smaller with Apple compared to the Control condition ($p < 0.01$). This indicates that Apple aroma inhibits the stress-induced cardiac SNS elevation. Also, the average change (decrease) in the HF component of HRV due to the task was significantly smaller with Apple compared to the Control condition ($p < 0.05$). This indicates that Apple aroma inhibits the stress-induced cardiac PNS suppression. Further, the decrease in HR during Rest2 was significantly larger with Apple compared to the Control condition ($p < 0.05$).

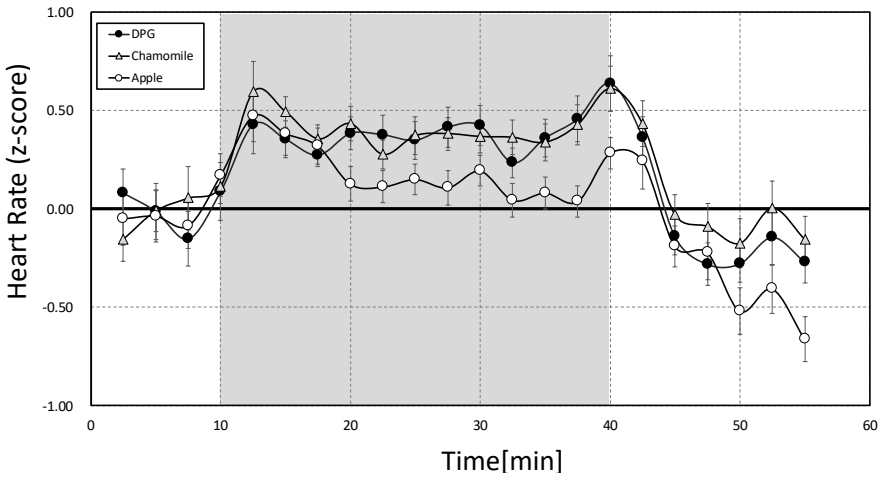


Figure 37: Changes in heart rate (mean±SE) with DPG, Apple, and Chamomile

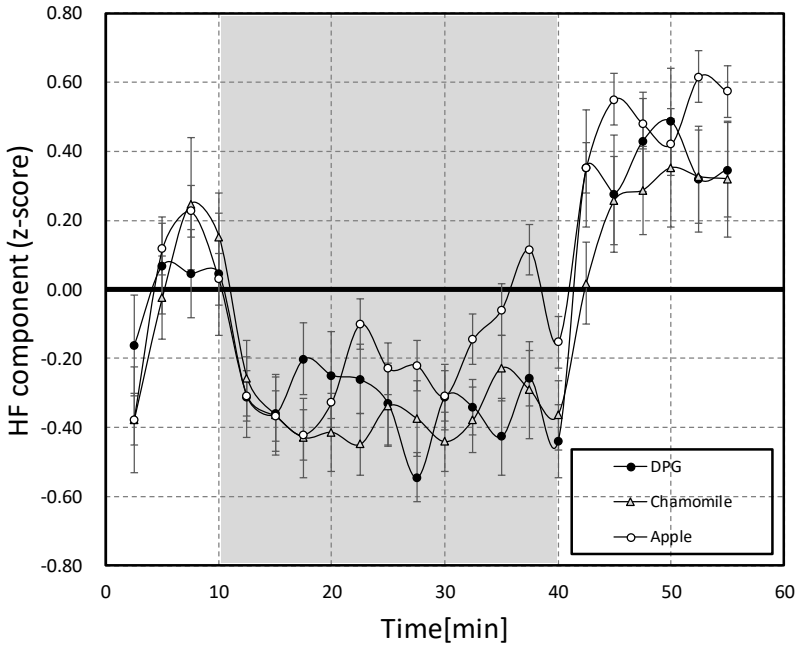


Figure 38: Changes in high-frequency component of heart rate variability (mean±SE) with DPG, Apple, and Chamomile

3.3.1.4 Nose Tip Temperature

Using the temperature of the forehead as a reference, the changes in the relative temperature at the tip of the nose (i.e. the difference between the nose and forehead temperatures) and the changes in the relative temperature at the dorsal hand (i.e. the difference

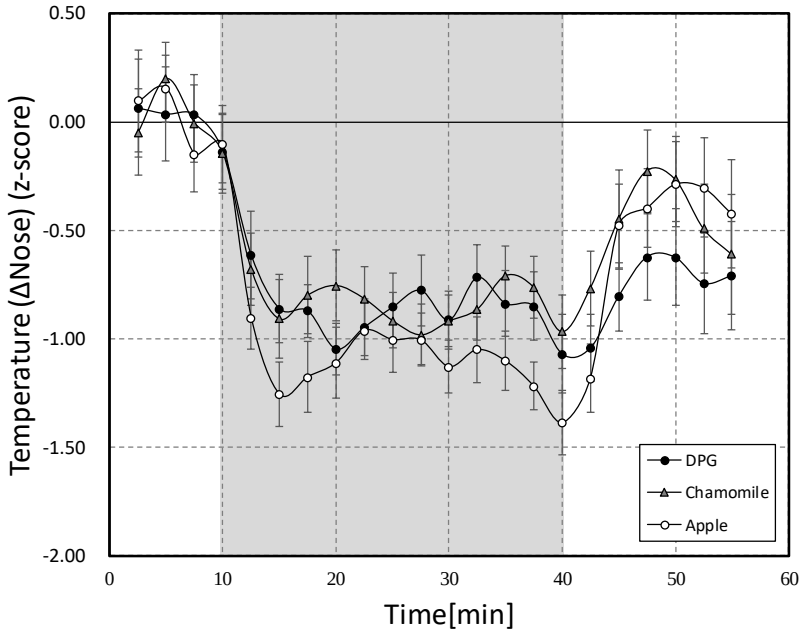


Figure 39: Changes in nose temperature (mean ± SE)

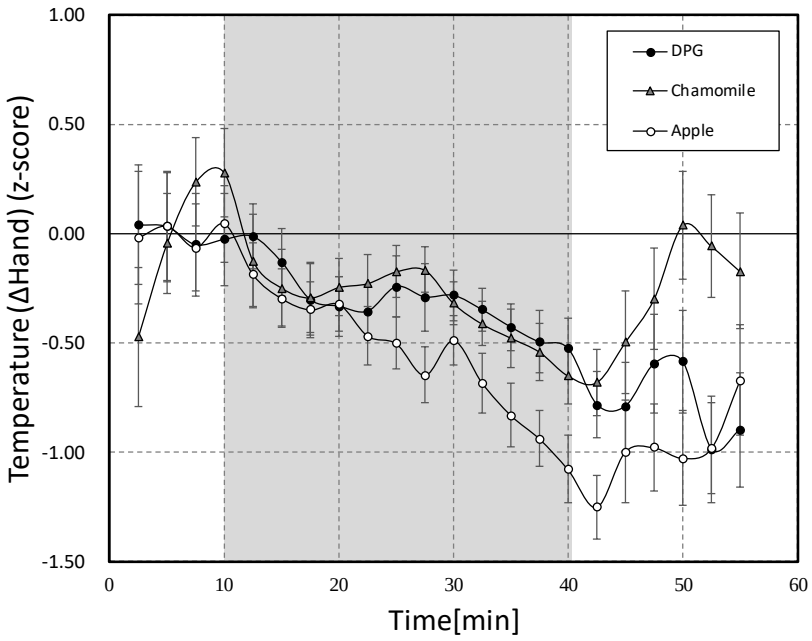


Figure 40: Changes in hand temperature (mean ± SE)

between the dorsal hand and forehead temperatures) are shown in Figure 39 and 40. The same standardization and baseline correction procedure used previously was applied here.

Compared to the Control condition, the changes in the relative temperature at the tip of the nose and that at the dorsal hand during the task were significantly larger with Apple aroma ($p < 0.01$), indicating that Apple aroma further promotes the reduction in skin temperature.

3.3.2 Discussion

3.3.2.1 Interpretation of the Results and Discrepancies within the Psychophysiological Effects

In Exp.3, we investigated the effects of Apple and Chamomile aromas on peripheral and cardiac autonomic nervous system activity under a short-term cognitive stressor, in a highly reproducible manner using an olfactometer.

With all aroma conditions, the VAS scores indicated that the calculation task employed in the study functioned as an acute stressor. The increase in HR and the decrease in the HF component of HRV during the task were significantly smaller with Apple compared to the Control condition. This result suggested an inhibition of cardiac SNS elevation and an inhibition of cardiac PNS suppression by Apple aroma. However, psychological measures in terms of VAS scores demonstrated no such beneficial effect of Apple. Contrasting with the findings reported on HR and HF, the nose and hand temperatures demonstrated a significant reduction

during the task with Apple compared to the Control condition. This indicated that Apple aroma further promotes the peripheral SNS activation. While verifying the efficacy of Apple aroma in alleviating cardiac stress response, the findings of Exp.3 revealed an interesting phenomenon associated with aromas which produces distinct effects on cardiac and peripheral responses.

Despite the significant differences in cardiac parameters discussed above, the differences cannot be visible through the graphs as clearly as in Exp.1 (e.g. Figure 37 compared to Figure 26). Having the same sample size (n=19) as of Exp.1, this could be attributed to the difference in the sample employed in Exp.3 in which the baseline HR was comparatively lower as can be seen in Appendix 7: Table 7.

3.4 Chapter Summary

In Exp.1, mild orange aroma inhibited the cardiac stress response. However, no significant differences were observed between conditions either in terms of VAS scores or nose temperature. In Exp.2, Cedarwood, Strawberry, Green tea, Apple, and Citrus ginger demonstrated potential inhibitory effects on cardiac stress response. However, no significant differences were observed in the VAS score with any of the aromas compared to the Control condition. Further, the results reported with SCL were also inconsistent with those on the cardiac response. In Exp.3, Apple remarkably inhibited the cardiac stress response. Contrastingly, Apple promoted the peripheral SNS activation, demonstrating significant decreases in nose and forehead temperatures. The VAS scores failed to demonstrate any remarkable psychological benefit.

The above discrepancies observed between the psychological and physiological responses as well as between the cardiac and peripheral responses have been discussed in detail in this chapter. Further, the results have been compared with those of the past studies taking into account the consistencies and discrepancies.

CHAPTER 4

GENERAL DISCUSSION AND CONCLUSION

4.1 Major Contributions to the Field of Study

Instead of employing conventional passive exposure administration methods which limit the experimental outcomes, the study has successfully introduced and utilized a proprietary olfactometer in the experimental series. Being among the very few studies that have paid thorough attention on precisely controlling the dose and duration of aroma administration, the study has presented a number of intriguing findings that the future researchers can rely on. At a time where the investigations on the discrepant phsychophysioloigcal effects of aroma remain in a preliminary stage, the study through conducting a series of individual well-controlled experiments, revealed a stimulus-specific nature of aroma that still leads to discrepant phsychophysioloigcal effects. While all the three experiments provide evidence for such discrepant effects, the findings of Exp.3 revealed a nature of Apple aroma that gives rise to remarkable positive effects on the cardiac activity and negative effects on the peripheral site. Exp.1 verified the efficacy of mild orange essential oil in alleviating cardiac stress response and its findings further stressed the significance of controlling the dose in aroma research concerning their phsychophysioloigcal effects. Exp.3 being a preliminary investigation on eight different aromas which were lacking scientific evidence on their psychophysiological effects, has provided the basis on which further research can be carried out.

Scientific experimentation which follows proper procedures is always encouraged within any field of study due to their immense contribution towards the advancement of the field. Findings of such experimentation are the foundation on which the successive developments can be built up. With regard to the field of olfactory psychophysiology, such applications range from consumer products to ambient odours used in service or living environments to clinical aromatherapy. Along with the recent advancements in mobile and ubiquitous computing, people are paying higher attention and focus on their healthcare and well-being than ever before. A number of healthcare and wellness apps are being emerged that can track the health indices and update the relevant parties in real time. Among the sense modalities including audition, vision, gustation, and tactile perception, the potential of olfaction in practical applications for enhancing quality of life is yet to be revealed. In realizing the above, the factors affecting the psychophysiological effects of olfactory stimuli, including the stimuli itself, the composition, concentration, and method of administration need to be thoroughly

investigated. In this regard, the findings of the present study would be of higher importance. They would be fundamental in the future developments including ambient feedback systems.

4.2 Limitations and Directions for Future Research

All the three experiments were carried out the subjects being male university students. The inclusion of such a homogenous study sample limited the generalizability of the experimental findings. Subjective impressions such as the preference for aromas vary based on factors including gender, ethnicity/cultural exposure to specific odours [42, 43], and age. Further, these factors affect the resulting psychophysiological effects. Therefore, employing a heterogeneous study sample representative of the above factors can be identified as a potential direction for future research that would enhance the generalizability of the findings and enable to explore the differences among sub-groups within the wider population. The scope of the present study was narrowed down, investigating the effects on a homogeneous study population of young Japanese males. This can be justifiable once we consider the extensively time and effort-consuming nature of the study. The experimental series comprised of 57 (3x19), 54 (9x6), and 57 (3x19) experiment sessions (in Exp.1, 2, and 3 respectively), the duration of each session being 55min. Meanwhile, the results were obtained without compensating on the statistical power or the systematic designing of scientific experimentations.

The small sample size (n=6) of the preliminary investigation (Exp.2) can be identified as another limitation of the study. Even though this is reasonable as for a preliminary investigation which still involved 54 (9x6) instances, the sample size is too small as for an experimental study.

As stated previously, the response to aromas is a complex process affected by multiple factors. These factors also include the individual experiences that create either pleasant or unpleasant memory associations [42]. Therefore, employing the data of the present study on subjective impression on familiarity as a preliminary data source, future research can be designed and developed that explores the influence of prior experiences on olfactory psychophysiology.

Having introduced a well-controlled experimental design while using an olfactometer which is a well-controlled method of aroma administration, this experimental set-up can be successfully employed in investigating the psychophysiological effects under various stressors other than the Kraepelin calculation task. Also, other aromatic stimuli ranging from aromatic compounds, aromas other than the ones considered in the present study, and mixed aromas can

be tested using the present experimental setup. Further, the findings of Exp.1 emphasize the importance of considering the variation in aroma concentration and thus it can be identified as a feature that is worth incorporating in future research. While the present study focused on olfaction, it can be successfully modified to suit with other sensory inputs including auditory (music) and/or visual inputs.

Where there are differences among certain physiological measures in terms of response latency (e.g. longer latency associated with Hypothalamic-pituitary-adrenal (HPA) axis compared to ANS [44]), the psychophysiological background associated with the discrepant effects reported in our study warrants further investigations.

4.3 Conclusion

In a series of experiments, we investigated the psychophysiological effects of aroma on peripheral and cardiac autonomic nervous system activity under a short-term cognitive stressor, controlling the dose and duration of aroma administration using a customized olfactometer. The effects of two different concentrations: 1 and 20% of Orange were investigated in Exp.1 whereas eight different aromas: Chocolate, Strawberry, Green tea, Apple, Citrus ginger, Chamomile, Cedarwood, and Musk were preliminarily investigated in Exp.2. Based on its findings, two prominent aromas: Apple and Chamomile were further investigated in Exp.3. The primary findings of Exp.1 indicated that the inhalation of mild orange essential oil inhibits the cardiac stress response. But no significant differences were observed between the conditions through psychological measures or the peripheral ANS response. The findings of Exp.2 revealed the potential of Cedarwood, Strawberry, Green tea, Apple, and Citrus ginger in inhibiting cardiac stress response, but no aroma showed a significant psychological effect compared to the Control. Further, the effects of the above aromas on peripheral response were inconsistent with those on the cardiac response. Exp.3 verified the efficacy of Apple aroma in inhibiting cardiac stress response with a remarkable suppression of the increase/decrease in HR and HF component of HRV during the task. However, such beneficial effect could not be observed through the psychological parameters. Meanwhile, Apple aroma demonstrated a remarkable negative effect on the peripheral site. The study revealed a dose-dependent effect and a stimulus-specific nature of aroma which still leads to discrepant psychophysiological effects even within a well-controlled experimental setting.

4.4 Chapter Summary

Taking into account the entire series of experiments, a general discussion is provided in this chapter along with the concluding remarks. Major contributions of the study for the field of olfactory psychophysiology have been discussed whereas the importance of the findings of this scientific research towards the advancement of the field of ambient biomedical engineering is discussed. Inclusion of a homogeneous study sample and the small sample size employed in the preliminary investigation have been identified as the major limitations of the study. A number of possible directions have been identified to proceed further, while suggesting ways to overcome the existing limitations of the study. Summarizing the results of the three experiments, conclusions have been made on the presence of a dose-dependent effect and a stimulus-specific nature of aroma which still leads to discrepant psychophysiological effects.

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Journal Publications

1. Sugeeswari Lekamge, Masaki Nakachi, Shu Sato, Kanetoshi Ito, Shusaku Nomura, "Psychophysiological Effects of Aroma Inhalation during a Short-term Cognitive Stressor: A Preliminary Study using Eight Different Aromas," *International Journal of Affective Engineering*. (Accepted)
2. Sugeeswari Lekamge, Masaki Nakachi, Shu Sato, Kanetoshi Ito, Shusaku Nomura, "Alleviation of the Acute Stress Response following Mild Orange Essential Oil Administration," *IEEJ Transactions on Electrical and Electronic Engineering*, Vol.12, No.S1, pp.158-163, 2017.6.5.
3. Sugeeswari Lekamge, Ashu Marasinghe, Pradeep Kalansooriya, Shusaku Nomura, "A Visual Interface for Emotion based Music Navigation using Subjective and Objective Measures of Emotion Perception," *International Journal of Affective Engineering*, Vol.15, No.2, pp.205-211, 2016.6.30.

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1. Sugeeswari Lekamge, Ashu Marasinghe, Pradeep Kalansooriya, "A Visual Interface for Music Navigation based on Subjective and Objective Measures of Music Emotion Perception," in Proc. The 1st International Symposium on Affective Science and Engineering (ISASE), Tokyo, Japan, pp. 1-5, 2015.03.23.
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3. Sugeeswari Lekamge, Ashu Marasinghe, "Emotion based Classification of Sri Lankan Folk Songs," in Abs. The 1st International Conference on Energy, Environment and Human Engineering (ICEEHE), Yangon, Myanmar, pg. 69, 2013.12.22.

APPENDICES

Appendix 1: Aroma Evaluation Questionnaire

香り評価アンケート

香りアンケート

実験中に感じた香りについてお聞きします。
当てはまる数字に〇を記入してください。

不快である　1　2　3　4　5　6　7　快い

全く感じられない　1　2　3　4　5　6　7　耐えられないほど強い

＊少しでも香りを感じたら２以上に〇を記してください。

好きな香りである　1　2　3　4　5　6　7　嫌いな香りである

リラックスする　1　2　3　4　5　6　7　目が覚める

この香りを　１．知っている(　　)　２．知らない

＊知っている場合は名前を記入してください。

１．と答えた方に質問です。

この香りをよく嗅ぐ　1　2　3　4　5　6　7　めったに嗅がない

＝＝＝＝＝＝＝＝＝＝実験者記入欄＝＝＝＝＝＝＝＝＝＝＝＝＝＝＝＝＝
実験コード：
実験日時：
被験者内観：

Appendix 2: Aroma Evaluation Questionnaire (English Translation)

Aroma evaluation questionnaire

This questionnaire is on the perceived aroma during the experiment

Please rate your impression on each of the following items

Uncomfortable 1 2 3 4 5 6 7 Comfortable

Weak 1 2 3 4 5 6 7 Strong

* Please rate above 2, if you can perceive an aroma

Like 1 2 3 4 5 6 7 Dislike

Relaxing 1 2 3 4 5 6 7 Activating

1. I know this aroma ( ) 2. I don't know this aroma

* Please name it if you know

For those who answered as 1:

The aroma is familiar to me 1 2 3 4 5 6 7 It is not familiar to me

========== For the use of the Experimenter ======

Experiment code:
Date and time:
Remarks:

Appendix 3: Pre-experiment Checklist

実験前チェックリスト

AP02　実験前チェックリスト

実験一時間前まで

□ 唾液冷蔵保存用の保冷剤の用意
□ 室温調整のため空調スイッチ
□ PC(BIOPAC 用、血圧用、オルファクトメーター用)の電源を入れる
□ 唾液用遠心機の準備
□ オルファクトメーターの香り条件の確認、香料のセット
□ オルファクトメーター動作確認
□ 実験報告書(エクセル)を準備（PC で記録）
□ ボンベの残量確認
□ 唾液採取用エッペンチューブの準備(11 個)
□ カニューレの準備
□ 各条件に合わせた香り刺激トリガ用ケーブル（1-5ch）をマルチチャンネル・オルファクトメータに接続
□ 空気ボンベ解放

実験開始 20 分前まで

□ 計測機器取り付け(ECG 電極→GSR→高精度 8 チャンネルデータロガーセンサ(額・鼻の頭・手の甲)→カニューレ)
□ ECG、RSP、GSR が正しく計測できるか確認
□ 体温計が正しく計測できるか確認
□ ケーブルなどで被験者に不自由がないか確認
□ 背部のケーブルをテープでまとめる
□ カニューレから空気が出ているかの確認
□ カニューレを顔にテープで固定
□ 温度・湿度確認
□ 質問紙
□ オルファクトメーターのプログラム選択（1-5ch 用）

実験開始 5 分前まで

□ 被験者の体調確認
□ 実験報告書に室温・湿度、被験者の体調状態などを記録
□ BIOPAC 記録開始
□ 高精度 8 チャンネルデータロガー測定開始

Appendix 4: Pre-experiment Checklist (English Translation)

AP02 Pre-experiment Checklist

One hour before the experiment

- ☐ Preparation of cold storage agent for saliva refrigeration preservation
- ☐ Air conditioning switch for room temperature adjustment
- ☐ Turn on the PC (for BIOPAC, for blood pressure, and for olfactometer)
- ☐ Preparation of salivary centrifuge
- ☐ Confirm the fragrance condition of the olfactometer, set the fragrance
- ☐ Olfactometer operation check
- ☐ Preparation of Experiment Report (Excel) (Recorded on PC)
- ☐ Check remaining amount of cylinder
- ☐ Preparation of Eppendorf tube for saliva collection (11 pieces)
- ☐ Preparation of cannula
- ☐ A scent stimulation trigger cable (1-5 ch) according to each condition is connected to the multichannel olfactometer
- ☐ Air cylinder release

20 minutes before the experiment

- ☐ Instrument installation (ECG electrode → GSR → High precision 8 channel data logger sensor (forehead / nose / dorsal hand) → cannula)
- ☐ Check whether ECG, RSP, and GSR, can be measured correctly
- ☐ Check whether the temperature can be measured correctly
- ☐ Confirm that the subjects are not inconvenienced by cables etc.
- ☐ Tape the cable on the back with tape
- ☐ Check if air is coming out from the cannula
- ☐ Fix the cannula to the face with tape
- ☐ Check the room temperature and humidity
- ☐ Questionnaire
- ☐ Program selection of the olfactometer (for 1-5 channels)

5 minutes before the experiment

- ☐ Physical condition check of the subject
- ☐ Record the room temperature / humidity, physical condition of the subject etc. in the experiment report
- ☐ BIOPAC recording start
- ☐ Start measurement of high precision 8-channel data logger

Appendix 5: Pre-screening Questionnaire

予備検討アンケート

香りに関する調査票

氏名：

日付：

性別：男性 / 女性

年齢：

アンケート

本日のあなたの状態を表した選択肢に〇をつけてください。

●カゼをひいていますか？　はい ・ いいえ

●本日、薬を服用しましたか？　はい ・ いいえ

●昨日、アルコールを摂取しましたか？　かなり ・ ふつう ・ かるく ・ いいえ

●本日の健康状態は？　良好 ・ どちらでもない ・ 不良

●その他　鼻が詰まっている、普段から匂いに敏感では ないetc...

例に従って、下記の評価をお願い致します。

例

嫌い ——— 好き

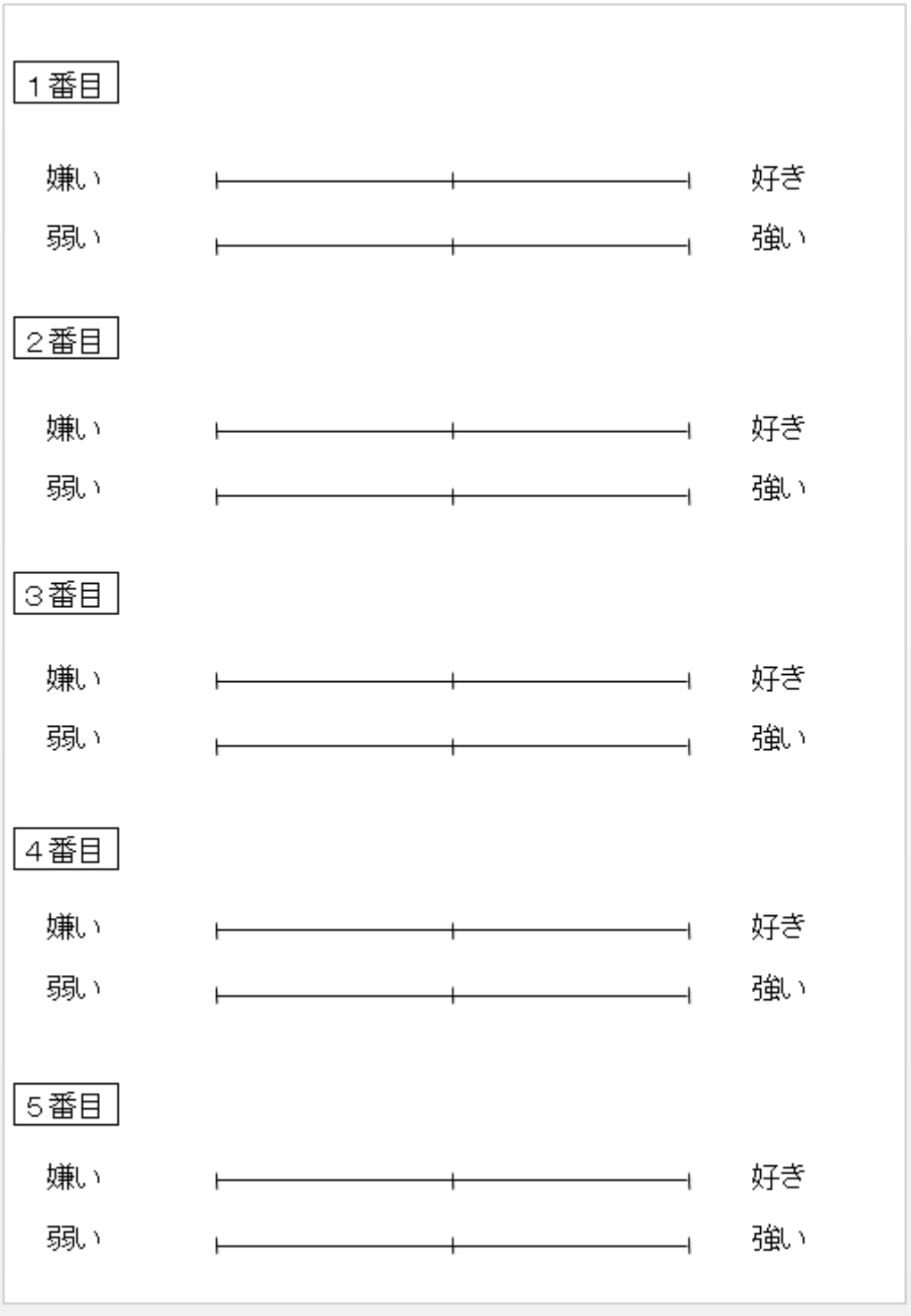
弱い ——— 強い

●注意

・香りは2-3回程度嗅ぎ、**第一印象**でさっと書く。

・アンケートを回収するまでは「**お互いに感想を述べ合わない**」。

予備検討アンケート(1/2)



予備検討アンケート(2/2)

Appendix 6: Pre-screening Questionnaire (English Translation)

Questionnaire on Aroma Perception

Name:

Date:

Gender: M/F

Age:

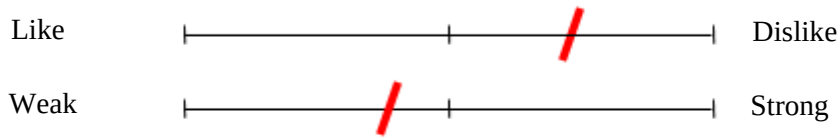
Please select the answer that best describes your present situation

- Are you having a cold? Yes No
- Did you take medicine today? Yes No
- Did you consume alcohol yesterday? High Moderate Low None
- How is your health condition today? Good Neither good nor bad Bad
- Other

stuffy nose, not sensitive to odour, etc.

Please see the example below and make your evaluations

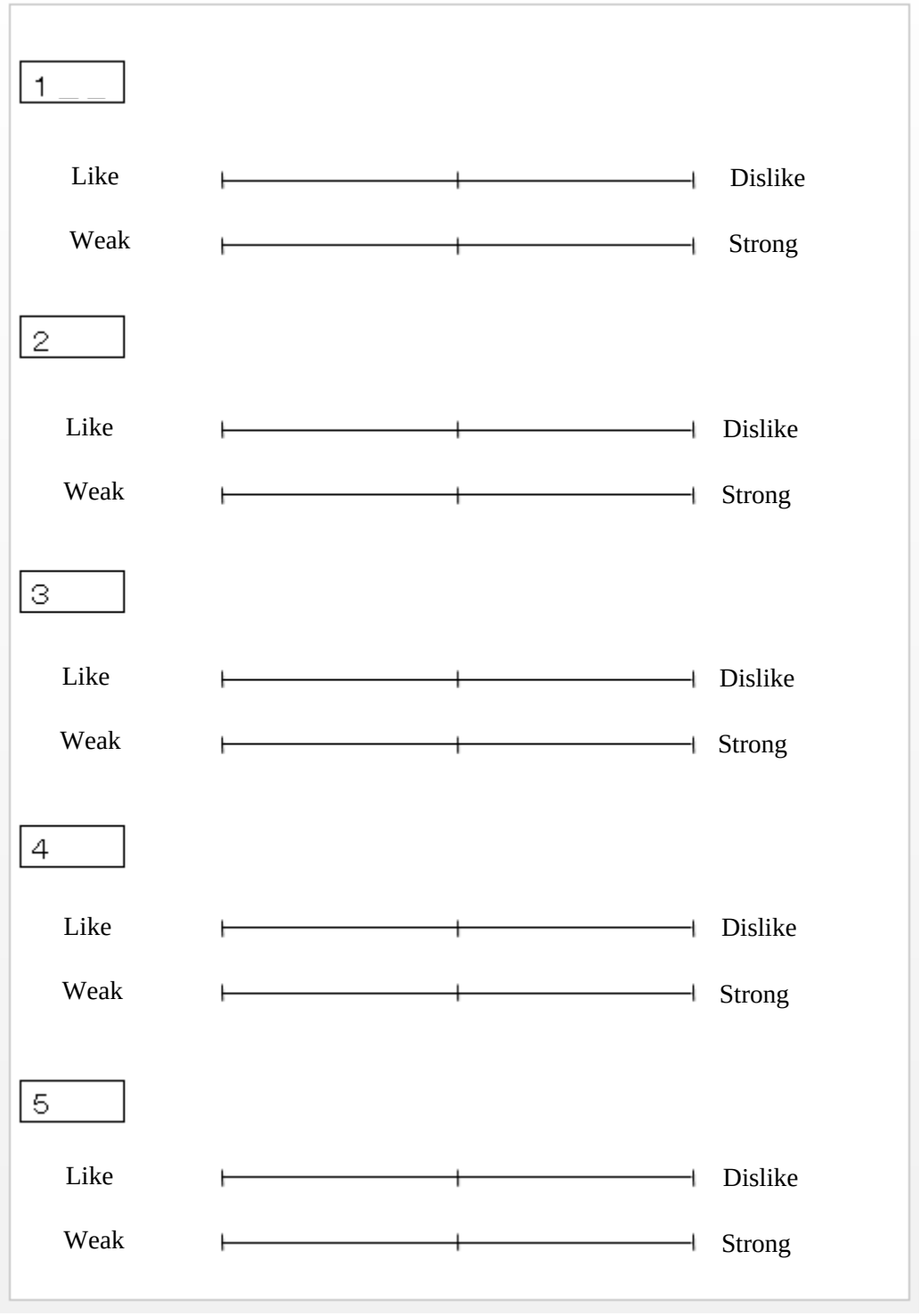
Eg:



- **Attention**

- Sniff the aroma for about 2,3 times, and rate your impression
- Please do not share your answers with others

Pre-screening Questionnaire (1/2)



Pre-screening Questionnaire (2/2)

Appendix 7: Raw Data

Exp.1

Table 1: Changes in heart rate (mean per 2.5 min)

	TEC (Ctl)	Orange1%	Orange20%
2.5	73.1	75.0	76.5
5	71.7	73.8	75.9
7.5	72.2	72.8	74.7
10	75.6	75.5	76.7
12.5	81.7	80.4	80.0
15	80.8	79.7	81.2
17.5	80.0	79.1	80.8
20	80.7	79.6	82.3
22.5	80.5	79.0	81.5
25	80.5	79.6	81.6
27.5	79.5	79.0	80.8
30	79.5	78.9	81.6
32.5	79.9	78.1	80.5
35	80.4	79.9	81.3
37.5	80.2	79.8	81.1
40	82.4	81.5	82.1
42.5	76.4	75.8	78.8
45	72.8	73.7	77.0
47.5	73.8	73.1	77.0
50	71.4	72.5	76.8
52.5	72.1	72.6	76.5
55	71.2	71.7	75.9

Table 2: Changes in high-frequency component of heart rate variability (mean per 2.5 min)

	TEC (Ctl)	Orange1%	Orange20%
2.5	688.26	479.21	545.33
5	786.34	590.78	595.27
7.5	736.06	611.89	615.11
10	793.34	662.21	663.68
12.5	339.94	335.69	366.05
15	374.87	317.52	303.33
17.5	307.23	333.74	320.21
20	283.33	287.76	300.69
22.5	317.15	330.85	307.7
25	328.09	288.16	290.24
27.5	343.99	297.6	347.65
30	290.96	291.29	289.65
32.5	338.07	312.23	321.96
35	349.92	307.64	312.19
37.5	347.35	339.76	307.58
40	266.94	301.69	264.8
42.5	703.76	441.02	439.97
45	805.23	499.29	593.18
47.5	683.24	514.72	568.99
50	706.18	587.18	531.65
52.5	797.08	550.02	581.56
55	872.33	572.51	559.73

Table 3: Changes in nose and forehead temperatures (mean per 2.5 min)

	TEC (Ctl)		Orange1%		Orange20%	
	Nose	**Forehead**	**Nose**	**Forehead**	**Nose**	**Forehead**
2.5	32.31	33.60	32.35	33.91	32.35	33.44
5	32.65	33.62	32.52	33.95	32.51	33.47
7.5	32.71	33.63	32.67	33.94	32.57	33.47
10	32.58	33.63	32.58	33.94	32.51	33.45
12.5	31.96	33.59	32.11	33.91	32.26	33.45
15	31.55	33.59	31.75	33.89	31.96	33.46
17.5	31.39	33.62	31.61	33.88	31.83	33.48
20	31.29	33.62	31.50	33.87	31.78	33.49
22.5	31.29	33.62	31.50	33.90	31.75	33.52
25	31.25	33.61	31.58	33.91	31.70	33.53
27.5	31.26	33.63	31.65	33.90	31.75	33.53
30	31.26	33.62	31.63	33.89	31.71	33.52
32.5	31.21	33.60	31.60	33.91	31.62	33.50
35	31.16	33.57	31.55	33.90	31.59	33.50
37.5	31.13	33.57	31.47	33.89	31.54	33.49
40	31.10	33.55	31.38	33.88	31.54	33.50
42.5	31.29	33.54	31.53	33.88	31.67	33.50
45	31.79	33.54	31.86	33.89	31.96	33.49
47.5	31.96	33.55	31.90	33.89	32.08	33.47
50	31.97	33.53	31.96	33.85	32.15	33.41
52.5	31.95	33.50	32.08	33.86	32.22	33.45
55	31.91	33.49	32.13	33.83	32.18	33.45

Exp.2

Table 4: Changes in heart rate (mean per 2.5 min)

	DPG (Ctl)	Cedarwood	Chamomile	Chocolate	Strawberry	Green tea	Apple	Citrus ginger	Musk
2.5	74.6	76.8	75.6	73.8	76.4	75.7	79.1	74.4	75.5
5	74.1	75.7	75.0	72.8	75.6	75.7	76.9	74.9	75.0
7.5	73.2	76.1	74.6	72.5	75.2	75.0	76.2	73.0	75.4
10	73.5	75.4	75.9	71.9	75.0	74.4	77.8	73.7	75.5
12.5	75.4	79.6	77.7	75.3	77.4	77.6	78.5	77.2	78.5
15	75.6	79.4	79.6	75.5	76.8	77.6	80.3	75.6	76.5
17.5	75.8	79.0	78.2	75.1	76.8	75.8	78.2	76.0	74.7
20	77.5	82.5	78.9	75.7	77.5	76.8	76.1	75.4	76.4
22.5	77.4	80.9	77.9	75.0	76.6	75.0	76.7	74.9	77.5
25	77.4	80.1	78.0	73.8	77.0	76.7	76.5	75.5	79.0
27.5	77.5	78.7	78.5	73.2	75.9	76.2	77.2	75.1	78.9
30	75.9	79.2	78.0	74.8	77.2	76.3	77.4	74.7	78.8
32.5	75.3	79.2	79.0	73.6	76.4	76.1	77.2	74.9	77.1
35	76.5	78.2	80.3	74.7	77.5	77.8	75.7	75.3	78.9
37.5	77.1	80.0	77.1	73.4	77.3	78.0	75.5	73.8	78.4
40	77.6	79.3	79.3	74.8	78.1	78.6	77.1	76.2	80.2
42.5	76.4	74.0	77.9	72.3	76.8	75.5	77.4	73.7	76.3
45	74.1	73.5	75.3	71.2	75.4	74.7	75.2	71.3	74.7
47.5	73.0	73.2	73.7	72.3	74.1	74.4	75.7	71.3	76.5
50	73.2	74.3	74.8	72.7	72.2	71.0	74.0	73.5	74.4
52.5	72.6	73.6	75.3	70.3	73.6	69.9	73.3	72.8	73.9
55	72.2	72.1	74.6	70.2	71.9	68.9	73.5	72.2	74.7

Table 5: Changes in high-frequency component of heart rate variability (mean per 2.5 min)

	DPG (Ctl)	Cedarwood	Chamomile	Chocolate	Strawberry	Green tea	Apple	Citrus ginger	Musk
2.5	606.68	343.10	622.73	393.16	430.66	698.06	352.89	695.40	374.89
5	487.89	505.80	642.17	446.32	529.18	483.63	481.91	694.50	477.28
7.5	507.38	477.58	911.63	691.49	601.67	521.37	595.37	764.10	390.02
10	576.06	462.68	818.20	673.20	626.17	529.60	525.98	772.80	453.37
12.5	636.44	293.32	609.75	375.87	504.30	522.83	731.18	832.03	277.99
15	476.97	376.54	747.71	598.70	541.07	526.04	516.56	834.58	272.01
17.5	527.24	443.91	608.27	628.81	473.68	543.81	878.06	971.89	423.07
20	538.79	507.33	613.53	594.98	506.74	480.83	1266.84	866.37	357.31
22.5	430.84	493.15	501.48	748.71	557.30	585.77	1223.55	940.78	263.19
25	484.05	520.24	481.28	725.63	485.07	473.03	1200.98	981.73	273.26
27.5	357.41	457.15	449.03	519.72	592.54	521.14	820.69	894.90	283.95
30	463.44	516.49	558.91	553.45	616.29	595.51	927.22	913.07	272.06
32.5	366.52	299.50	525.38	615.11	622.45	515.97	820.92	1026.27	271.08
35	411.76	491.30	721.12	554.59	502.57	442.97	891.14	823.43	293.47
37.5	470.25	369.15	503.25	687.28	609.47	414.96	890.07	885.24	311.15
40	572.90	499.90	585.93	486.51	484.31	517.74	782.79	738.10	235.08
42.5	460.29	691.54	551.49	773.88	622.80	648.54	497.23	865.52	476.72
45	465.13	607.63	667.21	759.36	651.25	764.18	888.07	1006.62	475.64
47.5	564.22	552.82	929.20	591.16	654.86	572.17	915.93	722.08	518.27
50	700.52	495.05	843.98	616.56	674.57	710.63	895.14	716.42	483.15
52.5	509.41	552.63	683.20	646.93	882.75	703.23	870.87	720.98	442.92
55	669.88	451.16	657.85	717.72	955.74	861.01	807.84	823.34	474.02

Table 6: Changes in skin conductance level (mean per 2.5 min)

	DPG (Ctl)	Cedarwood	Chamomile	Chocolate	Strawberry	Green tea	Apple	Citrus ginger	Musk
2.5	1.34	0.95	0.89	1.17	1.11	0.60	0.82	0.69	0.66
5	1.26	0.91	0.83	1.05	1.08	0.69	0.70	0.64	0.60
7.5	1.24	0.86	0.85	1.02	1.04	0.77	0.64	0.59	0.60
10	1.24	0.88	0.89	1.06	1.06	0.75	0.73	0.61	0.60
12.5	1.53	1.24	1.25	1.33	1.45	1.18	1.42	0.85	0.88
15	1.51	1.68	1.21	1.31	1.88	1.18	1.32	0.80	0.79
17.5	1.49	1.81	1.26	1.31	1.89	1.20	1.34	0.78	0.79
20	1.50	1.89	1.34	1.25	1.93	1.33	1.25	0.78	0.79
22.5	1.48	1.91	1.27	1.24	1.99	1.34	1.20	0.83	0.82
25	1.52	1.87	1.30	1.23	2.04	1.30	1.17	0.80	0.81
27.5	1.52	1.87	1.31	1.27	2.03	1.33	1.17	0.83	0.85
30	1.50	1.89	1.29	1.33	2.09	1.41	1.15	0.82	0.84
32.5	1.57	1.92	1.32	1.34	2.11	1.39	1.13	0.87	0.84
35	1.57	1.89	1.38	1.34	2.13	1.25	1.13	0.89	0.83
37.5	1.60	1.92	1.40	1.32	2.12	1.32	1.15	0.90	0.90
40	1.62	1.91	1.41	1.34	2.08	1.35	1.18	0.98	0.99
42.5	1.73	1.92	1.45	1.45	2.11	1.42	1.35	1.16	1.04
45	1.63	1.80	1.37	1.38	1.94	1.26	1.28	1.21	0.92
47.5	1.63	1.78	1.22	1.39	1.78	1.21	1.25	0.95	0.89
50	1.76	1.79	1.14	1.41	1.72	1.12	1.13	0.87	0.86
52.5	1.76	1.78	1.09	1.46	1.69	1.16	1.07	0.89	0.84
55	1.63	1.61	1.07	1.45	1.60	1.07	1.11	1.10	0.90

Exp.3

Table 7: Changes in heart rate (mean per 2.5 min)

	DPG (Ctl)	Chamomile	Apple
2.5	71.7	70.7	73.0
5	71.2	71.4	72.8
7.5	70.7	71.7	72.5
10	71.6	72.0	73.7
12.5	73.8	74.5	75.6
15	73.6	74.0	75.4
17.5	73.0	73.4	74.6
20	73.7	73.8	73.8
22.5	73.8	73.2	73.8
25	73.5	73.5	74.0
27.5	73.9	73.7	73.8
30	74.0	73.5	74.2
32.5	73.0	73.6	73.5
35	73.8	73.5	73.6
37.5	74.1	74.1	73.4
40	75.3	74.9	74.5
42.5	73.2	73.6	74.1
45	70.7	71.5	71.9
47.5	70.1	71.1	71.8
50	70.0	70.9	70.5
52.5	70.9	71.8	70.9
55	70.2	71.0	69.6

Table 8: Changes in high-frequency component of heart rate variability (mean per 2.5 min)

	DPG (Ctl)	Chamomile	Apple
2.5	518.97	521.46	417.59
5	462.50	502.09	458.92
7.5	442.05	588.31	488.70
10	485.31	572.78	436.17
12.5	401.92	472.67	416.16
15	348.84	491.72	362.81
17.5	425.24	454.26	438.25
20	388.10	441.25	538.06
22.5	394.97	417.63	563.79
25	383.76	430.04	534.32
27.5	340.46	411.94	459.60
30	372.14	439.95	457.51
32.5	378.26	430.80	491.15
35	340.38	501.50	498.40
37.5	390.44	429.38	518.19
40	362.90	436.68	469.43
42.5	499.18	489.15	469.05
45	501.40	572.08	595.21
47.5	583.78	624.73	553.87
50	613.93	620.70	571.83
52.5	534.63	595.19	573.80
55	534.18	562.69	565.81

Table 9: Changes in nose, hand, and forehead temperatures (mean per 2.5 min)

	DPG (Ctl)			Chamomile			Apple		
	Nose	**Hand**	**Forehead**	**Nose**	**Hand**	**Forehead**	**Nose**	**Hand**	**Forehead**
2.5	31.17	30.61	33.58	32.01	31.03	33.78	31.64	30.66	33.61
5	31.23	30.65	33.61	32.16	31.28	33.82	31.78	30.79	33.71
7.5	31.28	30.66	33.66	32.20	31.50	33.88	31.74	30.85	33.77
10	31.18	30.64	33.66	32.15	31.56	33.92	31.66	30.85	33.71
12.5	30.92	30.61	33.66	31.89	31.44	33.93	31.29	30.76	33.68
15	30.74	30.56	33.71	31.69	31.36	33.95	31.05	30.72	33.67
17.5	30.71	30.52	33.76	31.69	31.33	33.97	31.09	30.76	33.74
20	30.60	30.48	33.75	31.70	31.35	33.99	31.14	30.78	33.75
22.5	30.67	30.48	33.78	31.68	31.35	34.01	31.34	30.80	33.84
25	30.75	30.54	33.80	31.63	31.36	34.01	31.37	30.82	33.90
27.5	30.81	30.57	33.83	31.58	31.33	33.98	31.38	30.78	33.90
30	30.80	30.61	33.85	31.63	31.27	33.98	31.29	30.77	33.86
32.5	30.83	30.57	33.82	31.68	31.26	33.99	31.28	30.68	33.87
35	30.72	30.45	33.75	31.70	31.19	33.96	31.26	30.56	33.88
37.5	30.64	30.38	33.70	31.70	31.15	33.96	31.18	30.49	33.86
40	30.50	30.33	33.68	31.61	31.08	33.96	31.03	30.38	33.84
42.5	30.56	30.23	33.75	31.70	31.06	33.99	31.13	30.27	33.86
45	30.67	30.18	33.71	32.01	31.15	34.07	31.43	30.26	33.82
47.5	30.78	30.27	33.68	32.14	31.23	34.04	31.55	30.29	33.86
50	30.84	30.27	33.64	32.08	31.31	33.99	31.57	30.29	33.81
52.5	30.88	30.17	33.69	31.98	31.28	33.95	31.58	30.35	33.80
55	30.90	30.21	33.69	31.91	31.25	33.93	31.50	30.38	33.72